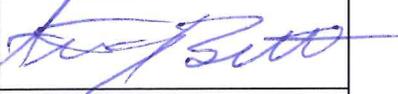


**QUALITY ASSURANCE PROJECT PLAN (QAPP)
FOR JAMES RIVER CHLOROPHYLL-a STUDY**

Special Study #14098

**Commonwealth of Virginia
Departmental of Environmental Quality
Central Office
629 East Main Street
Richmond, VA 23218**

Document Approval Initials:

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Ecology Office Director	John Kennedy	9/12/14	
Project Manager	Arthur Butt	9-12-14	
VADEQ Water Quality Monitoring Quality Assurance Coordinator	James Beckley	9-12-14	
ODU Project Leaders	Todd Egerton and Margie Mulholland		
VCU Project Leader	Paul Bukaveckas		
VIMS Project Leaders	Kimberly Reece and Ken Moore		
VIMS Project Leader (SONE Study)	Iris Anderson		

See Appendix 9 for signatures from Drs. Iris Anderson, Paul Bukaveckas, Todd Egerton, Ken Moore, Margie Mulholland, and Kimberly Reece

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VIMS Field Team Coordinator – SONE Study	Dr. Jennifer Stanhope

1. PROJECT MANAGEMENT

1.1. Project/Task Organization

1.1.1. Management Responsibilities

1.1.1.1. Water Division Director

The Water Division Director, Melanie Davenport, is responsible for the final approval of the Quality Assurance Project Plan (QAPP), including all associated monitoring costs.

1.1.1.2. Ecology Office Director

The Ecology Office Director, John Kennedy, is responsible for the approval of the project design.

1.1.1.3. Project Manager

The Project Manager, Dr. Arthur Butt, is responsible for project design and performing or overseeing project activities and data evaluation activities.

1.1.1.4. Water Quality Monitoring (WQM) Quality Assurance Coordinator

The WQM QA officer, James Beckley, works independently of the project manager and will report on the adequacy, status, and effectiveness of the QA program to the project manager, review audits of field activities, review laboratory QA performance, and ensure that corrective action, if necessary, is properly implemented and documented.

1.1.1.5. Project Leader

The project leaders, Drs. Harold Marshall and Todd Egerton (ODU Phytoplankton), Margie Mulholland (ODU Monitoring and Laboratory), Paul Bukaveckas (VCU), Ken Moore (VIMS), Kimberly Reece (VIMS), and Iris Anderson (VIMS) will supervise the implementation of the project related to their respective organizations including developing reports and management decisions.

1.1.1.6. Quality Assurance Officer

The Quality Assurance Officers, Michael F. Lane (ODU), Paul Bukaveckas (VCU), Ken Moore (VIMS Field), Kimberly Reece (VIMS Laboratory), and Iris Anderson (VIMS SONE Laboratory) are responsible for overseeing all quality assurance aspects of the project related to their respective organization. The Quality Assurance Officers will report deficiencies to their Project Leader. Significant QA deficiencies are to be reported to the DEQ Project Manager, Arthur Butt.

1.1.1.7. Field Team Coordinator (FTC)

The FTCs are Todd Egerton and Katherine Filippino (ODU), William Lee (VCU), Betty Neirkirk (VIMS), and Jennifer Stanhope (VIMS). The FTCs are responsible for monitoring and directing the field effort of the project for their respective organization including data entry. The FTC will ensure that the field technical staff is properly trained and equipped to execute the field sampling methods and procedures, and that the QA/QC procedures described in this QAPP are followed.

1.1.1.8. Field Staff

The field staff is responsible for all field activities including preparation and calibration of equipment, preparation and organization of sampling equipment and containers, collection of samples, packaging and preparation of samples for transportation to the contract lab and any other assignments as needed.

1.2. Problem Definition/Background

The U S Environmental Protection Agency (USEPA) listed the tidal James River as impaired under the Clean Water Act in 1999 for violation of Virginia's Water Quality Standards. Existing nutrient and sediment enriched water quality conditions within the entire tidal river were not supporting "balanced" populations of aquatic life protected under Virginia's state water quality standards regulations (9 VAC 25-260-10). These principal water quality impairments, which persist in the tidal James River, include excessive nutrients that nourish undesirable and nuisance algae, which, in combination with excessive sediments, greatly increase the risk of harmful algae blooms and create extremely poor water clarity conditions for underwater bay grasses. These water quality conditions in the tidal James River also don't support the desired aquatic life as identified within Virginia's proposed designated use for open-waters in the Chesapeake Bay and its tidal tributaries.

Recent determinations by EPA during the Chesapeake Bay TMDL development process call into question the conclusions and agreements reached during Virginia's 2005 rulemaking process for the chlorophyll criteria. The final nutrient allocations for the James River basin issued by EPA on December 31, 2010 are significantly more stringent than the levels that formed the basis for the state regulatory actions in 2005 for the chlorophyll criteria. Achieving these more stringent allocations would require additional expenditures estimated between \$0.5 to 1.0 billion to the restoration costs in the James basin. In addition, technological advancements in field monitoring since 2005 for the chlorophyll parameter provide greater understanding of the concentrations and variability of chlorophyll in the tidal James River. These advancements include "dataflow" monitoring which provides thousands of data points during a single monitoring cruise. Additional scientific research has since provided a greater understanding of the impact of algae blooms on aquatic life. It was concluded that additional scientific study is needed to

provide a more precise and scientifically defensible basis for setting the final nutrient allocations.

The Virginia Department of Environmental Quality (DEQ) is undertaking a comprehensive review of the existing site-specific numeric chlorophyll-a criteria (Table 1) and associated modeling framework for the tidal James River. This effort will be used to support a scientific study for a water quality standards review and potential rulemaking on the existing criteria.

A Science Advisory Panel was established by DEQ to provide recommendations on data and modeling needs for assessing the existing chlorophyll standards. Based on their review of the existing data, the Panel recommended that additional data were needed to characterize the occurrence of blooms (e.g., intensity, duration, and spatial extent) and to establish quantitative linkages between algal blooms and designated uses. A summary of their recommendations are outlined in the “Data and Modeling Needs for Assessing Numeric Chlorophyll-a (CHL_a) Criteria of the James River” (see following link).

http://www.deq.virginia.gov/Programs/Water/WaterQualityInformationTMDLs/WaterQualityStandards/RulemakingInfo.aspx#James_Chlorophyll_a_study

Table 1. Water Quality Standards

Parameter	Water Quality Standard
DO	Instantaneous > 4.0 mg/l, mean daily > 5.0 mg/l
pH	6.0 – 9.0 S.U.
Ammonia	Acute and Chronic, pH Dependent
Chlorophyll-a	March 1-May 31: 15 ug/l JMSTF1 & JMSTF2; 12 ug/l JMSMH & JMSPH; 10 ug/l JMSTF2
	July 1-September 3: 23 ug/l JMSTF1; 22 ug/l JMSTF2; 15 ug/l JMSTF2; 10 ug/l JMSMH & JMSPH

1.3. Monitoring History

1.3.1. DEQ Monitoring History

The Virginia Tributary Monitoring Program (VTMP) is an ongoing water quality-monitoring program implemented by the Commonwealth of Virginia and its contractors in the summer of 1988 as one component of the overall Federal-Interstate Chesapeake Bay Monitoring Program (CBMP) operating Bay-wide. The CBMP also includes long-term monitoring of phytoplankton communities, benthic communities, and submerged aquatic vegetation as well as occasional special monitoring for such things as sediment toxics or sediment nutrient fluxes. This comprehensive and coordinated monitoring of basic environmental aspects of the Bay provides a huge amount of information for determining important ecological inter-relationships. This scientific understanding provides the “sound science” basis for support and development of management actions. A full description of the VTMP is available through the VTMP Quality Assurance/Quality Control Project Plan (effective July 1, 2011).

1.3.2 Dataflow

Real-time mapping of algal blooms has been achieved through a method of on-board and underway monitoring (DATAFLOW) of CHL_a coupled with GPS navigation. Presently this technology is employed by the Hampton Roads Sanitation District (HRSD) scientists to map spatial variation in CHL_a for the meso- and poly- haline segments of the James, Elizabeth and Lafayette Rivers on a weekly basis. This method provides the most effective means for determining the size, intensity and location of algal blooms. Both VIMS and HRSD have established identical methodologies over the past several years and employ the latest QA/QC procedures (Quality Assurance Project Plan for the Water Quality Monitoring for Bay Water Quality Standards Assessment). The Dataflow system currently used by HRSD is modeled after the VIMS system and companion mapping runs were initially conducted to assure comparability of measurements. All HRSD and VIMS Dataflow data and visualizations of the data are served on the VIMS VECOS site (www3.vims.edu/vecos).

1.4. Project/Task Description

This section describes the project tasks and work schedule.

1.4.1. Project Tasks

Task 1. The project manager develops the Quality Assurance Project Plan (QAPP). The QAPP must describe the whole project in detail. The QAPP must be approved prior to implementation.

Task 2. The project manager establishes stations and determines sample collection logistics.

Task 3. The FTC will oversee the field staff in setting up the sampling runs, collecting field data, and shipping samples to the appropriate laboratory for analysis. The sampling procedures are presented in Section 2.2.

Task 4. The QA officer will validate sampling accuracy by comparing the results of split and blank samples to assess for bias, completeness, representativeness, and acceptable levels of precision and accuracy.

Task 5. The QA officer and/or FTC will audit field activities throughout the project. The QA officer will communicate with the laboratory as needed to verify laboratory performance. These technical systems audits are further described in Section 3.

Task 6. The project manager will submit the final report when the data have all been collected, validated, approved, and analyzed.

1.4.2. Work Schedule

Table 2 reflects the work schedule for this study.

Table 2. Work Schedule

Task	Description	Schedule
1	Develop QAPP	June 2012 -December 2013
2	Establish stations, determine sample collection logistics	January 2012-December 2013
3	Collect field data	January 2012 – December 2014
4	Laboratory analysis of water samples	January 2012 – December 2014
5	Data validation	January 2012 – December 2014
6	Auditing	January 2012 – December 2014
7	Submittal of the final report	Mid 2015

1.4.3. Estimated Project Costs

The estimated project costs for years one and two of the study are summarized in Table 3. The Virginia General Assembly authorized the expenditure of non-general funds to support the James River Chlorophyll Study. The total allocation was \$3 million with about \$1.2 million allocated for monitoring and data collection. Table 3 summaries the various studies being conducted in years one and two of this study.

Table 3. Estimated Project Costs for Years One and Two of the Study

Project Activity – Year One	Estimated Cost – Year One
Algal identification (ODU)	\$59,840
Harmful Algal Bloom Studies in lower James River (ODU)	\$136,999
Dataflow and bioassays in lower James River (VIMS)	\$ 182,378
Sediment, oxygen, and nutrient exchange study	\$83,027
Algal characterization, monitoring and grazing studies in upper tidal James River (VCU)	\$194,619
Project Activity – Year Two	Estimated Cost – Year Two
Algal identification (ODU)	\$82,929
Identification of algal bloom triggers in lower James River (ODU)	\$60,586
Dataflow and bioassays in lower James River (VIMS)	\$154,568
Experiments and bioassays to assess impact of harmful algae (VCU)	\$123,713

1.5. Quality Objectives and Criteria for Measurement Data

Data produced by this study are being used to help parameterize the site-specific James River water quality model as well as to characterize algal blooms, environmental factors that favor algal blooms, and to qualify and quantify their potential impacts on the aquatic life of the James River. Both field and laboratory personnel will work to achieve the highest possible level of confidence in the quality of study results by using established procedures to ensure the accuracy, precision, representativeness, comparability, and completeness of the data.

1.6. Special Personnel Training Requirement

No specialized training is required to conduct this study. The field and technical teams from ODU, VCU, and VIMS will follow standard sampling procedures as outlined in their respective SOP manuals included in Appendices 1 - 8.

1.7. Documentation and Records

Documentation of field and laboratory data is to be stored in DEQ's Comprehensive Environmental Data System (CEDS). Some of the parameters that may be stored in CEDS

can be found in Table 4. In addition, the QAPP and all final reports are to be stored on the database and hard copies kept on file. This section identifies the documents and reports to be generated throughout this study and the information to be included in those documents and reports.

Copies of the QAPP and any other interim or final reports are to be made available online at

<http://www.deq.virginia.gov/Programs/Water/WaterQualityInformationTMDLs/WaterQualityStandards/NutrientCriteriaDevelopment.aspx> under “James River Chlorophyll a Study”.

Records are stored electronically for an indefinite period of time.

1.7.1. Field Documentation

Field documentation requirements are described in section 2.2.4. In summary, the field team will be responsible for maintaining the following documents:

- (1) Field Data Sheet
- (2) Quality Control Checks for pre- and post-calibration checks of field equipment.
- (3) Sample container labels
- (4) Any other paperwork necessary for shipping or delivering to a laboratory

1.7.2. Laboratory Documentation

Laboratory documentation will include producing the following information:

- (1) Electronic data transfer to VADEQ of final certified data,
- (2) Printed copies of Certificates of Analysis when specifically requested to do so,
- (3) Any other data associated with the measurement process when specifically requested to do so.

1.7.3. Audits

The ODU, VCU, and VIMS QA officers will conduct field and technical audits as needed to ensure protocols as described in their respective SOPs are being followed (SOPs can be found in appendices 1- 8). DEQ will be notified of any issues and corrective actions taken.

1.7.4. Data Validation

All research and monitored data will conform to EPA CBP or DEQ specified data requirements unless noted otherwise. Only valid and certified data will be transferred to the VADEQ from the laboratories. Where applicable, data validation flags will be applied to those sample results that fall outside of specific limits. If necessary, DEQ will request the contracted laboratory QA officer to identify biases inherent in the data, including assessment of laboratory performance, and overall precision, accuracy, representativeness and completeness.

Table 4. STORET Parameter Codes

Parameter Code	Parameter Name
00010	Temperature C
00094	Specific Conductance (UMHOS/cm at 25 C)
00299	Dissolved Oxygen, probe (mg/L)
00400	Field pH (SU)
00610	Ammonia (mg/L as N)
00615	Nitrite (mg/L as N)
00620	Nitrate (mg/L as N)
00625	Nitrogen, Total Kleldahl (mg/L as N)
00630	Nitrogen, Total (mg/L as N)
00665	Phosphorus, Total (mg/L as P)
00671	Orthophosphorus, Dissolved (mg/L as P)
70507	Orthophosphorus, Total (mg/L as P)
32230	Chlorophyll a (mg/L

2. MEASUREMENT/ DATA ACQUISITION

2.1. Experimental Design

The James River Chlorophyll-a Study is designed to address a number of data needs identified by the Science Advisory Panel in 2011 (section 1.2). To address these data needs, DEQ awarded contracts to ODU, VCU, and VIMS to conduct field monitoring and laboratory studies. Detailed descriptions of the contracted work can be found in the following DEQ contracts: #15425 (ODU), #15433 (VCU), and #15427 (VIMS). A summary of this work is provided below.

2.1.1. Upper James River (Tidal Freshwater)

Characterizing Algal Blooms in the Upper James River (2012 and 2013): In the upper James River, Virginia Commonwealth University (VCU) will collect water quality data and water samples from 12 stations located between river miles 56 and 110 from May through October. Station locations and coordinates are provided in Table 5a. Water quality data are obtained by employing the Hydrolab sonde. Temperature, pH, conductivity and dissolved oxygen (concentration and saturation) are recorded at each site along with the time of sample collection. Water samples for TSS, CHLa and nutrient analyses are transferred to the VCU Environmental Analyses Lab (EAL) for analysis as per the VCU Bukaveckas Field and CHLa SOPs (Appendices 1 and 2). Phytoplankton samples are retained by EAL for

subsequent transport to Old Dominion University (ODU) for phytoplankton identifications as per the ODU Egerton SOP (Appendix 4) at the stations identified in Table 5a. Phytoplankton samples will be analyzed for HAB and non-HAB species, seasonal and spatial community compositions, as well as relationships between algal groups, individual species, and CHLa. For algal bioassay experiments, water will be collected from two stations (JMS75 and Rice Pier) during the weekly river monitoring cruises (VCU Bukaveckas Field SOP; Appendix 1).

Nutrient Limitation (2012): VCU will conduct algal bioassay experiments to identify limiting nutrients and to quantify light and nutrient effects on phytoplankton growth rates. Additionally, ODU will examine water column nitrogen regeneration and the sources of nitrogen fueling algal growth in the upper James River using stable isotopes as tracers. This work will quantify algal growth responses to variable light and nutrient conditions to provide a better understanding of the means by which algal blooms in this part of the river are initiated and sustained. (VCU Bukaveckas Lab SOP and ODU Mulholland SOPs; Appendix 3 and 5)

Top Down Effects - Grazing Experiment (2012): VCU will undergo a sampling program to identify the main consumers of algal biomass in the James River food web in order to determine the impact of grazing by benthic filter-feeders and the potential importance of grazing by fish on suspended algae. (VCU Bukaveckas Lab SOP; Appendix 3)

Characterize the Occurrence of Cyanotoxins (2012 and 2013): Water, sediment, and tissue samples will be collected for an analysis of microcystin concentrations which will be determined using an Enzyme-Linked Immunosorbent Assay (ELISA) kit (Abraxis). Collection of weekly water samples and the analysis of MC are described in the VCU Bukaveckas SOPs (Appendices 1, 2, and 3).

Longitudinal Study – the spatial extent of microcystin (2013): In order to assess the extent of toxin export from the tidal freshwater portion of the James River estuary, water samples will be collected from 11 James River DEQ stations (TF5.2, TF5.2A, TF5.3, TF5.5, TF5.5A, TF5.6, RET5.2, LE5.1, LE5.2, LE5.3, LE5.4; see tables 5a and 5c for station description) in July, August, and September and then analyzed for microcystin concentrations using an ELISA kit. Water samples will also be collected at each of these stations for phytoplankton analysis by ODU. (VCU Bukaveckas Field SOP and ODU Egerton SOP; Appendices 1 and 4)

Density of dominant fish species (2013): A survey of fish will be conducted in June and August using low and high electrofishing in order to estimate densities of dominant fish species in the tidal freshwater portion of the estuary. This data will be used in conjunction with previously derived estimates of CHLa ingestion for the purpose of determining the proportion of algae removed from the estuary by fish. (VCU Bukaveckas Lab SOP; Appendix 3)

Comparative study of toxin levels in fish (2013): Fish will be collected at three sites to assess the spatial extent of toxin exposure for living resources. The upper site is a region where toxin levels in the water are low; the middle site is in a region where toxin levels in the

water are elevated; and the most downstream site is in a region where the toxin levels are unknown. (VCU Bukaveckas Lab SOP; Appendix 3)

Dose-Response experiments to determine the effect of microcystin on health of selected native aquatic species (2013): 1) Effects of microcystin on zooplankton growth, survivorship, and reproduction and 2) Effects of microcystin on *Rangia* (wedge clams) grazing rates and 3) Effects of microcystin on growth and survivorship of early life stages of selected fish species and 4) Field experiments to assess chronic effects of microcystin exposure and 5) Laboratory experiments to assess the effects of acute exposure on Atlantic sturgeon (obtained from Canadian waters). (VCU Bukaveckas Lab SOP; Appendix 3)

2.1.2. Lower James River (oligohaline, mesohaline, polyhaline of the James River and including the Elizabeth and Lafayette tributaries)

Algal and water quality characterization using DATAFLOW (2012 and 2013): The DATAFLOW system allows the continuous measurement of dissolved oxygen, chlorophyll-a, turbidity, salinity, specific conductivity, temperature, and pH while underway in a small boat. VIMS will carry out DATAFLOW cruises in one Chesapeake Bay Program segment, the James River oligohaline (JMSOH). The Hampton Roads Sanitation District (HRSD) conducts weekly DATAFLOW cruises in the remaining saline segments of the James including the Elizabeth and Lafayette River tributaries. Both VIMS and HRSD have established identical methodologies and QA/QC procedures (Quality Assurance Project Plan for the Water Quality Monitoring for Bay Water Quality Standards Assessment.) Each DATAFLOW cruise has established verification stations. Chlorophyll and pheophytin, total suspended solids (TSS), volatile suspended solids (VSS), and dissolved oxygen are measured using a YSI 600 XLM sonde. Secchi depth, vertical profiles of photosynthetically available radiation (PAR) and temperature, dissolved oxygen, pH, salinity, and specific conductivity are also measured. In addition, bloom samples will be collected for phytoplankton enumeration by researchers at VIMS in the Reece laboratory and/or by researchers at ODU in Dr. Marshall's lab.

- VIMS DATAFLOW: DATAFLOW will be conducted in one Chesapeake Bay Program segment, the James River Oligohaline (JMSOH), by the Virginia Institute of Marine Science (VIMS). Data will be collected from 0.25 - 0.5m below the surface. VIMS DATAFLOW verification stations are listed in Table 5b. Cruises and collections, and analyses will be conducted as per the VIMS Moore SOP (Appendix 6).
 - 2012: Data collection will be performed twice a month from May through June during one spring and one neap tidal period. During the most intensive bloom period of July-September, the cruises will be conducted weekly and then in October they will be conducted twice monthly again.
 - 2013: Data collections will occur weekly during the spring bloom (expected onset is sometime in February or March) and then monthly until August; at which time frequency of cruises will be assessed dependent upon bloom conditions. Following the summer bloom, cruises will occur monthly through October.

- **HRSD/ODU DATAFLOW:** HRSD conducts weekly cruises in the mesohaline and polyhaline portions of the James as well as the Elizabeth and Lafayette Rivers. This study does not provide financial support to HRSD however the study does provide funding to ODU in 2012 and 2013 for their role in participating in and expanding the DATAFLOW program during scheduled weekly cruises as well as ad hoc cruises in response to storm events. In addition to the collection of samples for water quality parameters as outlined above, ODU will also be collecting samples for nutrient analyses. (ODU Mulholland SOP; Appendix 5)

Characterizing algal composition in the lower James River estuary (2012 and 2013): Water samples will be collected for phytoplankton analysis from the following stations: BC1, RET5.2, LE5.1, LE5.2, LE5.3, LE5.4, LE5.5, LE5.6, LFB01 (Table 5c). Samples will be analyzed for HAB and non-HAB species, seasonal and spatial community compositions, as well as relationships between algal groups, individual species, and CHLa. Additional phytoplankton samples will be collected during bloom events based on in-situ DATAFLOW chlorophyll readings. (ODU Egerton SOP; Appendix 4)

Quantifying variability in water quality using CONMON (2012 and 2013): In the lower James estuary water quality data and water samples are collected from three continuous monitoring (CONMON) stations. CONMON stations will quantify short-term variability and long-term changes in water quality constituents in specific shallow water areas by using a YSI extended deployment data sonde which will sample dissolved oxygen, pH, chlorophyll, turbidity, salinity, specific conductivity, and temperature. Station locations and coordinates are provided in Table 5b. (ODU Mulholland and VIMS Moore SOPs, Appendices 5 and 6)

Investigation of the distribution of major algal groups (2012): ODU will use diagnostic pigment analysis to identify the distribution of algal groups using diagnostic pigments. This data will provide a means for modelers to distinguish bulk chlorophyll impairments from the occurrence of potentially harmful algal bloom taxa. (ODU Mulholland SOP; Appendix 5)

Environmental factors that favor algal blooms (2012 and 2013): Another aspect of the study in the lower estuary is to determine the environmental factors that favor blooms. Both physical and nutritional factors promoting *Cochlodinium* blooms are being investigated. ISCO samplers will collect water before, during, and after storm events at several fixed stations including storm drains (Table 5b). Water samples will also be collected during ad hoc DATAFLOW cruises before, during, and after storm events. Data from these efforts will be used to determine relationships between physical and nutritional parameters and chlorophyll *a* concentrations. A complete description of these studies is available in the ODU Mulholland SOP (Appendix 5).

Evaluate potential adverse impacts to aquatic life (2012 and 2013): In order to evaluate potential adverse impacts to aquatic life, a series of bioassays will be conducted using both clonal cultures established for the important bloom organisms collected during the study and bloom samples. Test organisms include *Artemia salina*, *Ceriodaphnia reticulata*, *Crassostrea virginica*, and *Cyprinidon variegates*. Impacts will be determined through quantitative

measurements of morbidity and mortality caused by exposure to whole live cells and/or lysates. Impacts of harmful algal blooms on *Crassostrea virginica* will also be investigated through the deployment of oysters in cages near COMMON stations. During bloom events five to 10 oysters will be collected from these cages for histological analysis to determine if exposure causes pathological damage. (VIMS Reece SOP; Appendix 7)

Sediment Oxygen and Nutrient Exchange (2012-2013): VIMS will collect sediment cores at six sites in the James River (Table 5d) in order to measure sediment: water nutrient fluxes, metabolic rates and sediment characteristics. Collections will be made once in the summer of 2012 and once in the spring of 2013 at each of the six sites. This work will provide data for the James River water quality model. A complete description is available in the VIMS Anderson SOP (Appendix 8).

Table 5a. Upper James River Station Locations

Station	Nearest DEQ CBP Station	Description	Latitude	Longitude	Phytoplankton Sample-Year
Huguenot	-	James River – Huguenot Bridge	37.561583	-77.543806	N
Mayo's Bridge	TF5.2	James River – JMS 110	37.5305	-77.434	N
B168	-	James River	37.49606	-77.42354	N
B166	TF5.2A	James River – JMS 104	37.449835	-77.418533	N
B157	TF5.3	James River – JMS 99	37.40098	-77.387	Y-2012 and 2013
B150	-	James River	37.3789	-77.34747	N
B138	-	James River	37.382532	-77.257715	N
APP 1.5	App1.53	Appomattox – Rt 10 Bridge	37.3121	-77.296617	Y-2012
B107	TF5.5	James River – JMS 75	37.312422	-77.233105	Y-2012 and 2013
B91	TF5.5A	James River – JMS 69	37.301728	-77.128805	Y-2012
Rice Pier	-	VCU Rice Center	37.3252	-77.2044	N
B74	TF5.6	James River – JMS 56	37.272372	-76.990672	N

Table 5b. Lower James River DATAFLOW verification stations, COMMON stations, and storm drain stations

STATION	DESCRIPTION	LATITUDE	LONGITUDE
JMSOH1	DATAFLOW Verification	37.21335	-76.91730
JMSOH2	DATAFLOW Verification	37.20294	-76.78219
JMSOH3	DATAFLOW Verification	37.20297	-76.64833
JMSOH4	DATAFLOW Verification	37.22775	-76.79147
JMSOH5	DATAFLOW Verification	37.23980	-76.87915
Mesohaline Pier	FIXED Continuous Monitoring located on a private pier	37.04883	-76.50450
AC	Fixed Station Continuous Monitoring located on a pier off of Ashland Circle	36.88039	-76.27253
NYCC	Fixed Station Continuous Monitoring located on a pier belonging to Norfolk Yacht CC	36.90648	-76.30595
CP Storm Drain	Storm drain located near Colonial Place draining a residential neighborhood	36.8864	-76.2905
WHRO storm drain	Storm drain near WHRO radio station draining a mixed use area	36.8886	-76.3008

Table 5c. Station locations for phytoplankton identification

Station	Description	Segment/Salinity Region	Latitude	Longitude
BC1	Bailey's Creek- Hopewell Wastewater Treatment	James River- Tidal Fresh	37.2960	-77.2503
LE5.1	VIMS Slack Water; Red Buoy #36	James River-Oligohaline	37.20297	-76.64833
LE5.2	Buoy #C12-13	James River-Mesohaline	37.056	-76.59306
LE5.3	NH-15 James River Bridge; VIMS	James River-Mesohaline	36.99044	-76.47544
LE5.4	Buoy #9; Hampton Roads; Sampled By VADEQ	James River-Polyhaline	36.95486	-76.39275
LE5.5W	Mouth of the James River; 1000 Yards West of LE5.5	James River-Polyhaline	36.99903	-76.31328
LE5.6	Red Buoy #18	Elizabeth River-Polyhaline	36.90456	-76.33836
LFB01	VADEQ Station 2-LAF003.83. Lafayette River At Granby St. bridge.	Lafayette River-Mesohaline	36.89	-76.28333
RET5.2	Swann's Point; James River WQMP STA#19	James River-Oligohaline	37.20294	-76.78219
TF5.3	James River- Buoy #157 (JR WQMP Station #8)	James River-Tidal Fresh	37.4031	-77.39272

TF5.5	James River- Red Buoy #107 (JR WQMP Station #13)	James River-Tidal Fresh	37.31265	-77.23283
TF5.5A	James River-Buoy # 91	James River-Tidal Fresh	37.30165	-77.12840
App1.53	Near mouth of Appomattox River	Appomattox Tidal Fresh	37.3124	-77.2913

Table 5d. Station locations for sediment cores collected for SONE study

Site	Location	Water Depth (m; MSL)	Segment/Salinity Region	Latitude	Longitude	Nearest CBP Station
1	Tar Bay	1	James River Tidal Fresh	37.3058	-77.1847	TF5.5
2	Tar Bay	2	James River Tidal Fresh	37.3069	-77.1871	TF5.5
3	Chickahominy River near Simpson Island	1	Chickahominy River Tidal Fresh	37.3095	-76.8707	RET5.1A
4	Near 4-H Club above Jamestown Island and Jamestown-Scotland Ferry Pier	2	James River Oligohaline	37.2291	-76.7953	RET5.2
5	Near James River Country Club	2	James River Mesohaline	37.0448	-76.5066	Between LE5.2 and LE5.3
6	Lafayette River east of Hampton Road Bridge	1	Lafayette River Mesohaline	36.9021	-76.2988	--

2.2. Field Sampling Methods Requirements

This section briefly describes the field procedures for sample collection. A more detailed description is provided in the Standard Operation Procedures (SOP) manuals for the collecting organizations of VCU, ODU, and VIMS contained in Appendices 1, 4, 5, and 6.

2.2.1. Preparation for Field Work

Before field work begins, the Field staff will ensure that the necessary equipment is cleaned, calibrated and in good working order.

2.2.2. Sampling Preparation and Procedures

A specified series of procedures, summarized below, must be followed for sampling. However, in the event of extraordinary circumstances, whereby deviation from any established procedures is required, these deviations must be well-documented.

2.2.2.1. Sampling Preparation

All sampling equipment preparation, cleaning procedures, field calibration and testing procedures found in the SOPs referenced in section 2.2.

2.2.2.2. Sampling Procedures

All surface water sampling procedures found in the VCU, VIMS, and ODU SOPs will be followed. Flow measurements, when required, will be collected following manufacturer's instructions and SOP manuals.

2.2.3. Sample Containers, Preservation, and Holding Times

The required sample containers, preservation and maximum holding times are specified in the SOPs referenced in section 2.2.

2.2.4. Field Documentation

All necessary field documentation, including observations, measurements, and any other documentation pertaining to the survey will be kept by the field sampling team. Entries will be in blue or black indelible ink. Corrections will consist of a single line-out deletion and/or correction with initial of the person making the change.

2.2.5. Sample Numbering System

Standard sample identification procedures will be applied to each sample collected as outlined in the SOPs referenced in section 2.2.

2.2.6. Field Forms

The field sampling team will be responsible for maintaining field data sheets. An entry will be made on the field data sheet for each sample collected. The intent of the field data sheet is to document the place, date, time and depth for each sample collected. The same data sheet entry shall record any known deviation from the specified sampling described herein, and other pertinent field observations associated with the samples.

2.3. Field Corrective Action

Corrective actions will be initiated, at the direction of the QA officer, if the field team is not adhering to the sampling or documentation SOPs, or if laboratory results indicate interference, systematic contamination, or problems with sample handling protocols.

2.4. Sample Handling and Custody

The samples are preserved as described in the applicable SOP manual. Upon reaching the laboratory, samples will be handled in accordance with the laboratory sample handling procedures in the SOPs referenced in section 2.2.

2.4.1. Analytical method Requirements

All sample analyses will be conducted using either standard, approved analytical test methods or methods acceptable to DEQ. The analytical procedures and standard test methods used by the laboratory are identified in the SOPs referenced in section 2.2.

2.5. Quality Control Requirements

2.5.1. Field Quality Control

All field quality control samples will be collected in accordance with the applicable SOP manual. All QA samples will be entered into the online database.

2.5.2. Laboratory Quality Control

All laboratory samples will be analyzed in accordance with established standard laboratory methods, procedures and laboratory QA SOPs. This includes an evaluation of accuracy, precision, representativeness and comparability.

2.6. Instrument and Equipment Testing, Inspection, and Maintenance Requirements

The field staff will be responsible for the maintenance of equipment used to measure all the requested water quality field parameters, in accordance with the applicable SOP manual.

2.7. Inspection/Acceptance Requirements for Supplies and Consumables

The field staff will be responsible for inspecting incoming equipment and supplies to be used in the special study before placing them in service.

2.8. Data Management

Project data will include computer and handwritten entries. Field observations, measurements and records such as sample collection and shipping information will be recorded on hardcopy forms, and electronically. Data analyzed in the laboratory will be entered into the laboratory's sample database system (e.g. LIMS) by the responsible laboratory personnel. Following validation and approval, data is shipped electronically to DEQ.

3. OVERSIGHT AND ASSESSMENT

3.1. Technical System Audits (TSAs)

The QA officer or FTC will conduct TSAs of field activities. The contracted laboratory QA officer will conduct TSAs of laboratory operations as specified under the laboratory QA SOP and/or VELAP regulations.

Field TSAs focus on availability and proper use of field equipment; adherence to project-controlling documents for sample collection, identification, handling, and transport; proper collection and handling of QC samples. Laboratory TSAs include reviews of sample handling procedures, internal sample tracking, SOPs, analytical data documentation, QA/QC protocols, and data reporting.

4. DATA VALIDATION AND USABILITY

4.1. Data Review, Validation, and Verification

Collection and shipment of analytical samples to the appropriate laboratory will follow procedures outlined in the applicable SOP manual. To verify the accuracy of samples, the QA Officer will review calibration log sheets and the results of field equipment blanks and field splits to ensure they are within acceptable quality control limits. If quality control limits are exceeded, the QA Officer will communicate with the Project Manager to determine the necessary corrective action needed.

4.2. Validation and Verification Methods

The QA officer will perform data validation by reviewing and interpreting data. The analytical laboratory report will be reviewed for compliance with the applicable method and for the quality of the data reported. The data validation procedures will identify biases inherent in the data including assessment of laboratory performance, overall precision and accuracy, representativeness, and completeness. Data validation flags from the laboratory will be applied to those sample results that fall outside of the QC acceptance criteria.

4.3. Data Reduction, Analysis and Interpretation in Preparation of the Final Report

After the project is complete, the project manager is responsible for collecting all the data, analyzing the data and preparing a written final interpretation to be included in the final report.

5. REFERENCES

VA DEQ. 2011. Virginia Tributary Monitoring Program Quality Assurance/Quality Control Project Plan.

VA DEQ. 2011. Virginia Chesapeake Bay Tributary Water Quality Monitoring Program Standard Operating Procedures Manual.

6. APPENDICES

Appendix 1

VCU Bukaveckas Field Standard Operating Procedures

Virginia Commonwealth University
Environmental Analyses Laboratory
Standard Operating Procedure
River Water Quality Monitoring
Effective: July 1 2010
Revised: May 1 2012

Prepared by: Paul A. Bukaveckas & William M. Lee

This guide was developed to document data collection procedures for river monitoring performed by VCU personnel in the tidal freshwater James River. The procedures were implemented during monitoring activities conducted for the City of Richmond to assess water quality conditions. The document was revised in anticipation of additional data collection needs to be performed for the Virginia Department of Environmental Quality as part of the James River CHLa Study.

1.0 Scheduling, Station Locations and Sample Delivery

Sample runs are performed weekly on Tuesdays excluding the first Tuesday of each month. Decisions related to weather and boat safety are the responsibility of the boat captain who has the discretion to cancel all or part of the river run. In the event of cancellation, the run in its entirety is re-scheduled to Thursday of the same week and the respective lab managers (Environmental Analyses and Microbiology Labs) are notified.

Water quality data and water samples are collected from 13 stations located between river miles 56 and 110. Station locations are shown in Figure 1 and coordinates are provided in Table 1. The launch point is the VCU Rice Center and stations are sampled sequentially in an upstream direction beginning at the lowermost station (Buoy 74) and ending at B168. The samples are off-loaded at Osborne Landing and the final three stations are sampled from land (CSO, Mayo's Bridge and Huguenot Bridge) before returning samples to the lab. The elapsed time between collecting the first sample (Buoy 74) and the delivery of samples to the respective laboratories should not exceed 5 hours.

2.0 Pre-Cruise Preparation

In preparation for a sampling run, ensure that operating manual instructions have been followed concerning preventative maintenance and calibration for all equipment to be used. Hydrolabs are to be calibrated prior to each run. Be sure that the individual components (sonde, surveyor) are calibrated together and used as unit. Perform calibrations in the order by which they are specified by the manufacturer. Before preparing standards, consider the expected range of conductivity over the length of the study reach and select the appropriate KCl standards for the 2-point calibration. Maintain a calibration log book in which the calibrated values and maintenance procedures are recorded. Determine the quantity of samples bottles (1L HPDE) that will be required taking into account the number of sampling locations as well as blanks and duplicates. Blanks are used to ensure that sample bottles, sampling devices and filtration equipment have been cleaned effectively to prevent carry-over contamination. Duplicates are independent samples collected at the same time which are stored and analyzed separately to document the precision of the sampling process. Blanks are collected once per month, duplicates are collected weekly at a randomly selected site excluding weeks when a blank is performed.

Bottles for TSS, CHLa and nutrient analyses are acid-washed (10% HCl) and triple-rinsed with DI. Bottles for microbiology analyses are sterilized. Phytoplankton samples (250 ml HPDE bottles) are collected at 4 sites only (JMS99, APP1.5, JMS75 and JMS69). All bottles are labeled with date and site. Be sure to take enough ice in coolers to cool samples to 4°C and maintain them at that temperature during transport.

3.0 Field Procedures

At stations which are sampled by boat (excluding CSO, Mayo’s Bridge and Huguenot Bridge), water samples are collected using a 4L Kemmerer sampler. Samples are collected at depths 1 m below the surface and 1 m above the bottom. Care should be taken that the sampler is deployed vertically and does not disturb bottom sediments. The water sample is directly transferred to 1L sample bottles and to a 500 ml graduate. Sample bottles should be nearly full (~95% capacity) and tightly sealed.

Water quality data are obtained by placing the Hydrolab sonde into the 500 ml graduate. Temperature, pH, conductivity and dissolved oxygen (concentration and saturation) are recorded at each site along with the time of sample collection (see Figure 2 for sample Field Data Sheet). For the land (bridge) sampling locations, water is collected as a surface grab by bucket after pre-rinsing the bucket with local water. Hydrolab measurements are taken from the bucket after sample bottles have been filled. Blanks are obtained by bringing DI water into the field and transferring to the alpha bottle or surface grab bucket before filling sample bottles.

4.0 Post-Cruise Activities

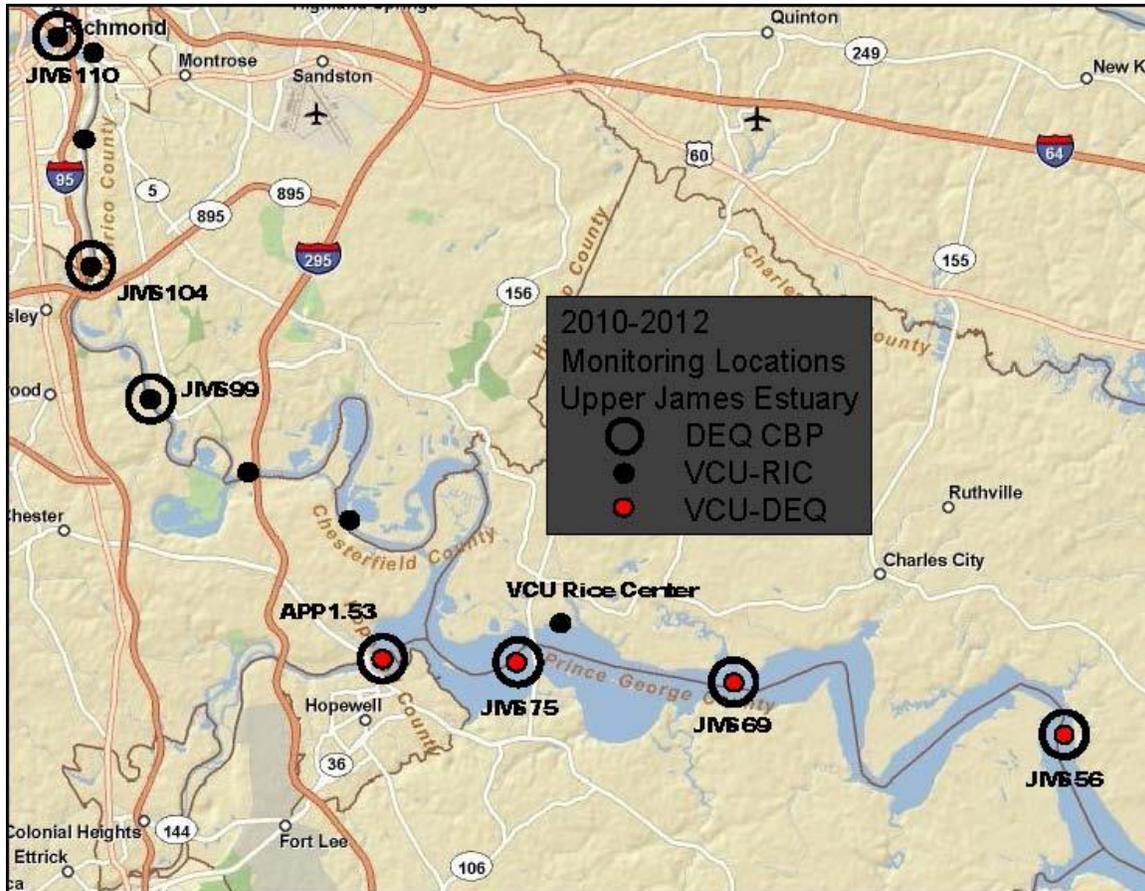
Upon return to the laboratory, samples for TSS, CHLa and nutrient analyses are transferred to the Environmental Analyses Lab (EAL) and samples for fecal coliforms and *E. coli* are delivered to the Microbiology Lab. Phytoplankton samples are retained by EAL for subsequent transport to ODU (Dr. Harold Marshall). Phytoplankton samples should be preserved immediately by the addition of 1.25 ml of Lugols solution.

Ensure that filtration equipment is clean by rinsing towers, tubing, filter flasks and graduated cylinders with DI. Sample water must be thoroughly mixed and transferred quickly to prevent settling of particulate matter and ensure that a representative sample is obtained. Filtration procedures are described in the VCU EAL SOP for TSS and CHLa analysis. Data entry for water quality measurements is performed by the Environmental Analyses Lab. Water quality data are reported along with results from TSS, CHLa and nutrient analyses.

5.0 Responsibilities and Contact Information

Position	Name	Responsibility
EAL Lab Manager	William Lee	Pre-Cruise Prep; TSS, CHLa, nutrient analyses, Data Entry
Microbiology Lab	Rima Franklin	<i>E. coli</i> , Fecal coliform analyses
Boat Captain	Dave Hopler	Boat operation, maintenance & safety
Oversight	Paul Bukaveckas	Project coordination, QAQC for EAL data

Figure 1. Map of tidal freshwater James River showing locations of monthly sampling locations for DEQ CBP long-term monitoring and weekly monitoring stations for VCU-City of Richmond and VCU-DEQ river monitoring.



Appendix 2

VCU Bukaveckas CHLa Analytical Standard Operating Procedures

10. Chlorophyll-a and Pheophytin (Similar to EPA 445.0 Sept 1997)

10.1 Scope and Application:

10.1.1 Chlorophyll data may be used to determine long-term trends in water quality and the trophic status of surface waters, to detect adverse effects of pollutants on plankton, and to provide estimates of studies attempting to estimate algal biomass and productivity.

10.2 Summary of Methods:

10.2.1 The two methods for determining Chlorophyll *a* given here are with 1) a scanning spectrophotometer and 2) a Turner Design fluorometer. The method used requires filtering a known quantity of water through a glass fiber filter. Acetone is also used to extract the filter into a 15 ml centrifuge tube with tight fitting cap. The sample is steeped at least 2 hours and not exceeding 24 hours at 4°C, in the dark. The samples are read on a fluorometer. If the samples can not be read within that time period, storage in the freezer at -20°C for a few days is acceptable. If pheophytin measurements are desired, the sample is acidified and read again.

10.3 Reagents:

NOTE: Use fresh ASTM TYPE I (DI) water. Label, date, and initial all reagents.

10.3.1 Saturated Magnesium Carbonate Solution: Add 10 gram magnesium carbonate to 1000 mL of deionized water. The solution is allowed to settle for a minimum of 24 hours. Only the clear "powder free" solution is used during subsequent steps.

10.3.2 Aqueous Acetone solution (90%) - Mix 90 parts acetone (Optima grade) with 10 parts Saturated Magnesium Carbonate Solution (10.3.1).

10.3.3 0.1 N Hydrochloric Acid solution: Add 8.5 mL of concentrated hydrochloric acid to 800 mL of deionized water in 1 liter volumetric flask. Adjust volume to 1 L with deionized water.

10.4 Equipment:

10.4.1 Fluorometer with appropriate excitation and emission filters in place.

The Fluorometer is equipped as follows:

10.4.1.1 Daylight White Lamp P/N 10-045

10.4.1.2 Filters (For Chlorophyll A and Pheophytin measurements)

Excitation: 340-500 nm P/N 10-050R

Emission: > 665 nm P/N 10-015R

10.4.1.3 Round cuvette tubes - 13x 100 mm culture tubes with tight fitting caps.

10.4.1.4 Filtration equipment - Vacuum pump (vacuum should not exceed 1/2 atm or 15psi), filter holder for 47mm filters, 47mm A/E filters, 2X4 ziplock bags, foil, or a dark storage containers (i.e. 15 ml centrifuge tubes).

10.5 Standards

10.5.1 Fluorometer BLANK: (Used as a "Filter Blank"). An unfiltered blank filter will be analyzed as if it were a sample, extracted as described in Volume V, Section 5.

10.5.2 Calibration Standard: Standards are obtained from Turner Instruments. Calibration standards consist of a minimum of one high standard and one low standard maintained in 90% acetone. An intermediate standard may be made by diluting the high standard appropriately with 90% acetone. This solution should be determined to be a high purity blank. Calibration Standards should be frozen at -20 °C prior to use.

10.5.3 Solid secondary standard – use before and after each sample run. Record the high and low values on instrument bench sheet. If concentrations have drifted more than 10%, make a note on the run sheet. Identify instrument issue. Re-calibrate instrument using primary standards. Available from Turner Designs: P/N 7000-994

10.6 Calibration of Fluorometer

10.6.1 Calibration Standards and a Calibration Blank (90% optima grade acetone) are required for this step

10.6.2 Proceed to Calibration set-up menu on the TD-700 fluorometer. A minimum of two standards should be used for the calibration. Three standards and a blank are optimal.

10.6.3 Use A multi-optional@ mode with / Direct Concentration/ug/L units

10.6.4 Calibration

10.6.4.1 Enter Highest Standard first. Key in the high concentration value. Insert test tube with 5 ml of the high standard in fluorometer. Press < * > when stable. The sensitivity

setting will be automatically set. The fluorometer will read the high standard then ask for subsequent standards.

10.6.4.2 When all standards have been read, insert the blank.

10.6.4.3 Press < 0 > when the value is stable.

10.6.4.4 Calibration will be completed

10.6.5 Acid Ratio Determination:

10.6.5.1 The acid ratio (the ratio of the fluorescence of any extract containing only chlorophyll a, before and after the addition of acid) should be determined for each instrument calibration.

10.6.5.2 Calibrate the fluorometer as described in Section 10.6.4.

10.6.5.3 Reread high, low, and/or intermediate calibration standard (R_b)

10.6.5.4 Acidify the standard with 0.170 ml of 0.1N HCL (for a 5ml sample). Mix. Wait 90 seconds.

10.6.5.5 Read sample as R_a .

10.6.5.6 Acid ratio

$$R = R_b / R_a$$

10.7 Procedure:

10.7.1 Reading on Fluorometer

10.7.1.1 Pipette 5.0 ml samples into fluorometric cuvettes.

10.7.1.2 Read sample in fluorometer. Results are read in direct concentration.

10.7.1.3 Read sample; record as R_b .

10.7.1.4 Acidify the standard with 0.170 ml of 0.1N HCL. Mix. Wait 90 seconds.

10.7.1.5 Read sample record as R_a .

10.8 Calculations:

10.8.1 For uncorrected Chlorophyll A using Method 445.0 with acidification:

(Instrument must be equipped with Excitation: 340-500 nm P/N 10-050R
Emission: > 665 nm P/N 10-015R)

10.8.1.1 $C_{E,u} = R_b \times F_s$

Where $C_{E,u}$ = uncorrected chlorophyll A concentration (ug/L) in the extract solution analyzed

R_b = fluorescence response of sample extract before acidification, and

F_s = fluorescence response factor for sensitivity setting S
(which = 1 for the TD-700 fluorometer)

10.8.1.2 Calculate the “uncorrected” concentration of chlorophyll A in the whole water sample as follows:

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

Where $C_{s,u}$ = uncorrected chlorophyll A concentration
(ug/L) in the whole water sample

Extract volume = volume (L) of 90% acetone used for extraction of the filter

DF = Dilution factor

Sample volume = volume (L) of whole water filtered through filter

10.8.2 For corrected Chlorophyll A using Method 445.0 with acidification:

10.8.2.1 $C_{E,C} = F_s (r/r-1) (R_b - R_a)$

Where $C_{E,C}$ = corrected chlorophyll A concentration
(ug/L) in the extract solution analyzed

F_s = response factor for sensitivity setting S,

r = the before to after acidification ratio of the pure chlorophyll standard

R_b = fluorescence of sample extract before acidification,
and

R_a = fluorescence of sample extract after acidification

10.8.2.2 Calculate the “corrected” concentration of chlorophyll A in the whole water sample as follows:

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

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

Extract volume = volume (L) of 90% acetone used for extraction

DF = Dilution factor

Sample volume = volume (L) of whole water filtered through filter

10.8.2.3 For corrected pheophytin using Method 445.0 with acidification:

$$P_E = F_s (r/r-1) (rR_a - R_b)$$

$$P_s = \frac{P_E \times \text{extract volume (L)} \times \text{DF}}{\text{Sample volume (L)}}$$

Where P_E = pheophytin concentration (ug/L) in the sample extract, and

P_s = pheophytin concentration (ug/L) in the whole water sample

10.8.3 For Corrected Chlorophyll A using Method 445.0 without acidification:
(Instrument must be equipped with Excitation: 436 nm P/N 10-113
Emission: 680 nm P/N 10-115)

10.8.3.1 Calculate the correct concentration of chlorophyll A in the whole water sample as follows:

$$C = \frac{C_c \times \text{extract volume (L)} \times \text{DF}}{\text{Sample volume (L)}}$$

Where C = corrected chlorophyll A concentration (ug/L) in the whole water sample

C_c = corrected chlorophyll A concentration (ug/L) in the extract solution analyzed

Extract volume = volume (L) of 90% acetone used for extraction of the filter

DF = Dilution factor

Sample volume = volume (L) of whole water filtered through filter

10.9 Reference(s):

Strickland, J.D.H., and Parson, T.R. 1972. A Practical Handbook of Seawater Analysis. Fish. Res. Bd. Canada 167:310.

TD-700 Laboratory Fluorometer Operating Manual. Version 1.8. July 7, 1999. Turner Designs, 845 West Maude Avenue, Sunnyvale, CA 94086.

EPA /600/ R-97/072 - Method 445.0. In Vitro Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence. Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices Revision 1.2. September 1997.

Using the Turner Designs Model 10 Analog, The 10AU Digital, Or the TD-700 Fluorometer with EPA Method 445.0. January 19, 1999. Turner Designs, 845 West Maude Avenue, Sunnyvale, CA 94086.

Appendix 3

VCU Bukaveckas Lab Standard Operating Procedures



Virginia Commonwealth University
River & Estuarine Ecology Laboratory
Standard Operating Procedures

Effective: May 1 2012

Updated May 30 2014

Prepared by: Paul A. Bukaveckas, William M. Lee, Maxwell Hasse,
David Elliot, Stephen McIninch, Greg Garman and Joseph Wood

This guide documents procedures for sample collection and experiments performed by VCU personnel conducting research on the James River. The procedures will be implemented in conjunction with the James River CHLa Study supported by the Virginia Department of Environmental Quality.

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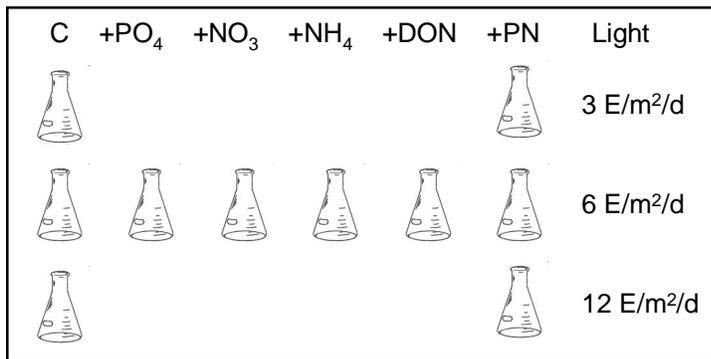
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2012 Data Collection Activities

1.0 Algal Bioassay Experiments

Water for algal bioassay experiments will be collected from two stations (JMS75 and Rice Pier) during the weekly river monitoring cruises (see VCU EAL Field SOP). Water is collected from 1-m below the surface using a Kemmerer water sampler and placed in a 20 L carboy which has been previously acid washed (10% HCl) and rinsed three times with DI. The carboy is further rinsed on site with river water prior to filling. Experiments will be performed on the first cruise in each month (June through October).

The experimental design entails single and combined nutrient amendments for a total of 6 treatments: Control, +NH₄, +NO₃, +DON (Urea), +PO₄, and Combined (DIN & PO₄). The



Control and Combined treatments are incubated at three light levels (3, 6 and 12 E/m²/d). The single nutrient additions are performed only for water collected from the Rice Pier and are limited to the middle light level. Each treatment consists of 3 replicates such that a total of 48 cultures are used in each experiment (both sites). Care should be taken to ensure that the contents of the carboy are well-mixed

before dispensing into experimental cultures to ensure uniform distribution of particulate matter. Cultures are comprised of 50% unfiltered river water and 50% filtered (GF/A) river water for a total volume of 150 mL in a 250 mL Erlenmeyer flask. Nutrient amendments are intended to double average ambient concentrations at these sites by adding 0.1 mg P/L (as Na₃PO₄) and 0.125 mg N/L (as NH₄Cl, or NaNO₃). Cultures are incubated on a shaker table at 50 RPM inside a Conviron Growth chamber at ambient river temperature (measured in the field on the day of water collection) with a 14:10 Light:Dark cycle. Light conditions are modified by proximity to the light source and use of shading. Light intensities are verified using a Li-500 light meter equipped with a deck (incident) light sensor.

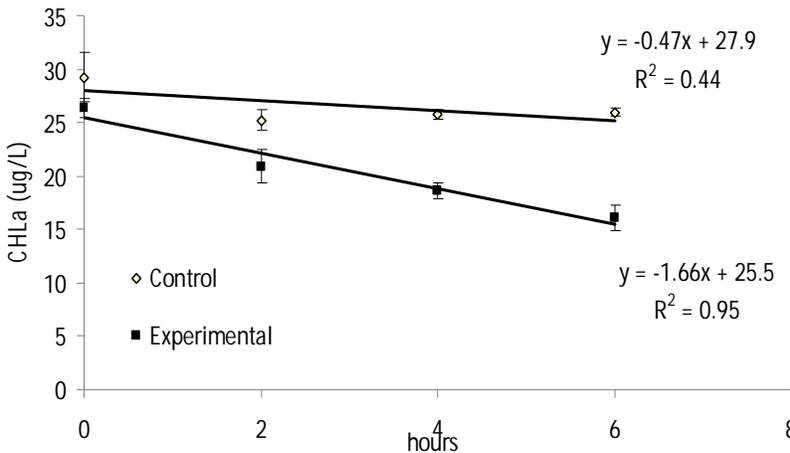
At the start and end (48 h) of the experiment, water samples are obtained from each culture for the determination of Chlorophyll-1 (CHL_a), particulate organic carbon (POC) and nutrient (N, P) concentrations according to protocols described in the EAL Lab SOP. Algal growth rates (*r*) are calculated as the slope of the natural logarithms of POC or CHL_a as a function of time.

Differences in growth rates among treatments are used to test *a priori* hypotheses regarding N vs. P limitation, the form of N limitation (NO₃, NH₄ or urea) and light limitation.

2.0 *Rangia* Grazing Experiments

Experiments will be performed to measure grazing rates by the benthic filter-feeder *Rangia cuneata* (wedge clam). Animals and water are collected monthly (June-October) from the James River in the region where persistent algal blooms have been documented (river miles 56-75). Animals are collected using benthic rakes or oyster tongs and cleaned by rinsing the exterior with river water. The animals are immediately returned to the lab along with a quantity of water

collected from the surface (~200 L). The animals are acclimated for ~16 h to one of two temperature regimes: Standard (20° C) and River Ambient (measured on day of collection).



Three to five animals of similar size are placed in each of three replicate mesocosms (containing 15 L of river water) for each temperature regime. Triplicate control mesocosms (no clams) are also maintained at the two temperature regimes (Total N = 12 mesocosms) to quantify changes in particulate matter concentrations in the absence of *Rangia*. Mesocosms at

the River Ambient temperature are kept in a Conviron Growth chamber while mesocosms at the Standard temperature are stored in a controlled temperature water bath housed at the VCU Aquatics Facility. All mesocosms are kept dark to minimize algal production. Temperature, and other water quality parameters, are monitored continuously using a Hydrolab sonde placed in one mesocosm in each of the two temperature regimes. A circulating pump is placed in each mesocosm to maintain particulate matter in suspension. Samples are taken at fixed intervals of 0, 2, 4, 6 and 24 h from each mesocosm to determine the rate of change in TSS, CHLa and POC concentrations. Differences in the rate of change for constituent concentrations between mesocosms with and without clams are used to estimate per capita net clearance rates by dividing the mass of material removed by the number of animals (see Figure at left). The size and weight of each animal is also recorded and used to develop size-weight regressions and to assess relationships between size and filtering rate.

3.0 Consumer Sampling & Gut Contents Analyses

Rangia, blue crabs and fish will be collected monthly (June-October) in the region of the CHLa maximum (river miles 56-75) for use in gut contents analyses and tissue Microcystin (MC) determinations. *Rangia* will be collected at the same time and by the same methods as those used for grazing experiments (see above). Blue crabs will be collected by seine or traps. Fish will be collected by electroshocking using protocols described in the Fish Ecology Laboratory SOP. Target fish species include Gizzard Shad, Threadfin Shad, Atlantic Menhaden and Juvenile blue catfish (<30 cm), though it is recognized that their availability will vary seasonally. Fish length and weight are recorded in the field. Stomach contents are removed in the field (large fish) or in the lab (small fish) and retained for determination of dry weight, CHLa and organic C. Fish and stomach contents will be kept on ice during processing and transport. For each fish, a homogenized (wet) gut contents sample will be divided into two aliquots and placed in pre-weighed containers to determine their wet weight. One aliquot is subsequently frozen and used for CHLa extraction following protocols for sediment samples described in the EAL SOP. Holding time from freezing to extraction will be three weeks or less as per EPA guidelines. The second aliquot is dried to determine bulk density (60° C for 48 h) and subsequently ground. A sub-sample is used to determine organic matter content via elemental analyses on a Perkin Elmer

CHN Analyzer (see EAL SOP). The results of these analyses will allow us to determine the dry weight, organic matter content, and CHLa content of fish gut contents on a per capita basis. In cases of very small fish (e.g., young-of-the-year), gut contents may be pooled among several individuals in order to obtain sufficient quantity for analyses.

4.0 Microcystin Analyses of Sediment and Tissues

Water, sediment and the tissue samples will be collected to determine Microcystin concentrations using an Enzyme-Linked ImmunoSorbent Assay (ELISA) kit (Abraxis). Collection of weekly water samples for MC analysis is described in the VCU EAL River Monitoring SOP. Samples are thawed and refrozen 2 times prior to analysis in order to release MC from cells. Sample analysis is performed in duplicate with appropriate standards and controls following manufacturer protocols. Results are expressed as $\mu\text{g MC/L}$.

Sediment samples are collected monthly (June-October) using an Eckman sampler at 3 shallow sites. Two sites are located in proximity to the CHLa maximum (Rice Pier, Tar Bay) and one site is located upstream (at Presquile or Jones Neck). Two benthic grab samples will be taken at each site and two replicate sediment cores (1 cm diameter) will be removed from each grab samples. The sediment cores are extruded to obtain the top 2 cm of sediment. Care should be taken when collecting the benthic grab sample that overlying water is also captured to ensure that the sediment-water interface is not disturbed. Sediment samples are measured for wet weight and then divided into two aliquots. One aliquot is frozen and subsequently used for CHLa analysis as per the EAL SOP. A second aliquot is dried (60°C for 48 h), re-weighed and then ground for use in organic matter analyses and MC determination. Organic matter content is determined by acid-fuming the sample and running on a Perkin Elmer CHN analyzer (see EAL SOP). Microcystin is extracted from sediments following the methods of Wilson et al (2008). Dried sediment is extracted first in 100% methanol for 60 minutes and secondly in methanol with acetic acid. The pooled extracts are centrifuged and filtered (1 μm glass fibre). Prior to ELISA analyses, samples are evaporated and re-diluted with DI to ensure that samples contain <5% methanol. Microcystin concentrations are expressed as mass per dry weight of sediment.

Tissue analyses will be performed monthly (June-October) for six target groups (dependent on availability) which include *Rangia*, blue crabs, blue catfish (juveniles and adults), gizzard shad, and Atlantic menhaden. For *Rangia* and blue crabs, samples of muscle and viscera will be analyzed separately. For fish, samples of muscle and liver will be analyzed separately. Microcystin extractions will be performed using the method of Garcia et al (2010). Dried tissue is homogenized, sonicated and placed in 100% aqueous methanol for 2 h extraction. The supernatant is collected after centrifugation and then a second extraction is performed for 24 h using 100% aqueous methanol with acetic acid. Supernatants are centrifuged combined with the first extraction. Extracts are filtered (1 μm glass fiber), evaporated, and re-dissolved in de-ionized water to reduce methanol content below 5% prior to ELISA analyses. Microcystin concentrations are expressed per dry weight of tissue.

To determine the efficiency of sediment and tissue extraction techniques, we will analyze a series of spiked samples. Samples are spiked with a known volume of 5 $\mu\text{g/L}$ Microcystin solution and percent recovery is determined. Prior studies have reported values ranging from 50% to 98% depending on tissue type.

2013 Data Collection Activities

1.0 Zooplankton Toxicity Assays

We will measure the effects of Microcystin on the growth, reproduction and survivorship of *Bosmina longirostris*, a common freshwater zooplankton occurring in the tidal fresh segment of the James River (Bukaveckas et al. 2011). Lethal and sub-lethal effects of microcystin will be assessed by monitoring growth, survival, and fecundity of animals in laboratory incubations. Exposure to Microcystin will be performed in a dosage dependent manner, to facilitate detecting thresholds for deleterious effects.

Zooplankton for experiments will be collected near the long-term plankton monitoring station (JMS75) using a 150 μm mesh net with non-filtering cod end. To ensure an adequate supply for experiments, animals will be kept in laboratory cultures. Cultures will be maintained under controlled environmental conditions approximating those at collection, and fed an algal diet of *Chlamydomonas reinhardtii*. Experiments will be performed in a Conviron growth chamber at 20°C with a 14 h light: 10 h dark cycle. Each animal will be separately incubated in its own 20 mL glass container filled with 0.45 μm filtered James River water and appropriate food. All experimental procedures and measurements described below will comply with accepted and best practice methodology according to the ICES Zooplankton Methodology Manual (Harris et al. 2000).

To evaluate the effects of dissolved Microcystin we will incubate zooplankton in water treated with 0.0, 0.4, 0.8, and 1.6 $\mu\text{g L}^{-1}$ dissolved Microcystin (as Microcystin-LR, available from Abraxis). Toxin concentrations in the James River reached 4.5 $\mu\text{g L}^{-1}$ (2011), though these are total concentrations with the dissolved fraction expected to be <30%. Thus our experimental treatments encompass the toxin range observed in this environment. Newly hatched juvenile animals will be collected to begin experiments, and these will be incubated for their entire lifespan (several weeks). The animals will be fed a diet of *C. reinhardtii* at *ad libitum* concentrations. To ensure consistent dissolved Microcystin and food concentrations throughout the experiment, individuals will be carefully transferred into containers with fresh water and algae every 1-3 days. Growth will be measured in two ways: (1) as increase in body mass through time (somatic growth, $\mu\text{g dry weight day}^{-1}$) and (2) as time from birth to reproductive maturity (development time, days). To estimate somatic growth, a calibrated microscope micrometer will be used to measure the length of each juvenile at the beginning of incubations and the length of each adult at the end of incubations. Lengths will then be used to estimate dry weight biomass using published regressions (Burkhill & Kendall 1982; Culver et al. 1985), and growth rates calculated as increase in biomass over the incubation period ($\mu\text{g dry weight day}^{-1}$). Development time will be determined from hourly monitoring with a video microscope, to determine the time when each animal produces its first brood of offspring. Survival, as total lifespan (longevity, days), will also be monitored hourly throughout experiments. In addition to quantification of growth, development, and survival, hourly monitoring will allow quantification of the number of offspring produced by gravid adult females during the experiment (fecundity, offspring female⁻¹ d⁻¹).

2.0 Rangia Toxicity Assays

Experiments will be performed to test the effects of dissolved (free) and dietary (particulate) exposure to the cyanotoxin, Microcystin, on filtering rates of *Rangia cuneata* (common wedge clam). *Rangia* will be obtained from the James River at a near-shore site near JMS75 (also used for 2012 feeding studies). Individuals are collected within 24 h of the start of each experiment and allowed to acclimate overnight. The length of each individual is measured to determine biomass from a previously-developed (2012) length-weight regression. Individuals are placed in 3-L mesocosms containing James River water (50% filtrate, 50% raw) spiked with Microcystin at a range of concentrations (0, 0.4, 0.8, 1.6 and 3.2 $\mu\text{g L}^{-1}$). The range was selected based on observed concentrations of total Microcystin in the James (2012: 0-1.5 $\mu\text{g L}^{-1}$; 2011: 0-4.5 $\mu\text{g L}^{-1}$). Aqueous Microcystin LR is available commercially (Abraxis); concentrations within the mesocosms will be confirmed through analysis of water samples using ELISA kits. Each treatment level will be performed in triplicate with experiments repeated in May, June, and August 2013 to assess responses before and during peak algal blooms. Mesocosms are incubated in the dark (to prevent algal growth) at 25° C and sampled for TSS, POC and CHLa at 0, 1, 2 and 3 hours. Clearance rates for CHLa, POC and TSS are determined for each experimental unit based on differences in concentration between mesocosms with and without clams. Slopes of these regression lines are used to determine clearance rates for CHLa, POC and TSS as mass/g clam dry weight/h as well as mass/individual/h (Coughlan 1969).

$$\text{Clearance rate (L/ g DW/ h)} = [(\text{slope}(\text{mg/L}) / \text{h}) * \text{mesocosm volume (L)}] / \text{clam dry mass}(\text{g DW}) / (\text{average concentration (mg/L)})$$

To assess the impacts of dietary Microcystin exposure we will measure clearance rates of *Rangia* collected from the James and incubated in water from the Pamunkey and James Rivers. Microcystin levels in the Pamunkey have not been previously measured, but we anticipate that this site will exhibit little to no Microcystin (Reay 2009) and therefore will be a suitable control for experiments using water from the James. Experiments were/will be performed in May, June, August and September 2013. We hypothesize that during pre-bloom conditions (May-June) similar clearance rates will be observed among clams filtering water from the Pamunkey and James Rivers whereas during bloom conditions (August-September), clearance rates will be lower among clams incubated in water from the James (i.e., in the presence of algae containing Microcystin). Water is collected on the day of the experiment from sampling locations in the tidal freshwater segment (Pamunkey TF 4.2, James at VCU Rice Pier). As prior work has shown that bivalve clearance rates are influenced by food concentrations, we will use 50% dilutions (with filtered river water) of water obtained from the James, in order to attain TSS, POC and CHLa levels comparable to those observed in the Pamunkey. For each experiment, twelve 3 L mesocosms are established with three replicates each for water from the two sources, and with/without clams. Samples for Microcystin analysis are taken at the beginning of each experiment and measured using ELISA kits. Clearance rates for CHLa, POC and TSS are determined for each experimental unit using methods described above.

3.0 Herring Egg and Larvae Assays

The experiments were designed to evaluate, under controlled conditions, fish egg and larvae viability following exposure to a range of Microcystin concentrations that may occur naturally in the tidal James River. The fish species used for the bioassay were anadromous blueback herring

(*Alosa aestivalis*) and American shad (*A. sapidissima*), both of which are native to the James and spawn in tidal freshwater reaches. Blueback herring and American shad eggs were obtained from wild, local sources in March and April, 2013, fertilized on-site, and transported to Harrison Lake National Fish Hatchery (Charles City, Virginia). Experiments were carried out with the assistance of Mr. Michael Odum, manager of the USFWS facility, who provided access to hatchery space, a supply of certified groundwater, and expertise with regard to holding and rearing alosine eggs and larvae. Eggs and larvae were maintained using standard hatchery procedures; organisms that were not used for exposure studies were stocked into grow-out ponds and may be used later for related (sentinel) experiments (see following section).

Egg and larval exposures were accomplished within a closed circulation-filtration system that



was modified from a design developed by Harrison Lake personnel and tested by VCU. The egg containment capsules were comprised of multiple PVC pipe pieces, couplings, and 200 μm nitex screen. The combination of these elements provided a chamber (51 mm diameter) in which eggs could be held and later recovered. Five egg containment capsules were placed in each of eight 19 L enclosures. This system provided a total of 40 separate egg containment capsules for each exposure trial. During each trial, filtration containers were placed in a shallow water trough supplied with a constant supply of water maintained at 17°C. All other environmental conditions were maintained at constant levels during experiments.

Following *in vitro* fertilization, eggs were rested for 24 h. This lag time allowed embryos to develop so that viable eggs could be identified visually (20X magnification) and selected for experiments. For each experimental trial, 10 viable eggs were placed in each containment capsule and exposed to hatchery water dosed with Microcystin (commercial source Abraxis) for 48 h (herring) or 72 h (shad), after which all eggs were recovered, evaluated for viability, and enumerated. Toxin concentrations were: 0.0, 0.4, 0.8, 1.6 and 3.2 $\mu\text{g/L}$. Four of the five levels were tested in each experiment with two replicate enclosures at each concentration. Target concentrations were confirmed through periodic testing of water samples taken from the enclosures. Four experiments were performed between mid-April and mid-May.

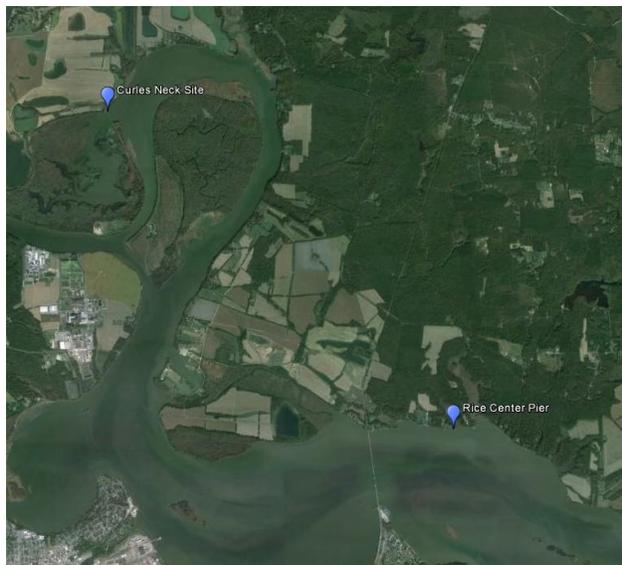


Larval exposures were conducted using the same 19 L enclosures and the same Microcystin treatments as were used in the egg exposures. Each enclosure held 50 larvae—selected for viability from hatchery stock—for a total of 400 larvae for a single trial. Larvae were exposed to hatchery water dosed with Microcystin for 48 h (herring) or 72 h (shad). Each dosage was replicated in each of four experimental trials (i.e., two containers per concentration). As for the egg exposures, enclosures were kept in a shallow water trough with a constant supply of water maintained at 17°C. At the conclusion of each of four larval trials,

larvae were recovered, evaluated for viability, and enumerated. Because only yolk-sac larvae were used in exposure studies, feeding prior to or during experimental trials was not necessary. Four experiments were performed between mid-April and mid-May.

4.0 Sentinel Fish Studies

The objectives of this study were to assess the effects of in situ exposure of James River fishes to Microcystin. Fish enclosures (~0.78 m³) were installed at two locations. One site was at the VCU Rice Center Research Pier (near JMS75), a section of the James where elevated Microcystin concentrations were observed in 2011 and 2012. A second location, near Curles Neck, was chosen as an area where Microcystin concentrations were expected to be low (based on low concentrations measured at JMS99). Three two-week experiments were conducted during the period when elevated Microcystin concentrations are typically observed (start dates:



August 8, August 23 and September 13). For each experiment, two replicate enclosures were used at each site. The four enclosures were each stocked with 20-30 fish. For the first experiment, gizzard shad (*Dorosoma sp.*) were obtained from the Harrison Lake Fish Hatchery. For the second experiment, threadfin shad (*Dorosoma petenense*) were obtained by electrofishing near the Curles Neck site. For the third experiment, juvenile (~3 mo) blueback herring (*Alosa aestivalis*) were obtained from the Harrison Lake Fish Hatchery. A subset from each group was retained to determine initial Microcystin concentrations in fish liver tissues. Water samples were collected at the start, middle and

end of each incubation to measure Microcystin levels at the two sites. At the conclusion of the two-week incubation period, fish were collected from the enclosures and dissected to obtain otoliths and liver tissue samples. Otoliths were used to compare daily incremental growth rates between the two sites. Polished otoliths were prepared using standard methods (Secor et al., 1991) and viewed under a light microscope to determine the number of annuli. Liver tissues were analyzed for Microcystin concentrations using previously described protocols (see 2012, Part 4).

5.0 Sturgeon Exposure Studies

Most U.S. populations of the Atlantic Sturgeon (ATS) were listed as *Endangered* under the federal ESA in April, 2012. The tidal James River is one of several Atlantic Slope coastal rivers that supports Atlantic Sturgeon—and the only Chesapeake Bay tributary where widespread spawning has been documented (Balazik, et al. 2012). The draft recovery plan for James River ATS recognizes several population threats, including habitat loss, ship strikes, and fishery by-catch losses. Critical habitats for both adult and juvenile ATS overlap spatially and temporally with James River *Microcystis* blooms, which may represent an additional threat to ATS that feed on benthic infauna (e.g. *Rangia* clams) or other riverine prey. Early juvenile ATS (ages 0-3) are

pre-migratory, year-round residents in tidal freshwater reaches of the James and would presumably experience greater exposures to cyanotoxins, compared to other life-history stages. Other benthic feeders (e.g. blue catfish, gizzard shad) in the tidal freshwater James River exhibit moderate (compared to planktivorous fishes) concentrations of MC in liver tissue (Wood et al. 2014). Because of their protected status, it is not possible to directly evaluate MC tissue concentrations and possible effects on James River ATS. However, access to Canadian-source ATS (no protected status) held at VCU's Aquatics Facility provides an opportunity to test hypotheses concerning ATS and MC exposure under laboratory conditions. The goal of this laboratory study is to emulate—to the extent possible—expected dietary consumption of MC by wild, early juvenile (age-0) ATS under natural riverine conditions, based on known toxin concentrations in putative James River prey, such as *Rangia* clams (Wood et al. 2014).

The primary research objectives of this study are to: 1) induce dietary exposures microcystin-LR in captive ATS; and 2) measure tissue (liver) concentrations in captive ATS across a range of dietary exposures. Given the moderate concentrations of MC proposed for ATS laboratory diets (modeled on MC levels in likely James River prey), and the relatively short exposure periods possible in the lab, we believe that lethal effects in this study are unlikely. Hence, a third study objective will evaluate possible sub-lethal effects of MC uptake for captive ATS under controlled conditions. Specifically, we hypothesize a positive dose-response relationship between cumulative MC ingestion and MC tissue concentrations, as well as an increase in sub-lethal effect in ATS with higher levels of MC ingestion. Uptake of cyanobacterial toxins by freshwater fish occurs primarily through dietary pathways, rather than across gill epithelia or via ingestion of water (Ernst et al. 2006), hence this study's focus on dietary uptake.

Diet Formulation: Pure microcystin-LR will be purchased as a dry powder from a commercial source (Abraxis BioScience) and incorporated into a standardized diet for captive ATS. MC powder (0.5mg) will be added to 125 mL of deionized water to yield a 4µg/mL solution of MC, which will be stored at 4°C. Feeding methods used in this study will be modified from Hirschfeld et al. (1970) to allow incorporation of chemical toxins with a semi-solid food matrix. Pellet formulation will minimize fragmentation of pellets and MC leaching in closed-system tanks during fish feeding bouts. Four separate diets will be prepared corresponding to the following treatment groups: 0.0 (control), 0.10, 0.25, 0.5 µg of MC/g wet weight of food. The range in MC treatment concentration is based on MC tissue concentrations in James River benthic invertebrates, including *Rangia* (Wood et al. 2014). The diets will be prepared adding 250 g of fish feed (Melick Aquafeed sinking No. 1 pellets, Melick Aquafeed, Catawissa PA, USA), 20 g granulated agar, 14 g unflavored gelatin, and microcystin solution (0 mL, 8 mL, 16 mL or 32 mL, depending on treatment group) to 1000 mL of deionized water. The water will be heated to 100°C at which point the ingredients will be mixed in until a uniform appearance is achieved. The solution will then be allowed to cool in a flat pan at 4°C. After 24 h, the food will be sliced into pellets of a size (1-3 mm²) appropriate for ingestion by age-0 ATS, placed in labeled containers, and stored at -20°C for less than 1 month. New batches of experimental pellets will be made as necessary during the experiment. Concentrations of MC in each dietary treatment will be confirmed by ADDA ELISA analysis (Abraxis; Warminster PA, USA) of a small subsample of pellets (Wood, et al. 2014).

Experimental Animals: Up to 250 age-0 (young-of-the-year; 40-100 mm FL) Atlantic Sturgeon from a Canadian wild source will be secured under an agreement between VCU and the Maryland Department of Natural Resources, transferred to VCU's Aquatics Facility, and

acclimated in freshwater at 16°C to an artificial diet (see above) for at least 1 month prior to the start of experiments. Fish will be held and used under conditions established by VCU's IACUC committee (protocol AD20127) and will not be released to the wild for any reason.

Leaching Experiment: A leaching experiment will be conducted prior to the main exposure experiment to test the assumption that the loss of MC from pellets during feeding bouts is nil. We will place 5 g of pellets (0.5 µg of MC/g) in 1.0 L of deionized water and remove aliquots (20 ml) of water at regular intervals for a period of 12 h after the food is first submerged. The solution will be gently stirred before each sample is taken. Samples will be held at 4°C and analyzed for MC (methods described in Wood et al. 2014) within 24 h. Detectable leaching of MC from pellets may require re-formulation of food matrix or a change in the duration of fish feeding bouts.

MC Exposure Experiment: A single experimental unit will consist of a clean, 38-liter glass tank (closed system) with a single age-0 ATS; water will be sourced from the Aquatics Lab supply line (treated city water). Treatment groups will be fed pellets containing 0.0 (control), 0.10, 0.25, or 0.50 µg MC/g wet weight of food. All other conditions (e.g. water quality, temperature, photoperiod) will be held constant and will be consistent among treatments. Each treatment will consist of 10 replicates, for a total of n=40 experimental units. Mass (to nearest 0.01 g; blotted wet weight) and fork length (mm) of each fish will be measured and recorded during transfer from the stock tank to experimental tanks and prior to the initial experiment feeding. Water changes of 50 % will be completed every 2 d, or as needed, for the duration of the experiment and solid waste will be siphoned from the tanks daily. Each fish will be fed a daily ration of 3% body weight, based on the mean mass of all experimental fish, once per day with the appropriate treatment diet. This daily ration is presumed to be a maintenance (zero net growth) ration for juvenile sturgeon (Albert Spells, USFWS, Charles City VA, personal communication). Preliminary feedings using the 0 µg MC/g wet weight diet demonstrated that age-0 Atlantic Sturgeon will consume a 3% daily ration of pellets within a 30-m feeding bout. All uneaten food will be removed from tanks and recorded. Mortalities during the experiment will be removed, evaluated, and stored at -20°C; dead fish will be replaced by new fish from the stock tank after the experimental tank has been thoroughly cleaned with a 10% Clorox solution and rinsed. The feeding experiment will proceed for consecutive 28 days, after which all remaining fish will be euthanized, evaluated, and stored at -20°C. Liver tissue will be removed from each fish used in the experiment and MC concentrations analyzed using the methods described in Wood et al. (2014). All data will be entered into an EXCEL spreadsheet and QA'd using a double-entry method. If resources permit, the experimental protocol will be replicated at least once prior to any conclusions about the objectives or hypotheses.

Responsibilities

Oversight	Paul Bukaveckas	Project coordination, QAQC, Data Reporting
	Greg Garman	Oversight of Fish Assays
EAL Lab	William Lee	CHLa, POC, nutrient analyses
	Aaron Porter	Microcystin Analysis
Research Staff	Joe Wood	Algal Bioassay Experiments, Rangia Toxicity Assays
	Dave Hopler	Boat operation, fish collection
	David Elliott	Zooplankton Toxicity Assays
	Maxwell Hasse	Fish Sentinel & Sturgeon Studies

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Appendix 4

ODU Egerton Standard Operating Procedures

**WORK/QUALITY ASSURANCE PROJECT PLAN FOR MONITORING
PHYTOPLANKTON AND PICOPLANKTON IN THE
LOWER CHESAPEAKE BAY AND TRIBUTARIES**

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For the Period: July 1, 2011 through June 30, 2012

Prepared for
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I. PROJECT DESCRIPTION (PHYTOPLANKTON COMPONENT):

This project is responsible for monitoring the composition and abundance of phytoplankton and the concentration of the autotrophic picoplankton located in the lower Chesapeake Bay and four rivers that enter the lower Bay. Emphasis is placed on the correct and consistent identification of species within the phytoplankton community, and the continuity in the use of methodologies that have been followed since the phytoplankton monitoring program began in 1985. This approach is essential to provide consistency and validity in data collections and in subsequent data analysis procedures for the evaluation of trends and any changes in these populations over time. To accomplish this consistency, five major resources are provided by this investigator. These are: 1.) Proven expertise (over 40 years) in phytoplankton systematics in the Chesapeake Bay and regional rivers, and phytoplankton that enter the Bay from the northeast U.S. coastal waters; 2) over 20 years experience in the Chesapeake Bay Program monitoring plankton concentrations in Bay and tributary waters; 3.) An extensive collection of voucher specimens of phytoplankton species from the areas mentioned above for comparative and verification requirements for phytoplankton species identification; 4.) A fully equipped phytoplankton laboratory, with seven inverted plankton microscopes, and two epifluorescence microscopes, and 5.) A complete series of identification reference keys for all phytoplankton categories. Additional resources available in the building include molecular genetic analysis capabilities, cell culture facilities, and a scanning electron microscopy component.

A. OBJECTIVES AND SCOPE OF PROJECT:

1. To determine the composition, and abundance of phytoplankton populations at stations in the lower Chesapeake Bay and stations in the Elizabeth, James, York (Pamunkey) and Rappahannock Rivers (Fig. 1). This requires knowledge of phytoplankton species from fresh water, estuarine, and marine populations.

2. To determine concentrations of the autotrophic picoplankton, at 7 stations in the lower Chesapeake Bay and 7 stations in the tributaries mentioned above.

3. To provide base line data that may be used for data interpretation and statistical analysis of the phytoplankton and picoplankton mentioned above.

4. To identify from these collections information on the seasonal abundance, occurrence, and distribution of potential toxin producing phytoplankters at these stations in the lower Bay and the tributaries (Marshall, 1994, 1996, 2007; Marshall et al. 2005, 2008, Marshall and Egerton, 2009).

5. To provide information regarding major algal bloom events of in the lower Bay and these tributaries that occurred during the collection period. Microcystin analysis will be conducted in major blooms events of the cyanobacteria *Microcystis aeruginosa*.

6. To establish a consistent, long term historical data-base that may be used in the future studies within the station locations mentioned above.

B. COORDINATION ACTIVITIES WITH CBP COMMITTEES:

A major value of this study is that it will be conducted in a similar time frame as the water quality collections (DEQ, ODU). This protocol provides a more meaningful basis to examine relationships that exist between these data sets, and for evaluations to other data sets in the Chesapeake Bay Program. Results obtained from phytoplankton, and picoplankton monitoring will have specific relevance and value to objectives of several Chesapeake Bay Program sub-committees. This study may provide information that can be used to study long-term trends of population growth, and eutrophic status. Additional information will be available on the presence and location of toxic and bloom producing phytoplankton, in addition to the abundance of picoplankton.

C. STUDY DESIGN:

1. Project Dates:

The time period for this study is from July 1, 2011 through June 30, 2012. In the Bay field collections for phytoplankton and picoplankton measurements will be from July 2011 through June 2012 and in the 4 Bay tributaries from July 2011 to October 2011, and March 2012 to June 2012.

2. Relationship to Background Information of this Project

The continuation of this project at Old Dominion University assures consistency and high levels of continual accuracy in the identification of the phytoplankton populations (with over 1400 species recognized in this region, Marshall, 1994, Marshall et al., 2005). A large analysis program of this size requires consistency and accuracy in the wide range of species identifications. These algae will represent the critical populations for studies regarding any long-term trends, to be indices to any water quality changes, and to note shifts, or interactions in local food webs.

3. Data Uses

The sampling and analysis procedures in this project provide essential data necessary to meet the objectives over this study

period. They also represent a continuation of previous methodology and assure the consistency in species identification necessary in this study. The methods followed will allow the incorporation of the proposed data set with the previous work for subsequent analytical interpretation and application.

4. Sampling Network Design Rationale:

The Chesapeake Bay is a plankton-driven ecosystem, the most important of which are the phytoplankters and autotrophic picoplankters, which represent the primary food and oxygen producers and the basis of all major food webs in these waters. The data set obtained in this project, combined with the previous data in the lower Chesapeake Bay Monitoring Program, will provide data sets on phytoplankton composition and trends and picoplankton abundance in the lower Chesapeake Bay to date. This project is designed to be an integral component of the CBP to associate relationships between the living resources and the water quality variables.

Long-term trend analysis of this data set will continue to provide information regarding associations that may have direct relationships to management decisions concerning nutrient entry into this region (Marshall et al. 2003, 2006, 2008, 2009). In addition, this data may determine specific relationships between the major producers (phytoplankton and picoplankton) in Chesapeake Bay to specific food web constituents and trophic exchanges in the system. Justification for this design is based on long term monitoring plans concerned with the ecological status and health of the Chesapeake Bay system.

5. Sampling Locations:

The contract identifies sampling seven stations in the lower Chesapeake Bay and seven stations in the four tributaries (Figure 1). The tributary locations were originally identified by the Virginia Department of Environmental Quality as representative of salinity regions in Virginia's rivers and includes tidal fresh, oligohaline, and mesohaline regions. The Bay stations were located throughout the Bay to provide representative sites along the central, eastern, and western Bay regions. These are as follows:

<u>Station</u>	<u>Description</u>	<u>Latitude</u>	<u>Longitude</u>
TF5.5	James R.	37.31265	77.23283
RET5.2	James R.	37.20294	76.78219
SBE5	Elizabeth R.	36.7675	76.2861
TF4.2	Pumunkey R.	37.57999	77.02128
RET4.3	York R.	37.50869	76.78889
TF3.3	Rappahan. R.	38.01847	76.90928
RET3.1	Rappahan. R.	37.91730	76.82220

CB7.4	Bay Mouth	36.9955	76.0208
CB7.3E	Eastern Shore	37.2285	76.0542
CB6.4	Central Bay	37.2364	76.2083
CB6.1	Main Channel	37.5883	76.1625
LE5.5W	Bay at Mouth James R.	36.9988	76.3135
WE4.2	Bay at Mouth York R.	37.2417	76.3867
LE3.6	Bay at Mouth Rapp. R.	37.5969	76.2853

6. Coordinated sampling:

At Bay stations and the Elizabeth River station, the plankton water samples are collected by ODU water quality personnel from the Department of Chemistry and Biochemistry from an ODU vessel. At the remaining 6 tributary stations, personnel from Virginia DEQ collect the tributary water samples to be analyzed in this program.

7. Parameters to Measure in the Phytoplankton Component:

- a. Phytoplankton composition and abundance.
- b. Autotrophic picoplankton abundance.

The phytoplankton populations that will be identified and counted in this study will include specifically when present the diatoms, dinoflagellates, cyanobacteria, chlorophyceans, euglenophyceans, cryptomonads, and other algal categories that appear in the samples. Identification will be to species level, or the lowest taxonomic category possible. The picoplankters to be monitored will consist of the autotrophic cells generally 0.2 to 2.0 microns in size. Taxonomic identifications of phytoplankters will be similar to those established by this principal investigator in the monitoring program since 1985 (See Marshall and Alden, 1990; Marshall, 1994), and for the autotrophic picoplankton by Marshall (1995).

8. Frequency of Collections:

Monthly water samples will be taken at the Bay stations (12 months). Collections in the tributaries will be taken monthly March through October (8 months).

9. Types of Samples:

All phytoplankton and picoplankton data will come from the analysis of water samples collected from a boat. See Section IV on Sampling Procedures.

II. PROJECT ORGANIZATION AND RESPONSIBILITIES

The processing and analysis of all samples, plus data computer entry, will be completed in the Phytoplankton Analysis Laboratory at Old Dominion University, under the direction of Dr. Harold G. Marshall (PI). Correspondence regarding this project would be addressed to the PI, Dr. Harold G. Marshall at the Department of

Biological Sciences, Old Dominion University, Norfolk, Va. 23529-0266. Phone: office 757-683-4204, lab-757-683-4994, FAX-757-683-5283, with direct e-mail hmarshall@odu.edu.

A. PROJECT MANAGER (Expert in phytoplankton collections and species identification): The Project Manager, Dr. Harold G. Marshall, will supervise the activities associated with this project. This includes the responsibilities of the Laboratory Supervisor and designated Laboratory staff. He will supervise the stages in the analysis of the samples, resolving problems that may arise, and assuring the satisfactory completion of the study. He is responsible for data review, submission of data, performance and systems audits. The project manager will review results of the analyses and approves the quality assurance/quality control protocols to insure the quality of results. The Project Manager will administer the financial and technical requirements of the project and be responsible for any reports concerning this project. He will also meet with members of the laboratory staff to discuss and review their responsibilities in relation to the project. The Project Manager will respond to questions by the contracting agencies regarding this project and project reports.

Harold G. Marshall is a phycologist and marine ecologist, with over 40 years of experience in the systematics and ecology of marine, estuary and fresh water phytoplankton. He has also studied and reported on the phytoplankton in the Chesapeake Bay region for the past 20 years, publishing over 160 articles on phytoplankton (plus an addition 190 abstracts), which include articles from the Chesapeake Bay, its rivers and from regional marine shelf waters. He is a recognized authority in phytoplankton systematics and ecology, and has published a phytoplankton identification manual (Marshall, 1986). His publications include over 40 articles and >110 abstracts specifically on phytoplankton in Chesapeake Bay and its tributaries, plus over 30 technical reports on these topics from this region. These past studies also include investigations of various toxic and bloom producing algae within the Chesapeake Bay (see Harold G. Marshall publications on the WEB)

B. QUALITY ASSURANCE OFFICER:

The Quality Assurance Officer (Michael F. Lane) will meet periodically with the principal investigator to discuss: 1. operation, sampling and analysis procedures, 2. data entry, and 3. any problems that may arise that would delay data entry. He is responsible for approving the QA/QC protocol used in this project and advises the principal investigator on procedures, in addition to logistics, or other related concerns that may influence the sampling, or data analysis.

C. PHYTOPLANKTON FIELD/LABORATORY SUPERVISOR:

This position is held by Mr. Todd Egerton. Mr. Egerton has a M.S. in Biology and is in the Ph.D. degree program in Biological Sciences at ODU, with 8 years of experience as a phytoplankton investigator he is responsible for sampling operation, supervising lab personnel, preparation of collection bottles, collection of water samples, and custody of samples from each cruise to the phytoplankton laboratory. He also oversees laboratory analysis and QA/QC, and data processing. He reports to the principal investigator. The backup person for this position is Mr. Mathew Semcheski. Laboratory phone: 757-683-4994.

D. PHYTOPLANKTON LABORATORY TRAINED EXPERTS IN ADDITION TO H.G. MARSHALL AND T.A. EGERTON:

1. Mathew Semcheski (M.S.) is a trained phytoplankton specialist and a graduate student at ODU in the Ph.D. program.

2. Cory Gall (B.S.) is a trained phytoplankton specialist and a graduate student at ODU in the Masters program.

3. Sharon Vaturi (B.S.) is a trained phytoplankton specialist and a graduate student in the Masters program at ODU.

E. GRADUATE ASSISTANTS:

The Phytoplankton Analysis Laboratory has maintained since 1965 graduate research assistants who have been trained by the principal investigator in phytoplankton systematics. This practice continues at the present time with new graduate students added to the program each year as needed.

F. SUB-CONTRACTS:

No sub-contracts are included in this project. The use of sub-contractors for analysis is not practical or justified with the high caliber of expertise on phytoplankton systematics already in this laboratory, and the experience and an extensive historical record on the capability in analyzing large quantities of samples monthly.

G. ADDITIONAL RESPONSIBILITIES:

Each step of the laboratory analysis will be routinely reviewed by H. Marshall (PI) and the laboratory supervisor. This includes examining the raw data sheets, data entry procedures and the review of the final station data sets. Routine species checks will also be made of the species identified in the laboratory by the PI and laboratory supervisor.

III. QUALITY ASSURANCE OBJECTIVES AND CRITERIA

A. OBJECTIVES AND DATA USAGE:

The objectives of the QA standards are to assure that an accurate estimate and characterization of phytoplankton and picoplankton populations are provided by maintaining the consistent and established protocols, with the appropriate checks of quality control. The goal is to meet the objectives stated for this study.

The standards of comparability and the representation of the data collected during this study will be maintained by the adherence to the sampling, analysis, and data entry procedures. QA/QC will be enhanced through procedures that include examining a composite sampling base that are tested by verification of sample identifications and cell counts by individuals in the laboratory, and having any new phytoplankton identifications made by trained specialists. In-lab verifications of identification and cell counts are conducted by the re-examination of ca. 5% of the sample concentrates as indicated in Section XII. Values of cell concentrations will be the reporting units given for data analysis. Protocol followed is given in regard to quantitative discrepancies in the Section XIII on Corrective Action. If an incorrect identification is noted during the in-lab sample analysis, the correction is made at that time to prevent any mis-identifications in future analyses. If more than 2 incorrect identifications are made in a sample, the technician is provided further information to clarify the identification, and the sample is recounted. Any major discrepancies in cell counts or identification of the major phylogenetic categories or dominant taxa (< or > 40%) would require a sample recount.

These sample analysis standards are enhanced by the training and experience in working with phytoplankton by the laboratory personnel and the PI, plus the repeated quality control checks on the analysis and data entry. The protocol followed represents an accuracy estimate of ca. 80-85% (Venrick, 1978).

A permanent record of the taxon identification's cell counts made will initially be made on raw data sheets, which will be kept as a permanent record. Upon completion of all sample analyses, the raw data sheets are reviewed for possible code or mathematical errors before data entry to a computer program takes place. These data sheets are filed in the laboratory and may contain additional notes with any additional information pertinent to the analysis results. These raw data sheets are archived and kept in the Phytoplankton Laboratory.

B. POTENTIAL CONTAMINATION:

It is the routine practice to properly rinse carboys and pump apparatus between stations by the personnel making the collections. All collection bottles to be washed after usage. All glassware and settling chambers are cleaned according to standard laboratory

practice.

C. PHYTOPLANKTON:

There are two major objectives for obtaining valid phytoplankton data. The first objective is the correct identification of the species. The other is to obtain an estimate of their concentrations in the water column. Unlike most training programs for analyzing various nutrients, etc.; there is a long-term indoctrination process necessary to train individuals to identify phytoplankton species accurately. This can only be done by working with trained and experienced specialists in the broad area of phytoplankton systematics. This type of program has been conducted in the Phytoplankton Laboratory at Old Dominion University since 1965, where graduate students and technicians are given this type of training and experience. During the last two decades, a set of over 700 voucher specimens, with data records of over 1400 species of phytoplankters, have been collected from this region and are used within the laboratory for reference in addition to our laboratory library of identification reference texts and journals to assure consistency and provide verification of identifications (Marshall et al. 2005).

There are 7 inverted plankton microscopes and two epifluorescence microscopes, plus several compound microscopes in the Phytoplankton Laboratory. An electron microscope suite is located three doors away down the corridor, and includes a scanning electron microscope, which may be used in questions of species verification.

D. PICOPLANKTON:

Separate samples are collected at each station and stored for autotrophic picoplankton analysis. Samples are taken at the same time as the phytoplankton collections. Standard epifluorescence microscopy procedures are followed to count these cells (Hobbie et al., 1977; Porter and Feig, 1980; Davis and Sieburth, 1982; Marshall, 1995). Since 1989, autotrophic picoplankton cells have been reported in this monitoring program.

IV. SAMPLING PROCEDURES.

A. ORGANIZATIONAL PLAN:

All project activities are based on established protocols for field and laboratory activities. These represent specific and detailed directions established by the PI. Past protocol of these specific assignments provide for consistent comparability and compatibility, and points for reference, for all tasks associated with field sampling and laboratory analysis.

B. PROJECT OBJECTIVES AND BACKGROUND:

To obtain representative water samples for phytoplankton and picoplankton measurements. Background information is provided in Section I on Project Description. It is based on the historical usage of these monitoring sites in the CBP since 1985.

C. ANALYSIS OF EXISTING DATA:

The PI has analyzed and reported published results of phytoplankton studies from the lower Chesapeake Bay and several of its rivers since 1964, and from the Bay Monitoring Program since 1985. As the PI of this current monitoring program, he has consistently submitted analysis of this data-base, has produced technical reports, published results, and made numerous presentations at professional meetings of these results (see references, publication records). To date this PI has published in scientific journal >40 articles based on phytoplankton results from the Bay Monitoring Program, in addition to >110 abstracts from presentations at professional scientific meetings. Numerous technical reports have also been made (ca. 30). This practice will continue.

D. ANALYSES OF INTEREST:

There are numerous components of this project that have distinct ecological importance and their presence and development patterns will be stressed in the project. These include dominant and bloom producing species, toxin producers, concentrations of cyanobacteria and dinoflagellates, and those species that may be used as indices to changing water quality conditions and trophic (health) status within the Bay system (Marshall et al., 2005, 2008, 2009). Emphasis will also be placed on relationships between these components and the water quality conditions (Marshall et al. 2009).

E. DEQ-ODU CO-ORDINATED TRIBUTARY COLLECTIONS:

DEQ personnel will collect surface (<1M) samples for all phytoplankton and picoplankton water collections from 6 river stations (TF3.3, RET3.1, TF4.2, RET4.3, TF5.5, and RET5.2). Two sets of collections are made. The collection hose is used to collect the sample to fill two carboys each (15 liters). When completed, agitate the carboys to mix the contents, and fill a 500 ml bottle containing 2 ml of Lugol's solution, and a 125 ml bottle the glutaraldehyde preservative from each carboy. The bottles are properly labeled regarding date and station site, with the 125 ml samples placed in a cooler with ice. DEQ personnel will deliver the samples in a timely manner on the day of collection to personnel from the ODU Phytoplankton Analysis Laboratory at a designated mutually agreed upon site. Prior phone contact by DEQ personnel to ODU Phytoplankton personnel is required to inform of any delay or cancellation, and to confirm delivery time and the transfer of these samples at specified locations. DEQ personnel will pick up

the collection bottles that will be provided by ODU at the ODU lab.

F. CHESAPEAKE BAY COLLECTIONS

1. Phytoplankton:

a) Prior to the Bay collections a series of vertical conductivity measurements will determine the depth of the pycnocline, with water samples taken above and below the pycnocline. The phytoplankton and picoplankton samples will be taken by personnel in the water quality component of the Bay monitoring program.

b) At each Bay station, two vertical, composite series of five 3-liter water samples are taken above and below the pycnocline at approximate equidistant depths between samples of the water column. In each of these collection sets, these waters are placed in two carboys from each depth region. These water samples are collected using a pump, connected to a hose lowered to the appropriate depths. Appropriate time limits (2 minutes) will be established for each depth pumped prior to taking the sample to assure that water from that depth is being sampled. When finished, each carboy will contain 15 liters from this pumping action. Each carboy is then gently, but thoroughly mixed, then followed by removing a 500 ml sub-sample from each carboy (2) from the upper water column series into two pre-labeled sample bottles, each containing 4 ml of Lugol's solution as a fixative. This process is repeated from the carboys (2) taken from the lower water column series.

c) The Bay protocol provides 2 sub-samples each from both the upper and lower regions of the water column that will represent the replicate composite samples from these depths. Station information is recorded on the label for each sample. Prior to sampling at a new station, the carboy and pump-hose system is repeatedly rinsed.

The samples (containing the Lugol preservative) are placed in a cooler for protection and transportation to the phytoplankton laboratory. Between stations, the carboys will be repeatedly rinsed before being used again. No additional preservation steps are required at this time. The samples are provided by the water quality personnel to phytoplankton laboratory personnel for analysis. The pump and hose is to be flushed after and before each pumping, and rinsed thoroughly after each cruise, and be checked routinely for maintenance needs. A backup system for the pump, battery, and hose will be available on each cruise. In total, for phytoplankton analysis, initially 336 water samples are collected annually from the Bay.

2. Picoplankton:

Water sample collections will be taken at the same 7 stations in the lower Chesapeake Bay and the 7 stations in the four rivers as mentioned above. These will be sub-samples taken from the same

carboys containing the composite water used for the phytoplankton collections. Sub-samples will be taken from composite collections from both the upper and lower regions of the water column as described above. A 125 ml sub-sample, each containing 2 ml of glutaraldehyde, will be collected from each of the four carboys in Nalgene plastic bottles. The station information is placed on each bottle label, and the bottles are then placed on ice in an ice cooler until their return to the phytoplankton laboratory. A total of 336 picoplankton samples will be collected annually from the Bay stations and 112 from the tributary stations (8 months) to total 448 annually.

V. SAMPLE PROTOCOL:

A. FIELD SAMPLING PROCEDURES:

1. Preparation of collection gear. This includes maintaining a fully operable pump system, functional hose, and fully charged storage battery by DEQ and ODU. A back-up system for each of these items is necessary for each cruise.

2. Preparation of sample bottles. Prior to usage, all previously used sample bottles are washed, rinsed and then labeled. Each label is to be inscribed with the date, station number, water stratum, and from which carboy it came from. Samples will then be boxed and returned to the phytoplankton laboratory. These samples are then in the custody of the phytoplankton laboratory supervisor.

3. Additional precautions need to be followed with the water samples taken for picoplankton analysis. Once taken, these samples are kept on ice in a cooler and transported directly to the phytoplankton laboratory. The picoplankton samples will be placed in a refrigerator in the phytoplankton laboratory.

B. LABORATORY PROCEDURES:

The phytoplankton laboratory supervisor will be responsible for the custody of all phytoplankton and picoplankton samples delivered to the laboratory. The labels for all of these samples will be checked for accuracy and completeness. The picoplankton samples are placed in the refrigerator and are normally analyzed by laboratory personnel within 7-14 days. The phytoplankton samples will be processed through a settling and siphoning procedure, with the final concentrate placed in a previously non-used storage vial for microscopic analysis (Marshall and Alden, 1990). This analysis will be conducted within a short period of time following collection to prevent any loss or distortion of cells in storage. The normal sinking rate of phytoplankton cells is enhanced with Lugol's solution the fixative used in these samples. Analysis of these cells is programmed to be completed within 3-4 weeks of collection. Label information is transferred from the sample bottle

to the label on the storage vial. The laboratory supervisor assigns vials for analysis to laboratory personnel.

Upon return to the laboratory, each water sample will be preserved with 5 ml of buffered formaldehyde. The 500 ml replicate sample sets are mixed (1000 ml), then 500 ml are withdrawn from this composite sample and placed on a settling table. This will begin a settling/siphoning protocol of steps for subsequent analysis. A similar composite will be obtained from the picoplankton sample sets and provide the source for their analysis.

C. SETTLING/SIPHONING PROTOCOL:

The siphoning and settling protocol used to obtain the concentrate for analysis is as follows: The sinking rate for even the smaller algae is noted as ca. 0.25 cm/hour (Admiraal et al. (1994)). The recommended settling times for phytoplankton using the Utermöhl method is also based on the height the cells have to settle in the container. Nauwerck (1963) recommends 4 hours per cm of height, Margalef (1969) recommends the sedimentation time in hours of at least 3 times the height of the container in cm, while Hasle (1978) in the UNESCO Phytoplankton Manual recommends at least 40 hours in reference to a 100ml chamber 20cm in height. For this same chamber height (20cm) Willen (1976) recommends 48 hours, with chamber heights of 10 cm (50ml). Several other references have indicated lesser time periods for settling. More specifically, the siphoning and siphoning steps are as follows:

1. The initial settling period prior to siphoning is at least 72 hours to siphon ca. one/half of the 500 ml sample water from our standard bottle ca.13.5 cm in height. This amounts to siphoning water from the upper 6.75 cm of the water sample, and is at least ca. 45 hours in excess of the recommended times indicated for settling, noted above. Siphoning is done slowly, by natural gravity flow (no pump), with the end of the siphon tube kept always just below the water's surface. No agitation to the settled sample should be made, if so the settling period would have to be repeated.
2. The second settling of the undisturbed bottom half of the sample occurs after another 48 hours (actually a total of ca. 93 hours over the total settling period needed). This remaining amount is drawn down to a concentrate of ca. 40 ml which is transferred to a glass vial. This settling period is in excess to the highest recommended values for the settling process. Siphoning is done slowly, with the end of the siphon tube kept always just below the surface of the water. No agitation to the settled sample should be made, if so the settling period would have to be repeated.

3. In the presence of a high density of cells, etc. and to assure a clear microscopy analysis of the sample, a measured fraction of the concentrate would be necessary to be removed from the concentrate for microscopic analysis. This is placed in the Utermöhl chamber, and allowed to settle undisturbed for a minimum of 16-24 hours prior to counting. Again, this exceeds the recommended settling time ca. 5-8 fold (Willen, 1979). Routine counting follows with minimum number of cells counted and fields examined.
4. Siphoning is done by placing the siphon end directly below the water's surface; the siphon should not be lowered to other depths, or to agitate the water. The siphon end is modified to contain a siphoning plug that allows water to slowly enter the siphon through several mm openings 1 cm above the siphoning plug. Flow from the siphon is by gravity, and not by any pumping or vacuum device, representing a slow and constant flow. Our staff individually receives instructions on these procedures and they recognize the importance of conducting this procedure with care and consistency.
5. To assure no significant amount of cells are lost in this Process, routine analysis of the supernatant from the siphoning is conducted. Laboratory studies to date have not indicated any significant loss of cells occur using this procedure.

After analysis, the storage vials are kept for six months, after which they may be discarded through protocols of the State of Virginia and the university Health and Safety Officer. The exception is that the laboratory still retains storage vials from station CB6.4 from the beginning of the project for future reference.

D. FINAL EVIDENCE FILE:

A record of custody for each sample analyzed will be kept on file in the phytoplankton laboratory. This will consist of the original raw data sheets, in addition to a computer data file that will be available for future reference.

E. PRESERVATIVES:

All preservatives and fixatives used in this project will be prepared by the Old Dominion University Phytoplankton Analysis Laboratory from standard stock supplies. Use of all materials that are hazardous will be secured by standards acceptable by the University, and the federal and state guidelines. This operation is routinely inspected by the University Health and Safety Officer. This Officer requires specific laboratory storage practices, and

safety practices be followed for all chemicals used in this project.

F. CUSTODY OF SAMPLES:

After they are collected, the sample custody passes directly to the laboratory supervisor, who assigns their analysis to specific laboratory personnel. The raw data sheet used for each sample is kept on file in the final evidence file in the laboratory.

VI. CALIBRATION PROCEDURES AND FREQUENCY:

A. LABORATORY OPERATIONS:

Light and epifluorescence microscopes have an annual maintenance schedule, and are repaired whenever needed.

VII. ANALYTICAL PROCEDURES:

A. JUSTIFICATION AND COMPATIBILITY OF DATA:

Procedures for the field and analysis parameters used in this project concerning the identification and measurements associated with the phytoplankton and picoplankton abundance are similar as those used consistently since 1985 in the Virginia Chesapeake Bay Monitoring Program in this laboratory. For modifications see Appendix A. These protocols have been retained to guarantee a continuity and consistency in data acquisition and analysis, and species identification. Results from these analyses will provide comparable data sets that will be essential for long-term statistical data analysis within Virginia and this region. The methods used have included specific QC objectives addressed in this proposal (section III). References used include: Hobbie et al. (1977), Davis and Seiburth (1982), Marshall (1986, 1994, 1995, and others), Marshall and Alden (1990), Marshall and Nesius (1996), Marshall et al. (2003), and Venrick (1978). Operation and all activities in the Phytoplankton Laboratory will be in accordance to Health and Safety regulations followed at Old Dominion University and agree with those for the federal government and the Commonwealth of Virginia.

B. PHYTOPLANKTON:

1. The standard Utermöhl method of phytoplankton analysis, using inverted plankton microscopes, is used in this project, and follows internationally accepted protocol for phytoplankton analysis, and is the same method used since 1985 in Virginia (Marshall 1994, 1955; Marshall and Alden 1990; Marshall and Nesius 1996). This method is essential to preserve the broad representation of species and consistency in the analysis of this community.

At high magnification (600X), and examining 20 random fields of the settling chamber only representative cells within the size

categories of the following groups will be counted. These are: a) Diatoms <10 microns in length for pennate diatoms, and <10 microns diameter for centric diatoms; b)Cryptomonads <10 microns in length; c)Unidentified "green cells" <10 microns in size; and d) Unidentified microflagellates <10 microns in size. At 300X magnification, all common representatives of the phytoplankton not included above or at the lowest magnification (described below) will be counted. At this magnification, at least 200 cells will be counted using a minimum of 10 random fields. In addition, a revised classification size breakdown will be applied to recording cell counts for unidentified diatom categories, and recording cyanobacteria filaments. The protocol followed will be as follows: a)In the unidentified pennate and centric diatoms, the following categories will be divided within these measurements: 10-30, 31-60, and >60 microns in size; also b)All sizes of trichomes and colonies will be counted at this 300X magnification. The third and lowest magnification used in the sample analysis is at 125X. At this magnification a scan of the entire settling chamber bottom plate will be conducted. Taxa identified and counted will only be the large sized taxa, often less abundant in the sample, and not included in the two other analyses described above. These taxa are generally few in number compared to others in the sample and stand out for more accurate enumeration and identification at this magnification. Common constituents in this group would be large diatoms and dinoflagellates. Lab policy is to begin analysis in a timely manner upon delivery to prevent cell loss and distortion.

Prior to counting, a work sheet is prepared, where information from the sample vial label will be transferred to the data sheet and verified. All species will be counted at only one of these magnifications. Calculations will be made from these data at the different magnifications to determine the cell concentrations per unit volume (e.g. cells/l). Identification will be based on internationally accepted identification keys, and checked against voucher specimens and/or identification keys in the ODU phytoplankton analysis laboratory. This assures a high degree of consistency and continuity in species identification that has been maintained at our laboratory. New taxa would be verified by H. Marshall, and included in the voucher records.

3. In the analysis, all taxa observed and their cell counts are initially recorded on (raw) data sheets for each station set, and entered to the computer. All raw data sheets are archived and kept on file and available for later reference. These sheets also represent the source of significant observations concerning blooms, predators, or any unusually conditions worthy of recording.

4. All preserved water sample vials that are analyzed are

archived for a minimum of 6 months. These are kept in case any follow-up examination of the samples is required.

C. PICOPLANKTON:

When brought to the phytoplankton laboratory, samples (125 ml) will be stored in a refrigerator at 4°C and the counting procedures will be completed within 7-14 days after their collection date.

Using a millipore apparatus, a backing 0.45 um nuclepore filter, wetted with distilled water, is placed on the millipore stem. Then a blackened 0.20 um nuclepore filter, is placed over the other filter. 1-2 ml of the composite water sample is added to the filter apparatus. Using a pump, and a maximum vacuum of 10 cm of Hg, the sample is filtered until the meniscus disappears from the top filter. The 0.2 um nuclepore filter is removed and placed immediately on a glass slide previously moistened with breath. A drop of immersion oil (Cargille type A, refractive index 1.515) is placed at the center of the filter, then a cover glass is added, followed by another drop of immersion oil to the cover glass. The slide is examined immediately with an epifluorescence microscope equipped with a 100-W Hg lamp and a 100X oil immersion objective (Neofluar 100/1.30) at 1000X magnification.

Using an appropriate filter set random field counts are made with a minimum coverage of 20 fields for each slide. Cells not counted here are those previously identified and counted with the phytoplankton sample at 300x or 600X (e.g. some *Merismopedia* spp.). Mean cell counts of replicate samples are computer entered and cell concentrations determined (cells/liter). The raw data sheets are also archived.

D. MICROCYSTIN AND BLOOM SPECIES ANALYSIS: During major cyanobacteria blooms of Virginia rivers with *Microcystis aeruginosa* concentrations exceeding 50,000 cells per ml, this laboratory will use ELISA microcystin analysis procedures to analyze for microcystin.

E. ANALYTICAL COSTS BASIS:

The project plan stipulates a total of 448 phytoplankton and 448 picoplankton samples will be collected for subsequent processing procedures over the 12 month period to give a grand total of 896 samples collected.

F. LABORATORY FACILITIES:

The Old Dominion University Phytoplankton Analysis Laboratory is located in the Mills Godwin Life Science Building on the campus of Old Dominion University in Norfolk, Virginia. It occupies

approximately 600 sq. ft. and has additional storage space for equipment, and supplies, with an accompanying cell culture laboratory. It has been one of the most active and fully equipped laboratories for phytoplankton analysis in the United States. It contains 7 inverted plankton microscopes, two epifluorescence microscopes, and several compound microscopes, with an active staff annually of typically 4 lab specialists. It possesses all necessary supplies and support material for phytoplankton, picoplankton and related studies. The facility also contains a personal computer system (2) for data entry into a mainline system. Additional features include an extensive laboratory library of identification keys, manuals and publications for all the major phytoplankton categories.

The laboratory also contains an extensive photographic and electron micrograph reference (voucher) record of phytoplankton from the Chesapeake Bay and adjacent coastal waters that are used to verify species and maintain consistency of identifications.

In the near vicinity of the Phytoplankton Laboratory is an electron microscope suite that is available for additional species examination and has an SEM and TEM, plus cell culture facilities. The Phytoplankton Laboratory has been in operation for over 30 years. Phytoplankton studies centered in this laboratory have emphasized the Chesapeake Bay, Virginia lakes and rivers, bloom producing species, and toxin producing species. Other studies have included phytoplankton studies from the northeastern Atlantic coastal waters of the United States, the Delaware Bay Basin, the Caribbean, and the eastern equatorial Pacific. This laboratory has extensive experience in analyzing large quantities of phytoplankton samples (ca. 900-1200 annually), preparing data analysis reports, and presenting the results.

VIII. INTERNAL QUALITY CONTROL CHECKS:

A. FIELD CHECKS:

All sample bottles are screened prior and after usage on station, in regard to proper labeling and that the bottles contain the proper preservative. During collections, checks are made by the collection personnel to assure picoplankton and productivity sample bottles are kept on ice in an ice cooler and the phytoplankton bottles are stored properly.

B. LABORATORY CHECKS:

1. Identification Protocol:

All species identification will be supervised by the principal investigator who is a phytoplankton specialist with over 40 years of experience in phytoplankton systematics and ecology. Other personnel are technicians, or graduate assistants trained by the

PI. In addition, the laboratory contains extensive identification keys, voucher records and other data of previous phytoplankton collections from the Chesapeake Bay and the region that are used for taxonomic correctness and consistency in identifications. The PI has regular meetings with the entire laboratory staff to discuss the program, data results and current populations in the samples.

Phytoplankton checks are done by a second member of the lab personnel and never by the individual being checked. This assures there will be less chance of miss-identifications being made. A record is maintained of the results of the QC sample analysis. Upon completion of all sample analyses, the raw data sheets are initially reviewed for possible code or mathematical errors before data entry takes place by the lab supervisor or his designee. These data sheets are filed in the laboratory. See Sections IX, XI, and XIII on Performance Audits, Data Reduction, and Corrective Action for more specific details. After data entry the entered station data is again checked for any entry errors.

IX. EXTERNAL QUALITY CONTROL CHECK:

The Old Dominion University Phytoplankton Analysis Laboratory has routinely interacted with other phytoplankton laboratories regionally, nationally, and internationally regarding confirmation of species identification and cooperative studies involving the Chesapeake Bay Monitoring Program, plus numerous joint publications with these other laboratories. This practice will continue as a component of the program. This laboratory has also participated in split sample analysis protocols for confirmation of species present and their concentrations.

X. PERFORMANCE AND SYSTEMS AUDITS:

1. A member of the ODU laboratory staff will meet with DEQ's field team once every 1-2 year period to ensure there is consistency between agencies in the collections. The QA/QC performance comparisons of the plankton samples will also be conducted and checked for errors in taxa identification and/or their cell counts, under the direction of the PI and/or laboratory supervisor on ca.5% of the samples. This assures that the identity of any species identified by the first examiner will be directly observed and verified, and that the species cell counts of species identified can be compared. Upon completion of sample analyses, the raw data sheets are reviewed for possible code or mathematical errors before data entry takes place, and are filed in the laboratory. In a similar fashion ca. 5% of the concentrated samples will be re-analyzed in house for data comparison checks.

3. The laboratory protocol will be under the supervision of the principal investigator and the phytoplankton laboratory

supervisor. A review of raw data sheets, etc. will be done monthly.

XI. PREVENTIVE MAINTENANCE:

A. FIELD COLLECTIONS:

All collection gear are to be routinely cleaned and examined for wear or breakage by the users (ODU or DEQ). Proper maintenance of pumps, collection apparatus, and all back up gear is the responsibility of the collection agency. DEQ assumes all responsibilities during tributary, or other, sample collections made on the water, and sample delivery to ODU personnel.

Contingency Plan: Back up pumps, hose, batteries, and other support gear are to be maintained by the collection agency.

B. LABORATORY:

The laboratory supervisor will oversee the care and maintenance schedule of the laboratory microscopes and computer system. The microscopes are serviced annually, or when needed, and the computers whenever needed.

Contingency Plan: A back up computer system is available in the Phytoplankton Laboratory and in the office of the PI. Additional back up inverted plankton microscopes and epifluorescence microscopes are available in the laboratory.

XII. DATA REDUCTION AND REPORTING:

Data transcription, validation and reporting procedures are designed to produce data sets that been verified as reproducing all information from each raw data analysis sheet for phytoplankton and picoplankton measurements.

A. REDUCTION:

1. Raw Data Sheets:

A raw data sheet is prepared for each sample analysis. Cell counts are assigned to a taxonomic code for each species within the sample and these counts are calculated into numbers of cells per liter using the following formula:

$$\frac{\text{no. cells}}{\text{no. fields}} \times \frac{\text{constant}}{1} \times \frac{1}{\text{conc.}} \times \frac{1}{\text{vol.}}$$

Upon completion of the analysis, species code numbers and calculations are spot checked monthly by the laboratory supervisor.

2. Data Entry, Confirmation and Submission:

Cell counts from the raw data sheets are entered into a Microsoft Foxpro relational database. The database is constructed to minimize potential data entry errors. In addition, the database is designed to require visual confirmation of all fields prior to submission processing. Data entry and visual confirmation is performed when data is entered. The Foxpro database application also generates the required data sets for submission to the EPA. These data sets are comma delimited ASCII format text files designed to comply with the current data submission requirements specified in the Chesapeake Bay Program.

Once the final submission data sets are created, a series of SAS programs are used to conduct an additional check for consistency of dates, station locations and other important fields between submission data sets generated for the plankton programs. If corrections are required, appropriate changes are made to the Foxpro database and the ASCII files. After all final checks are completed, the ASCII format data sets are transferred to the Chesapeake Bay Program office using an FTP data transferal protocol. Finalized data sets are converted into SAS format and appended to an existing long-term SAS data set for use in data analysis.

3. Data Storage and Backup:

All finalized data are stored in three separate formats: 1) as permanent records in the Foxpro database; 2) as ASCII format text files and 3) as a SAS format data set. Backup of all relevant files occurs routinely. Monthly backup records containing all files are retained for one year and an annual backup is retained permanently. In addition, permanent data sets and programs are copied to read-only CD-ROMS on an annual basis. Copies of the CD-ROMS are kept in two separate locations. The original raw data sheets are also archived as a final backup.

B. REPORTING:

1. Raw Data:

Data will be submitted to the Chesapeake Bay Data Center via tape or file semi-annually as requested. Data sets and data requests will be formatted and verified in a manner consistent with the most recent versions of the Chesapeake Bay Program Data Management Plans.

2. Reports:

Reports will be submitted to the Virginia Department of Environmental Quality Project Officer when requested. These may include raw data summaries, a brief narrative of progress, any QA/QC problems, and suggestions for improvement. DEQ will be informed of publications and presentations made at professional meetings regarding the program.

XIII. DATA REVIEW SOP:

A set procedure is established to review all data entry. This includes field sampling, labeling, the transfer of label information to vials, and raw data sheets as the initial stages. These stages are followed by the analysis and checking of data on the raw data sheets prior to transfer to computer entry by the laboratory supervisor and the PI. Data entered into the computer is screened after each station entry to check for double entry, species codes, or any other errors. These values are checked against the raw data sheets.

XIV. CORRECTIVE ACTION:

The principal investigator and/or the laboratory supervisor are responsible for evaluating initial phytoplankton identifications by laboratory personnel until proficiency has been established in identification by an individual. No new species is accepted as valid until verified by the PI. The co-investigator is responsible for corrective action involving the productivity measurements.

Laboratory personnel are trained in a rigorous program of sample preparation and species identification prior to their data entry. We have voucher photographs and illustrations, plus an extensive identification library in our laboratory for species verification and reference. Consistency in our identifications has been our philosophy for over two decades of plankton studies. Throughout this training program their identifications are checked routinely. Personnel are trained to seek confirmation about any questionable taxon from the lab supervisor or the principal investigator for confirmation. There have been systematic changes in accordance to a normal nomenclature revisions.

1. All work by laboratory personnel will be routinely checked by the PI or the Laboratory Supervisor for identification and total counts. If there are inconsistencies (in any recount procedures, Section III), the sample will be re-analyzed. Refer to Section III, for standards. In general, any major discrepancy in cell count (< or > 40%) will be recounted, and any mis-identification of taxa will be immediately corrected, and in either case the samples would be re-analyzed.

2. Out of Control Situations. The nature of this project should not produce "out of control" situations. Any unexpected event that would occur would be approached with a definite plan to remedy the situation, without jeopardizing the project. Exceptions that may occur involve weather conditions that would prevent sample collections. Under these conditions, an alternative collection date, if feasible, is scheduled.

XV. QUALITY ASSURANCE:

The principal investigator will be responsible for preparing any requested reports on QA/QC results associated with this project. The PI evaluates the results of the data analysis. If there are QA problems, the PI is responsible for their correction.

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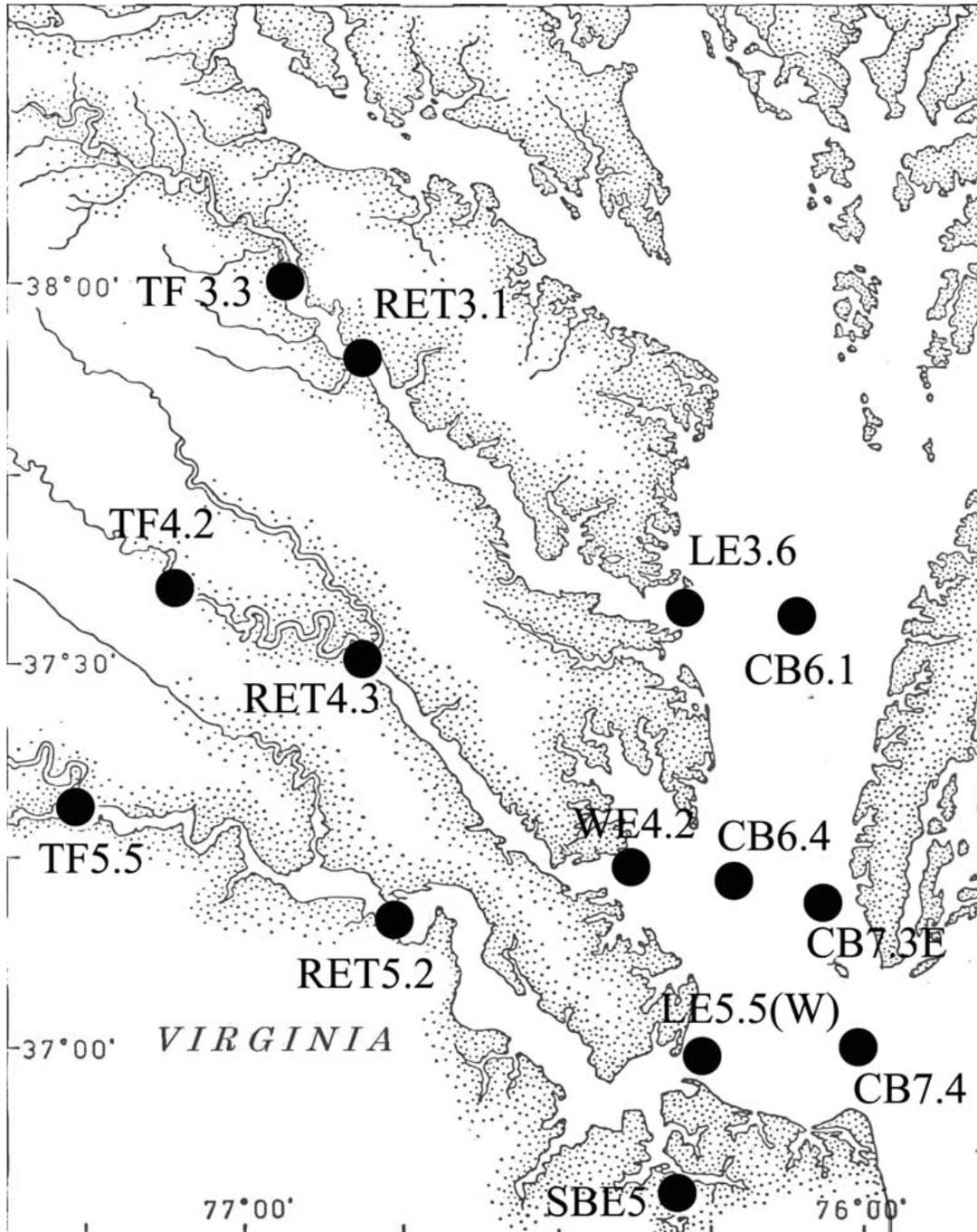


Figure 1. Map of Chesapeake Bay and tributaries, showing station locations.

APPENDIX A.

HISTORICAL MODIFICATIONS TO THE VIRGINIA PHYTOPLANKTON COMPONENT OF THE CHESAPEAKE BAY MONITORING PROGRAM SINCE JULY 1985

July 1985: The Virginia phytoplankton monitoring program began this month. It consisted of monitoring phytoplankton composition and abundance at 7 lower Chesapeake Bay stations. All microscopic analysis were conducted by ODU personnel. Composite samples from above and below the pycnocline would be taken for analysis. Collections were twice monthly March through October, and monthly November through February at stations CB6.1, CB6.4, CB7.3e, CB7.4, LE3.6, LE5.5, and WE4.2. Bay collections were taken by ODU personnel.

March 1986: Seven tributary stations were added for monthly phytoplankton collections by ODU personnel. Stations were TF3.3, RET3.1, RET4.1, RET4.3, TF5.5, and RET5.2.

March 1987: Collections at the Pamunkey River station RET4.1 were cancelled and subsequent sampling was then established at station TF4.2 for phytoplankton sample collections and analysis by ODU personnel.

January 1988: Autotrophic picoplankton abundance analysis by ODU personnel was added at stations in James River and lower Bay (TF5.5, RET5.2, LE5.5, CB7.4, and CB7.3e).

February 1989: Stations SBE2 and SBE3 in the Elizabeth River were added for phytoplankton analysis and collection by ODU.

July 1989: Collections for and analysis of autotrophic picoplankton abundance began at all Virginia tributary and Chesapeake Bay stations by ODU personnel.

July 1989: Water sample collections for the measurement of primary phytoplankton productivity were added at all Virginia tributary and Chesapeake Bay stations by ODU personnel.

October 1990: The twice monthly Virginia collections in Chesapeake Bay from March through October were reduced to monthly collections.

November 1995: Station SBE2 in the Elizabeth River was dropped for phytoplankton, picoplankton, and productivity measurements.

September 1996: Station LE5.5, at the mouth of the James River was moved 0.6 miles west and changed to LE5.5-W.

January 2002: Monthly collections for January, February, November, and December at tributary stations in the James, York, and Rappahannock rivers were discontinued in the program. Monthly collections of SBE3 will continue.

August 2002: ODU purchased new inverted plankton microscopes changing their mid- and high magnification from 315/500X to 300/600X, with magnification constants adjusted accordingly.

January 2003: DEQ personnel replaced ODU personnel in the collection of samples used in phytoplankton, autotrophic picoplankton, and productivity measurements at tributary stations TF3.3, RET3.1, TF4.2, RET4.3, TF5.5, and RET5.2. The shift from ODU to DEQ plankton collections resulted in slight location changes. TF3.3 was moved from latitude 38.0186 and longitude -76.9083 to 38.01847 and -76.90928; RET3.1 from 37.9200 and -76.8300 to 37.91730 and -76.82220; TF4.2 from 37.5797 and -77.0219 to 37.57999 and -77.02128; RET4.3 from 37.5067 and -76.7883 to 37.50869 and -76.78889; TF5.5 from 37.3128 and -77.2331 to 37.31265 and -77.23283; and RET5.2 was moved from 37.2067 and -76.7933 to 37.20294 and -76.78219. These samples would be picked up by ODU personnel from DEQ the same day of the collections for analysis.

2003: Maryland phytoplankton lab changed from the high magnification (500X) to 312X magnification as the preferred magnification for primary species analysis. This would then be similar to what ODU uses.

2005: Both ODU and Maryland agreed to the following protocols to follow in their analysis. 1. Maryland will add a scan at 125X for species records (as ODU has been using); 2. ODU will count cells in cyanobacteria filaments rather than filaments (as Maryland does); 3. Maryland agreed to count 10 random fields, with a 200 cell minimum as done by ODU at mid-magnification; 4. Both laboratories agreed to change protocols to count 20 random fields at high magnification with no cell minimum only the following taxonomic groups: centric diatoms <10 µm, pennate diatoms <10 µm, cryptomonads <10 µm, unidentified green algal spherical cells 3-5µm, and unidentified micro-phytoflagellates <10µm.

January 2010: Productivity measurements were discontinued for the Virginia tributaries and Chesapeake Bay stations. No phytoplankton or picoplankton samples were taken during months of January and February.

July 2010: Phytoplankton and picoplankton collections were re-instated for future January and February months for Chesapeake Bay. Sub-surface water sample collections for phytoplankton and picoplankton analysis were discontinued for the tributaries (James, York, Pamunkey, Elizabeth, and Rappahannock rivers).

**WORK/QUALITY ASSURANCE PROJECT PLAN FOR MONITORING
PHYTOPLANKTON AND PICOPLANKTON IN THE
LOWER CHESAPEAKE BAY AND TRIBUTARIES**

by

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April 15, 2013

For the Period: July 1, 2013 through June 30, 2014

Prepared for
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I. PROJECT DESCRIPTION (PHYTOPLANKTON COMPONENT):

This project is responsible for monitoring the composition and abundance of phytoplankton and the concentration of the autotrophic picoplankton at stations located in the lower Chesapeake Bay and four rivers that enter the lower Bay. Emphasis is placed on the correct and consistent identification of species within the phytoplankton community, and the continuity in the use of methodologies that have been followed since the phytoplankton monitoring program began in 1985. This approach is essential to provide consistency and validity in data collections and in subsequent data analysis procedures for the evaluation of trends and any changes in these populations over time. To accomplish this consistency, major resources are provided: These include: 1.) Proven expertise in phytoplankton systematics in the Chesapeake Bay and regional rivers, and phytoplankton that enter the Bay from the northeast U.S. coastal waters; 2) decades of experience in the Chesapeake Bay Program monitoring plankton concentrations in Bay and tributary waters; 3.) A fully equipped phytoplankton analysis laboratory, 4.) A complete series of identification reference keys for the phytoplankton categories, and 5.) An on-going program of graduate research assistants trained in phytoplankton systematics. Additional resources available include molecular genetic analysis, plus cell culture and scanning electron microscopy facilities. Previous modifications to this analysis program since its inception are described in Appendix A.

A. OBJECTIVES AND SCOPE OF PROJECT:

1. To determine the composition, and abundance of phytoplankton populations at 7 stations in the lower Chesapeake Bay and 7 stations in the Elizabeth, James, York (Pamunkey) and Rappahannock Rivers (Fig. 1). This requires knowledge of phytoplankton species from fresh water, estuarine, and marine populations.

2. To determine concentrations of the autotrophic picoplankton, at 7 stations in the lower Chesapeake Bay and 7 stations in the tributaries mentioned above.

3. To provide base line data that may be used for data interpretation and statistical analysis of the phytoplankton and picoplankton mentioned above (e.g. Marshall et al. 2005, Marshall et al. 2009).

4. To identify from these collections information on the seasonal presence and abundance of potential toxin producing phytoplankters at these stations in the lower Bay and the tributaries (e.g. Marshall, 1994, 1996, 2007; Marshall et al. 2003, 2005, 2006, 2008, 2009; Marshall and Egerton, 2009).

5. To provide information regarding major algal bloom events of in the lower Bay and these tributaries that occurred during the collection period (Marshall and Egerton 2009).

6. To establish a consistent identification protocol, providing a

long term historical data-base that may be used in the future statistical and trend studies in the Bay and its tributaries (e.g. Marshall et al. 2005; 2006; 2009).

B. COORDINATION ACTIVITIES WITH CBP COMMITTEES:

This study will be conducted in a similar time frame as the water quality collections (DEQ,ODU). This protocol provides a more meaningful basis to examine relationships that exist between these data sets, and for evaluations to other current and previous data sets in the Chesapeake Bay Program. Results obtained from phytoplankton, and picoplankton monitoring will have specific relevance and value to objectives of several Chesapeake Bay Programs. This study would provide information used in the phytoplankton IBI, to study long-term trends of phytoplankton development and related trophic status in these waters. Additional information will be available on the presence and location of toxic and bloom producing phytoplankton, in addition to long term trends of the picoplankton and their contribution to Bay productivity.

C. STUDY DESIGN:

1. Project Dates: The time period for this study is from July 1, 2013 through June 30, 2014. In the Bay field collections for phytoplankton and picoplankton measurements will be between July 2013 through June 2014 (10 collections), and in the 4 Bay tributaries from July 2013 to October 2013, and March 2014 to June 2014. For details see section IV, Sampling Procedures.

2. Relationship to Background Information of this Project and Data Uses

The continuation of this project at Old Dominion University provides consistency in the identification of the phytoplankton populations (with over 1400 species recognized in the Chesapeake Bay region (Marshall 1994, Marshall et al., 2005). An analysis program of this size requires consistency and accuracy in the wide range of species identifications. These algae will represent the major populations for studies regarding any long-term trends, response indices to major water quality changes, and to note trophic shifts, or interactions in local food webs.

3. Sampling Network Design Rationale:

The Chesapeake Bay is a plankton-driven ecosystem, the most important of which are the algae, composed of phytoplankters and picoplankters, which are the primary food and oxygen producers, and the basis of major food webs in these waters. The data sets obtained in this and on-going projects, and combined with the previous data in the lower Chesapeake Bay Monitoring Program, will provide and enhance data sets on phytoplankton composition and picoplankton abundance in the lower Chesapeake Bay and these

rivers. This project is designed to be an integral component of the CBP to associate relationships between these living resources and various water quality variables, including algal bloom development.

Long-term trend analysis of this data set will continue to provide information regarding associations that may have direct relationships to management decisions concerning nutrient entry into this region (Marshall et al. 2003, 2006, 2008, 2009). This data offers specific relationships between the major producers (phytoplankton and picoplankton) to specific food web constituents, water quality, and trophic exchanges. Justification for this design is based on long term monitoring plans concerned with the ecological status and health of the Chesapeake Bay system.

4. Sampling Locations:

The contract identifies sample analysis from seven stations in the lower Chesapeake Bay and seven stations in the four tributaries (Figure 1). The tributary locations were originally identified by the Virginia Department of Environmental Quality as representative of salinity regions in Virginia's rivers and include tidal fresh, oligohaline, and mesohaline regions. The Bay stations were located to provide representative sites along the central, eastern, and western Bay regions. These are as follows:

<u>Station</u>	<u>Description</u>	<u>Latitude</u>	<u>Longitude</u>
TF5.5	James R.	37.31265	77.23283
RET5.2	James R.	37.20294	76.78219
SBE5	Elizabeth R.	36.7675	76.2861
TF4.2	Pumunkey R.	37.57999	77.02128
RET4.3	York R.	37.50869	76.78889
TF3.3	Rappahan. R.	38.01847	76.90928
RET3.1	Rappahan. R.	37.91730	76.82220
CB7.4	Bay Mouth	36.9955	76.0208
CB7.3E	Eastern Shore	37.2285	76.0542
CB6.4	Central Bay	37.2364	76.2083
CB6.1	Main Channel	37.5883	76.1625
LE5.5W	Bay at Mouth James R.	36.9988	76.3135
WE4.2	Bay at Mouth York R.	37.2417	76.3867
LE3.6	Bay at Mouth Rapp. R.	37.5969	76.2853

5. Coordinated sampling:

At Bay stations and the Elizabeth River station, the plankton water samples are collected by ODU water quality personnel from the Department of Chemistry and Biochemistry from an ODU vessel. At the remaining 6 tributary stations, personnel from Virginia DEQ collect the tributary water samples to be analyzed in this program.

6. Parameters to Measure in the Phytoplankton Component:
 - a. Phytoplankton composition and abundance.
 - b. Autotrophic picoplankton abundance.

The major phytoplankton taxa to be identified and counted in this study will include when present: the diatoms, dinoflagellates, cyanobacteria, chlorophyceans, euglenophyceans, cryptomonads, and other algal categories that appear in the samples. Identification will be to species level when possible, or the lowest taxonomic category. The picoplankton to be monitored and counted will consist of the autotrophic cells generally 0.2 to 2.0 microns in size. Taxonomic identifications of phytoplankters will be similar to those established in the monitoring program since 1985 (See Marshall and Alden, 1990; Marshall, 1994; Marshall et al. 2005), and for the autotrophic picoplankton by Marshall (1995).

7. Frequency of Collections:

Ten monthly water samples will be taken at the Bay stations during the sampling year. Collections in the tributaries will be taken during 8 months of the year.

8. Types of Samples:

All phytoplankton and picoplankton data will come from the analysis of water samples collected from a boat. See Section IV on Sampling Procedures.

II. PROJECT ORGANIZATION AND RESPONSIBILITIES

The processing and analysis of all samples, plus data computer entry, will be completed in the Phytoplankton Analysis Laboratory at Old Dominion University.

A. PROJECT MANAGER. The Project Manager, Dr. Harold G. Marshall, will supervise the activities associated with this project that include overseeing responsibilities of the Laboratory Supervisor and designated Laboratory staff. He will supervise the stages in the analysis of the samples, resolving problems that may arise, and assuring the satisfactory completion of the study. He is responsible for data review, and submission of data. The project manager will review results of the analyses and approves the quality assurance/quality control protocols to insure the quality of results. The Project Manager will administer the financial and technical requirements of the project and be responsible for any reports concerning this project. He will also meet with members of the laboratory staff to discuss and review their responsibilities in relation to the project. The Project Manager will respond to questions by the contracting agencies regarding this project and project reports.

Harold G. Marshall is a phycologist and marine ecologist, with over 40 years of experience in the systematics and ecology of marine, estuary and fresh water phytoplankton. He has published over 170 articles on phytoplankton (plus an addition 190 abstracts), which include articles from the Chesapeake Bay, its rivers and from regional marine shelf waters. His publications include over 40 articles and >110 abstracts specifically on phytoplankton in Chesapeake Bay and its tributaries, plus over 40 technical reports on these topics from this region, including investigations of various toxic and bloom producing algae within the Chesapeake Bay (Marshall 1996, Marshall et al. 2005, Marshall et al. 2008, Marshall et al. 2009, Marshall and Egerton 2009).

B. QUALITY ASSURANCE OFFICER:

The Quality Assurance Officer (Michael F. Lane) will meet periodically with the principal investigator to discuss: 1. operation, sampling and analysis procedures, 2. data entry, and 3. any problems that may arise that would delay data entry. He is responsible for approving the QA/QC protocol used in this project and advises the principal investigator on procedures, in addition to logistics, or other related concerns that may influence the sampling, or data analysis.

C. PHYTOPLANKTON FIELD/LABORATORY SUPERVISOR AND ASSOCIATE PROJECT MANAGER: This position is held by Todd Egerton who will receive his Ph.D. this year at ODU. He has 10 years of experience as a phytoplankton investigator with 13 publications and numerous presentations at professional meetings regarding phytoplankton within these waters. He is responsible for sampling operation, statistical data analysis, supervising lab personnel, preparation and custody of samples from each cruise. He also oversees laboratory analysis and QA/QC, and data processing.

D. PHYTOPLANKTON LABORATORY STAFF

1. Mathew Semcheski (M.S.) is a trained phytoplankton specialist and a graduate student at ODU in the Ph.D. program.

2. Matthew Muller (M.S.) is a trained phytoplankton specialist and a graduate student at ODU.

3. Sharon Vaturi (B.S.) is a trained phytoplankton specialist and a graduate student in the Masters program at ODU.

4. Kathryn Rogers (B.S.) is a trained phytoplankton specialist and a graduate student in the Masters program at ODU.

E. GRADUATE ASSISTANTS:

The Phytoplankton Analysis Laboratory has maintained since 1965 graduate research assistants who have been trained in phytoplankton systematics.

F. SUB-CONTRACTS:

No sub-contracts are included in this project. The use of sub-contractors for analysis is not practical or justified with the high caliber of expertise on phytoplankton systematics already in this laboratory, and the importance of maintaining consistency in analyzing these populations.

G. ADDITIONAL RESPONSIBILITIES:

Each step of the laboratory analysis will be routinely reviewed by the Project Manager and the laboratory supervisor. This includes examining the raw data sheets, data entry procedures and the review of the final station data sets. Routine species checks will also be made of the species identified in the laboratory by the PI and laboratory supervisor.

III. QUALITY ASSURANCE OBJECTIVES AND CRITERIA

A. OBJECTIVES AND DATA USAGE:

The objectives of the QA standards are to assure that an accurate estimate and characterization of phytoplankton and picoplankton populations are provided by maintaining consistent and established protocols, with appropriate checks of quality control to meet the objectives stated for this study. In-lab verifications of identification and cell counts are conducted by the re-examination of ca. 5% of the sample analysis concentrates as indicated in Section XII. Values of cell concentrations will be the reporting units given for data analysis. Protocols followed are given in regard to quantitative discrepancies in Section XIII on Corrective Action. If an incorrect identification is noted during the in-lab sample analysis, the correction is made at that time to prevent any mis-identifications in future analyses. If more than 2 incorrect identifications are made in a sample, the technician is provided further information to clarify the identification, and the sample is recounted. Any major discrepancies in cell counts or identification of the major phylogenetic categories, or dominant taxa (< or > 40%) would require a sample recount. These sample analysis standards are enhanced by the training and experience provided by laboratory personnel and the PI, which is supported by repeated quality control checks on the analysis protocol and data entry. The protocol followed represents an accuracy estimate of ca. 80-85% (Venrick, 1978).

A permanent record of the taxon identification's cell counts made will initially be made on raw data sheets, which will be kept as a permanent record. Upon completion of all sample analyses, the

raw data sheets are reviewed for possible code or mathematical errors before data entry to a computer program takes place. These data sheets are filed in the laboratory and may contain additional notes regarding information pertinent to the analysis findings. These raw data sheets are archived and kept in the Phytoplankton Laboratory.

B. POTENTIAL CONTAMINATION:

It is the routine practice to properly rinse carboys and pump apparatus between stations by the personnel making the collections. All collection bottles are to be washed after usage. All glassware and settling chambers are cleaned according to standard laboratory practice.

C. PHYTOPLANKTON:

There are two major objectives for obtaining valid phytoplankton data. The first objective is the correct identification of the species, the other is to obtain an estimate of their concentrations in the water column. Unlike most training programs for analyzing various nutrients, etc.; there is a long-term indoctrination process necessary to train individuals to identify phytoplankton species accurately. This can only be done by working with trained and experienced specialists in the broad area of phytoplankton systematics. This type of program has been conducted in the Phytoplankton Laboratory at Old Dominion University since 1985, where graduate students and technicians are given this training and experience. During the last two decades, a set of over 700 voucher specimens, with data records of over 1400 species of phytoplankters, have been collected from this region and are used within the laboratory for reference in addition to our laboratory library of identification reference texts and journals to assure consistency and provide verification of identifications (Marshall et al. 2005).

There are 7 inverted plankton microscopes and two epifluorescence microscopes, plus several compound microscopes in the Phytoplankton Laboratory. An electron microscope suite is located three doors down the corridor, and includes a scanning electron microscope, which may be used in questions of species verification.

D. PICOPLANKTON:

Separate samples are collected, preserved at each station and stored for autotrophic picoplankton analysis. Samples are taken at the same time as the phytoplankton collections. Standard epifluorescence microscopy procedures are followed to count these cells (Hobbie et al., 1977; Porter and Feig, 1980; Davis and Sieburth, 1982; Marshall, 1995). Since 1989, autotrophic picoplankton cells have been reported in this monitoring program.

IV. SAMPLING PROCEDURES.

A. ORGANIZATIONAL PLAN:

All project activities are based on established protocols for field and laboratory activities. These represent specific and detailed directions established by the PI. Past protocol of these specific assignments provide for consistent comparability and compatibility, and points for reference, for all tasks associated with field sampling and laboratory analysis.

B. PROJECT OBJECTIVES AND BACKGROUND:

To obtain representative water samples for phytoplankton and picoplankton measurements. Background information is provided in Section I on Project Description. It is based on the historical usage of these monitoring sites in the CBP since 1985.

C. ANALYSIS OF EXISTING DATA:

The PI has a record of reporting and publishing results of phytoplankton studies from the lower Chesapeake Bay and several of its rivers since 1964 and from the Bay Monitoring Program since 1985, which have also included technical reports and numerous presentations at professional meetings. To date this includes as published in scientific journal >40 articles based on phytoplankton results from the Chesapeake Bay Monitoring Program, in addition to >110 abstracts from presentations at professional scientific meetings. Numerous technical reports have also been made (ca. 30), in addition to publications by Todd Egerton, and numerous presentations by the research assistants in the laboratory. This practice will continue.

D. ANALYSES OF INTEREST:

There are numerous components of this project that have distinct ecological importance and their presence and development patterns will also be stressed in the project. These include identifying the dominant and bloom producing species, the toxin producers, the presence of newly recorded invasive species to these sites, the increasing presence of cyanobacteria and specific dinoflagellates, and species as indices to changing water quality conditions and trophic (health) status within the Bay system (Marshall et al., 2005, 2008, 2009, Marshall and Egerton 2009).

E. TRIBUTARY COLLECTIONS: DEQ-ODU CO-ORDINATED

Monthly samples are taken from March to October. DEQ personnel will collect surface (<1M) samples for all phytoplankton and picoplankton water collections from 6 river stations (TF3.3, RET3.1, TF4.2, RET4.3, TF5.5, and RET5.2). Two sets of replicate collections are made. The collection hose is used to collect the

sample to fill two carboys each (15 liters). When completed, each carboy is gently, but thoroughly mixed, then a 500 ml bottle containing 2 ml of Lugol's solution are filled (phytoplankton sample), plus a 125 ml bottle (picoplankton sample) containing the glutaraldehyde (1 ml) preservative from each of the two carboys. The bottles are labeled regarding date and station site. DEQ personnel will deliver, and transfer the samples in a timely manner on the day of collection to personnel from the ODU Phytoplankton Analysis Laboratory at a designated mutually agreed upon site. Prior phone contact (757-683-4994) by DEQ personnel to ODU Phytoplankton personnel is required of any delay or cancellation, and to confirm delivery time and the transfer of these samples at specified locations. DEQ personnel will pick up the collection bottles that will be provided by ODU at the ODU lab. The tributary collections total annually 112 water samples for the phytoplankton and 112 picoplankton water samples, totaling 224 samples.

F. CHESAPEAKE BAY COLLECTIONS

1. Phytoplankton:

a) Collections are made by personnel from the Old Dominion University Department of Chemistry and Biochemistry under the supervision of Dr. John Donat. Monthly phytoplankton and picoplankton collections will be taken February through October, plus a collection will be made either during December or January, which will be determined by weather conditions, for a total of 10 collection months.

b) Prior to the Bay collections on station, a series of vertical conductivity measurements will determine the depth of the pycnocline. At each Bay station, two vertical, composite series of five 3-liter water samples are to be taken above and below the pycnocline at approximate equidistant depths between samples of the water column. From each of these collection two sets the water taken is placed in two carboys from each depth. The water samples are collected using a pump, connected to a hose lowered to the appropriate depths. Time limits (ca. 2 minutes) will be established for each depth pumped prior to taking the sample to assure that depth is being sampled. When finished, each carboy will contain 15 liters from this pumping action. Each carboy is then gently, but thoroughly mixed, then followed by removing a 500 ml sub-sample from each carboy (2) from the upper water column series into two pre-labeled sample bottles, each containing 2 ml of Lugol's solution as a fixative. This process is repeated from the carboys (2) taken from the lower water column series below the pycnocline.

c) The Bay protocol provides 2 sub-samples each from both the

upper and lower regions of the water column and represent the replicate samples from these depths. Station information is recorded on the label for each sample. Prior to sampling at a new station the carboy and pump-hose system is repeatedly rinsed. The samples will then be forwarded to the Old Dominion University phytoplankton laboratory for analysis. Between stations, the carboys will be repeatedly rinsed before being used again. No additional preservation steps are required at this time. The samples are provided by the water quality personnel to phytoplankton laboratory personnel for analysis. The pump and hose are thoroughly flushed after and before each pumping, and rinsed thoroughly after each cruise, and are to be checked routinely for maintenance needs. A backup system for the pump, battery, and hose will be available on each cruise. In total, for phytoplankton analysis, initially 280 water samples are collected annually from the Bay.

2. Picoplankton:

Water sample collections will be taken at the same 7 stations in the lower Chesapeake Bay (and the 7 stations in the four rivers) as mentioned above. These will be sub-samples taken from the same carboys containing the composite water used for the phytoplankton collections. Sub-samples will be taken from composite collections from both the upper and lower regions of the water column as described above. A 125 ml sub-sample, each containing 2 ml of glutaraldehyde, will be collected from each of the four carboys in plastic bottles. The station information is placed on each bottle label, and the bottles are subsequently forwarded to the Old Dominion University phytoplankton laboratory for analysis. A total of 280 picoplankton samples will be collected annually from the Bay stations.

V. SAMPLE PROTOCOL:

A. FIELD SAMPLING PROCEDURES:

1. Preparation of collection gear. This includes maintaining a fully operable pump system, functional hose, and fully charged storage battery by DEQ and ODU. A back-up system for each of these items is necessary for each cruise.

2. Preparation of sample bottles. Prior to usage, all previously used sample bottles are washed, rinsed and then labeled. With each collection, each label would contain the date, station number, water stratum, and from which carboy it came from. After samples are returned to Old Dominion University they are then in the custody of the phytoplankton laboratory supervisor.

B. LABORATORY PROCEDURES:

The phytoplankton laboratory supervisor is responsible for protocols associated with the processing the samples for laboratory analysis. The labels for all of these samples will be checked for accuracy and completeness. The picoplankton samples are stored in the laboratory refrigerator and are normally analyzed by laboratory personnel within 7-14 days. The phytoplankton samples will be processed through a settling and siphoning procedure with the final concentrate placed in a non-used storage vial prior to microscopic analysis (Marshall and Alden, 1990). This analysis will be conducted within a short period of time following collection to prevent any loss or distortion of cells in storage. The normal sinking rate of phytoplankton cells is enhanced with Lugol's solution the fixative used in these samples. Analysis of these cells is programmed to be completed within 3-4 weeks of collection.

Upon return to the laboratory, each water sample will be preserved with 5 ml of buffered formaldehyde. The 500 ml replicate sample sets are mixed (1000 ml), then 500 ml are withdrawn from this composite sample and placed on a settling table. This will begin a settling/siphoning protocol of steps for subsequent analysis. A similar composite will be obtained from the picoplankton sample sets and provide the source for their analysis.

C. SETTLING/SIPHONING PROTOCOL:

The siphoning and settling protocol used to obtain the concentrate for analysis from the 500 ml sample is as follows: The sinking rate for even the smaller algae is noted as ca 0.25 cm/hour (Admiraal et al. (1994). The recommended settling times for phytoplankton using the Utermöhl method is also based on the height the cells have to settle in the container. Nauwerck (1963) recommends 4 hours per cm of height, Margalef (1969) recommends the sedimentation time in hours of at least 3 times the height of the container in cm, while Hasle (1978) in the UNESCO Phytoplankton Manual recommends at least 40 hours in reference to a 100 ml chamber 20cm in height. For this same chamber height (20cm) Willen (1976) recommends 48 hours, with chamber heights of 10 cm (50ml). Several other references have indicated lesser time periods for settling. More specifically, the siphoning and siphoning steps are as follows:

1. The initial settling period prior to siphoning is at least 72 hours to siphon ca. one/half of the 500 ml sample water from our standard bottle ca.13.5 cm in height. This amounts to siphoning water from the upper 6.75 cm of the water sample, and is at least 45 hours in excess of the recommended times indicated for settling, noted above. Siphoning is done slowly,

by natural gravity flow (no pump), with the end of the siphon tube kept always just below the water's surface. No agitation to the settled sample should be made, if so the settling period would have to be repeated.

2. The second settling of the undisturbed bottom half of the sample occurs after another 48 hours (actually a total of ca. 93 hours over the total settling period needed). This remaining amount is drawn down to a concentrate of ca. 40 ml which is transferred to a glass vial. This settling period is in excess to the highest recommended values for the settling process. Siphoning is done slowly, with the end of the siphon tube kept always just below the surface of the water. No agitation to the settled sample should be made, if so the settling period would have to be repeated.
3. In the presence of a high density of cells, etc. and to assure a clear microscopy analysis of the sample, a measured fraction of the concentrate would be necessary to be removed from the concentrate for microscopic analysis. This is placed in the Utermöhl chamber, and allowed to settle undisturbed for a minimum of 16-24 hours prior to counting. Again, this exceeds the recommended settling time ca. 5-8 fold (Willen, 1979). Routine counting follows with minimum number of cells counted and fields examined.
4. Note: Siphoning is done by placing the siphon end directly below the water's surface; the siphon should not be lowered to other depths, or to agitate the water. The siphon end is modified to contain a siphoning plug that allows water to slowly enter the siphon through several mm openings 1 cm above the siphoning plug. Flow from the siphon is by gravity, and not by any pumping or vacuum device, representing a slow and constant flow. Our staff individually receives instructions on these procedures and they recognize the importance of conducting this procedure with care and consistency.
5. To assure no significant amount of cells are lost in this process, routine analysis of the supernatant from the siphoning is conducted. Laboratory studies to date have not indicated any significant loss of cells occur using this procedure.

After analysis, the storage vials are kept for six months, after which they may be discarded through protocols of the State of Virginia and the university Health and Safety Officer. The exception is that the laboratory still retains storage vials from station CB6.4 from the beginning of the project for future reference.

D. FINAL EVIDENCE FILE:

A record of custody for each sample analyzed will be kept on file in the phytoplankton laboratory. This will consist of the original raw data sheets, in addition to a computer data file that will be available for future reference.

E. PRESERVATIVES:

All preservatives and fixatives used in this project will be prepared by the ODU Phytoplankton Analysis Laboratory from standard stock supplies. Use of all materials that are hazardous will be secured by standards acceptable by the University, and the federal and state guidelines. This operation is routinely inspected by the University Health and Safety Officer. This Officer requires specific laboratory storage practices, and safety practices be followed for all chemicals used in this project.

F. CUSTODY OF SAMPLES:

After they are collected the sample custody passes directly to the laboratory supervisor, who assigns their analysis to specific laboratory personnel. The raw data sheet used for each sample is kept on file in the final evidence file in the laboratory.

VI. CALIBRATION PROCEDURES AND FREQUENCY:

A. LABORATORY OPERATIONS:

Light and epifluorescence microscopes have an annual maintenance schedule, and are repaired whenever needed.

VII. ANALYTICAL PROCEDURES:

A. JUSTIFICATION AND COMPATIBILITY OF DATA:

Procedures for the field and analysis parameters used in this project concerning the identification and measurements associated with the phytoplankton and picoplankton abundance are similar as those used consistently since 1985 in the Virginia Chesapeake Bay Monitoring Program in this laboratory. For modifications see Appendix A. These protocols have been retained to guarantee a continuity and consistency in data acquisition and analysis, and species identification. Results from these analyses will provide comparable data sets that will be essential for long-term statistical data analysis within Virginia and this region. The methods used have included specific QC objectives addressed in this proposal (section III). References used include: Hobbie et al. (1977), Davis and Seiburth (1982), Marshall (1986, 1994, 1995, and others), Marshall and Alden (1990), Marshall and Nesius (1996), Marshall et al. (2003), and Venrick (1978). Operation and all activities in the Phytoplankton Laboratory will be in accordance to Health and Safety regulations followed at Old Dominion University and agree with those for the federal government and the

Commonwealth of Virginia. The analytical protocols followed are those previously approved by a joint committee composed of Virginia and Maryland participants conducting the laboratory analysis of phytoplankton in the Program, plus representatives of the Chesapeake Bay Monitoring Program and invited persons of interest.

B. PHYTOPLANKTON:

1. The Utermöhl method using inverted plankton microscopes, is used in this project, and generally follows internationally accepted protocol for phytoplankton analysis, and is basically the same method used since 1985 in Virginia (Marshall 1994, 1955; Marshall and Alden 1990; Marshall and Nesius 1996). This method is essential to preserve the broad representation of species and consistency in the analysis of this community.

At high magnification (600X), and examining 20 random fields of the settling chamber only representative cells within the size categories of the following groups will be counted. These are: a) Diatoms <10 microns in length for pennate diatoms, and <10 microns diameter for centric diatoms; b) cryptomonads <10 microns in length; c) Unidentified "green cells" <10 microns in size; and d) Unidentified microflagellates <10 microns in size. At 300X magnification, all common representatives of the phytoplankton not included above or at the lowest magnification (described below) will be counted. At this magnification, at least 200 cells will be counted using a minimum of 10 random fields. In addition, a revised classification size breakdown will be applied to recording cell counts for unidentified diatom categories, and recording cyanobacteria filaments. The protocol followed will be as follows: a) In the unidentified pennate and centric diatoms, the following categories will be divided within these measurements: 10-30, 31-60, and >60 microns in size; also b) All sizes of trichomes and colonies will be counted at this 300X magnification. The third and lowest magnification used in the sample analysis is at 125X. At this magnification a scan of the entire settling chamber bottom plate will be conducted. Taxa identified and counted will only be the larger sized taxa, often less abundant in the sample, and not included in the two other analyses described above. These taxa are generally few in number compared to others in the sample and stand out for more accurate enumeration and identification at this magnification. Common constituents in this group would be large diatoms and dinoflagellates. Lab policy is to begin analysis in a timely manner upon delivery to prevent cell loss and distortion.

Prior to counting, a work sheet is prepared, where information from the sample vial label will be transferred to the data sheet and verified. All species will be counted at only one of these magnifications. Calculations will be made from these data at the

different magnifications to determine the cell concentrations per unit volume (e.g. cells/l). Identification will be based on internationally accepted identification keys, and checked against voucher specimens and/or identification keys in the ODU phytoplankton analysis laboratory. This assures a high degree of consistency and continuity in species identification that has been maintained at our laboratory. New taxa would be verified by the Project Manager and included in the voucher records.

3. In the analysis, all taxa observed and their cell counts are initially recorded on (raw) data sheets for each station set, and entered to the computer. All raw data sheets are archived and kept on file and available for later reference. These sheets also represent the source of significant observations concerning blooms, predators, or any unusually conditions worthy of recording.

4. All preserved water sample vials that are analyzed are archived for a minimum of 6 months. These are kept in case any follow-up examination of the samples is required.

C. PICOPLANKTON:

When brought to the phytoplankton laboratory, samples (125 ml) will be stored in a refrigerator at 4°C and the counting procedures will be completed within 7-14 days after their collection date.

Using a millipore apparatus, a backing 0.45 um nuclepore filter, wetted with distilled water, is placed on the millipore stem. Then a blackened 0.20 um nuclepore filter, is placed over the other filter. 1-2 ml of the composite water sample is added to the filter apparatus. Using a hand pump, and a maximum vacuum of 10 cm of Hg, the sample is filtered until the meniscus disappears from the top filter. The 0.2 um nuclepore filter is removed and placed immediately on a glass slide previously moistened with breath. A drop of immersion oil (Cargille type A, refractive index 1.515) is placed at the center of the filter, then a cover glass is added, followed by another drop of immersion oil to the cover glass. The slide is examined immediately with an epifluorescence microscope equipped with a 100-W Hg lamp and a 100X oil immersion objective (Neofluar 100/1.30) at 1000X magnification.

Using an appropriate filter set random field counts are made with a minimum coverage of 20 fields for each slide. Cells that are not counted here are those previously identified and counted with the phytoplankton sample at 300x or 600X (e.g. some *Merismopedia* spp.). Mean cell counts of replicate samples are computer entered and cell concentrations determined (cells/liter). The raw data sheets are also archived.

D. MICROCYSTIN AND BLOOM SPECIES ANALYSIS: During major cyanobacteria blooms of Virginia rivers with *Microcystis aeruginosa* concentrations exceeding 50,000 cells per ml, this laboratory will use ELISA microcystin analysis procedures to analyze for microcystin.

E. ANALYTICAL COSTS BASIS:

The project plan stipulates a specific number of samples will be provided to this laboratory for analysis.

F. LABORATORY FACILITIES:

The Old Dominion University Phytoplankton Analysis Laboratory is located in the Mills Godwin Life Science Building on the campus of Old Dominion University in Norfolk, Virginia. It occupies approximately 600 sq. ft. and has additional storage space for equipment, and supplies, with an accompanying cell culture laboratory. It has been one of the most active and fully equipped laboratories for phytoplankton analysis in the United States. It contains 7 inverted plankton microscopes, two epifluorescence microscopes, and several compound microscopes, with an active staff annually of typically 4 lab graduate student trained specialists. It possesses the necessary supplies and support material for phytoplankton, picoplankton analysis and related studies. The facility also contains a personal computer system (2) for data entry into a mainline system. Additional features include an extensive laboratory library of identification keys, manuals and publications for the major phytoplankton categories.

The laboratory also contains an extensive photographic and electron micrograph reference (voucher) record of phytoplankton from the Chesapeake Bay and adjacent coastal waters that are used to verify species and maintain consistency of identifications.

In the near vicinity of the Phytoplankton Laboratory is an electron microscope suite that is available for additional species examination and has both an SEM and TEM, plus a near by cell culture facility. The Phytoplankton Laboratory has been in operation for over 30 years. Phytoplankton studies centered in this laboratory have emphasized the Chesapeake Bay, Virginia lakes and rivers, and both bloom and toxin producing species. Other activities have included phytoplankton studies from the northeastern Atlantic coastal waters of the United States, the Delaware Bay Basin, the Caribbean, and the eastern equatorial Pacific. This laboratory has extensive experience in analyzing large quantities of phytoplankton samples (ca. >1200 annually), preparing data analysis reports, and presenting the results.

VIII. INTERNAL QUALITY CONTROL CHECKS:

A. FIELD CHECKS:

All sample bottles are screened prior and after usage on station, in regard to proper labeling and that the bottles contain the proper preservative. During collections, checks are made by the collection personnel to assure bottles are stored properly.

B. LABORATORY CHECKS:

1. Identification Protocol:

All species identification will be supervised by the principal investigator. In addition, the laboratory contains extensive identification keys, voucher records and other data of previous phytoplankton collections from the Chesapeake Bay and the region that are used for taxonomic correctness and consistency in identifications.

Phytoplankton checks are done by a second member of the lab personnel and never by the individual being checked. This assures there will be less chance of miss-identifications. A record is maintained of the results of the QC sample analysis. The raw data sheets are initially reviewed for possible code or mathematical errors before data entry takes place. These data sheets are filed in the laboratory. See Sections IX, XI, and XIII on Performance Audits, Data Reduction, and Corrective Action for more specific details. After data entry the entered station data is again checked for any entry errors.

IX. EXTERNAL QUALITY CONTROL CHECK:

The Old Dominion University Phytoplankton Analysis Laboratory has routinely interacted with other phytoplankton laboratories regionally, nationally, and internationally regarding confirmation of species identification via exchanges with the Principal Investigator, plus numerous joint publications with these other laboratories. This practice will continue as a component of the program. This laboratory has also participated in several split sample analysis evaluations of species identification and quantification protocols with personnel conducting the Maryland phytoplankton component of the Chesapeake Bay Monitoring Program. These have established consistencies in analytical protocols used.

X. PERFORMANCE AND SYSTEMS AUDITS:

1. A member of the ODU laboratory staff will meet with DEQ's field team once every 1-2 year period to ensure there is consistency used by agencies in the collection protocol. The QA/QC performance comparisons of the plankton samples will also be conducted and checked for errors in taxa identification and/or their cell counts, under the direction of the PI and/or laboratory supervisor on ca.5% of the samples. This assures that the identity

of any species identified by the first examiner will be directly observed and verified, and that the species cell counts of species identified can be compared. Upon completion of sample analyses, the raw data sheets are reviewed for possible code or mathematical errors before data entry takes place, and are filed in the laboratory. In a similar fashion ca. 5% of the concentrated samples will be re-analyzed in house for data comparison checks.

XI. PREVENTIVE MAINTENANCE:

A. FIELD COLLECTIONS:

All collection gear are to be routinely cleaned and examined for wear or breakage by the users (ODU or DEQ). Proper maintenance of pumps, collection apparatus, and all back up gear is the responsibility of the collection agency. DEQ assumes all responsibilities during tributary, or other, sample collections made on the water, and sample delivery to ODU personnel.

Contingency Plan: Back up pumps, hose, batteries, and other support gear are to be maintained by the collection agency.

B. LABORATORY:

The laboratory supervisor will oversee the care and maintenance schedule of the laboratory microscopes and computer system. The microscopes are serviced regularly, or when needed, and the computers whenever needed.

Contingency Plan: A back up computer system is available in the Phytoplankton Laboratory and in the office of the PI. Additional back up inverted plankton microscopes and epifluorescence microscopes are available in the laboratory.

XII. DATA REDUCTION AND REPORTING:

Data transcription, validation and reporting procedures are designed to produce data sets that have been verified as reproducing all information from each raw data analysis sheet for phytoplankton and picoplankton measurements.

A. REDUCTION:

1. Raw Data Sheets:

A raw data sheet is prepared for each sample analysis. Cell counts are assigned to a taxonomic code for each species within the sample and these counts are calculated into numbers of cells per liter using the following formula:

$$\frac{\text{no. cells}}{\text{no. fields}} \times \frac{\text{constant}}{1} \times \frac{1}{\text{conc.}} \times \frac{1}{\text{vol.}}$$

Upon completion of the analysis, species code numbers and

calculations are spot checked monthly by the laboratory supervisor.

2. Data Entry, Confirmation and Submission (Provided by Michael Lane, ODU Quality Assurance Officer):

The phytoplankton raw data is entered into a Microsoft Foxpro relational database. The database is constructed to minimize potential data entry errors. In addition, the database is designed to require visual confirmation of all fields prior to submission processing. Data entry and visual confirmation is performed when data is entered. The Foxpro database application also generates the required data sets for submission to the EPA.

These data sets are comma delimited ASCII format text files designed to comply with the current data submission requirements specified in the Chesapeake Bay Program.

Once the final submission data sets are created, a series of SAS programs are used to conduct an additional check for consistency of dates, station locations and other important fields between submission data sets generated for the plankton programs. If corrections are required, appropriate changes are made to the Foxpro database and the ASCII files. After all final checks are completed, the ASCII format data sets are transferred to the Chesapeake Bay Program office using an FTP data transferal protocol. Finalized data sets are converted into SAS format and appended to an existing long-term SAS data set.

3. Data Storage and Backup:

All finalized data are stored in three separate formats: 1) as permanent records in the Foxpro database; 2) as ASCII format text files and; 3) as a SAS format data set. Backup of all relevant files occurs routinely. Monthly backup records containing all files are retained for one year and an annual backup is retained permanently. In addition, permanent data sets and programs are copied to read-only CD-ROMS on an annual basis. Copies of the CD-ROMS are kept in two separate locations. The original raw data sheets are also archived as a final backup.

B. REPORTING:

1. Raw Data:

Data will be submitted to the Chesapeake Bay Data Center semi-annually, or as requested. Data sets and data requests will be formatted and verified in a manner consistent with the most recent versions of the Chesapeake Bay Program Data Management Plans.

2. Reports:

Reports will be submitted to the Virginia Department of Environmental Quality Project Officer when requested. These may include raw data summaries, a brief narrative of progress, any QA/QC problems, and suggestions for improvement. DEQ will be

informed of publications and presentations made at professional meetings regarding the program.

XIII. DATA REVIEW SOP:

A set procedure is established to review all data entry. This includes field sampling, labeling, the transfer of label information to vials, and raw data sheets as the initial stages. These stages are followed by the analysis and checking of data on the raw data sheets prior to transfer to computer entry by the laboratory supervisor and the PI. Data entered into the computer is screened after each station entry to check for double entry, species codes, or any other errors. These values are checked against the raw data sheets.

XIV. CORRECTIVE ACTION:

The principal investigator and/or the laboratory supervisor are responsible for evaluating initial phytoplankton identifications by laboratory personnel until proficiency has been established in identification by an individual. No new species is accepted as valid until verified by the PI. The co-investigator is responsible for corrective action involving the productivity measurements.

Laboratory personnel are trained in a rigorous program of sample preparation and species identification prior to their data entry. Consistency in our identifications has been our philosophy for over two decades of plankton studies. Throughout this training program their identifications are checked routinely. Personnel are trained to seek confirmation about any questionable taxon from the lab supervisor or the principal investigator for confirmation. There have been systematic changes in accordance to the normal nomenclature revisions.

1. All work by laboratory personnel will be routinely checked by the PI or the Laboratory Supervisor for identification and total counts. If there are inconsistencies (in any recount procedures, Section III), the sample will be re-analyzed. Refer to Section III, for standards. In general, any major discrepancy in cell count (< or > 40%) will be recounted, and any mis-identification of taxa will be immediately corrected, and in either case the samples would be re-analyzed.

2. Out of Control Situations. The nature of this project should not produce "out of control" situations. Any unexpected event that would occur would be approached with a definite plan to remedy the situation, without jeopardizing the project. Exceptions that may occur involve weather conditions that would prevent sample collections. Under these conditions, an alternative collection date, if feasible, is scheduled.

XV. QUALITY ASSURANCE:

The principal investigator will be responsible for preparing any requested reports on QA/QC results associated with this project. The PI evaluates the results of the data analysis. If there are QA problems, the PI is responsible for their correction.

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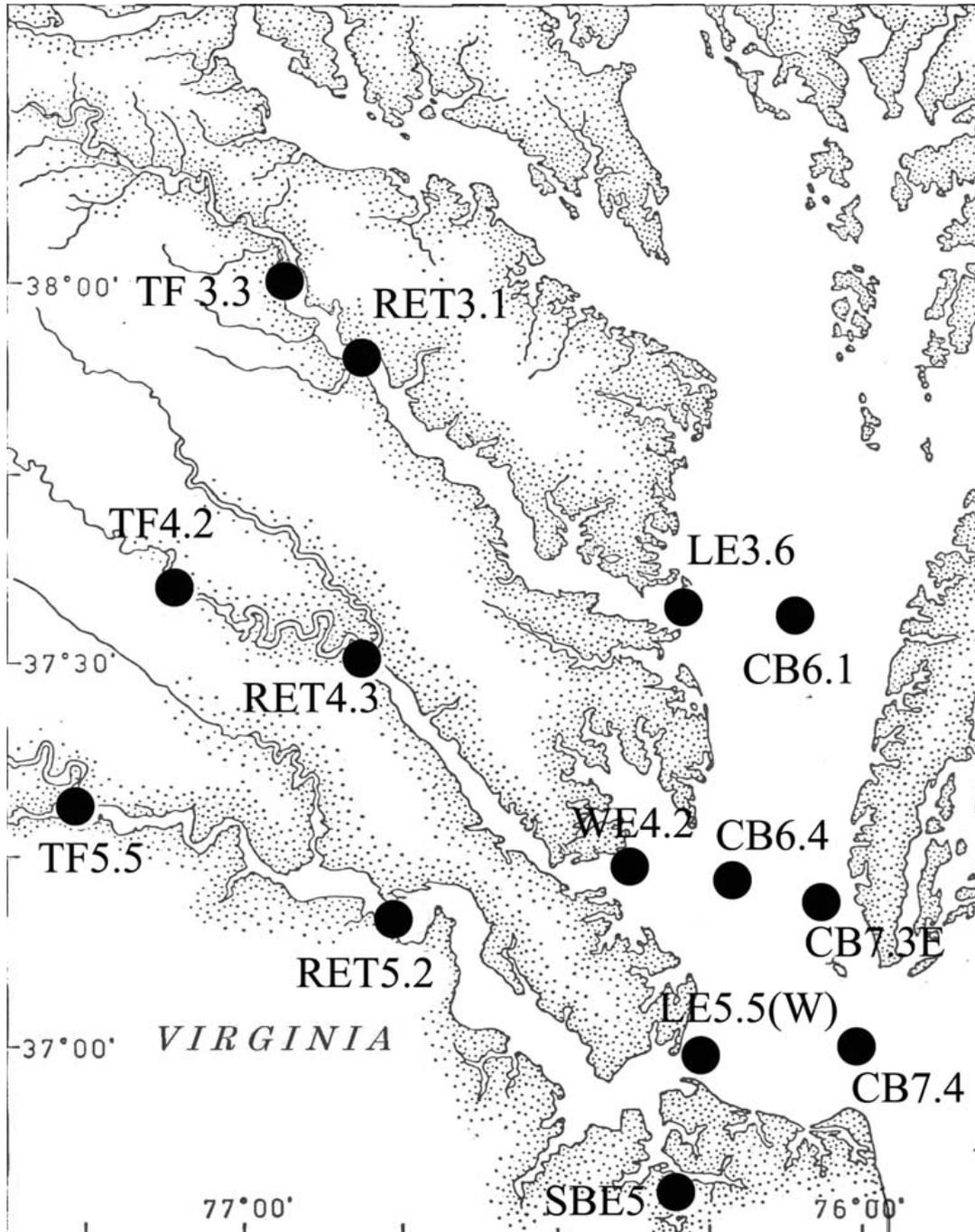


Figure 1. Map of Chesapeake Bay and tributaries, showing station locations.

APPENDIX A.

HISTORICAL MODIFICATIONS TO THE VIRGINIA PHYTOPLANKTON COMPONENT OF THE CHESAPEAKE BAY MONITORING PROGRAM SINCE JULY 1985

July 1985: The Virginia phytoplankton monitoring program began this month. It consisted of monitoring phytoplankton composition and abundance at 7 lower Chesapeake Bay stations. All microscopic analysis were conducted by ODU personnel. Composite samples from above and below the pycnocline would be taken for analysis. Collections were twice monthly March through October, and monthly November through February at stations CB6.1, CB6.4, CB7.3e, CB7.4, LE3.6, LE5.5, and WE4.2. Bay collections were taken by ODU personnel. H.G. Marshall as Principal Investigator.

March 1986: Seven tributary stations were added for monthly phytoplankton collections by ODU personnel. Stations were TF3.3, RET3.1, RET4.1, RET4.3, TF5.5, and RET5.2.

March 1987: Collections at the Pamunkey River station RET4.1 were cancelled and subsequent sampling was then established at station TF4.2 for phytoplankton sample collections and analysis by ODU personnel.

January 1988: Autotrophic picoplankton abundance analysis by ODU personnel was added at stations in James River and lower Bay (TF5.5, RET5.2, LE5.5, CB7.4, and CB7.3e).

February 1989: Stations SBE2 and SBE3 in the Elizabeth River were added for phytoplankton analysis and collection by ODU.

July 1989: Collections for and analysis of autotrophic picoplankton abundance began at all Virginia tributary and Chesapeake Bay stations by ODU personnel.

July 1989: Water sample collections for the measurement of primary phytoplankton productivity were added at all Virginia tributary and Chesapeake Bay stations by ODU personnel.

October 1990: The twice monthly Virginia collections in Chesapeake Bay from March through October were reduced to monthly collections.

November 1995: Station SBE2 in the Elizabeth River was dropped for phytoplankton, picoplankton, and productivity measurements.

September 1996: Station LE5.5, at the mouth of the James River was moved 0.6 miles west and changed to LE5.5-W.

January 2002: Monthly collections for January, February, November, and December at tributary stations in the James, York, and Rappahannock rivers were discontinued in the program. Monthly collections of SBE3 will continue.

August 2002: ODU purchased new inverted plankton microscopes changing their mid- and high magnification from 315/500X to 300/600X, with magnification constants adjusted accordingly.

January 2003: DEQ personnel replaced ODU personnel in the collection of samples used in phytoplankton, autotrophic picoplankton, and productivity measurements at tributary stations TF3.3, RET3.1, TF4.2, RET4.3, TF5.5, and RET5.2. The shift from ODU to DEQ plankton collections resulted in slight location changes. TF3.3 was moved from latitude 38.0186 and longitude -76.9083 to 38.01847 and -76.90928; RET3.1 from 37.9200 and -76.8300 to 37.91730 and -76.82220; TF4.2 from 37.5797 and -77.0219 to 37.57999 and -77.02128; RET4.3 from 37.5067 and -76.7883 to 37.50869 and -76.78889; TF5.5 from 37.3128 and -77.2331 to 37.31265 and -77.23283; and RET5.2 was moved from 37.2067 and -76.7933 to 37.20294 and -76.78219. These samples would be picked up by ODU personnel from DEQ the same day of the collections for analysis.

2003: Maryland phytoplankton lab changed from the high magnification (500X) to 312X magnification as the preferred magnification for primary species analysis. This would then be similar to what ODU uses.

2005: Both ODU and Maryland agreed to the following protocols to follow in their analysis. 1. Maryland will add a scan at 125X for species records (as ODU has been using); 2. ODU will count cells in cyanobacteria filaments rather than filaments (as Maryland does); 3. Maryland agreed to count 10 random fields, with a 200 cell minimum as done by ODU at mid-magnification; 4. Both laboratories agreed to change protocols to count 20 random fields at high magnification with no cell minimum only the following taxonomic groups: centric diatoms <10 µm, pennate diatoms <10 µm, cryptomonads <10 µm, unidentified green algal spherical cells 3-5µm, and unidentified micro-phytoflagellates <10µm.

January 2010: Productivity measurements were discontinued for the Virginia tributaries and Chesapeake Bay stations. No phytoplankton or picoplankton samples were taken during months of January and February.

July 2010: Phytoplankton and picoplankton collections were re-instated for future January and February months for Chesapeake Bay. Sub-surface water sample collections for phytoplankton and picoplankton analysis were

discontinued for the tributaries (James, York, Pamunkey, Elizabeth, and Rappahannock rivers).

July 20, 2013: Monthly phytoplankton and picoplankton collections for analysis in the Bay have been reduced from 12 to 10. The November collections have been eliminated, plus collections taken either during December or January (to be determined by weather conditions).

Appendix 5

ODU Mulholland Standard Operating Procedures

Quality Assurance Project Plan for addressing data needs for DEQ subtasks 1.1, 1.2, and 1.3 in the James River estuary

Proposed work includes:

- 1) Environmental factors promoting algal blooms in the Lower James River estuary – Year 1 and Year 2
- 2) Nutrient regeneration as a means of initiating and sustaining algal blooms in the James River estuary – Year 1
- 3) Diagnostic pigments as a means of tracking the distribution of algal functional groups within the James River – Year 1

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I. Lab description, data collected, personnel

A. Lab purpose

The laboratory in the Ocean, Earth, and Atmospheric Sciences at Old Dominion University is run by Dr. Margaret Mulholland. This laboratory has the capability of analyzing dissolved and particulate constituents in fresh and marine waters, including nutrients, biomass indicators (particulate nitrogen and carbon), algal biomass, and algal pigments. The main focus of the laboratory is researching nitrogen and carbon cycling within natural phytoplankton assemblages in aquatic systems with the use of stable isotopes. Sampling techniques, methods, and data handling and analysis employed in the laboratory and by the laboratory staff and students are all described within the Quality Assurance Project Plan (QAPP).

B. Data collected

Data collected from this study will consist of surface chlorophyll *a* (chl *a*), temperature, salinity, and dissolved oxygen (DO) mapping of the meso- and polyhaline James River, and its tidal tributary, the Lafayette River, surface nutrient mapping of the Lafayette River, stratification indices of the meso- and polyhaline James River, and the Lafayette River following storm events, and water column uptake and regeneration of nutrients in the Upper and Lower James River, and the Lafayette River.

C. Personnel

PI: Dr. Margaret Mulholland, ODU, mmulholl@odu.edu, (757) 683-3972

ODU Personnel: Dr. Katherine C. Filippino, Field and Lab Coordinator, kcfilipp@odu.edu, (757) 683-5654

Mr. Peter Bernhardt, Lab Manager, QA/QC Officer, pbernhardt@odu.edu, (757) 683-5603

HRSD Team Leader: Mr. Will Hunley, HRSD Liason, whunley@hrsd.com, (757) 633-2776

HRSD Personnel: Mr. Bruce Weckworth, Field Coordinator, bweckworth@hrsd.com, (757) 814-3366

II. Environmental factors promoting algal blooms in the Lower James River estuary – Year 1 and Year 2

A. Continuous Water Quality Monitoring in the Lafayette River - Year 1 and Year 2

Background: The study proposal entitled “*Environmental factors promoting algal blooms in the Lower James River estuary (Year 1 and Year 2)*” contains provisions for the deployment of two continuous monitoring sites (YSI) where algal blooms are known to initiate. This document contains the specifics related to the CMON data collections. A summary of the monitoring parameters is provided in Table 1.

1. Fixed Monitoring Stations Description – Year 1 and Year 2

Two fixed continuous monitoring stations are located in the Lafayette River. These fixed stations are used to continuously monitor water quality parameters including depth, water temperature, salinity, pH, chlorophyll, turbidity, and dissolved oxygen using YSI 6600EDS V2 data sondes. These stations will provide long term measurements that can be supplemented with shorter term monitoring, such as the DATAFLOW cruises, and other process-oriented studies.

The fixed stations are deployed off of existing piers within an attached 4 inch PVC housing. This housing is perforated to allow flow of water freely to the instrument while at the same time protecting the instrument from large pieces of floating debris. Within the PVC housing, a YSI 6600EDS V2 data logging sonde is secured 1-2 meters above the bottom. The PVC pipe structure is cleaned with a chimney sweep type tool at each switch out to reduce fouling and the collection of sediment on the inside of the pipe.

2. Locations of Fixed Monitoring Stations and Sample Frequency – Year 1 and Year 2

There are a total of two continuous monitoring stations in the Lafayette River (**Appendix 1, Maps 1 - 3**).

Locations of Fixed Stations within the Lafayette River

Fixed Station	Location	CBP Segment	Latitude (decimal degrees)	Longitude (decimal degrees)
AC	Ashland Circle	LAFMH	36.880390	-76.272525
NYCC	Norfolk Yacht and CC	LAFMH	36.906483	-76.305945

Individual YSI 6600EDS V2 sondes will be deployed for a maximum of 14 days on permanently established stations, where they will log data at 15-minute intervals. Deployment length is adjusted for areas and periods of high fouling. At the end of the deployment period each YSI sonde is returned to the lab for downloading of data, post calibration, cleaning, and re-calibration. A second YSI 6600EDS V2 is deployed following retrieval of the original sonde in order to maintain a continuous record of data. The two sondes are placed adjacent to one another for 20 minutes to allow for post comparison of the individual units and to allow for no loss of monitoring data. Note: Prior to July 15, 2012 there was only one YSI 6600EDS V2 available for each site. During this time period the instruments were retrieved, serviced, and re-deployed as soon as practical.

3. Water Quality Instrumentation – Year 1 and Year 2

The fixed stations utilize the YSI 6600EDS V2 equipped with the Clean Sweep Extended Deployment System (EDS) and with sensors including a ROX 6150 Optical DO probe, a YSI 6560 conductivity/temperature probe, a 6561 pH probe, a 6136 turbidity probe, and a 6025 chlorophyll probe. The EDS is comprised of a brush that at set intervals sweeps across the sensors to dislodge any fouling organisms or material that has settled on the sensors. This feature ensures better quality data over longer deployment periods in areas with high fouling rates. The new YSI ROX DO probe utilizes the luminescence-lifetime technique to provide DO measurements which are less likely to be affected by fouling or low DO environments.

Specifications for YSI Data (YSI 6600EDS V2)

PARAMETER	UNITS	PRECISION	ACCURACY	MDL
ODO	% Saturation	0.1%	±1%	0 %
ODO	mg/L	0.01mg/L	±0.1mg/L	0 mg/L
Salinity	ppt	0.01ppt	0.1ppt	0 ppt
Temperature	°C	0.01°C	±0.15°C	-5°C
pH	unit	0.01units	±0.2units	0 units
Turbidity	NTU	0.1NTU	2 NTU	0 NTU
Chlorophyll	µg/L Chl	0.1µg/L Chl	-	0 µg/L Chl

4. Calibration and Maintenance – Year 1 and Year 2

All calibrations and maintenance are completed in accordance with the YSI, Inc. operating manual methods (YSI 6-series Environmental Monitoring Systems Manual; YSI, Inc. Yellow Springs, OH). Refer to HRSD CMAP documentation for more detailed information.

5. Sampling Procedures – Year 1 and Year 2

a. Verification Samples

Field verification of pH, salinity, dissolved oxygen and temperature is made during each deployment and retrieval interval with a YSI 6920. Water samples are also collected for verification during each deployment and retrieval interval for Winkler DO and chlorophyll-a with a VanDorn water sampler. These water samples are collected at the depth of the YSI 6600 EDS sensor array. A secchi depth measurement is also performed. Chlorophyll water samples are collected in amber sample containers and field filtered on site. Immediately after field filtration of Chl *a*, and HRSD employee folds and wraps the filters into quarters onto a piece of lab foil. Once folded in foil, the filters are placed in labeled petri dishes which are then placed on ice in a cooler and returned to the laboratory where they are stored at -20°C. The use of the amber containers, folding filters in foil and placing in coolers in the dark all serve to reduce the effect of light as much as possible. During sample processing in the lab additional steps are taken to address incident light. Chl *a* samples are analyzed at the HRSD laboratory using the EPA 445.0 method. Winkler DO samples are stored in the shade after preservatives are added. Samples are then delivered to ODU for processing and all measurements are kept in a log book. Refer to HRSD CMAP documentation for details concerning Winkler DO determinations and secchi depth measurements. Note: Prior to July 1, 2012 field verification of the hydrographic measurements were made with a YSI 556.

The data being gathered by YSI 6600EDS V2 are also verified by comparison measurements collected by the HRSD Chlorophyll Monitoring and Assessment Program (CMAP). Weekly (or more frequent) CMAP cruises are routinely conducted in the Lafayette River study area and provide additional opportunities for validation. These CMAP comparison data consists of YSI 6600 instrument readings collected from Dataflow while on station at the CMONs as well as fixed site QA station data. Refer to HRSD CMAP documentation for details regarding procedures at these sites. Note: During March 2012 HRSD added two fixed QA site locations at NYCC and Ashland Circle sites for this purpose.

b. Vertical Profile of Dissolved Oxygen, Water Temperature, and Salinity

A vertical profile of the water column is accomplished using a YSI 6920 which includes a ROX 6150 Optical DO probe, a YSI 6560 conductivity/temperature probe, and a 6561 pH probe. This profile includes water temperature, specific conductivity, salinity, dissolved oxygen and pH. This is accomplished by placing the sensor at a 0.1 meter depth, letting it equilibrate and recording the measurements. The same procedure is then followed at successive 0.25 m intervals thereafter until the final reading is taken approximately 5 cm above the bottom. All calibrations and maintenance on the YSI 6920 sonde is completed in accordance with the YSI, Inc. operating manual methods (YSI 6-series Environmental Monitoring Systems Manual; YSI, Inc. Yellow Springs, OH). Note: Prior to July 15, 2012 vertical profiles were not collected.

c. Paired particulate N, particulate C, and Chl *a* comparisons – Year 1 only

Beginning in June 2012, particulate N (PN) and C (PC) samples are collected during the weekly CMAP cruises at 5 water quality stations to begin to determine a PN:Chl and PC:Chl relationship. Samples are filtered through a GF/F filter (0.7 μm) and the filter is placed in a labeled cryo vial and frozen. Frozen samples are delivered with chain of custody to ODU for processing (See **Appendix 2**).

6. Data Review and Quality Assurance – Year 1 and Year 2

The data from the CMONs are compared to (a) fixed site data collected during deployment and retrieval intervals, (b) Dataflow readings collected by CMAP that coincide with CMON deployment, and (c) fixed site data QA data collected by CMAP that coincide with CMON deployment. The data from these three sources will be used in the process of validating and error coding with established EPA error codes (**Appendix 3**) prior to submitting the data to VIMS for VECOS web posting.

7. Processing of Data – Year 1 and Year 2

The continuous monitoring data is downloaded and backed up. Graphs are created of each parameter and outliers are examined as well as any data trends which indicate trouble with individual probes. Depth data is compared with the rest of the data so that data can be tagged if the sonde was out of water and optical data is closely examined for periods where wipers may have seated over optics, etc. The same issues with the data/probes that are addressed in CMAP (Dataflow) are examined in the continuous monitoring dataset. The continuous monitoring for each site is joined to form a seamless file for the year's deployment. The times associated with any missing data are inserted and the appropriate error codes are placed in the cells to explain the absence of data. As with the Dataflow, YSI post calibration data is also checked to be sure that any drifts in the probes are within acceptable ranges. Once these checks have been accomplished, the data can be put into the final form for submission and the appropriate error codes are used to tag erroneous observations. (For acceptable drifts see CMAP documentation)

Missing data due to YSI maintenance (down time) are inserted into the spreadsheet and are denoted by time stamps with no records. Edited files are merged to contain one full year of data for each station. Computer programs are used to screen the data set for outliers (values which fall outside the range that the instrument is designed to measure) and other erroneous data. Suspect data are evaluated, edited, and documented. A summary of the QA/QC screening protocols used for the continuous monitoring is provided in **Appendix 4**. This table reflects the stated maximum upper limits for the YSI probes by the manufacturer. When these values are exceeded the values get an automatic "NQR" code. The CMON data are subjected multiple reviews for erroneous or questionable results. Prior to posting of final results to VECOS the data are thoroughly reviewed and EPA error codes are entered.

8. Field Documents and Records – Year 1 and Year 2

All field data are recorded on specially prepared field data sheets. The name of the person recording the data is recorded on each data sheet. The raw data sheets are reviewed for possible missing data values due to sample collection problems prior to data entry. The field sheets for deployment and retrieval intervals (**Appendix 5**) are stored at HRSD. The field sheets for CMAP portion are also stored at HRSD.

Table 1. Summary of Monitoring Parameters

PARAMETER	COLLECTION PROCEDURE	PRESERVATION	PERFORMS ANALYSIS	DETECTION LIMITS	CBP (CIMS) METHOD (unless noted otherwise)
Temperature	YSI 6600V2/6920		Field		F01
pH	YSI 6600 V2/6920		Field		F01
Dissolved Oxygen	YSI 6600 V2/6920		Field		F01
Specific Conductivity	YSI 6600 V2/6920		Field		F01
Salinity	YSI 6600 V2/6920		Field		F01
Fluorescence	YSI6600 V2		Field		NA
Chlorophyll	YSI6600 V2		Field		F01
Turbidity	YSI6600 V2		Field		F01
Secchi Depth	Secchi Disk		Field		F01
Chlorophyll a	GF/F Filter 25 mm diameter 0.7 um pore size	ICE	ODU Lab	0 ug/l	L03
Winker dissolved oxygen	HRSD SOP	Shaded	ODU Lab		NA

PARAMETER	COLLECTION PROCEDURE	PRESER- VATION	PERFORMS ANALYSIS	DETECTION LIMITS	CBP (CIMS) METHOD (unless noted otherwise)
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B. Weekly N and C uptake experiments in the Lower James and Lafayette River – Year 1

Background: The study proposal entitled “*Nutrient regeneration as a means of initiating and sustaining algal blooms in the James River estuary*” discusses the scope and purpose of examining the uptake and regeneration of N and C compounds by the natural microbial assemblages in the Lafayette and lower James Rivers. Below is a brief description of sampling location, duration, and all details pertaining to N and C uptake experimentation can be found in **Appendix 2**.

1. Sampling Stations Description

Two fixed sampling stations were chosen, one in the Lafayette River at the Ashland Circle pier, and one in the Lower James River, at the Huntington Beach Pier. The Lafayette River site is already designated as a fixed station used to continuously monitor water quality parameters including depth, water temperature, salinity, pH, chlorophyll, turbidity, and dissolved oxygen using YSI 6600EDS V2 data sondes, as described above. The James River site is frequented during the DATAFLOW cruises weekly, providing continual monitoring information.

2. Locations of uptake sampling stations

There is one uptake sampling station in the Lafayette River and one in the James River (**Appendix 1, Maps 3 & 4**).

Locations of Fixed Stations within the Lafayette River

Fixed Station	Location	CBP Segment	Latitude (decimal degrees)	Longitude (decimal degrees)
AC	Ashland Circle	LAFMH	36.880390	-76.272525
HP	Huntington Beach Pier	JMSMH/PH	37.005294	-76.459351

3. Sampling protocols, measurements taken, data management

Each week (Wednesday) samples will be collected from the pier and the AC dock in the morning using a clean bucket and carboy. DIC samples will be collected on-site (See **Appendix 2**) and temperature will be measured in-situ. The carboys will be transported back to the lab where experiments will be conducted immediately. Please refer to **Appendix 2** for all details regarding sample handling, experimental design, and data management.

C. Storm event sampling in the Lafayette River - Boat Operations – Year 1 and Year 2

Background: The study proposal entitled “*Environmental factors promoting algal blooms in the Lower James River estuary*” contains provisions for HRSD and ODU to collect additional measurements in the Lafayette River around storm events. These additional sampling events will be conducted prior to storms and at intervals of 1 day, 3 days, and 7 days after storms. This document describes the details of this data collection effort. A summary of the monitoring parameters is provided in Table 2.

1. Cruise Track

Water quality data will be collected along the cruise tracks indicated in **Appendix 6**.

2. Dataflow collection

The HRSD research vessel will be outfitted with a Dataflow system to collect water quality data along the cruise track using the methods outlined in the HRSD CMAP documentation. One exception to regular CMAP operations is that the boat speed is significantly reduced from approximately 25 knots to less than 3.5 knots. A reduction in boat speed was needed to allow processing time for on-board nutrient determinations as described below.

3. Field Documents and Records

All field data are recorded on specially prepared field data sheets. The name of the person recording the data is recorded on each data sheet. The raw data sheets are reviewed for possible missing data values due to sample collection problems prior to data entry. The field sheets will be stored at HRSD.

Table 2. Summary of Monitoring Parameters

PARAMETER	COLLECTION PROCEDURE	PRESE- RVATION	PERFORMS ANALYSIS	DETECTION LIMITS	CBP (CIMS) METHOD (unless noted otherwise)
NH ₃ -N	Pump, clean tubing	0.2 µm filtered and frozen	Field and ODU lab	See Appendix 2	See Appendix 2
NO ₂ +NO ₃ -N	Pump, clean tubing	.2 µm filtered and frozen	Field and ODU lab	See Appendix 2	See Appendix 2
PO ₄	Pump, clean tubing	.2 µm filtered and frozen	Field and ODU lab	See Appendix 2	See Appendix 2
Refer to HRSD CMAP documentation for associated Dataflow and QA site parameters					

D. Storm event sampling in the Lafayette River – Land Based Operations – Year 1 and Year 2

Background: The study proposal entitled “*Environmental factors promoting algal blooms in the Lower James River estuary*” contains provisions for ODU and HRSD to collect additional measurements in the Lafayette River around storm events with automatic sampling equipment at fixed sites. This document describes the details of this component of the data collection effort. A summary of the monitoring parameters is provided in Table 3.

1. Site Locations

Automatic sampling equipment will be used to collect water quality samples at the two fixed site locations in the Lafayette River described above in section II.A above.

2. Sampling Details

At each sampling site (Norfolk Yacht Club and Ashland Circle) an ISCO Model 6712 sequential sampler will be configured and programmed to collect a series of river water samples as follows:

- 2012 - Collect samples every 6.21 hour interval (i.e. half a tidal cycle); 2013 – Collect samples every 30 minutes for first 2 hours, then every two hours for next 6 hours, then every 6 hours for remainder of event (48 hours total)
- At each interval collect whole samples held on ice. On same day as sample collection pre-filter these samples through a 47mm GFF 0.45 µm filter. Freeze the filtrate.
- When possible the samplers will be programmed to begin the sampling regime at the next nearest high or low tide based on tidal predictions for the Lafayette River using the “delayed start” option.

3. Sampling Criteria

The activation of the samplers will be established upon forecast storm activity. For the purposes of this study a forecasted storm is defined as expected rainfall exceeding 0.5” in 2012, in 2013 a rain event was considered at least 0.25” of rain. Upon forecast of an approaching storm the samplers will be set up 24 hours in advance.

In Year 1, if the storm actually occurs (>0.5”) within the first 50 hours of the first sample it will be continued until the storm ends. Sampling will be further continued 24, 48, 72 and 120 hours (1, 2, 3, and 5 days) after the end of the storm (or the end of the previous sampling cycle, whichever is greater). In Year 2, sampling will cease at 48 hours. A minimum of 72 hours will be used to separate one rain event from another (0.1” is considered a rain event).

If the storm does not occur as forecast within the first 50 hours of sampling but forecasts appear promising (definite rain) the sampler will be reset for another 25 hour period. Otherwise, the sampling will be discontinued until the next storm event.

4. Quality Assurance Measures

Equipment decontamination procedures- HRSD will decontaminate the sampling equipment and containers using procedures described in the HRSD CMAP documentation for nutrients and chlorophyll.

Field blanks- Field blanks will be collected on site prior to activating the sampler. Blank water will be processed through the sampler and filters while in the field in the same manner as project samples. Field blanks will be processed each time a sampler is deployed to collect a set of samples. Artificial seawater will be used for the blanks because it is required by the analytical methods.

5. Measured Parameters

The collected samples will be analyzed for the parameters indicated in Table 3.1 by methods described in (**Appendix 2**).

6. Field Documents and Records

All sampler servicing activities (set up, retrieval of samples, etc.) will be documented on field sheets maintained by HRSD. The log book will document the date, time, investigator, and other relevant field comments. The collected samples will be transferred to the ODU laboratory for analysis with log book.

Table 3. Summary of Monitoring Parameters

PARAMETER	COLLECTION PROCEDURE	PRESE- RVATION	PERFORMS ANALYSIS	DETECTION LIMITS
NH ₃ -N (dissolved)	Clean bucket and carboy – 2012; ISCO sampler - 2013	0.2 µm filter and freeze	ODU Lab	See Appendix 2
NO ₂ +NO ₃ -N (dissolved)	Clean bucket and carboy – 2012; ISCO sampler - 2013	0.2 µm filter and freeze	ODU Lab	See Appendix 2
PO ₄ (total and dissolved)	Clean bucket and carboy – 2012; ISCO sampler - 2013	0.2 µm filter and freeze	ODU Lab	See Appendix 2
Total Dissolved Nitrogen	Clean bucket and carboy – 2012; ISCO sampler - 2013	0.2 µm filter and freeze	ODU Lab	See Appendix 2

E. Storm event sampling in the Lafayette and Lower James River – N and C uptake Year 1

Background: The study proposal entitled “*Nutrient regeneration as a means of initiating and sustaining algal blooms in the James River estuary*” discusses the scope and purpose of examining the uptake and regeneration of N and C compounds by the natural microbial assemblages in the Lafayette and lower James Rivers, particularly after large storm events. Sampling location is at Ashland Circle dock and Huntington Pier, and details can be found in Section II.B above. Sampling will occur 1 -2 days following a large storm event as described in Sections II.C and II.D above.

F. Storm event sampling in the Lafayette River – Storm water – Year 1 and Year 2

Background: The study proposal entitled “*Environmental factors promoting algal blooms in the Lower James River estuary – Year 1 and Year 2*” contains provisions for ODU and HRSD to perform storm water sampling of discharges to the Lafayette River. This document describes the details of this component of the data collection effort. The parameters of measurement are provided in **Appendix 2**.

1. Site Locations

Automatic sampling equipment will be used to collect storm water samples at the two sampling locations on the Lafayette River (refer to **Appendix 1, Maps 5 & 6**).

2. Sampling Details

At each sampling site an ISCO Model 6712 sampler and ISCO 4250 flow module will be configured and programmed to collect flow weighted composite samples of storm water.

3. Sampling Criteria

The activation of the samplers will be established upon forecast storm activity. For the purposes of this study a forecasted storm is defined as expected rainfall exceeding 0.5” (2012) and 0.25” (2013). All sampling will be performed consistent with EPA guidelines regarding amount and duration.

4. Quality Assurance Measures

Equipment decontamination procedures- HRSD will decontaminate the sampling equipment and containers using standard procedures (HRSD FC 1130 using methods appropriate for nutrients).

Field blanks- Field blanks will be collected on site prior to activating the sampler. Blank water will be processed through the sampler while in the field in the same manner as project samples. Field blanks will be processed each time a sampler is deployed to collect a set of samples.

5. Measured Parameters

The collected samples will be assessed for the parameters indicated in **Appendix 2** according to the specifications provided.

6. Field Documents and Records

All sampler servicing activities (set up, retrieval of samples, etc.) will be documented in a log book maintained by ODU. The log book will document the date, time, investigator, and other relevant field comments. The collected samples will be transferred to the ODU laboratory for analysis with chain of custody (**Appendix 2**).

G. Daily pre-bloom and diel bloom sampling at fixed locations on the Lafayette River – Year 2

Background: The study proposal entitled “*Environmental factors promoting algal blooms in the Lower James River estuary –Year 2*” contains provisions for ODU to perform pre-bloom daily sampling at fixed locations (AC and NYCC) and a 24 hour diel study at AC during a bloom event. This document describes the details of this component of the data collection effort. The parameters of measurement are provided in **Appendix 2**.

1. Site Locations

Automatic sampling equipment will be used to collect river samples daily at a fixed time of the day at AC and NYCC, 10 am and 10:30 am, respectively. Using the ISCO samplers, water will be pumped into clean 1 L PETG bottles and brought back to the lab for nutrient analysis. Water will also be pumped into bottles containing lugols for phytoplankton enumeration. Stratification of the water column will be assessed daily using the CastAway CTD (refer to **Appendix 1, Maps 1, 2 & 3**). Sampling will begin in June 2013, and continue until the bloom has been identified. Once the bloom has been established, diel sampling will be conducted at site AC. Stratification will be measured and samples for nutrients, phytoplankton enumeration, and N and C stable isotope uptake experiments will be conducted during daylight hours (6 am, 10 am, 2pm, and 6pm). Nutrient and phytoplankton enumeration collections will be similar to those described above. N and C uptake experiments will be conducted as described in section **II.B.3** above and in **Appendix 2**. In addition to nutrient grab samples, the ISCO sampler will be set to collect nutrient and preserved Lugol’s samples (for phytoplankton enumeration) every 2 hours,

beginning at 6 am and ending at 4 am the following day. Bottles will be stored in the ISCO on ice and nutrient samples will be filtered (0.2 μm) in the laboratory.

2. Sampling Details

At each sampling site an ISCO Model 6712 sampler will be configured and programmed to collect riverine samples.

3. Sampling Criteria

The activation of the samplers will be conducted by hand on a daily basis. No programming will be necessary and a ‘grab’ sample will be collected from the ISCO to ensure that all samples are collected in the same manner at the same depth each time.

4. Quality Assurance Measures

Equipment decontamination procedures- ODU will use acid cleaned carboys and bottles for collection devices and HRSD will maintain sampling equipment using standard procedures (HRSD FC 1130 using methods appropriate for nutrients).

5. Measured Parameters

The collected samples will be assessed for the parameters indicated in **Appendix 2** according to the specifications provided.

6. Field Documents and Records

All sampler servicing activities (set up, retrieval of samples, etc.) will be documented in a log book maintained by ODU. The log book will document the date, time, investigator, and other relevant field comments. The collected samples will be transferred to the ODU laboratory for analysis with chain of custody (**Appendix 2**).

III. Nutrient regeneration as a means of initiating and sustaining algal blooms in the James River estuary – Year 1

Background: In conjunction with the sections above (II.B and II.E) which addresses the proposal entitled “*Nutrient regeneration as a means of initiating and sustaining algal blooms in the James River estuary*” in the Lower James River, this section describes the N and C uptake experiments in the Upper James River estuary.

A. Sampling Stations Description and Location

Sampling will occur monthly (second Tuesday of each month) from June to October at Virginia Commonwealth University’s Rice Center Pier (37.271048, -77.079735). The pier is located in the freshwater portion of the James River. Experiments will be conducted simultaneously with the Bukaveckas group at VCU.

B. Sampling protocols, measurements taken, data management

Each month (2nd Tuesday) samples will be collected from the dock in the morning using a clean water sampler and carboy. DIC samples will be collected on-site (See **Appendix 2**) and temperature will be measured in-situ. The carboy will be transported back to the lab where experiments will be conducted immediately. Please refer to **Appendix 2** for all details regarding sample handling, experimental design, and data management.

IV. Diagnostic pigments as a means of tracking the distribution of algal functional groups within the James River – Year 1

Background: This section addresses the proposal entitled “*Diagnostic pigments as a means of tracking the distribution of algal functional groups within the James River*” in the James River.

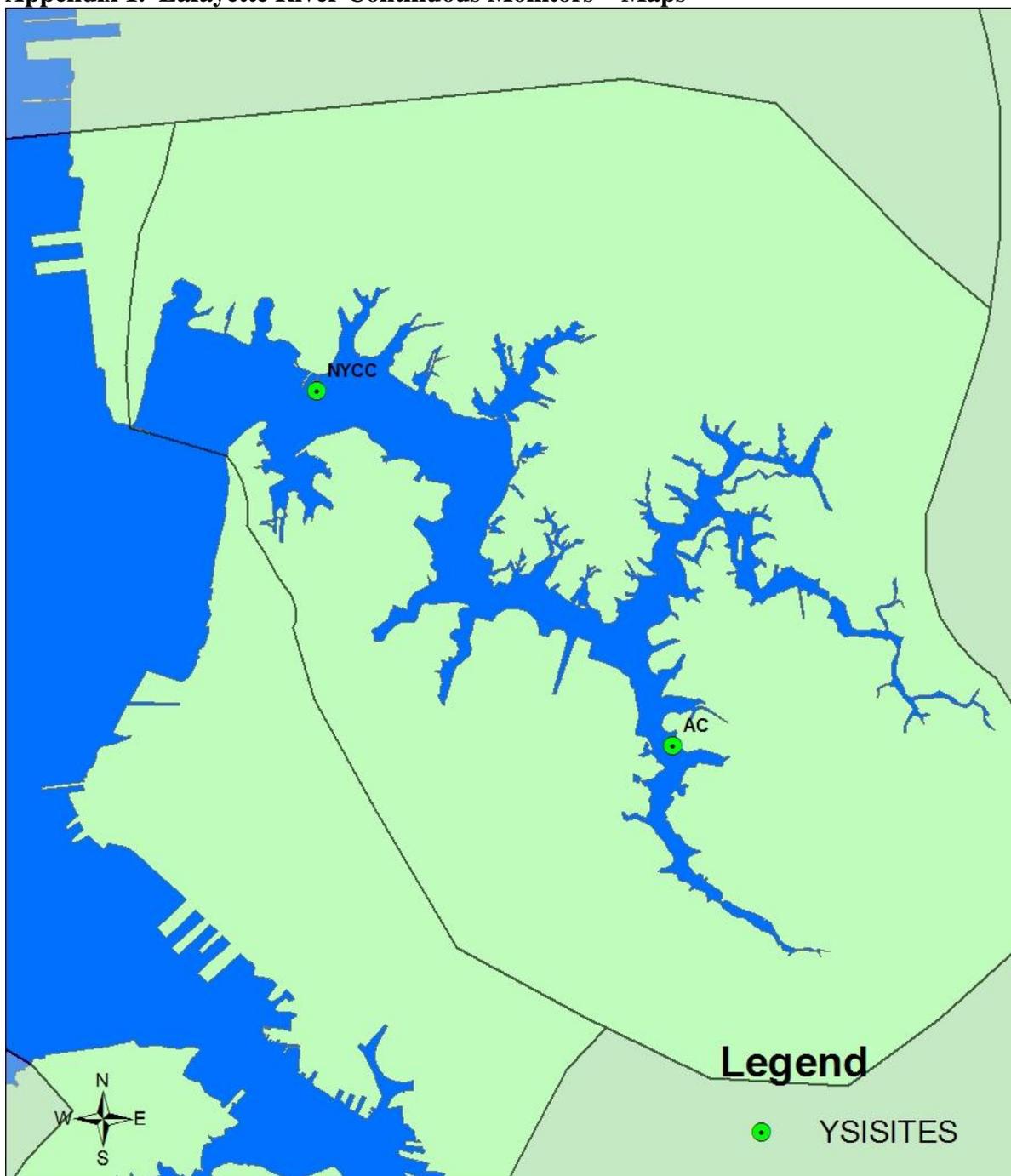
A. Sampling Stations Description and Location

Samples for pigment analysis will be taken during HRSDs weekly CMAP cruises. Location of sampling is contingent upon the chl *a* criteria established by the CMAP program, therefore pigment samples will only be taken when chl *a* concentrations exceed the 15 $\mu\text{g L}^{-1}$ criteria.

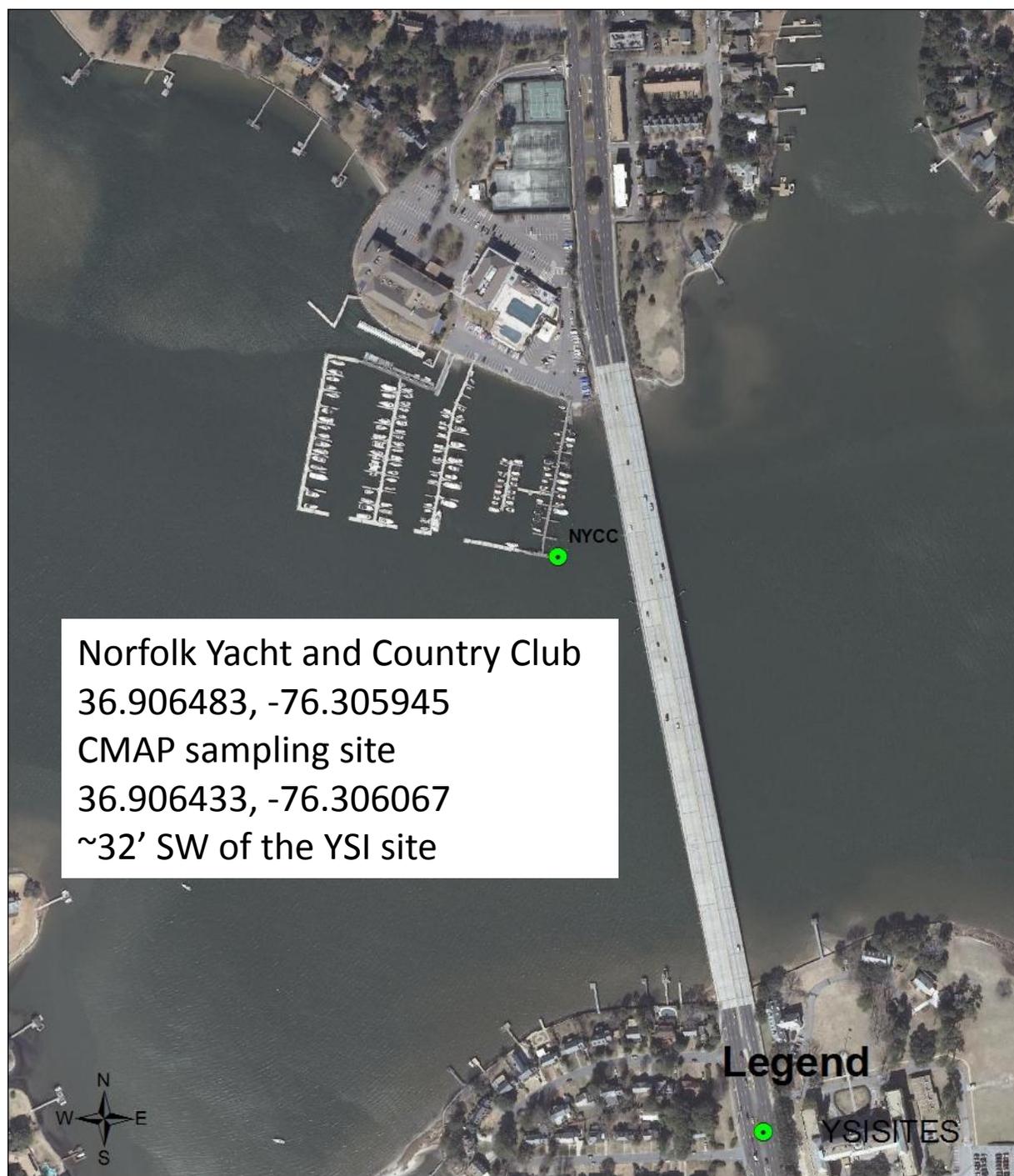
B. Sampling protocols, measurements taken, data management

Using a Swinnex filter holder, acid-clean syringe and a GF/F (nominal pore size 0.7 μm) filter, water is filtered and the filter is placed in a cryovial and immediately put into liquid nitrogen. Sample location, time, volume and ID are recorded on a chain of custody worksheet. Samples are delivered to ODU in liquid nitrogen weekly, along with the chain of custody, and placed in a -80 °C freezer until analysis. For further details regarding sample analysis and data management, refer to **Appendix 2**.

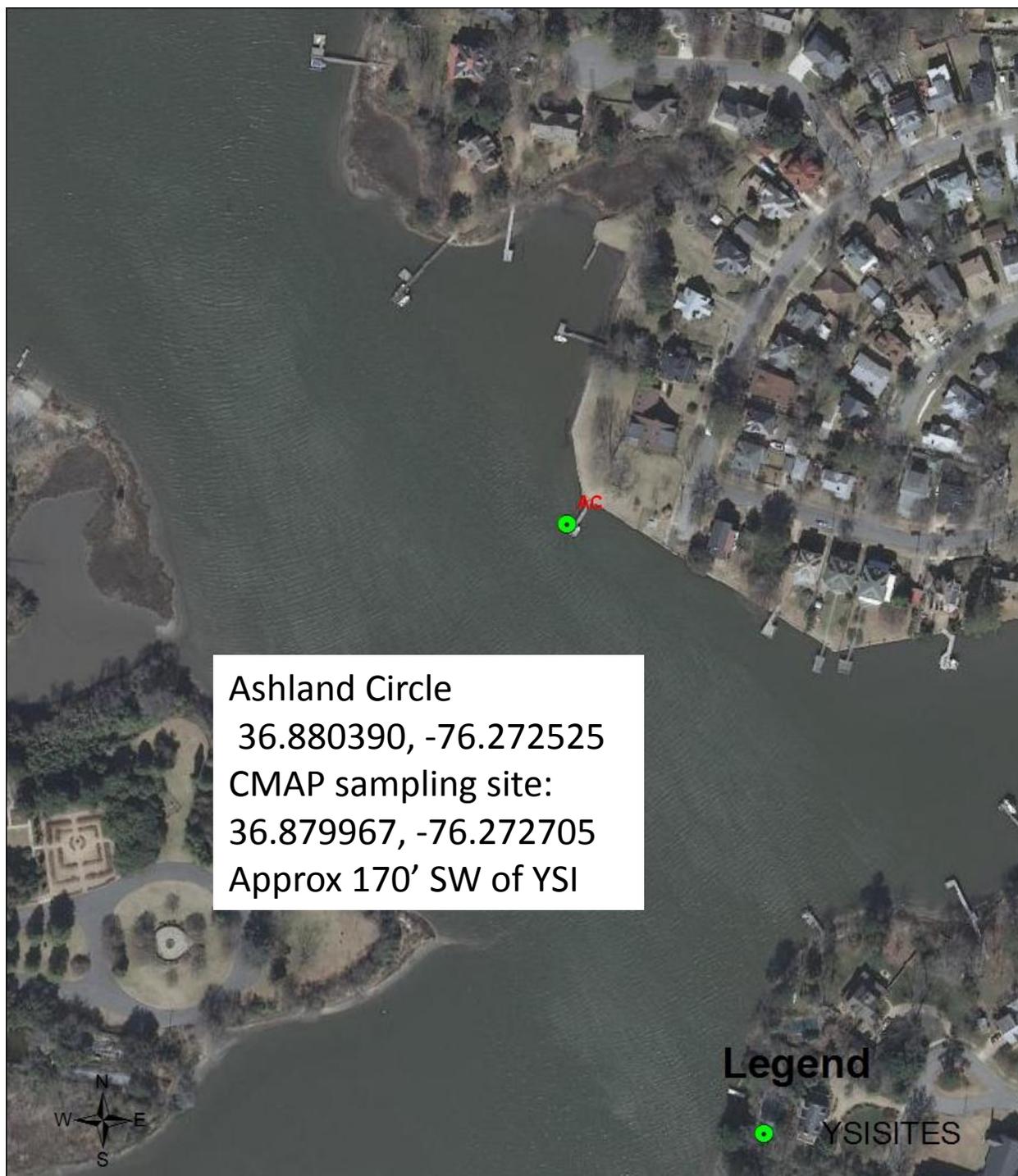
Appendix 1. Lafayette River Continuous Monitors – Maps



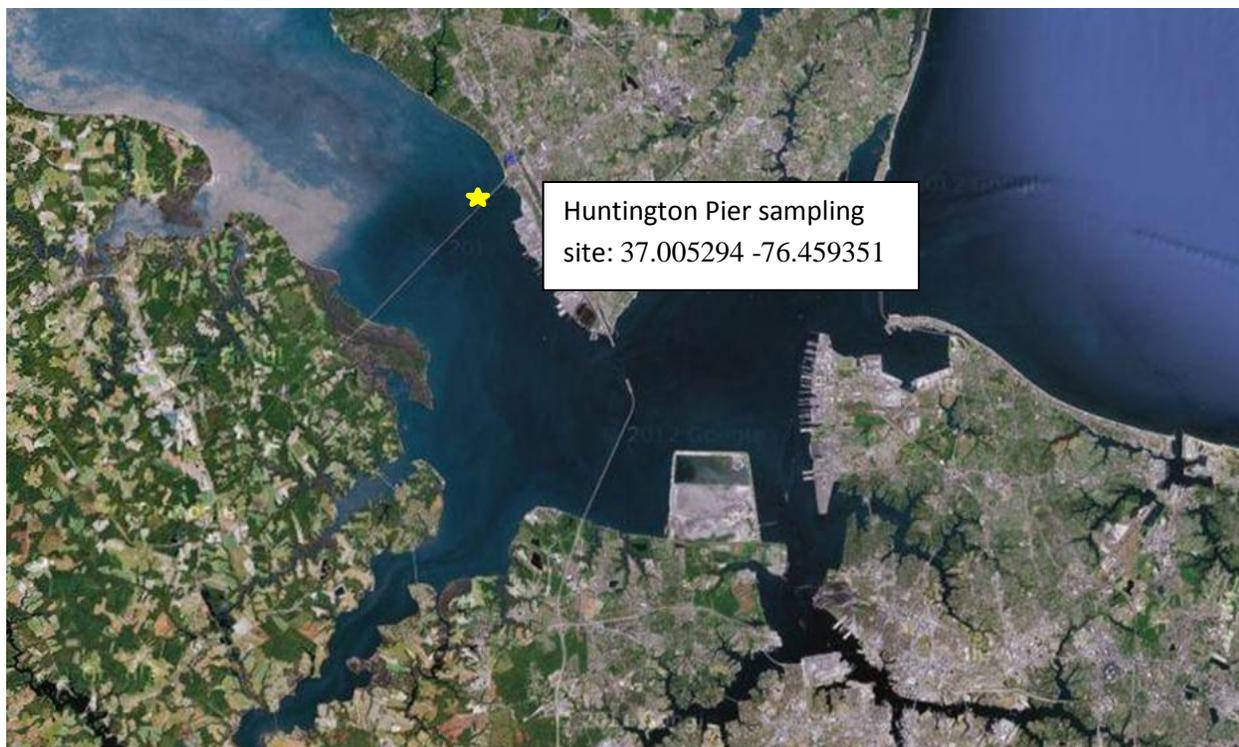
Map 1. YSI Sites, Norfolk Yacht and Country Club (NYCC), Ashland Circle (AC)



Map 2. YSI site (NYCC) and CMAP sampling site.

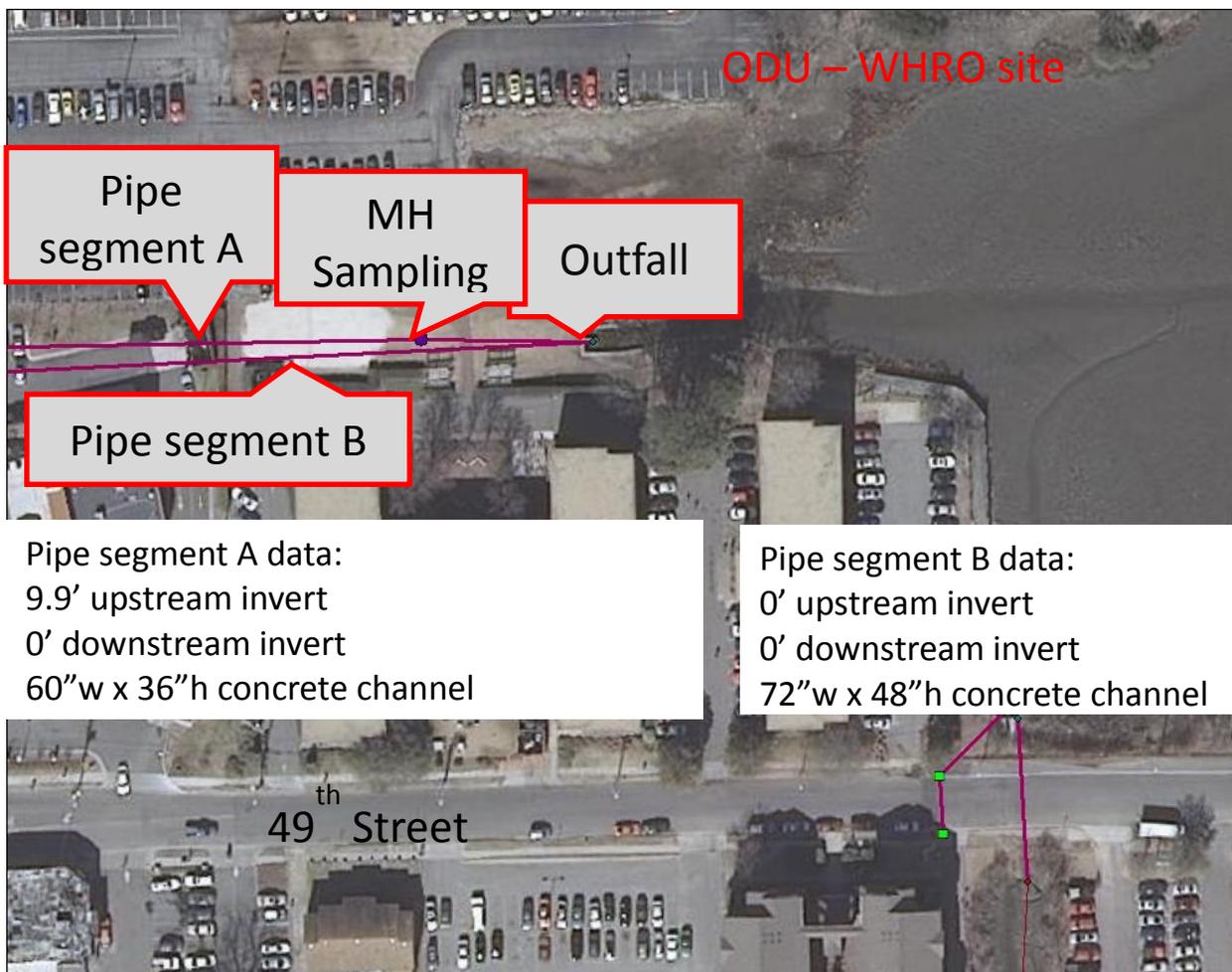


Map 3. YSI site (AC) and CMAP sampling site.



Map 4. Huntington Pier sampling site for N and C uptake experiments.

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Map 5. Stormwater sampling site, ODU – WHRO site

Colonial Place site



Map 6. Stormwater sampling site, Colonial Place.

Appendix 2. ODU Standard operating procedures (SOPs)

Mulholland Lab 2012 - 2013

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I. Sample Collection

A. Cleaning and equipment preparation

Cleaning Procedures

Rinse all material to be cleaned with Barnstead Water (BW) before cleaning.

If large particles are present then clean with Liquinox soap and then proceed to next step.

- Soak all glassware, plasticware, tubing, caps, etc. in 10% HCL bath for at least one hour or rinse with 10% HCL from carboy.
- Rinse 4X with BW.
- Allow to dry on clean bench covered with drying paper.
- If plastic, cap and put away for future use.
- If glass (except **volumetric flasks**) cover with aluminium foil and combust in oven at 450°C for at least 6 hours.
- Any lids corresponding to combusted glass should be soaked in MeOH for one hour and then rinsed with BW and dried in drying oven at 40°C.

Equipment preparation

If necessary, pre-label all bottles with station ID, date, and sample type. Pack all necessary equipment depending upon the type of sampling being undertaken. Make sure all pumps are in proper working order, and bring lab notebook and pens for recording. Combust GF/F filters (450°C for two hours) to be used for particulates and uptake experiments. If bringing samples home make sure there is adequate ice in coolers, and/or liquid nitrogen.

B. Dock-side sample collection

To filter for chl a, particulate carbon (PC), particulate nitrogen (PN), and pigments:

- Water sample were collected using an acid cleaned bucket attached to a rope (2012) and via the ISCO samplers installed at AC and NYCC (2013)
- Surface water only is collected.
- Water is either pumped or poured into an acid-clean carboy or PETG bottle and the carboy or bottle is rinsed three times with the surface water
- Carboys or bottles are then covered (kept in dark) and transported back to the lab for filtration.
- In lab, ensure samples are kept in dark, set up filters, using GF/F's on 6-seater rig fitted with filter towers (25mm),
- Measure using a graduated cylinder an appropriate volume to filter, note volume filtered:
 - o Riverine waters with high biomass = 5 – 25 mL
 - o Coastal waters = 50 – 100 mL
 - o Oceanic waters = 500 – 1000 mL or more
- Turn on vacuum pump (not to exceed 5 psi) and filter water, don't let filter go dry
- Turn off pump and collect filters using clean forceps
- Chl a filters are folded in half and placed in sterile 15 mL falcon tubes, PC/PN and pigment filters are folded in quarters and placed into sterile micro-centrifuge tubes and immediately placed in the dark in a freezer (-20°C)
- Pigment samples are folded in half and placed in cryo vials and put immediately into a liquid N dewar and place in -80°C freezer upon return to lab

To filter for nutrients (nitrate, nitrite, TDN, phosphate, ammonium, urea, silicate, dissolved free and combined amino acids [DFAA/DCAA]):

- Water from carboys or bottles is filtered using filter towers with Supor filters (0.2 μm)
- Pour water into towers, apply vacuum (< 5 psi) and filter into acid cleaned, plastic side arm flasks
- Pour filtrate into labeled acid cleaned plastic (~250 mL) bottles, do not fill to the top, leave room for expansion when the sample is frozen
- Aliquot 2 mL from each bottle into 2.5 mL cryovials, labeled accordingly for DFAA and DCAA
- Store bottles and cryovials in freezer until analysis (-20°C)

To sample for dissolved inorganic carbon (DIC):

- Attach acid-cleaned tubing, fitted with small rigid tubing at the end to carboy
- Place tubing in bottom of combusted glass flask (30 or 60 mL) and start filling very slowly, do not introduce bubbles
- Over fill bottles with sample
- Add mercuric chloride (0.02%) with pipette
- Carefully place stopper on vial so as not to introduce any air bubbles
- Seal bottles shut with crimp sealer
- Refrigerate upon return to lab

II. Experimental Designs

A. $^{15}\text{N}/^{13}\text{C}$ stable isotope uptake experiments

^{15}N has been used to trace N in the marine environment since the 1960's. In recent years, stable isotopes have been used more commonly to measure N uptake and cycling, and now we use ^{13}C dually with ^{15}N in some compounds. One reason for this is that isotope ratio mass spectrometers are more widely available and have been interfaced with a variety of inlets to make a variety of experiments feasible.

Three important aspects of planning your experiment:

- Determining how much ^{15}N or ^{13}C to add, it is important not to add so much as to “perturb” the system. In practice, we calculate this to be about 10% of ambient nutrient pool.
- Determining the volume of our incubations is important because we need enough mass of N and C on the filter to satisfy the detection limit of the ANCA unit on the front end of the IRMS. It is best to have at least 5 μg N on the filter.
- Getting all the samples you need to make a calculation.
 - o isotope enrichment of the source pool (at T_0)
 - o isotope enrichment of the target pool (at T_0)
 - o isotope enrichment of the tracer added
 - o amount of tracer added
 - o Concentration of the ambient pool that you're enriching
 - o Concentration of the particulate pool
 - o isotopic composition of the dissolved pool after incubation (especially to calculate isotope dilution)
 - o isotopic composition of the particulate pool after incubation

In general, tracer experiments begin with the addition of a known quantity of ^{15}N isotopically labeled compound. The movement of tracer into various particulate and dissolved pools is then monitored over time. This movement is monitored by measuring the change in the isotopic enrichment ($^{15}\text{N}/^{14}\text{N}$) in the substrate and particulate fractions over a time interval. Depending on the pools monitored, these measurements can allow for estimation of N remineralization, regeneration, nitrification, denitrification, N uptake by microbes, etc. Limitations of these methods arise when measuring processes in nature because many processes affecting substrate and particulate pools occur concurrently.

Experimental protocol will vary among systems because organisms mediating N transformations vary, as does their density and environmental factors affecting rate processes. It is important to consider: the *size fraction* (grazers or no grazers), the *length of incubation*, the *volume of the incubation* and the *amount of the tracer addition* relative to the ambient pool. The incubation container is also important. Polycarbonate or other plastics are usually preferred over glass because of potential trace metal contamination.

- *Size fraction*: grazers can substantially alter recycling processes and affect the particulate pool.
- *Incubation time*: simultaneously occurring processes can result in isotope dilution or equilibrium in incubation bottles. Usually do time courses to determine optimum incubation length. You always want to calculate whether the addition was “used up” during the incubation and you generally do not want more than about $\frac{3}{4}$ of the isotope added to have moved to the target pool.
- *Incubation volume*: Need enough mass (instrument dependent) to get a signal
- *Tracer addition*: Do you want a maximum potential or actual rate?

Basic experimental design:

- Sample collection - 250 mL, 500 mL or 1 L unfiltered samples are collected from various depths (generally surface, chl *a* max, and bottom)
- Labeled substrates are added in separate, triplicate, PETG bottles, available substrates are: $^{15}\text{NO}_3^-$, $^{15}\text{NO}_2^-$, $^{15}\text{NH}_4^+$, ^{15}N and ^{13}C urea, ^{15}N and ^{13}C glutamine, and ^{15}N and ^{13}C amino acids
- Bottles are placed in Plexiglas incubators with a flow through seawater system (or taken back to the lab if doing small-boat or dock sampling)
- Screens are placed over the bottles to allow for varying levels of light to enter the bottles
- After an appropriate incubation time (30 minutes for high biomass water, 2 hours or more for coastal or oceanic water), the entire incubation bottle volume is filtered onto combusted (450°C for 2 hours) GF/F's
- Filters are removed from the towers with forceps, folded into quarters, put into labeled cryovials and placed on ice
- Samples are frozen back at the lab until analysis

B. ^{13}C primary productivity

Primary productivity estimates are made based on the same principle as the $^{15}\text{N}/^{13}\text{C}$ stable isotope experiments and conducted at the same time.

Basic experimental design:

- Sample collection - 250 mL, 500 mL or 1 L unfiltered samples are collected from various depths (generally surface, chl *a* max, and bottom)

- Labeled bicarbonate ($H^{13}CO_3$) is added to separate, triplicate, PETG light and dark bottles
- Bottles are placed in Plexiglas incubators with a flow through seawater system (or taken back to the lab if doing small-boat or dock sampling)
- Screens are placed over the bottles to allow for varying levels of light to enter the bottles
- After an appropriate incubation time (12 – 24 h), the entire incubation bottle volume is filtered onto combusted ($450^{\circ}C$ for 2 hours) GF/F's
- Filters are removed from the towers with forceps, folded into quarters, put into labeled cryovials and placed on ice
- Samples are frozen back at the lab until analysis

C. Peptide hydrolysis

Using lucifer yellow anhydride (LYA) labeled peptides, peptide hydrolysis can be estimated.

Basic experimental design:

- Sample collection – 30 or 60 mL unfiltered samples are collected in acid-cleaned PET incubation bottles with caps from various depths (generally surface, chl *a* max, and bottom)
- LYA substrates (LYA-ala4 or LYA-AVFA) are added to a final concentration of 100nM
- Immediately obtain a subsample for time zero (T0) concentration (~2ml of sample, NO LESS THEN 1ml) by filling a clean 3 mL plastic syringe using Eppendorf pipette, careful not to cross-contaminate
- Attach a 0.2 μm syringe filter to the syringe and filter and collect into a labeled 2.0 ml microcentrifuge tube.
- Continue to subsample (as outlined above) over a time course (15 min, 30 min, 1 hr, 2 hr, 4 hr, & 6 hr) to trace the disappearance of the parent compound and the appearance hydrolysis products
- Freeze cryovials until analysis
- At end of the hydrolysis incubation filter 1/2 of sample for chl *a* and PN/PC, make sure to note the volumes.
- Freeze chl *a* in centrifuge tubes and PN/PC in microcentrifuge tubes as described above

III. Sample Storage

Upon collection, chl *a*, PN/PC, nutrients, stable isotope samples, and peptide hydrolysis samples are placed on ice or in a freezer (if available). All of these samples are placed in freezers upon return. Chl *a* samples must be analyzed within 2 weeks of collection. Pigment samples are placed in a dewar filled with liquid N and placed in a $-80^{\circ}C$ freezer upon return. DIV samples are placed on ice or refrigerated (if available) upon collection and placed in a refrigerator upon return to the lab.

IV. Sample analysis

A. Chl *a*

Chl *a* analysis is conducted fluorometrically with a 10-AU Turner fluorometer using the non-acidification method as described by Welschmeyer (1994).

Sample Preparation:

- Add 10ml of 90% acetone to each centrifuge tube and place in freezer for ~24hrs.

- Remove from freezer and sit in dark (drawer or box) allowing samples to reach room temperature.
- Turn on fluorometer and allow to warm up for at least ten minutes. Be sure to use the fluorometer set up for non-acidification method.

Instrument calibration (using sold standard):

- Place solid standard (in drawer below fluorometer) in sample chamber with the letter “L” on your left-hand side. Make sure the metal pin is completely seated in the notches of the 13mm round cuvette adapter.
- Allow reading to stabilize for a few seconds and then press <*> to read value. Wait until “Done” is displayed next to value. Record this as the low concentration on the calibration record sheet.
- Now pull out standard, rotate 180 degrees and reinsert with “H” on left side.
- Allow reading to stabilize and press <*>, record value as high concentration.

Calibration (using known concentration):

Note: only needs to be done when sample concentrations are outside of set calibration range. See table 1. Check calibration record sheet to determine last calibration range.

Table 1. Recommended Standard Concentration and Fluorometer Range

Sample concentration range ($\mu\text{g L}^{-1}$)	Standard concentration ($\mu\text{g L}^{-1}$)	Fluorometer concentration range
0 – 3.75	2.1 – 3.75	LOW
0 – 37.5	21 – 37.5	MED
0 – 100	75 – 100	HIGH
0 – 250	200	HIGH

Blanking:

Always have concentration range set to manual. Blank is 90% acetone.

- Set concentration range control: from screen 2.0, press <4> to bring up screen 2.4, then <3> to bring up screen 2.43 (set control using ENT to toggle between choices)
- Set Concentration range: From screen 2.4, press <2> to bring up screen 2.42 and press ENT to toggle between choices. Set range according to estimated sample range. You can change range when running samples if the actual concentration is outside of range.
- Run Blank: from screen 2.0 press <1> to access screen 2.1. Make sure #2 on screen reads YES. From screen 2.1, press <1> to call up screen 2.11. Fill a clean 13mm cuvette with 90% acetone. Put blank in light chamber. After Blank % reading is stable and assuming Blank % is less than 200%, press <0>. When “Finished” appears, press ESC.
- Remove blank.

Reading Samples:

Always use a clean glass cuvette. Clean between samples using 90% acetone from squirt bottle and wipe outside with Chem-wipe. Be careful not to touch sides with fingers, fingerprints will affect readings. Once samples reach room temperature and with lights off centrifuge all samples on setting 4, for 5 minutes before reading. When pouring sample into cuvette, pour slowly being careful not allow any debris to enter cuvette.

- Place sample in light chamber. Allow for reading to stabilize and then press <*>. If display reads “OVER” then change the concentration range control to the next highest setting. Reading will first read “DELAY”, then “AVG” and then “DONE”. Record value from “DONE” in provided data sheet

- Remove Cuvette and dispose of sample in appropriate waste container. Clean cuvette and pour in next sample. Repeat step 1.
- Repeat steps 1-2 until all samples are read.
- If display reads “OVER” and the concentration range is HIGH, sample must be diluted. Pipette 1 mL of sample into a clean cuvette and dispense 8 mL 90% acetone into cuvette. Note dilution on sample sheet.
- Turn off fluorometer

These readings are the actual concentration of extracted chlorophyll a in the cuvette. To arrive at the environmental chlorophyll a, for each sample you must correct for the volume of water filtered and the volume of 90% acetone used in the extraction.

B. PN/PC

PN/PC samples are prepared by the individual collecting the samples and run on an automated N and C analyzer (ANCA) by the lab manager (Peter Bernhardt).

Preparing samples for mass spectrometer analysis:

- Dry filters by removing all caps and placing them in cryovial containers (9 x 9) in a 40° C for at least 48 hours
- Clean pelletizing area with methanol, including forceps, pelletizer, and platform
- Wear gloves
- Record sample ID in provided sample sheet
- Remove filter from vial and place in tin disc, rolling disc and pushing in sides with forceps
- Place tin disc ‘burrito’ into pellet press and press down
- Invert pellet holder and press again to remove the pellet
- Place pellet in clear plastic pellet tray, **ensure that pellet is placed in the correct numbered spot in tray that is represented on the sample sheet**
- Repeat for all filters in group
- Place lid on plastic pellet tray, with fine-tipped Sharpie number each spot where there is a filter accordingly. Place tape securely on all sides to hold lid in place. Label the plastic tray and tape with the ID of the bulk samples (initials, date, sample type)
- Place plastic tray in desiccator and sample log sheet in appropriate folder
- Samples will be analyzed by lab manager

C. Pigments

The Mulholland Laboratory follows the method of Van Heukelem and Thomas (2001) for the determination of phytoplankton pigments. This method is also thoroughly described in Hooker et al. (2005). Samples are run on a Shimadzu reverse phase high performance liquid chromatography (HPLC) system using an Eclipse XDB C8 column. Samples for phytoplankton pigment analyses were obtained by filtering whole water onto GF/F filters. After filtration, the filters were immediately frozen in liquid nitrogen and then transferred to a -80°C freezer for storage until analysis.

Each filter was placed in a heavy walled, 5mL conical glass tube; 3mL of 95% acetone, containing approximately 0.025 µg/mL⁻¹ of the internal standard vitamin E acetate (Fluka 95250), was added to the glass tube using a Dispensette Organic re-pipette set to deliver 3.00

ml. Each tube was then covered with Parafilm, placed in an ice bath, and kept in the dark until a set of filters (20 to 40) was processed in the same manner.

Each filter was then individually disrupted using an ultrasonic probe for approximately 20 seconds with an output of 25 W. Each tube was submerged in a beaker of ice during disruption to prevent heat accumulation. After each filter was disrupted and the tube was again tightly wrapped in Parafilm, the entire filter set was placed in a freezer (-15°C) for 4 h. Each sample slurry was then well mixed on a vortex mixer, and centrifuged. The supernatant was transferred to a 5mL syringe, and the extract was clarified by pushing the contents through a Teflon HPLC syringe cartridge filter (Scientific Resources, Inc.) with a $0.45\ \mu\text{m}$ pore size. The extract was collected in a 7mL glass vial.

After mixing well, 285 μL of extract was placed in an HPLC vial along with 715 ml of buffer (90:10, 28mM tetra butyl ammonium acetate (TBAA), 6.5 pH; methanol) and placed directly into the autosampler compartment. The remaining extract was stored in a freezer (-15°C) until the HPLC analysis was complete. Vials of solvent blanks, samples, and calibration standards were placed in the autosampler compartment tray (maintained at a constant 4°C) and analyzed over a time period not exceeding 24 h.

After sample injection, separation was achieved using an Eclipse XDB C8 HPLC column (part number 963967-906), $4.6\text{mm}\times 150\text{mm}$ (diameter by length), manufactured by Agilent Technologies with gradient elution using a linear gradient from 5–95% solvent B in 22 min. Solvent B was methanol, and solvent A was (70:30) methanol:28mM aqueous tetrabutyl ammonium acetate, 6.5 pH. An isocratic hold on 95% solvent B was necessary from 22–29 min for elution of the last pigment ($\beta\beta$ -carotene) at approximately 27 min. After a return to initial conditions (5% solvent B) by 31 min, the column was equilibrated for 5 min prior to the start of the next injection cycle and analysis. The flow rate was 1.1mLmin^{-1} and the column temperature was $60\pm 0.8^{\circ}\text{C}$.

Pigments were detected at 450 and 665nm (20nm bandwidths for both) with the photodiode array detector. The signal at 665nm was used to quantify monovinyl and divinyl chlorophyll *a*, phaeophytin *a*, and phaeophorbide *a*; the signal at 450nm was used to quantify all other pigments. Peaks were integrated using the automated functions of the Shimadzu Class VP software. All peaks were visually inspected to verify the automated integrator had drawn peak baselines correctly and in a fashion consistent with the peak integrations of calibration standards. External calibration pigment standards were purchased from DHI Lab Products, Denmark. The pigment standard concentrations were determined using absorption coefficients and absorbance measured with a dual beam, monochromator-type spectrophotometer (Shimadzu Scientific Instruments, Inc., model UV-2401-PC). A spectral bandwidth of 2 nm was used, the sample was corrected for turbidity at 750 nm, and the standards were sufficiently concentrated such that the absolute absorbance of each pigment fell from 0.1–1.0 (in absolute units) for greatest spectrophotometer accuracy.

After the pigment standard concentrations were determined, each standard was injected individually in a series of dilutions to determine chromatographic purity, peak purity, and the response factor, *R*.

The signal at 665nm (± 10 nm) was used for quantification of Chl *a* and DVChl *a*, because they respond similarly (and strongly) at this wavelength and the linear regression for Chl *a* was used for both Chl *a* species. The signal at 450nm (± 10 nm) was used for quantification of all other pigments.

Pigment concentrations (ng l^{-1}) were automatically calculated within the Shimadzu Class VP software based on retention times and standard calibration files for each pigment quantified. Quantitation followed the equation:

$$C_p = (A_p * R * v_{\text{ext}} * 10^3) / (v_{\text{inj}} * v_{\text{filt}} * B)$$

Where A_p is peak area, v_{ext} is the volume of solvent used for extraction, v_{inj} is the volume of sample injected onto the column, v_{filt} is the volume of sample filtered, and B is the buffer dilution factor (< 1.0).

For determining the contribution of MVChl *a* and DVChl *a* to TChl *a*, the peak areas of all products (including all allomers and epimers) were summed and used in the calculations.

Table 2. Pigment retention times based on injections of calibration standards purchased from DHI, Denmark. Pigment abbreviations follow SCOR/UNESCO standards.

Pigment	Retention time (min)
Chl c3	4.704
Chl c2	8.15733
Perid	13.8827

But-fuco	16.3947
Fuco	16.9867
Neo	17.196
Pras	17.78
Hex-fuco	17.862
Viola	17.91
Diad	19.19
Anth	19.3573
Allo	20.056
Diato	20.738
Zea	21.274
Lut	21.315
Cantha	22.0427
Chl b	24.2693
Croc	25.103
Chl a'	26.2293
DV-Chl a	26.416
Chl a	26.5973
BE-Car	28.0613
BB-Car	28.176

D. Nutrients

1. NH_4^+ :

NOTE: When performing all nutrient analysis, reagents and artificial seawater should be allowed to warm to room temperature before use. For reagents that are only stable for one day to a week, make only enough as needed for samples. Date and ID all reagents made. Always perform standards and samples in duplicate when possible. Always use BW for reagents and/or standards (except when using artificial seawater which should be made with BW). Any salts used should be dried in oven at 40°C for ~24hrs before use.

Source: ([Solorzano 1969](#))

Reagent Preparation

Use dedicated bottles with dispensers for solutions. Kept clean in hood across from HPLCs in 418.

- Phenol-alcohol solution – *stable for 1 week at 4°C*
 - o Using a plastic volumetric pipette, dispense 22ml (11ml) of liquid phenol into 200ml (100ml) of 95% EtOH or reagent alcohol (Store in amber bottle). Take care with phenol.
- Sodium nitroprusside solution – *stable 1 month at 4°C*
 - o Dissolve 1.0g of sodium nitroprusside ($\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}\cdot 2\text{H}_2\text{O}$) in 200ml of BW (Store in amber bottle)
- Oxidizing solution– *prepare fresh daily*

- Alkaline reagent - Dissolve 100g of sodium citrate and 5 g of sodium hydroxide in 500ml of BW - *stable indefinitely*
- Sodium Hypochlorite solution (bleach)
- Mix 100ml of alkaline reagent with 15ml of sodium hypochlorite solution.

Prepare standards

- Prepare primary standard of 10 mM NH_4Cl – *Store in freezer.*
- Prepare secondary standard 0.1mM (100 μM) NH_4Cl
 - Add 1.0ml of primary standard to 100ml volumetric flask
 - Add BW or ASW with appropriate salinity up to the 100ml line with a squirt bottle
- Prepare working standards – make in a range that encompasses that of the samples, in triplicate. Direct addition method made directly in test tubes, use 15ml Falcon plastic centrifuge tubes with blue caps (See Table 3).

Table 3. How to prepare NH_4^+ standards using direct addition method.

Desired Conc. (μM)	Addition of 2° (μl)	BW or ASW with appropriate salinity in test tube (ml)
Reagent blank	0	10
0.1	10	10
0.2	20	10
0.5	50	10
1.0	100	10
2.0	200	10
3.0	300	10

*Can use any range of concentrations. Higher concentrations must be run using a 1 cm cell.

Adding reagents and processing standards and samples

- Add reagents in a fume hood, due to the phenol reagent
- Add 10ml of sample to sterile tube using and Eppendorf pipette
- Add 0.4ml of phenol solution from automatic dispenser
- Cap and Vortex or shake vigorously
- Add 0.4ml of sodium nitroprusside solution from automatic dispenser
- Cap and Vortex
- Add 1.0ml of oxidizing solution from automatic dispenser
- Cap and Vortex
- Allow test tubes to develop in a dark drying oven ($\sim 40^\circ\text{C}$) for one hour, no less.
- Remove and place in dark to cool to room temperature
- Measure absorbance at 640 nm on spectrophotometer (turn on spectrophotometer 1 hr before use)
- Blank instrument against air (use a 10cm or 1cm depending on concentrations)
- Record measurements in logbook.

- Keep samples in dark before measurement, take one sample out of dark, pour into cell using a final, close lid, wait 5 sec., read sample, suck out sample using pump with tubing attached.
- Take next sample out of dark, etc.
- Dispose of all waste in appropriately labeled container.

Standard curve linear regression should yield r values of > 0.99.

2. Auto Analyzer nutrients, NO_2^- , NO_3^- , PO_4^{3-} , urea, TDN, silicate:

Things to consider:

- *Which nutrients to run?* The AA can only run 3 chemistries at a time, so plan accordingly. Available chemistries in place are $\text{NO}_3^- + \text{NO}_2^-$, NO_2^- (both on same channel), TDN, PO_4^{3-} , urea, and silicate is available but not on-line.
- *What is the matrix?* If samples were taken in full strength seawater, make up artificial seawater with the appropriate salinity. Alter for varying salinities based on sample salinity. The sample wash and standards must have similar salinities as your standards. There is a cheat sheet in rm. 420 (above the balances) telling you how much of which salt to add to 10L for varying salinities. The only exception is TDN, due to the presence of a dialyzer, TDN wash and standards must be made in BW only, otherwise a precipitate forms after the digestion reagent when too much salt is introduced to the sampling stream.
- *What is the concentration range for each chemistry?* Think about the water type your samples came from. If they are estuarine, concentrations will be high, if they are oceanic, concentrations will be low. There is a cheat sheet on top of the computer next to the AA with different suggestions for standards based on sample type. Prepare them accordingly.

Preparation:

- Once the chemistries have been decided upon, determine if any reagents need to be made prior to running your samples. Refer to the cook book recipes available from Astoria Pacific for each chemistry and each reagent. A summary of what is needed to run each chemistry is below.

NO_x & NO_2 :

Source: ([Parsons et al. 1984](#), [Price & Harrison 1987](#)):

Sulfanilamide – Kept in amber bottle in fridge, stable until it is used up.

NED – Kept in amber bottle in fridge, stable until it is used up.

Working imidazole buffer – Kept in small plastic rectangular bottle in cabinet above AA. Replenish with buffer (in 2L glass bottle in cabinet above AA) and Brij (in small plastic container on fridge) as necessary. Re-make buffer when 2L glass bottle is empty)

PO_4 :

Source: (Parsons et al. 1984, Price & Harrison 1987)

Working molybdic acid reagent – Must be made fresh Daily 30 mL molybdic acid reagent (in cabinet above AA, brown bottle) plus 30 mL BW. Molybdic acid stock is stable for 3 months.

Working hydrazine sulfate reagent – Rectangular plastic bottle kept in fridge, replenish with stock (kept in glass bottle in fridge, stable indefinitely) and Dowfax (kept

in small bottle in shelf above AA- when cook book says SLS, use Dowfax instead) as necessary.

Urea:

Source: (Parsons et al. 1984, Price & Harrison 1987)

Working color reagent – In rectangular container kept in cabinet above AA, make fresh daily with stock thiosemicarbazide (in small bottle, foil covered bottle in cabinet above AA) and diacetyl monoxime (chemical found in Rm 420)

Working acid reagent – In rectangular container kept in cabinet above AA, make fresh daily with stock 54% sulfuric acid (found in cabinet above AA) and stock ferric chloride (found in small bottle found in cabinet above AA)

Water with Brij – found in 1L PET bottle next to AA. If running high salinity samples, add 50g NaCl to 1L BW, and Brij. For freshwater samples, just use BW and Brij.

SiO₄:

Source: (Parsons et al. 1984, Price & Harrison 1987)

Working molybdic acid reagent – In rectangular container kept in cabinet above AA. Use stock solution (in 1L PET bottle in cabinet above AA), but must remake if the sides become white, it will not all dissolve, don't know why.

Working tartaric acid reagent – In rectangular container kept in fridge. Replenish with stock solution in plastic bottle in fridge, stable indefinitely.

Working stannous chloride reagent – In rectangular container kept in cabinet above AA. Make fresh daily with stock stannous chloride (kept in 2nd tall freezer on right in back of 418 in small PET bottle in door).

TDN:

Source: (Valderrama 1981)

Alkaline Persulfate Digestion reagent – in 250 mL PET bottle, make up as needed. Add 3 g of potassium persulfate (Sigma Aldrich brand only, other stuff contaminates) to 200 mL DI, then add 0.5 mL 10 N NaOH. Mix well, make sure persulfate is dissolved. 10 N NaOH is kept in 250 mL glass bottle in cabinet, add 100 g slowly to 200 mL water in an ice bath. Bring to 250 mL when cool. Store indefinitely.

Buffer solution – Kept in rectangular bottle in cabinet. Use the recipe for the stock ammonium hydroxide-EDTA buffer, pH 8.5 under Operating Notes #6 in the recipe booklet. Do in the hood, nasty stuff. The working buffer is this plus a small amount of brij.

Color reagent – Kept in glass bottle in refrigerator. Needs to be remade if the reagent turns pink. Follow instructions in recipe booklet.

**Again for all details regarding reagent making, see the cook book recipes next to the computer. Please put initials and date on any bottle of any reagent that you make up. Most stock reagents are in cabinet above AA, or in the fridge as necessary.

- Make up necessary standards. All primary and secondary standards are in freezer (2nd tall one on right in back of 418) in the door.

- Make sure the appropriate salinity wash water is made up. 1 carboy on sink is for BW the other is for artificial seawater, whatever salinity is needed, just make sure the salinity is marked on the carboy.
- When adding Brij or Dowfax, a few drops will suffice, don't want to add too much, but if flow does not look right, add a little more.

Start-up of the AA:

- First thing to do on day of analysis is to take all samples out of the freezer to thaw, remove all necessary standards and reagents from fridge. Samples, standards, and reagents must be at room temp. before analysis.
- On AA, make sure all pump tubing is stretched across both sides of the pump and put down all platens. On the mini-pump on the sampler, make sure the tubing is latched in (bottom latch) and turn pump on (slide button up). Make sure all tubing is in BW water and turn pumps on.
- Make sure the sample line from the auto-sampler is connected to the correct chemistries you are running that day, you will need splitters depending on which chemistries you are running. Place all sample lines that aren't in use in the BW rinse bottle.
- Once sufficient BW pumping has occurred (10 – 15 min) place reagent tubing in appropriate surfactant containers. *** For TDN analysis, please follow start up and shut down procedures in the recipe booklet, starting on Page 11. TDNs are different than the others.*** NO_x, and Urea go into the water with Brij container (make sure it is fresh and full) and PO₄³⁻ and SiO₄ go into the water with Dowfax container (make sure it is fresh and full). You only need to place the tubes in the Brij or Dowfax for the chemistries you are using that day, leave the rest in the BW.
- Allow to pump for a while and monitor bubbles. Ensure there are consistent bubble patterns for the chemistries in use. If not, determine what the problem is, could be flattened pump tubing (replace if necessary), clogged lines, etc.
- Once sufficient bubble pattern is established, place appropriate reagent lines in reagents. Ensure good bubble pattern again.
- Turn on detector, switch on back of 305D detector.
- Open up FastPac on desktop. Hit enter, there is no password. Connect the computer with the AA by clicking on the lower left-hand plug in the software.
- Pick appropriate chemistries from the drop down menu.
- Ensure the calibration curves are correct, that they match your standards. Do general look through on software to make sure all the settings are how you want them. Generally pecking is off, sip times are set appropriately, etc.
- Make sure there are no bubbles in the flow cell. Get them out by pinching the waste line.
- When using Cd coil, never let air go through it. Always keep it stored in BW, as long as the pH is neutral. Flush it a few times with BW, then just leave BW in it. If it is necessary to re-activate it, just push through 10 –20 mL of BW, followed by 10 mL of CuSO₄, slowly add this, let it sit, then flush again with 10 – 20 mL of BW. These procedures are the same for both Cd coils, the longer coil is for the NO_x channel, the shorter coil is for the TDN channel.

Shut down procedures:

- ***For TDN, follow specific shut down procedures in recipe booklet on page 9.

- If using heaters, turn them off.
- If using Cd coil, take off line and rinse with BW, store with BW.
- Take all sample lines out of reagents and place in BW wash.
- Let the instrument rinse for 15 minutes
- Shut off pumps, loosen platens and pump tubing from both the main pump and the sampler pump.
- Turn off detector
 - Make sure data is saved.
- If reagents are stored in the fridge, put them in. Put all standards in fridge. Place samples in freezer or dispose.
- Export data: Go to C drive, program files, Astoria Pacific, FPX folder, appropriate year, save in a new folder as the date samples were run. Export both the .fpx and .xls files. Back this up on the Jdrive under mmlab, auto analyzer, appropriate year and month.

Troubleshooting:

- If there is an air bubble in the flow cells, pinch the waste line coming from the flow cell and allow the air bubble to come out.
- For TDN analysis, when running with saltwater, you **will** get a white precipitate in the lines, if you notice a slowdown of flow prior to the heater, remove all lines and place them in 10% HCl for at least 15 minutes, find the blockage and make sure it is gone, you may even have to cut or replace tubing if necessary, then flush with DI for at least 25 minutes. Always run rinse and standards in BW.
- Always observe the bubble patterns, almost all problems can be diagnosed by following the flow of the bubble patterns, if it doesn't look right, make sure all reagent lines are flowing freely, and sample lines are flowing freely.
- Do not let the rinse water, either BW or seawater, become empty, always monitor the levels.
- Do not let your washes get empty.
- Insert many washes throughout your run, it will help if your baseline starts to drift.

3. DFAA:

Sample preparation and hydrolysis:

The method employed to analyze dissolved free amino acids (DFAAs) by high pressure liquid chromatography (HPLC) is the O-phthaldialdehyde (OPA) amino acid method.

Source: (Lindroth & Mopper 1979, Kuznetsova & Lee 2002, Kuznetsova et al. 2004)

Chemical recipes:

	1mL	10mL
<i>OPA solution:</i>	25mg OPA	250mg
	0.5ml MeOH	5ml
	0.5ml 1.0 M Borate Buffer (F.W. 61.83g)	5ml
	25 ul β -mercaptoethanol	250 μ l

Age in freezer at least 24 hours before use. Good for up to two weeks. Light sensitive.

Eluent A: 0.025 M Sodium Acetate, F.W. 136.1g (13.61g/4L)
2% Tetrahydrofuran (80ml/4L)
0.05% Brij (2ml/4L)

pH to 7.1 with acetic acid and filter before adding THF and Brij. Good for two weeks at room temperature.

Eluent B: 100% MeOH

Amino acid standard mix: 0.5ml amino acid mix (25nmol.ml)*
0.025ml 0.5mM GLN, ASN*
0.020 ml 1.0 M KOH (add .020ml, ck pH, add more if necessary)*

*this is for 50ml at 250nM, adjust accordingly for other concentrations or volumes.

Make in 50 ml volumetric flask and check pH (7) by dropping onto a pH strip.

Amino acid standards: 0.025 ml of 0.5mM solution (in freezer)
0.020 ml of 1.0 M KOH

Make up in 50 ml volumetric flask and check pH (7) by dropping on pH strip.

Sample preparation:

- Samples are thawed and two 1.0 mL aliquots from each sample are transferred to (acid rinsed and pre-combusted) 2 mL hydrolysis vials and placed in the HPLC rack.
- First two vials should be blanks, BW or ASW
- Next set of vials are triplicate standards, 37.5 nM, 75 nM, 125 nM
- Sample IDs and sample rack location are recorded in the HPLC log book
- 2 or 3 vials are filled with the OPA solution to be used for derivitization in the HPLC

High performance liquid chromatography (HPLC):

The HPLC method was based on Mopper and Furton (1991) and Fitznar et al (1999). The column in use is an Adsorposphere OPA HR 5u, 150 mm x 4.6 mm. Gradient conditions are stored in the software. Load the DFAA method into the software.

4. DIC/Alkalinity:

A new coulometer and titrator are being set up in the Mulholland lab, no protocols are available for analysis as of this time.

E. Peptide hydrolysis

Instrumentation: Shimadzu HPLC system equipped with Class-VP 4.2 Chromatography software, SCL-10A System Controller Module, 2 LC-10AS Solvent Delivery Modules, a SIL-10A Automatic Sample Injector with a refrigerated autosampler (4°C), a McPherson FL-750BX Spectrofluorescence Detector (424nm excitation, 470nm Emission) and a Beckman ODS 5µm 4.6 mm x 250mm column (Alltech #235329) with a Beckman Ultrasphere ODS 5µm 4.6 mm x 45 mm guard column (#243533).

Protocols for using HPLC to analyze LYAala4 and/or LYAAVFA samples:

- Flip all switches on the HPLC to ON, except pump C
- Turn on computer
 - o Open VP software
 - o Choose Shimadzu 1 or 2
 - o Must hear a beep, then hit OK

If switching DFAA column to LYA column, follow directions below, if not, start at the *

- Change out column while methanol is pumping through it
- Change out eluent B
- Need to rinse column
 - o Under File, Method, Open method
 - o Choose the method under Projects, HPLC1, Method, Rinse
 - o Make sure channel is correct, under pull down menu choose RF-10Ax1
 - o Download method, pumps will begin to change based on set parameters
- DI rinse
 - o Use appropriate syringe with DI water through pump B, place syringe into pink needle and push water through to rinse the outside of the pump heads
- Purge line
 - o Attach empty syringe to needle on Pump A
 - o Open valve ½ turn on Pump A
 - o Hit purge button
 - o Repeat twice

*If not switching columns start here

- Choose appropriate method
 - o Under File, Method, open new method
 - o Method is under Project, HPLC1 (HPLC2), Method, LYAala4 (LYAAVFA)
- Let detector warm up for ~30 min.
- Set up a data folder
 - o On desktop, go to My Network places, VP folder, Projects, HPLC1 (HPLC2), Data, LYAala4 (LYAAVFA)
 - o Then pick appropriate year and make a new file if need be for a new project
- Load samples
 - o Take out rack and load samples appropriately
 - o The first two samples are standards (44.92 nM LYAala2/112.8 nM LYAala4 or 50 nM LYAAV/100 nM LYAAVFA)
 - o Number samples according to how they have been done in the past, see log book

- Log everything in the log book
- Place rack back into autosampler and lock in
- Make sure detector is on
- Make a batch
 - File, Sequence, Sequence wizard
 - First menu, make sure method is correct
 - Second menu, leave sample ID blank, Change data path to where folder was set up on the desktop, under Data file choose Increment and Date & Time, add up total amount of samples (70 samples in rack + standards that are interspersed every 10 samples + rinse + stop)
 - Third menu, First vial = 0, injection volume = 200 uL
 - Finish
- In sequence file, go to sample ID and fill in samples according to how they were placed in rack
 - Every 10th sample will be a standard, so must change the vial # to either 0 or 1 to send sampler back to standards, then continue on with samples in rack and repeat standard every 10th sample. Alternate between sipping from vial 0 and vial 1.
 - Each run must end with a rinse and a stop
 - Name the sample RINSE, vial # = -1 and sample volume = 0
 - Change the method to Rinse.met
 - Name the next sample STOP, vial # = -1 and sample volume = 0
 - Change the method to stop.met
 - Save sequence
 - Go under file, sequence, save as, Projects, HPLC1 (HPLC2), sequence, LYAala4 (LYAAVFA), find the appropriate folder and save as the date
- Start sequence by hitting the double green arrows
- Wait and make sure green line appears on screen (~1-2 min)
- Check on instrument periodically to make sure there is enough eluents, and clean the pump heads with BW water
- Once run is over, turn off instrument or leave on if running more samples

V. Data handling and analysis

A. Lab protocol for log books and data backup

While running samples on any lab equipment, HPLC, GC, Mass Spectrometer, Auto Analyzer, etc., it is important to keep a detailed and accurate log of your use. Please be ensure to include the date, time of use, procedures used, any problems and a detailed description of the samples run and where they were saved to on the computer. This will be helpful if the data will ever need to be found at later date when you may not be available. Here is a sample log entry:

04/27/06 HPLC 1 DFAA method

0800 Ran a set of standards using the dfaa.met method. Used following standards:

Vial	ID
0	dummy water sample

1	75nM amino acid sample
2	125nM amino acid sample
3	250nM amino acid sample
4	Rinse method
5	Stop method

Batch run saved as: 042706A

Data saved in HPLC 1 folder, DFAA folder, Data folder, test folder

1230 Standard batch successful. Ran set of Brown Tide Public Landing DFAA samples. Batch saved as 042706B. Samples saved by increment number and date.

Example: 001 042706 12:32.dat

Sample Table:

<u>Vial</u>	<u>ID</u>
0	dummy water
1	75nM amino acid standard
2	125nM amino acid standard
3	250nM amino acid standard
4	BT PL 071003 Diel A
5	BT PL 071003 Diel B
6	Etc

Note: pressure seemed to increase during final run, possible bad column?

Batch finished on 042806 at 1830.

This is just an example of a log book entry. For each instrument there will be slightly different entries but please be sure to include, the date, times used, method, samples run (even just test runs), problems, and where the data was saved.

Also please be sure to back up ALL data to the MMLAB folder on the K drive. This can be accessed by logging into the network. The computer in 420 has quick access to this drive if your office computer is slow.

Rm 420:

Log in using

Username: oeas

Password: department

Then click on the Novell symbol and login using your Novell username and password, takes less than a minute. Then click on My Computer. From here you can access the K drive at Oeas on 'Almond\.....

Then click on the MMLAB folder. Use the corresponding folder for each instrument. Again be sure to use the date of when the samples were run as the name of the data.

If you like you can also send the files to me (Peter) and I will save them to the appropriate place on the K drive.

B. Mass Spec. Calculations

THE INSTRUMENT YIELDS AN ATM % OR A DELTA VALUE THAT IS NOT MASS DEPENDENT!

^{15}N (atom %) in a sample:

$\% \text{ } ^{15}\text{N} = \text{amt. of } ^{15}\text{N} \text{ (by atoms or wt) / total N (by atoms or wt)}$

We will get data in terms of atom percent:

$\% \text{ } ^{15}\text{N} = [(\text{mass } 30) + 1/2(\text{mass } 29)] / [\text{mass } 30 + \text{mass } 29 + \text{mass } 28]$

To calculate the atom % excess, the natural isotopic abundance of ^{15}N (typically about 0.3663 (atmospheric)) is subtracted from the atom % sample. Alternatively, the natural abundance itself can vary so this might be something that one would need to measure, particularly for C because the natural abundance can be quite variable in the environment.

Background N (on filter) – run blanks (we won't blank correct because N is very low on pre-combusted filters)

Calculating Uptake Rate

$$^{15}\text{N} = \frac{(\text{atom \% PON})_{\text{final}} - (\text{atom \% PON})_{\text{initial}}}{\text{Uptake} \quad (\text{atom \% N source pool} - \text{atom \% PON})_{\text{initial}} * \text{time}} \times [\text{PON}], \quad (1)$$

$$^{13}\text{C} = \frac{(\text{atom \% POC})_{\text{final}} - (\text{atom \% POC})_{\text{initial}}}{\text{Uptake} \quad (\text{atom \% C source pool} - \text{atom \% POC})_{\text{initial}} * \text{time}} \times [\text{POC}], \quad (2)$$

$$\text{H}^{13}\text{CO}_3^- = \frac{(\text{atom \% POC})_{\text{final}} - (\text{atom \% POC})_{\text{initial}}}{\text{Uptake} \quad (\text{atom \% enrichment HCO}_3^- - \text{atom \% POC})_{\text{initial}} * \text{time}} \times [\text{POC}] \quad (3)$$

$$(\text{atom \% POC})_{\text{final}} = F * (\text{atom \% source})_{\text{initial}} + (1 - F) * (\text{atom \% POC})_{\text{initial}}$$

$$(\text{atom \% POC})_{\text{final}} = F * (\text{atom \% source})_{\text{initial}} + (\text{atom \% POC})_{\text{initial}} - F * (\text{atom \% POC})_{\text{initial}}$$

$$(\text{atom \% POC})_{\text{final}} - (\text{atom \% POC})_{\text{initial}} = F * [(\text{atom \% source})_{\text{initial}} - (\text{atom \% POC})_{\text{initial}}]$$

$$F = [(\text{atom \% POC})_{\text{final}} - (\text{atom \% POC})_{\text{initial}}] / [(\text{atom \% source})_{\text{initial}} - (\text{atom \% POC})_{\text{initial}}]$$

Derived from a mixing model where:

$$V \text{ (h}^{-1}\text{)} = \text{atom \% excess} / (\text{atom \% enrichment} * \text{incubation duration (h)})$$

$$\text{Atom \% enrichment} = (\text{enrichment of } ^{15}\text{N added} + \text{what was there}) / (\text{total mass } ^{15}\text{N added} + \text{total mass ambient N substrate})$$

Enrichment of ^{15}N added = \square g-at N added * enrichment (e.g., 99%)

Enrichment of ambient N = $\mu\text{g-at N ambient}$ * enrichment (e.g., 0.3663 %)

Total mass in \square g-at N

For example: if you have 1 $\mu\text{M NH}_4^+$ this is equal to 1 $\mu\text{mol/l NH}_4^+$ or 1 $\mu\text{g-at N/l}$ (because there is 1 atom of N in NH_4^+). If your incubation volume is 50 ml (= 0.05 l) then the total mass of N in your incubation is 1 $\mu\text{g-at N/l}$ * 0.05 l = 0.05 $\mu\text{g-at N}$.

For urea and amino acids, this varies. Urea has 2 N atoms and so 1 $\mu\text{M urea}$ is equal to 2 $\mu\text{mol N/l}$ or 2 $\mu\text{g-at N/l}$ (because there is 2 atoms of N in urea)

Specific uptake rho (ρ):

$$\rho (\mu\text{mol l}^{-1} \text{h}^{-1}) = V * \text{PN}$$

$$\rho (\mu\text{mol l}^{-1} \text{h}^{-1}) = V * \text{PC}$$

Where PN or PC is the particulate N or C (from the instrument you will get a mass in $\mu\text{g N}$ or C).

To convert to $\mu\text{mol N}$ divide mass by the elemental mass (for N this is 14g/mol). To convert to $\mu\text{mol C}$ divide mass by the elemental mass (for C this is 12g/mol).

To get in terms of units mass/volume divide by the volume filtered (that is on your filter).

You can lose or spill sample and still get the correct atm % because you don't fractionate when you spill! But, to calculate rho, you do need to know the exact PN of the sample that was analyzed. If you lose some mass (e.g., from spilling a sample), there must be an independent measure of mass per unit volume to calculate specific uptake. So, you can analyze the sample as usual to retrieve an isotopic ratio but, in the uptake calculation, use the mass number from a sample of known volume (to calculate the correct mass per unit volume).

Check to see if isotope was used up in an experiment:

Atom % xs ($^{15}\text{N}/(^{15}\text{N}+^{14}\text{N})$) * [PN] /100 . Check to see if this approaches what you added to the incubation.

Calculating Production of one compound from another:

$$\rho (\mu\text{mol l}^{-1} \text{h}^{-1}) = ((\text{atm \% xs product pool}) * [\text{concentration of product pool}] / (\text{atm \% enrichment of pool} * \text{incubation time}))$$

Issues:

1. Correcting for carrier (recovery of NH_4^+ through columns is not 100% (about 35%) (another time)

2. Calculating movement through pools (e.g., trophic transfer experiments) – adding exponential decay or formation functions

$$[^{15}\text{N}]_{\text{prey avg}} = \frac{(\text{Atm \% PN} * [\text{PN}])_{\text{prey final}} - (\text{Atm \% PN} * [\text{PN}])_{\text{prey initial}}}{\ln((\text{Atm \% PN} * [\text{PN}])_{\text{prey final}} / (\text{Atm \% PN} * [\text{PN}])_{\text{prey initial}})}$$

$$\text{Atm \% PN}_{\text{prey final}} [\text{PN}]_{\text{prey final}} = (\text{Atm \% PN})_{\text{prey initial}} * [\text{PN}]_{\text{prey initial}} * e^{(\mu-g)t}$$

$$g \text{ (time}^{-1}\text{)} = - (\ln(\text{Atm \% PN}_{\text{prey final}} [\text{PN}]_{\text{prey final}}) - \ln(\text{Atm \% PN}_{\text{prey initial}} * [\text{PN}]_{\text{prey initial}}) - \ln((\text{Atm \% PN}_{\text{control final}} [\text{PN}]_{\text{control final}}) / (\text{Atm \% PN}_{\text{control initial}} * [\text{PN}]_{\text{control initial}}))) / t$$

For ammonium release from N_2 fixation:

$$\frac{\text{Rate (time}^{-1}\text{)}}{[^{15}\text{N}]_{\text{PN avg}}} = \frac{(\text{Atm \% N4})_{\text{final}} * [\text{N4}]_{\text{final}} - (\text{Atm \% N4})_{\text{initial}} * [\text{N4}]_{\text{initial}}}{[^{15}\text{N}]_{\text{PN avg}} * \text{incubation time}}$$

$$[^{15}\text{N}]_{\text{PN avg}} = \frac{(\text{Atm \% PN} * [\text{PN}])_{\text{final}} - (\text{Atm \% PN} * [\text{PN}])_{\text{initial}}}{\ln((\text{Atm \% PN} * [\text{PN}])_{\text{final}} / (\text{Atm \% PN} * [\text{PN}])_{\text{initial}})}$$

3. Correcting for difference in uptake between 0.2 μm and GF/C filters (1.2 μm).

0.2 μm filters contain mass on GF/C filters + mass < GF/C and > 0.2 μm .

- a. Must calculate mass of ^{15}N or ^{13}C in PN or PC, respectively from the GF/C and 0.2 μm filters.

$$\text{Mass } ^{15}\text{N (or } ^{13}\text{C)} = \frac{(\text{atm \% xs} * \text{mass [e.g., } \mu\text{mol l}^{-1}\text{)])}{100}$$

- b. Subtract GF/C ^{15}N or ^{13}C mass from 0.2 μm mass to get < 1.2 μm mass.

$$\text{< 1.2 to > 0.2 } \mu\text{m } ^{15}\text{N or } ^{13}\text{C mass} = \text{Mass } ^{15}\text{N or } ^{13}\text{C (0.2 } \mu\text{m filter)} - \text{Mass } ^{15}\text{N or } ^{13}\text{C (GF/C filter)}$$

- c. Subtract GF/C mass (PN or PC) from 0.2 μm mass to get < 1.2 μm mass.

$$\text{< 1.2 to > 0.2 } \mu\text{m Total PN or PC mass} = \text{Mass N or C (0.2 } \mu\text{m filter)} - \text{Mass N or C (GF/C filter)}$$

$$(\mu\text{mol N or C l}^{-1})$$

- d. ^{15}N or ^{13}C atm % xs for < 1.2 - > 0.2 μm size fraction.

$$\text{Atm \% xs (< 1.2 to > 0.2 } \mu\text{m)} = \frac{\text{< 1.2 to > 0.2 } \mu\text{m } ^{15}\text{N or } ^{13}\text{C mass}}{\text{< 1.2 to > 0.2 } \mu\text{m Total PN or PC mass}} * 100$$

- e. Recalculate uptake for < 1.2 - > 0.2 μm size fraction by using the revised mass and revised atm % xs

$$\text{Uptake (h}^{-1}\text{) (< 1.2 to > 0.2 } \mu\text{m)} = \frac{\text{Atm \% xs (< 1.2 to > 0.2 } \mu\text{m)}}{\text{Atm \% enrichment} * \text{incubation time}}$$

$$\text{Uptake } (\mu\text{M h}^{-1}\text{) (< 1.2 to > 0.2 } \mu\text{m)} = \text{V (< 1.2 to > 0.2 } \mu\text{m)} * (\text{< 1.2 to > 0.2 } \mu\text{m } ^{15}\text{N or } ^{13}\text{C mass})$$

Calculating Natural abundance:

$\delta^{15}\text{N}$, the per mil ^{15}N excess (‰ ^{15}N):

$$\delta^{15}\text{N} (\text{‰}) = \frac{\text{R(spl)} - \text{R(std)}}{\text{R(std)}} * 1000\text{‰}$$

Standard is Atmospheric N (0.3663)

So, if you have a sample that is 0.3700 and your standard is atmospheric (0.3663) then your $\delta^{15}\text{N}$ is 10.1

The $\delta^{15}\text{N}$ of an N_2 fixing plant is something on the order of 0.

Get isotopic fractionation (discrimination) naturally: equilibrium effects, kinetic effects (e.g., enzymatic reactions, etc.)

You are what you eat plus 1-3.

$\delta^{13}\text{C}$, the per mil ^{13}C excess (‰ ^{13}C):

$$\delta^{13}\text{C} (\text{‰}) = \frac{\text{R(spl)} - \text{R(std)}}{\text{R(std)}} * 1000\text{‰}$$

Standard is the PD Belemite (0.0112372) or 1.12372‰

Stable isotope compositions of most natural materials of biological interest range from 0 to –110‰ versus the PDB.

Inorganic C in seawater, freshwater, and carbonates is relatively enriched in ^{13}C . Organic C is generally depleted in ^{13}C as a result of biological fractionation during ps.

C3 plants are –27‰

C4 plants are –13‰

CAM plants –10 to –28 ‰

Anthropogenic CO_2 is –26‰

Rural CO_2 is –7.8‰

Urban CO_2 is –7.8 – 12‰

Salt marsh plants –27 to –13‰

DIC 0‰

DOC –23‰

Seagrasses – 10‰

Macroalgae – 15‰

Marine Phytoplankton – 22‰

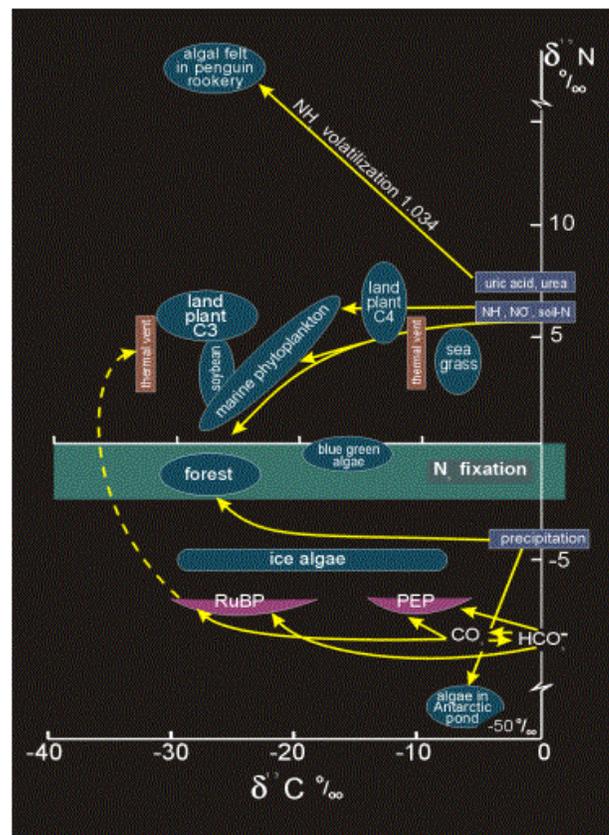
(Our values should be values around 1.101% ???)

Zooplankton – 20‰

FW plankton –42 to –26‰

(values would be 1.083 to 1.097% ???)

FW DIC – 10 to –5‰



C. Peptide hydrolysis data analysis

- Once all data is collected
 - o Make sure the appropriate method is open (LYAala4 or LYAAVFA), if not, open method
 - o Open up first standard file
 - o Under Method, Int. events – Delete all data in rows, close
 - o Under Method, Peaks – Delete all data in rows, close
 - o Define threshold
 - On menu buttons at bottom of screen, click on 2nd button, threshold

- Use cursor and click on baseline just before the LYAala2 (LYAAV) peak, then click about $\frac{3}{4}$ of way up peak, click Add to table
- Define width
 - On menu buttons at bottom of screen, click on 1st button, width
 - Use cursor and click on baseline before first peak, then baseline after the 1st peak, click Analyze Now
- Define peaks
 - On menu buttons on bottom of screen, find define peaks button and click
 - Use cursor and click on baseline before first peak and on baseline after 2nd peak
 - Go to Method, Peaks, go to ID and name LYAala2 (LYAAV), and LYAala4 (LYAAVFA) for each peak, then scroll over to Level 1 and put in the concentrations for each peak, save, close
- Calibration
 - Go to Analysis, single level cal, check the calibration box, and click on clear all calibration
- Go to Method, adv. exp., enabled
 - Clear the export files under My network places, VP, Projects, HPLC1 (HPLC2), Data, LYAala4 (LYAAVFA), export folder
- Now you can go through all of the data, start with all of the standards, and they will be organized at the top of the final spreadsheet
 - Open up a standard file, make sure it looks good and defines the appropriate peaks, click on Analysis, Analyze, wait for a beep, open up next folder
 - Repeat this for all samples
- Go to My Network Places, VP, Projects, HPLC1 (HPLC2), Data, LYAala4 (LYAAVFA), find where the data you just exported was placed
 - Open up bottom files in Excel, one is area, and one is concentration, save them both onto 2 different sheets in same Excel file
 - Save file on hard drive, then wherever else it is needed

VI. QA/QC

A. Quality Control

- All holding vials and sample bottles used in the laboratory are factory cleaned to assure no sample contamination. Glass ware is combusted (450°C, >6 hours). Supplies are ordered from reputable manufacturers and inspected upon arrival for breakage or contamination. Gases are certified by the suppliers for minimum contaminant levels and their QA/QC protocols are available to us for our inspection. All equipment is calibrated prior to each day's use in accordance to manufacturer's directions. Calibration logbooks are maintained for each instrument.
- Lab duplicates are performed on a minimum of 10% of samples. Field or Lab Blanks are run in each sample batch with a minimum of one in each 10 samples. Investigators responsible for sample collection are encouraged to split one of each 10 samples submitted to the laboratory as a blind duplicate.
- Unacceptable variance among lab duplicates will be defined as data points which differ by more than one standard error of the mean from mean data value for each sample pair.

B. Mass Spec QA/QC

Daily QA/QC for the Isotope Ratio Mass Spectrometer (IRMS)

- Daily tuning scans are performed for both nitrogen and carbon using reference gas samples on continuous flow. IRMS tuning parameters are adjusted to achieve maximum sensitivity and stability.
- Blank runs are performed to ensure that there is no system contamination.
- Standard curves are run using prepared standards of a sucrose/ammonium sulfate solution. Standard mass ranges are prepared according to the estimated mass of unknown samples to be analyzed. Acceptable R^2 values are >0.99 .
- Sample batches are prepared with reference and reference check samples every 8 unknown samples to ensure that both reference mass and natural abundance values are within acceptable values. Blank runs are performed at the beginning and end of every batch of samples to test for system contamination.

Monthly QA/QC

- ^{15}N and ^{13}C atom % values in the sucrose/ammonium sulfate standards are established by testing against certified standards.
- Nitrogen and carbon mass standards and instrument stability are tested by comparison to NIST (National Institute of Standards and Technology) certified standards and by inserting certified standards in a batch as unknown samples. Acceptable ranges are within $\pm 5\%$ of NIST values.

C. Data Validation

If unacceptable variance is observed, a report will be made within 24 hours to the Project QA/QC officer detailing:

- Sample type and responsible investigator
- Reasons for suspecting compromised data quality
- Possible reasons (if known) for compromised data quality.

The QA/QC officer may then, in consultation with the Lab Manager and the responsible investigator:

- Order the samples re-analyzed following re-calibration of the instrument or some such action as is deemed appropriate to correct the errors AND alert the responsible investigator to obtain new samples if possible to replace the compromised data.
- Call a meeting of the appropriate individuals to reconsider sampling and analytical methods to avoid errors in the future.

VII. References

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Appendix 3. DATAFLOW & Continuous Monitoring Specific Error Codes

<u>Category</u>	<u>Code</u>	<u>Description</u>
Specific Probe Code	PSW	Salinity Calibrated to Incorrect Level
Specific Probe Code	PDP	DO poisoning (anoxia)
General Probe Code	GBO	Blocked Optic
General Probe Code	GWM	Wiper Malfunction
General Probe Code	GWL	Wiper Lost
General Probe Code	GSC	Seal Compromise
General Probe Code	GPF	Probe Failure
General Probe Code	GPC	Post Calibration Out of Range
General Probe Code	GNV	Negative Value
Non-Probe Codes	NPF	Power Failure
Non-Probe Codes	NOW	Instrument Out of Water
Non-Probe Codes	NNF	Ram Clogged/No Flow
Non-Probe Codes	NND	No Data
Non-Probe Codes	NIS	Invalid Data Due to Incorrect YSI Setup
Non-Probe Codes	NIR	Instrument Removed
Non-Probe Codes	NQR	Data Rejected Due to QA
Comments	CTS	Time Skip
Comments	CBF	Biofouling
Comments	CWD	Instrument at Wrong Depth
Comments	CTF	Temperature Probe Failure
Comments	CSW	Salinity Level Calibrated Incorrectly
Comments	CLF	Flow Low
Comments	CDB	Disturbed Bottom
Comments	CTC	Time Change
Comments	CTW	Turbid Water
Comments	CFK	Fish Kill
Comments	CSC	Site Location Change
Comments	CAS	Algal Sample Taken
Comments	CAB	Algal Bloom – No Sample Taken

See http://www.chesapeakebay.net/pubs/cbwqdb2004_RB.PDF for any questions parameter units, other error codes, method codes, etc

Appendix 4. Continuous Station QA/QC Screening Protocol

Step 1. Insert appropriate parameter_qualifiers

Parameter	<	>
Temp	-5	45
SpCond	0	100
Salinity	0	70
DO sat	0	500
DO mg/l	0	50
Depth	0	9.10
PH	0	14
Turb	0	1000
Chl	0	400
Fluor	0	100

Step 2. Identify negative “-“ values

All negative values are flagged with a “GNV” in the appropriate parameter_a column

Step 3. Identify time periods when the datalogger or certain probes were not deployed.

In the appropriate parameter_a column, flag all non-deployments with a “NND”.

Step 4. Identify and flag single spike NTU values

In the NTU_a column, flag all single spike NTU values >300 with “NQR”.

Step 5. Identify and flag single spike Chl values

In the Chl_a column, flag all single spike Chl values >400 with “NQR”.

Step 6. Identify and flag single spike %Fluor values

In the Fluor_a column, flag all single spike Fluor values >100 with “NQR”.

Step 7. Identify and flag probe failures

In the appropriate parameter_a column, flag time intervals with “GPF”.

Note: This would apply to DO membrane punctures, pH bulb breakage etc.

Step 8. Identify and flag time periods in which specific probes failed to meet acceptable post calibration criteria

In the appropriate parameter_a column, flag time intervals with “GPC”.

Note: This would apply to DO drift and similar issues. If you can not clearly identify where the problem began, flag the entire deployment interval.

Step 9. Identify and flag time periods in which entire instrument or specific probes were out of water.

In the appropriate parameter_a column, flag affected time intervals with “NOW”.

Note: If entire instrument is out of water, all parameter_a columns must be flagged.

Step 10. Identify and flag time periods affected by a time skip.

In the comment column, flag effected time period with “CTS”.

Step 11. Identify and flag time periods affected by station maintenance.

In the appropriate parameter_a column, flag affected time interval with “NQR”

Note: This was seen in some of our turbidity and depth data.

Step 12. Link deployments and look for probe drifts, significant mismatch, etc

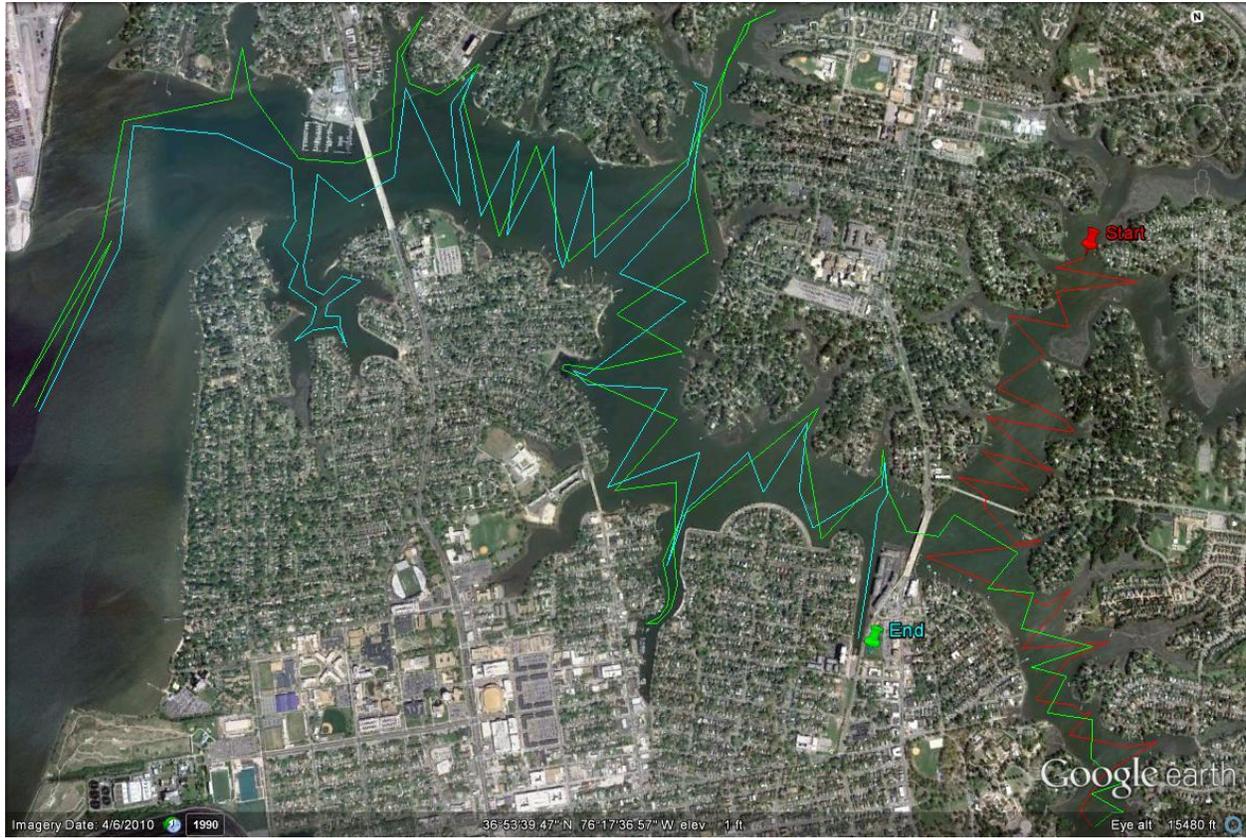
In the appropriate parameter_a column, flag affected data with the appropriate error code.

Appendix 5. Field Sheet – Quality Assurance Data for CMONs (Deployment and Retrieval Intervals)**INVESTIGATOR:** _____**SERVICE TYPE: DEPLOY, RETRIEVE, OR ADDITIONAL CHECK (CIRCLE ONE)****SITE:** _____**DATE:** _____**QA DATA COLLECTION:**

	TIME	RESULT
Temp. (deg. C)		
Salinity (ppt)		
pH (su)		
Secchi depth (m)		
YSI DO (mg/L)		
Winkler DO (mg/L)		
Chl a (mg/L)		

COMMENTS:**DATA REVIEWED AND VALIDATED BY (SIGNATURE):** _____

Appendix 6. Storm event sampling in the Lafayette River – Cruise tracks



Appendix 6

VIMS Moore Standard Operating Procedures

**Quality Assurance Project Plan for the Project:
Fulfilling Data Needs for Assessing Numeric CHLa Criteria of the Lower
James River Estuary, 2012**

(For the Period: May 1, 2012 through April 30, 2013)

Virginia Institute of Marine Science
School of Marine Science
College of William and Mary
Gloucester Point, Virginia 23062

Dr. Kenneth A. Moore, Project Manager, VIMS Date

Mrs. Betty B. Neikirk, Quality Assurance Officer, VIMS Date

Dr. Arthur Butt, Program Officer, Va. DEQ Date

1. PROJECT DESCRIPTION

1.1 Project Definition and Background

The VA DEQ is undertaking a comprehensive review of the existing Site-Specific Numeric Chlorophyll-a (CHLa) criteria and associated modeling framework for the tidal James River. This effort will provide the scientific basis for a potential water quality standards rulemaking process, which may result in revisions to nutrient allocations contained in the Chesapeake Bay TMDL. A Science Advisory Panel was established by DEQ to provide recommendations on data and modeling needs for assessing the existing CHLa standard. The Panel reviewed existing data resources and modeling capacity to identify knowledge gaps in characterizing the occurrence of algal blooms in the tidal James River and associated impairments to designated uses. The Panel's recommendations provided an overall framework for addressing these needs as well as specific tasks for data collection and model development.

The Lower James River Estuary (inclusive of the oligo-, meso- and poly- haline regions) experiences algal blooms that are more ephemeral in time and place. Given the large spatial area of the Lower James, and the sporadic incidence of algal blooms, a greater proportion of data collection activities must be allocated to characterizing the frequency and extent of blooms. Advanced technologies including continuous, fixed-station monitoring and continuous on-board monitoring will be needed to map their spatial extent and identify zones of bloom initiation. Assessing impairments in the Lower James is also challenging because the blooms are typically comprised of dinoflagellates which are known to cause harmful effects, though these may not be linked to the occurrence of specific toxins.

Algal blooms occurring in the Lower James River Estuary are ephemeral in nature and unpredictable in their timing, location and duration. Algae have the capacity to bloom quickly and to be transported by currents. As a result, sites of bloom initiation may be geographically distinct from areas where blooms develop and cause detrimental effects on water quality and living resources. The distinction between sites of initiation and impact is important because mitigation actions designed to prevent blooms would need to be focused at sites of bloom initiation whereas actions aimed at mitigating bloom impacts would need to focus on sites where blooms accumulate. Fixed station monitoring, such as the program carried out by DEQ for the CBP, is not designed to locate, map and track these events. Thus, alternative monitoring strategies are needed to characterize the occurrence of algal blooms in the Lower James. A method of on-board and underway monitoring (DATAFLOW) of CHLa can be used in conjunction with GPS navigation to provide real-time mapping of algal blooms. Presently this technology is employed by the Hampton Roads Sanitation District (HRSD) scientists to map spatial variation in CHLa for the meso- and poly- haline segments of the James, Elizabeth and Lafayette Rivers on a weekly basis. This method provides the most effective means for determining the size, intensity and location of algal blooms. The Panel recommended that these efforts should be expanded to include the oligohaline segment of the James (Appendix 3).

There is also a need to complement CHLa mapping efforts with fixed-station, continuous monitoring (CMON) to enhance temporal coverage and bloom detection capabilities. Specifically, CHLa sensors should be deployed in potential "hot spots" for bloom initiation that are identified from previous mapping efforts and/or their proximity to nutrient inputs. One site in the JMSMH segment will be sampled using this protocol (Figure 4). The JMSMH location represents a region where algal blooms are often first observed either by initiation and/or hydrodynamic transport. One Chesapeake Bay Program (CBP) segment, the James River Oligohaline (JMSOH) will be sampled by this program in 2012-13 using water

quality mapping (DATAFLOW) sampling to complement other DATAFLOW sample currently conducted by HRSD.

1.2. Objectives and Scope of Project (Fulfilling Data Needs for Assessing Numeric CHLa Criteria of the Lower James River Estuary, 2012. Subtask 1.1 – Expand Monitoring Network)

- 1) Collect data to be used in assessing numeric water quality standards for Chlorophyll.
- 2) Collect data for diagnosing reasons for any non-attainment of these water quality criteria.
- 3) Collect data to improve overall understanding and modeling of processes influencing these water quality criteria.
- 4) Provide calibration data for refined James River Model simulations of water clarity, and phytoplankton that will be completed over the next three years.
- 5) Provide continuous water quality data from a site in the JMSMH in conjunction with in situ plantings of oysters to assess biological impairments as they relate to the duration and intensity of exposure to bloom events.

DATAFLOW sampling in 2012 will be conducted in one Chesapeake Bay Program segment, the James River Oligohaline (JMSOH). Collection of data from 0.25-0.5m below the surface will be performed twice a month from May through June during one spring and one neap tidal period. During the most intensive bloom period of July-September the cruises will be conducted weekly and twice monthly again in October. The DATAFLOW system allows the continuous measurement of dissolved oxygen, chlorophyll, turbidity, salinity, specific conductivity, temperature, and pH while underway in a small boat. The data collected in any one day can then be interpolated to provide a complete surface “map” of water quality conditions throughout the segment that can then be compared against water quality criteria.

The specific goal of the fixed station continuous monitoring system is to assess water quality standards as well as quantify short-term variability and long-term changes in water quality constituents in specific shallow water areas. One fixed continuous monitoring (CONMON) station for 2012 will be located in the lower James River at or near the James River Country Club (Appendix A). The station will consist of a YSI extended deployment datasonde, which will sample dissolved oxygen, chlorophyll, turbidity, salinity, specific conductivity, temperature, and pH.

Concomitant data on chlorophyll and suspended sediment conditions are collected at single point sites during the dataflow mapping cruises and when the fixed stations sondes are exchanged. These data, for dataflow verification stations, will include: chlorophyll and pheophytin, total suspended solids (TSS), volatile suspended solids (VSS), dissolved oxygen using a YSI 600 XLM sonde, secchi depth, and vertical profiles of photosynthetically available radiation (PAR) and temperature, dissolved oxygen, salinity, specific conductivity, and pH. For the fixed station these data will include: chlorophyll and pheophytin, total suspended solids (TSS), volatile suspended solids (VSS), dissolved oxygen by a YSI 6920 sonde, secchi depth, as well as a vertical profile for temperature, dissolved oxygen, salinity and pH and vertical profiles of PAR. These data will be used to help assess the ecological conditions affecting the water quality criteria and provide enhanced monitoring data for refined James River model simulations of chlorophyll and HAB concentrations.

1.3. Project Location Descriptions

The James River Oligohaline segment begins at the boundary of the mesohaline segment near Carter's Grove Home, about 1.25 miles southeast of Grove Creek on the north shoreline to 0.7 miles north of Hunnicut Creek, south of Hog Island on the southern shoreline. It then extends upriver to adjoin the mouth of the Chickahominy River and upward to Tettington on the north shore and 0.3 miles downstream of Sloop Point on the southern shore. The segment includes Hog Island, Surry Nuclear Power Plant, and Jamestown Island.

1.4. Project Dates

The James River Mesohaline segment begins at the boundary of the polyhaline segment extending from Newport News Point just south of Lincoln Park on the north shore to the US Army Disposal Area at Craney Island on the south shore. It extends upriver to near Carter's Grove Home, about 1.25 miles southeast of Grove Creek on the north shoreline to 0.7 miles north of Hunnicut Creek, south of Hog Island on the southern shoreline. This segment includes the Warwick, Pagan, and Nanesmond Rivers as well as Mulberry Island.

The time period for this study is from May 1, 2012 through April 2013. Status reports will be delivered in accordance with the dates stipulated under deliverables in the contract.

1.5. Data Quality Objectives

The main objective of this program is to collect data of sufficient quantity and quality to assess James River standards for chlorophyll. This data must be representative and comparable across all the monitored tributaries. The greater spatial and temporal density of data which can be used to assess surface water quality criteria and standards is an important component of this monitoring program. Another strength of this study is the comparability of data with that collected by HRSD for lower segments in the James River, as well as ongoing data collections by other Chesapeake Bay Monitoring Programs. Through the use of the same Chesapeake Bay Program approved protocols, instrumentation, quality control checks, and communication, an integrated net of data is generated for this system.

2. PROJECT ORGANIZATION AND RESPONSIBILITIES

2.1. Project Organization

The collection and preparation of samples, plus data entry and management will be completed at the Virginia Institute of Marine Science under the direction of Dr. Kenneth A. Moore (PI). All water quality analyses (Chl a and suspended solids) are performed by VIMS Analytical Services Center (ASC).

2.2. Principal Investigator

The principal investigator (PI), Dr. Kenneth Moore (moore@vims.edu, 804-684-7384), will supervise all activities associated with this project. This includes fieldwork, data management and report writing. He will be responsible for all stages of the analysis of samples, resolving problems that may arise, and assure the satisfactory completion of the study. He is responsible for data review and oversight and submission of data. The PI will review the results of the analyses and approve the quality assurance/quality control protocols to insure the validity of the results. The PI will administer the financial and technical requirements of the project and be responsible for preparing the data and progress report and the final

report to be submitted at the end of the project. He will also meet, at regular time intervals, with the other members of staff to discuss and review their responsibilities in relation to the project. The PI will respond to questions by the contracting agencies regarding the completion of different stages of the project and the reports that have to be submitted as part of the deliverables outlined in the project contract. Dr. Kenneth Moore holds a B.S. degree in Biology from The Pennsylvania State University, a M.S. degree in Marine Science from the University of Virginia and a Ph.D. degree in Marine, Environmental and Estuarine Science from the University of Maryland.

2.3. Personnel Qualification Summary

Mrs. Betty Neikirk (betty@vims.edu, 804-684-7400) is a Marine Scientist Supervisor at VIMS and holds a B.S. in Biology from Randolph-Macon College and a M.S. in Marine Science from the College of William and Mary. Mrs. Neikirk is the Quality Assurance Officer for the Shallow Water Monitoring Program and Field Manager. She has been involved with water quality research and monitoring at VIMS for 27 years and oversees the DATAFLOW and CMON (Continuous Monitoring) field programs, has responsibility for data analysis, coordinates and schedules all field operations, as well as participates in the field operations. She reports to the principal investigator.

Mr. David Parrish (parrishd@vims.edu, 804-684-7835) is a GIS Developer/Scientist II at the Virginia Institute of Marine Science and holds a B.S. in Integrated Science and Technology from James Madison University and an M.S. in Natural Resource Management from Central Washington University. He will serve as Data Manager. He conducts spatial and statistical analysis of water quality data, provides technical support to the program, and manages the VECOS (www.vecos.org) database and website.

Mrs. Joy D. Austin (justjoy@vims.edu, 804-684-7307) is a Laboratory & Research Specialist II/Laboratory Supervisor at the Virginia Institute of Marine Science. She is currently in charge of the day-to-day activities of the water quality and fixed station monitoring at Chesapeake Bay National Estuarine Research Reserve in Virginia (CBNERRVA) and is familiar with all aspects of CBNERRVA monitoring. She will be responsible for overseeing the continuous, fixed station monitoring activities including activities such as sonde calibration, QA/QC of data and data management, sampling coordination, purchases of equipment and supplies. She will coordinate fixed station activities with Mrs. Betty Neikirk.

Ms. Alynda Miller (alynda@vims.edu, 804-684-7576) is a Laboratory and Research Specialist II at VIMS. She has a B.S. in Biology from the Millersville University of Pennsylvania. She will aid in the fixed monitoring program including activities such as sonde calibration, QA/QC of data and data management, sampling coordination, purchases of equipment and supplies.

Ms. Lisa Ott (lott@vims.edu, 804-684-7576) is Laboratory and Research Specialist II at VIMS. She has a B.S. in Biology from Christopher Newport University. She will aid in the fixed monitoring program including activities such as sonde calibration, sample filtration, and maintaining equipment and preparation of field supplies.

Mr. Jim Goins (goins@vims.edu, 804-684-7559) is a Laboratory & Research Specialist II /Field Manager at VIMS. He will help conduct the field sampling activities associated with this project including; Dataflow mapping, water quality verification sampling and fixed station sampling and participates in other monitoring programs as needed. He has extensive small vessel handling experience, and holds US Coast Guard Basic Seamanship and Safety and VIMS small vessel certification

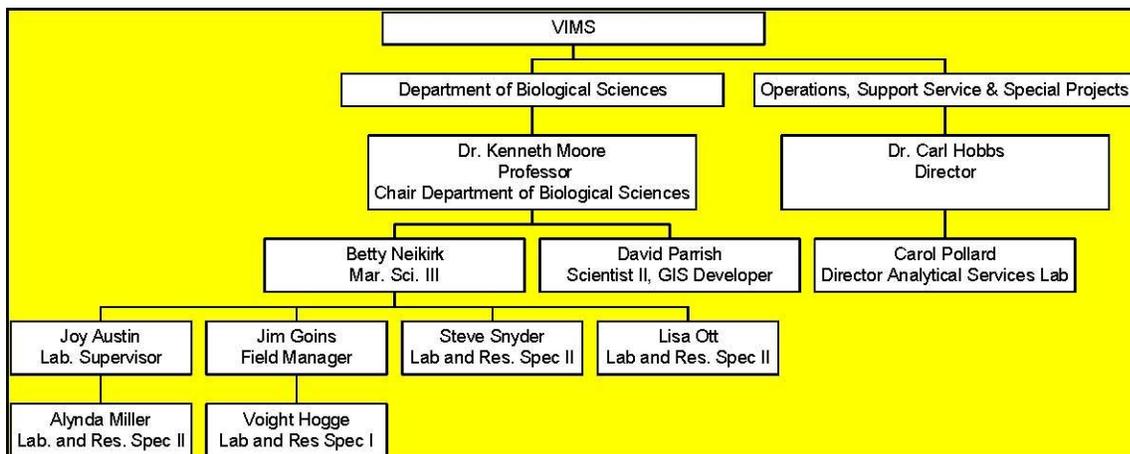
Mr. Steve Snyder (snyder@vims.edu, 804-684-7442) is a Laboratory and Research Specialist II at VIMS who holds an Associates Degree in Applied Science from Thomas Nelson Community College. He assists in the field and laboratory work related to this project including the dataflow mapping, water quality verification sampling and the calibration of the instruments. He also has extensive small vessel handling experience and holds US Coast Guard Basic Seamanship and Safety and VIMS small vessel certification.

Mrs. Carol Pollard (pollard@vims.edu, 804-684-7213) is a Laboratory Director/Scientist II (Supervisor). She has a B.S. in Environmental Science from Florida Institute of Technology and a M.S. in Marine Science from the College of William and Mary. Mrs. Pollard is the Director of the Analytical Services Center at the Virginia Institute of Marine Science. Her responsibilities include lab goals, budget, coordinating personal and maintaining safety and quality control. Mrs. Pollard also coordinates with the Virginia Department of Environmental Quality and the U.S. Environmental Protection Agency in blind audits, cross laboratory calibration samples and the Chesapeake Bay Program’s Coordinated Split Sample Program (CSSP).

All of the above personnel are located at VIMS, P.O. Box 1346, Gloucester Point, VA 23062.

2.4. Organizational Chart

Table 1. Organizational Chart



2.5. Special Training and Certification

Vessel certification is required for use of all small boats at VIMS. This includes successful completion of US Coast Guard approved small vessel navigation and safety course and a yearly renewable, vessel-specific certification by the VIMS Vessels Center. A current list of approved small vessel operators can be found at the web site: <http://www.vims.edu/admin/vessels>.

3. SAMPLING PROCEDURES

3.1. Continuous Surface Water Quality Mapping in the James River Oligohaline

3.1.1. DATAFLOW Mapping system

DATAFLOW is a compact, self-contained surface water quality mapping system, suitable for use in a small boat operating at speeds of about 25 KT. The system collects water through a pipe ("ram") deployed on the transom of the vessel, pumps it through an array of water quality sensors, and then discharges the water overboard. The entire system from intake ram tube to the return hose are shielded from light to negate any effect high intensity surface light might have on phytoplankton in the flow-through water that is being sampled. A blackened sample chamber is also used to minimize any effect of light on measurements by the fluorescence probe.

3.1.2. Area of Operations, Cruise Tracking, and Sample Frequency

The area of operations will include the James River Oligohaline area (JMSOH). This area includes of the EPA Chesapeake Bay Program Office's designated Chesapeake Bay segments (see http://www.chesapeakebay.net/maps/map/chesapeake_bay_segmentation_scheme_for_303d_listing_92_segments for description of CBP segments).

2012 JMSOH cruises (Appendix A) are scheduled to take place twice a month in May, June and October during one spring and one neap tidal period. Cruises will be conducted weekly from July through September. All cruise dates are dependent on weather conditions optimum for the safety of the field crew, tidal stage, and ability to attain quality data. Cruises which have to be cancelled due to weather conditions will be rescheduled immediately as weather permits. Cruises will take place during the mid day, over an approximate four to five hour interval beginning at approximately 0900 to 1000.

Operations follow different cruise tracks depending on the morphology of the area segment being monitored and the amount of navigable shallow water. In the lower segment of the river, where the width of the river is normally wide, a series of tracks running parallel to the shoreline along fixed depth contours is followed. For example, the track may follow the shoreline down river along the 2 meter depth contour, then up river along a mid depth contour (approximately 5 meters), then down river along the channel (>10 meters depth), then finish up along the other shoreline in the shallows (Appendix A).

The DATAFLOW mapping system collects a sample once every 3-4 seconds. The resulting distance between samples is therefore a function of vessel speed. Vessel speeds vary throughout the cruise depending on depth of water, navigational hazards, weather conditions and the slowing of the vessel approaching or leaving verification stations. Average speed underway is approximately 20 knots, which results in an observation collected every 30 meters. As speeds decrease this means samples will occur closer together, but for the most part when underway between the speeds of 10-20 knots samples will occur every 15-30 meters. A total of five stations for verification samples will also be sampled at intervals along the cruise track in JMSOH (Appendix A; Table 3).

3.1.3. Water Quality Instrumentation

The DATAFLOW system has a YSI 6600 sonde equipped with a flow-through chamber. The sensors include a Clark-type 6562 DO probe, a 6561 pH probe, a 6560 conductivity/temperature probe, a 6136 turbidity probe, and a 6025 chlorophyll probe. The sonde transmits data collected from the sensors directly to a Panasonic Toughbook, ruggedized laptop computer using a data acquisition system created with LabView software (National Instruments, Inc.). Custom software written in the Labview environment provides for data acquisition, display, control, and storage. Real-time graphs and indicators provide feedback to the operator in the field, ensuring quality data is being collected. All calibrations and maintenance on the YSI 6600 sondes are completed in accordance with the YSI, Inc. operating manual methods (YSI 6-series Environmental Monitoring Systems Manual; YSI, Inc. Yellow Springs, OH).

Table 2. Quality of YSI Data (6600)

PARAMETER	UNITS	PRECISION	ACCURACY	MDL
DO	% Saturation	0.1%	± 2%	0 %
DO	mg/L	0.01mg/L	±0.2mg/L	0 mg/L
Salinity	ppt	0.01ppt	0.1ppt	0 ppt
Temperature	°C	0.01°C	±0.15°C	-5°C
pH	unit	0.01units	±0.2units	0 units
Turbidity	NTU	0.1NTU	2 NTU	0 NTU
Chlorophyll	µg/L Chl	0.1µg/L Chl	-	0 µg/L Chl

3.1.4. Positioning, Depth Information and Data Acquisition

The DATAFLOW system is equipped with a Garmin GPSMAP 540s with sounder. This unit serves several functions including chart plotting, position information, and depth. The unit is WAAS (Wide Area Augmentation System) enabled providing a position accuracy of better than three meters 95 percent of the time. The NEMA 0183 data sentence containing all pertinent position and depth information is output to the SBC data acquisition system.

The DATAFLOW system utilizes a SBC data acquisition system for data collection and storage. The system is based on a Panasonic Toughbook, ruggedized laptop computer. Custom software written in a Labview environment provides for data acquisition, display, control, and storage. All data is collected simultaneously in one file, removing the chore (and possible errors) of merging separate files into one.

3.1.5. Cruise Scheduling

Two cruises per month will be scheduled in May, June and October during one spring and one neap tidal period for the 2012 season. During the most intensive bloom period of July-September the cruises will be conducted weekly. As stated previously cruise dates are subject to change due to inclement weather conditions or mechanical problems with the research vessel. A schedule for activities for each day of the individual cruises is submitted to the PI and other members of staff. Cruises that are cancelled due to weather or mechanical problems with the research vessel are rescheduled.

3.1.6. Sampling Procedures

3.1.6.1. Real-Time Field Verification

The DATAFLOW system provides field personnel with a real time display of parameters as the data is being collected. The field crew, who are knowledgeable and experienced in estuarine water quality monitoring, accesses this data in real time. The crew has a working knowledge of the normal water quality conditions and how weather patterns, diurnal patterns, seasons, and events such as algal blooms might affect data. When unusual results are observed, action is taken to investigate the cause of the unusual data and either note the cause or take action to correct any equipment issues that may be

contributing factors. There are various factors which can lead to erroneous data including; fouling of the ramjet intake, wiper malfunction on the optical probes, probe failure, etc. When these conditions are observed the affected part of the data record is noted in the field notes. During the data review the Quality Assurance Officer takes these notes into account and flags the data accordingly. At verification stations, dissolved oxygen, pH, salinity and temperature are verified using a YSI 600 XLM. If there is a greater than 0.5mg/l difference in dissolved oxygen, 0.2 SU difference in pH, or greater than 1 degree Celsius difference between instruments this is investigated and corrective action is taken. This usually involves switching the YSI 6600 for the spare that is carried on board the research vessel. Close attention is also given to YSI 6600 chlorophyll and turbidity and if readings are questionable the YSI 6600 is switched out with the spare on board.

3.1.6.2. Verification Sample Collection

A total of five verification stations (Appendix A and Table 3) will be sampled along the cruise track in JMSOH. Stations are selected to maximize the range of values that are seen along a track (e.g. when moving up a tributary with a salinity gradient, samples are taken to get a high, medium, and low salinity value). Within each segment, the fixed monitoring stations from this program as well as the stations from the VA Department of Environmental Quality's tributary monitoring program will be utilized as verification stations. Extra sampling supplies will be available to sample more stations under special conditions such as in areas of large blooms. At each station the boat is stopped and water samples are collected from the effluent tubing of the DATAFLOW System (sampling water depth of approximately 0.25 - 0.50 m) for total suspended solids (TSS), volatile suspended (VSS), chlorophyll, and pheophytin. At these stations secchi depth, a vertical profile of photosynthetically available radiation (PAR), as well as a vertical profile for temperature, dissolved oxygen, salinity and pH are also done (See Appendix D for further details). Samples for TSS, VSS, chlorophyll and pheophytin are collected in darkened bottles, which are rinsed three times with ambient water before filling. Samples for chlorophyll and pheophytin are filtered on board the vessel. (See Appendix D for further details). These are then packed on ice in a darkened cooler, and returned to the laboratory where they are stored at -20°C. Samples for TSS and VSS are packed on ice, in a darkened cooler, and returned to the laboratory where they are filtered immediately upon return and frozen (Appendix D). Samples are then delivered to the VIMS Analytical Service Center for further processing.

3.1.6.3. Vertical Profile of Dissolved Oxygen, Water Temperature, and Salinity

A vertical profile of the water column is accomplished using a YSI 600 XLM which includes a Clark-type YSI 6562 DO probe, a YSI 6560 conductivity/temperature probe, and a 6561 pH probe. This profile includes water temperature, specific conductivity, salinity, dissolved oxygen and pH. At the shallow water verification stations (water depth less than 2 meters), this is accomplished by placing the sensor at a 0.1 meter depth, letting it equilibrate and recording the measurements. The same procedure is then followed at successive 0.25 m intervals thereafter until the final reading is taken approximately 5 cm above the bottom. At deep-water verification stations the YSI is lowered until the bottom is reached. This depth will be rounded to the nearest meter and then the sonde will be pulled to 1 meter above the bottom and allowed to equilibrate. Measurements will be recorded and then the sonde will be raised through the water column stopping at each 1 meter interval to allow equilibration and recording of readings until a depth of 1 meter below the surface has been reached. All calibrations and maintenance on the YSI 600 XLM sonde is completed in accordance with the YSI, Inc. operating manual methods (YSI 6-series Environmental Monitoring Systems Manual; YSI, Inc. Yellow Springs, OH).

3.1.6.4. Field Duplicates

At one verification station, during each individual sampling event, a duplicate sample for all sample types and measurements will be taken. The duplicate information will be used to assess the precision of the sample results and measurements. Field duplicates are taken in quick succession with the initial sample from the outflow of the DATAFLOW system, in separate sample bottles. The precision of field duplicate pairs will be assessed by a combination of relative percent difference (RPD) based on the previous year's average RPD, and absolute difference. If a duplicate's RPD falls outside of two (2) standard deviations of the average it will be considered a warning, outside of three (3) standard deviations is considered failure. Under both circumstances, investigation into the possible source of the issue will ensue.

3.1.6.5. Field Blanks

Artificial sample contamination in the field represents an issue of concern for chlorophyll sampling equipment and bottles. To address these concerns the sample bottles and equipment are prepared and cleaned according to the procedures indicated in Section 4.1.2. Field blanks are collected by processing DI water through the filtration apparatus (including filters) while on location at one verification station per individual field event. Field results will be used to determine if contamination has occurred in the sampling process. Decisions to invalidate associated project data on the basis of artificial sample contamination will be made based on a comparison of the field blank with the lowest analytical standard in the calibration curve and the detection limit for each parameter. If the concentration of a field blank exceeds the lowest analytical standard in the calibration curve field and/or laboratory contamination is suspected, corrective action will be initiated. This will include investigation of possible contamination sources (both in the field and in the laboratory) as well as potential procedural modifications to alleviate the problem.

3.1.6.6. Field Documentation and Records

All field data are recorded on specially prepared field data sheets. The raw data sheets are reviewed for possible missing data values due to sample collection problems prior to data entry. These sheets are initialed and filed by the Quality Assurance Officer/Data Manager.

3.2. Fixed Station Water Quality Monitoring in the Lower James River

3.2.1. Fixed Monitoring Stations Description

One continuous fixed monitoring station is located at along the JMSPH north shore littoral zone (Appendix A; Figure 3). The fixed station is used to continuously monitor water quality parameters including depth, water temperature, salinity, pH, chlorophyll, turbidity, and dissolved oxygen using YSI 6600EDS V2 data sondes. These stations will provide long term measurements that can be supplemented with shorter term monitoring, such as the DATAFLOW cruises, and other process-oriented studies.

The fixed station is designed to be deployed off of an existing pier or other structure within a locked 4 inch PVC housing. This housing is perforated to allow flow of water freely to the instrument while at the same time protecting the instrument from large pieces of floating debris. The stations are placed at a depth of 1.0 to 1.5 meters MLW. Within the PVC housing, an YSI 6600EDS V2 data logging sonde is secured 0.25-0.5 meters above the bottom. The PVC pipe structure is cleaned with a chimney sweep type tool at each switch out to reduce fouling and the collection of sediment on the inside of the pipe.

3.2.2. Locations of Monitoring Station and Sample Frequency

There is one continuous monitoring station located along the north shoreline of segment JMSMH (Appendix A, Table 3).

Table 3. Locations of All Sampling Stations within James River Oligohaline and Mesohaline Segments

Station ID	Type	CBP Segment	Latitude	Longitude
JMSOH1	DATAFLOW Verification	JMSOH	37.21335	-76.91730
JMSOH2	DATAFLOW Verification	JMSOH	37.20294	-76.78219
JMSOH3	DATAFLOW Verification	JMSOH	37.20297	-76.64833
JMSOH4	DATAFLOW Verification	JMSOH	37.22775	-76.79147

An individual YSI 6600EDS V2 sonde will be deployed for a maximum of 14 days on permanently established stations, where they will log data at 15-minute intervals. Deployment length is adjusted for areas and periods of high fouling. At the end of the deployment period, the YSI sonde is returned to the lab for downloading of data, post calibration, cleaning, membrane replacement, and re-calibration. A second YSI 6600EDS V2 is deployed following retrieval of the original sonde in order to maintain a continuous record of data. The two sondes are placed adjacent to one another for 20 minutes to allow for post comparison of the individual units and to allow for no loss of monitoring data.

3.2.3. Water Quality Instrumentation

The fixed station utilizes the YSI 6600EDS V2 equipped with the Clean Sweep Extended Deployment System (EDS) and with sensors including a ROX 6150 Optical DO probe, a YSI 6560 conductivity/temperature probe, a 6561 pH probe, a 6136 turbidity probe, and a 6025 chlorophyll probe. The EDS is comprised of a brush that at set intervals sweeps across the sensors to dislodge any fouling organisms or material that has settled on the sensors. This feature ensures better quality data over longer deployment periods in areas with high fouling rates. The new YSI ROX DO probe utilizes the luminescence-lifetime technique to provide DO measurements which are less likely to be affected by fouling or low DO environments.

Table 4. Quality of YSI Data (YSI 6600EDS V2)

PARAMETER	UNITS	PRECISION	ACCURACY	MDL
ODO	% Saturation	0.1%	±1%	0 %
ODO	mg/L	0.01mg/L	±0.1mg/L	0 mg/L
Salinity	ppt	0.01ppt	0.1ppt	0 ppt
Temperature	°C	0.01°C	±0.15°C	-5°C
pH	unit	0.01units	±0.2units	0 units
Turbidity	NTU	0.1NTU	2 NTU	0 NTU
Chlorophyll	µg/L Chl	0.1µg/L Chl	-	0 µg/L Chl

3.2.4. Schedule of Maintenance and Sonde Exchange

Sondes are deployed for a maximum of 14 days. Upon return to the laboratory the sondes are post calibrated, cleaned, and routine maintenance is performed as needed. The sondes are then stored until they are calibrated just prior to their next deployment. All calibrations and maintenance are completed in

accordance with the YSI, Inc. operating manual methods (YSI 6-series Environmental Monitoring Systems Manual; YSI, Inc. Yellow Springs, OH). See Appendix C for more detailed information.

3.2.5. Sampling Procedures

3.2.5.1. Verification Samples

Field verification of pH, salinity, dissolved oxygen and temperature are taken during the deployment/retrieval procedure with a YSI 6920 sonde. Water samples for total suspended solids (TSS), volatile suspended (VSS), chlorophyll-a, and pheophytin are taken when the YSIs are switched out at the depth of the instrumentation. Chlorophyll and pheophytin water samples are immediately filtered and the filter stored in sterile Whirlpak bags (See Appendix D for further details). These are then packed on ice and returned to the laboratory where they are stored at -20°C. Samples for TSS and VSS are packed on ice and returned to the laboratory where they are filtered immediately upon return and frozen (Appendix D). Samples are then delivered to the VIMS Analytical Service Center for further processing. At these stations secchi depth, a vertical profile of photosynthetically available radiation (PAR), as well as a vertical profile for temperature, dissolved oxygen, salinity and pH are also done (See Appendix D for further details).

The data being gathered by the original YSI 6600EDS V2 is also verified by placing the new YSI 6600EDS V2 into the water beside it for a 20 minute time period at the end of its deployment. The two data sets can then be compared to determine that the YSIs are functioning correctly.

3.2.5.2. Vertical Profile of Dissolved Oxygen, Water Temperature, and Salinity

A vertical profile of the water column is accomplished using a YSI 6920 which includes a ROX 6150 Optical DO probe, a YSI 6560 conductivity/temperature probe, and a 6561 pH probe. This profile includes water temperature, specific conductivity, salinity, and dissolved oxygen and pH. This is accomplished by placing the sensor at a 0.1 meter depth, letting it equilibrate and recording the measurements. The same procedure is then followed at successive 0.25 m intervals thereafter until the final reading is taken approximately 5 cm above the bottom. All calibrations and maintenance on the YSI 6920 sonde is completed in accordance with the YSI, Inc. operating manual methods (YSI 6-series Environmental Monitoring Systems Manual; YSI, Inc. Yellow Springs, OH).

3.2.5.3. Summary Table of Monitoring Parameters

Table 5.

PARAMETER	COLLECTION PROCEDURE	PRESERVATION	PERFORMS ANALYSIS	DETECTIN LIMITS	CBP (CIMS) METHOD (unless otherwise noted)
Temperature	YSI 6600V2/6920	N/A	Field		F01
pH	YSI 6600 V2/6920	N/A	Field		F01
Dissolved Oxygen	YSI 6600 V2/6920	N/A	Field		F01
Specific Conductivity	YSI 6600 V2/6920	N/A	Field		F01
Salinity	YSI 6600 V2/6920	N/A	Field		F01
Fluorescence	YSI6600 V2	N/A	Field		NA
Chlorophyll	YSI6600 V2	N/A	Field		F01
Secchi Depth	Secchi Disk	N/A	Field		F01
PAR Light Attenuation	Li-Cor	N/A	Field		F01
Total Suspended Solids	Whole water	ICE	ASC	1.4 mg/l	L01
Fixed Suspended Solids	Whole water	ICE	ASC	2.5 mg/l	L01
Volatile Suspended Solids	Whole water	ICE	ASC	2.5 mg/l	L01
Chlorophyll a	GF/F Filter 47 mm	ICE	ASC	0.50 ug/L	L03

Phaeophytin a diameter 0.7 um pore size GF/F Filter 47 mm diameter 0.7 um pore size ICE ASC 0.50 ug/L L03

3.2.5.4. Field Documents and Records

All field data are recorded on specially prepared field data sheets. The initials of the person recording the data are recorded on each data sheet. The raw data sheets are reviewed for possible missing data values due to sample collection problems prior to data entry. These sheets are filed with the Laboratory Supervisor.

4. DATA MANAGEMENT PROCEDURES

4.1. QA/QC Field Checks

The objectives of QA/QC standards are to provide accurate measurement of water quality relative to phytoplankton levels and composition. Over the 15 years that the VIMS water quality research group has been making similar measurements, consistent protocols have been developed in the field. Together with laboratory procedures already in place and used by the Analytical Services Center (ASC) and data management procedures, the data obtained can be analyzed and interpreted so that the final report submitted will meet the objectives stated for this study. Information derived from the report will be useful to managers making decisions concerning these areas, as well as comparable to water quality data being generated in other areas and by other partners such as the Hampton roads Sanitary District (HRSD).

4.1.1. Preparation of Field Gear

During the few days prior to initiating a DATAFLOW or sonde deployment/retrieval cruise all the necessary equipment involved in the collection of water samples and of physical water quality data are inventoried according to "checklists." All equipment is checked to insure that it is fully operational and has been properly cleaned. The equipment is packed into containers that provide for easy transport and loaded aboard the research vessel. The "checklist" is then re-examined to verify the presence of all necessary gear. Standards and reagents used in the calibration of instrumentation are made according to a schedule of shelf life or if the supply is exhausted. All chemicals are handled, prepared, and stored in accordance with standard laboratory practices.

4.1.2. Potential Contamination

During the course of a research cruise, different steps are taken to insure that the chances for contamination are minimized. All containers used to collect bulk raw water are rinsed three times with sample (station) water before they are filled and are thoroughly cleaned with an acid wash and rinsed with deionized water and dried at the end of the cruise. All glassware associated with the preparation of standards and reagents is cleaned with deionized water and acid washed when appropriate.

Another area of potential contamination is the DATAFLOW system itself. Though we have never seen fouling or growth in the tubing, we do take measures to prevent any contamination. During each use, large volumes of water are pumped at a very high velocity through the system which prevents growth. After each use the intake, hoses and flow through cells are taken apart and allowed to dry completely and stored in the laboratory until they are reassembled for the next use. Periodically the components are rinsed with freshwater. The system is examined before each use for any fouling or growth.

4.1.3. Calibration Procedures and Frequency

All instruments (YSI 6600, YSI 6600 V2, YSI 600, and YSI 6920) are maintained in accordance with manufacturer’s specifications. Sensors involved in the collection of water quality data (temperature, conductivity, salinity, pH, dissolved oxygen, turbidity, and chlorophyll) are calibrated just prior to each deployment. Standards and reagents involved in the calibration of instrumentation are made according to a schedule of shelf life (i.e. daily, weekly or seasonally) or if the supply is exhausted. All chemicals are handled, prepared and stored in accordance with standard laboratory practices. If any apparent problems arise the instrument is removed from use until the malfunction can be diagnosed and remedied.

The manufacturer calibrates temperature when the instrument is returned for service. The thermistor is also checked yearly against a NIST certified thermometer. For the Continuous Monitoring Program the Conductivity standard has a Specific Conductance of 10 mS/cm and is purchased from Fischer Scientific. For the Dataflow program a 0.2 molar standard solution of potassium chloride is made using DI water and Mallinckrodt Granular Potassium Chloride (FW 47.56) purchased from VWR. The solution is made by weighing out 29.82g of KCl (which has been dried and desiccated) using an analytical balance and adding it to a volumetric flask. The level of DI water is then brought up to 1000mL resulting in a standard of 24.82mS/cm. A 3-point calibration is used to calibrate the pH sensor, this includes a pH of 4.0, 7.0, and 10.0 (VWR Buffer Solutions). Dissolved oxygen calibration incorporates a standard air calibration based on air temperature and barometric pressure or for the Optical DO probe an air saturated water calibration technique. A 2-point calibration is used for calibration of the transmissometer (turbidity sensor): deionized water (0 NTU) and a YSI NTU turbidity standard at a level of 123 NTU. A 2-point calibration is also used for the fluorometer: deionized water (0 ug/l) and an upper standard made with Rhodamine WT and deionized water (See Appendix C for more detailed information).

For transmittance and fluorescence, the manufacturer also recommends that the instrument be verified against in-situ properties measured in the field. This involves collecting verification samples during deployment that are analyzed for total and active chlorophyll-a and total suspended solids concentrations. These field standards are related to sensor readings via multiple regression procedures.

At all verification stations light attenuation measurements are taken using a Li-COR LI192SA sensor to relate to turbidity measurements gathered from YSI 6600 turbidity sensor as well as to the total suspended solid numbers.

Table 6. Quality of Verification Data

PARAMETER	UNITS	PRECISION	MDL	METHOD
PAR	μ mol/sec/m ²	-	1 μ mol/sec/m ²	LI-COR 190SA
Light Attenuation	-	-	0.5% @ 100% light	Li-COR 192SA
Secchi Disk	m	-	0.1m	
TSS	mg/L	6 mg/L	2 mg/L	Gravimetric
Chlorophyll	μg/L	3.29μg/L	0.95 μg/L	Fluorescence

(See appendix D for more information on laboratory SOPs and methodologies)

4.1.3.1. Continuous Surface Water Quality Mapping (DATAFLOW)

4.1.3.1.1. Documents and Records

A cruise checklist is filled out prior to each cruise to ensure all field equipment is in order. A calibration sheet is filled out for both the YSI 600 and 6600 sondes. Data generated from the DATAFLOW System is continuously written to an ASCII text file on the hard disk of the unit. After each cruise the data is copied to backup disk as well as stored on the centrally managed VIMS shared file space that is backed up daily. Calibration station data are first recorded on data sheets, which are copied and stored with the Quality Assurance Officer, then transferred to electronic files, which are stored in an Access database. All field data and observations are recorded on specially prepared field data sheets. The initials of the person recording the data are recorded on each sheet. These sheets are filed with the Quality Assurance Officer. (See Appendix B for copies of all documents for field and lab data).

4.1.3.2. Fixed Station Water Quality Monitoring

4.1.3.2.1. Documents and Records

A calibration sheet will be filled out for each YSI 6600 V2 as it is prepared to be placed out into the field. Verification samples and any information gathered during the retrieval/deployment process, including vertical profiles of dissolved oxygen, salinity and water temperature, are recorded on data sheets. These are initialed by the field personnel and filed by the Laboratory Supervisor.

4.2. Description of Individual Data Sets

4.2.1. Continuous Surface Water Quality Mapping (DATAFLOW) in the James River Oligohaline

Two data sets contain the continuous surface water quality measurements.

-The sampling station field verification data set contains: date, time, latitude, longitude, total depth; concurrent instrument readings for temperature, specific conductivity, salinity, dissolved oxygen, pH, fluorescence, and turbidity; laboratory analyses for active chlorophyll-a, total chlorophyll-a, TSS, VSS, secchi depth, light attenuation and dissolved oxygen, water temperature, pH, and salinity for the vertical profile.

-The continuously measured DATAFLOW screened data set contains: date, time, depth, latitude, longitude for each record of water temperature, specific conductivity, salinity, dissolved oxygen, pH, chlorophyll and turbidity.

4.2.2. Fixed Station Water Quality Monitoring in the James River Mesohaline

Two data sets are generated at the fixed stations.

-The sampling station field verification data set contains: date, time, latitude, longitude, total depth; concurrent instrument readings for temperature, salinity, dissolved oxygen, pH, fluorescence, and turbidity; laboratory analyses for active chlorophyll-a, total chlorophyll-a, TSS, VSS, secchi depth, light attenuation, and dissolved oxygen, water temperature, pH, and salinity for the vertical profile.

-The continuously measured data set contains: date, time, water temperature, salinity, dissolved oxygen, pH, chlorophyll, turbidity and depth values.

4.3. General Information Related to Data Sets

4.3.1. Continuous Surface Water Quality Mapping (DATAFLOW)

4.3.1.1. Raw Data Set Retrieval

The data are continuously transferred electronically from the YSI 6600 to the computer hard drive during each day of sampling. The data are stored as an ASCII text file. This file is retained indefinitely as the original record of the data collection. Upon completion of the cruise, data is retrieved from the Panasonic Toughbook laptop using a USB drive.

4.3.1.2. Initial QA/QC of Data Set

Very little post-processing is required before the data can be used. A macro has been developed that takes the raw downloaded dataflow text file and breaks it into columns, puts headers on the columns, changes the latitude and longitude to decimal degrees and highlights cells that have incorrectly formatted data (e.g. if there is text where there should be numbers). The data manager then looks over the data file, first focusing on the cells the macro has highlighted. The next step is comparing field notes to the data and highlighting areas of “bad” data (e.g. increased turbidity due to being too shallow while coming onto station or time spans of a clogged intake etc). Each parameter is then graphed to look for things such as single high spikes in the optical data that are questionable, strange trends in DO data (e.g. steadily decreasing levels that could indicate degradation in the electrolyte solution), or any other issues with probes that might appear in the data. These are all highlighted so that the associated error codes can be attributed to the data. Another step when dealing with both sets of data is to examine the YSI post calibration data and be sure that any drifts in the probes are within acceptable ranges. These ranges are listed in the table below. Once these checks have been accomplished, the data can be put into the final form for submission and the appropriate error codes are used to tag erroneous observations. (See Appendix E for a more detailed protocol for the processing of DATAFLOW data.)

Table 7. Allowable drift in YSI sensor readings

PARAMETER	VALUE
Chlorophyll	<input type="checkbox"/> +/-5 % of true value
Dissolved Oxygen	<input type="checkbox"/> +/-0.5 mg/L
pH	<input type="checkbox"/> +/-0.2 pH units
Specific Conductance	<input type="checkbox"/> +/-5 % of true value
Turbidity	<input type="checkbox"/> +/-5 % of true value
Water Temperature	<input type="checkbox"/> +/-0.2 °C

4.3.1.3. Further Data Review

As indicated in section 3.1.6.1. of this document, project data is routinely monitored on a real-time basis for potential problems. Additional data reviews are conducted subsequent to the completion of the monitoring events as follows: As soon as the cruise is completed the Quality Assurance Officer/Data Manager downloads the raw data file and reviews the field documentation for legibility, accuracy, and

completeness. Any issues are discussed with the field crew and the field sheets are initialed as being complete. Once reviewed the verification station information contained in the field sheets are transcribed onto an electronic version (Excel or ACCESS) and these files are subsequently examined for accuracy and completeness. The information contained on the electronic versions of the field sheets is then incorporated into the verification data database. Subsequent QA/QC of this dataset, as well as data analysis of the data by the Quality Assurance Officer (QAO) offers yet another opportunity to detect and correct any remaining errors which may have been over looked in the reviews and/or audits.

4.3.1.4 Comparisons between DATAFLOW and Laboratory Derived Results

As previously described, the total chlorophyll values as determined by the YSI 6600 sondes can be corrected by results obtained in the laboratory as well as with turbidity values. The goodness of fit (measured as a regression R² value) between laboratory derived chlorophyll a and corrected YSI will be statistically evaluated. Light attenuation (K_d) will be predicted by determining statistical relationships between YSI turbidity (NTU) and measured LiCor results (K_d) determined at the verification stations. The goodness of fit (measured as a regression R² value) between LiCor measurements and YSI turbidity will be statistically evaluated.

4.3.1.5. Processing of Data

After the data is examined and coded, a final version is stored on the centrally managed VECOS database that is stored in VIMS shared file space and is backed up daily. The data is then imported into ArcMap and maps of the surface water quality conditions are produced using GIS software (ESRI ArcInfo). (See Appendix F for a more detailed GIS protocol to generate interpolations).

4.3.2. Fixed Station Water Quality Monitoring

4.3.2.1. Raw Data Set Retrieval

The data from the YSI 6600EDS V2 are uploaded to a Personal Computer (IBM compatible) upon return to the lab. Files are uploaded to a PC in a comma-delimited format. These are then backed up.

4.3.2.2. Initial QA/QC of Data Set

For the continuous monitoring stations, the data is downloaded into Ecowatch. In this program, graphs are created of each parameter and outliers are examined as well as any data trends which indicate trouble with individual probes. Depth data is compared with the rest of the data so that data can be tagged if the sonde was out of water and optical data is closely examined for periods where wipers may have seated over optics, etc. The same issues with the data/probes that are listed in the dataflow portion are examined in the continuous monitoring dataset. With the continuous monitoring stations, the dataset must also be joined to form a seamless file for the year's deployment. The times associated with any missing data are inserted and the appropriate error codes are placed in the cells to explain the absence of data. As with the Dataflow, YSI post calibration data is checked to be sure that any drifts in the probes are within acceptable ranges. Once these checks have been accomplished, the data can be put into the final form for submission and the appropriate error codes are used to tag erroneous observations. (For acceptable drifts see table above in section 4.3.1.2.) (See Appendix G for summary of QA for the Fixed Station data)

4.3.2.3. Processing of Data

Missing data due to YSI maintenance (down time) are inserted into the spreadsheet and are denoted by time stamps with no records. Edited files are merged to contain one full year of data for each station.

Microsoft Excel macros provided by the NERR Centralized Data Management Office are used to discover data set outliers (values which fall outside the range that the instrument is designed to measure) and other erroneous data. Suspect data are evaluated, edited, and documented in each site's metadata document (data documentation)

4.4. Analytical Methods QA/QC

4.4.1 Analytical Services Center (ASC)

The Analytical Services Center (ASC) at VIMS provides water quality analyses to University, State and Federal agencies. As part of the laboratory's QA/QC program, ASC will participate in cross calibration exercises with other institutions and agencies whenever possible. Some examples include:

- Quarterly cross calibration exercises with the Chesapeake Biological Laboratory (CBL) and Old Dominion University (ODU) in conjunction with the Chesapeake Bay Program's Coordinated Split Sample Program (CSSP).

- Environmental Protection Agency (EPA) unknown audits for various nutrients have been conducted.

- EPA audits of known nutrients were analyzed using samples in different salinity water while looking for possible matrix effects.

- The Chesapeake Bay blind audit sample program which includes dissolved nutrients, particulate carbon, nitrogen and phosphorus, and chlorophyll.

As part of the Chesapeake Bay Tributary Monitoring Program, the laboratory analyzed approximately ten percent of the total sample load for QA/QC checks. These samples included laboratory duplicates and spike analyses.

4.4.2 "Bottle Blanks"

"Bottle Blanks" are necessary for the Whirlpak bags, which are used to collect sample filtrate in the field. Whirlpak bags are tested for contamination by selecting a number of bags (5) from each lot, filling them with deionized water, freezing them, and then analyzing the water for ammonium, nitrate, nitrite, and orthophosphate.

4.4.3. Sample Custody

Upon arrival at the laboratory, samples are counted, observed for potential problems (melting, broken containers, etc.), processed, and placed in a freezer until analysis. Sample information and date of arrival, times of filtering and storage by freezing are recorded on a log sheet.

4.4.4. Instrument Maintenance

Analytical instruments are maintained on a regular basis and records are kept of hours of operation, scheduled maintenance, pump tube changes, etc. A critical spare parts inventory is maintained for each instrument. Instrument down time is minimized by troubleshooting instrument problems telephonically with manufacturers and service representatives. Spare parts can be received within 24 hours via next-day air service. Backup YSI 6600EDS V2 sondes are also maintained as backup instruments.

4.4.5. Data Handling

Data are entered in a pre-determined format in an Access Database on a computer. Any necessary corrections are noted and made. Any data errors are given specific error codes. All data are backed up on tape on VIMS server. Specific data handling procedures are provided above and will be provided in the data management protocol.

4.4.6. Data Analysis

Preliminary data analysis is performed in which data from the DATAFLOW system is interpolated over 25m cells for the given study sites using kriging techniques with the software package Geostatistical Analyst (ArcGIS). This data is served, as interim data, on the program website www.vecos.org.

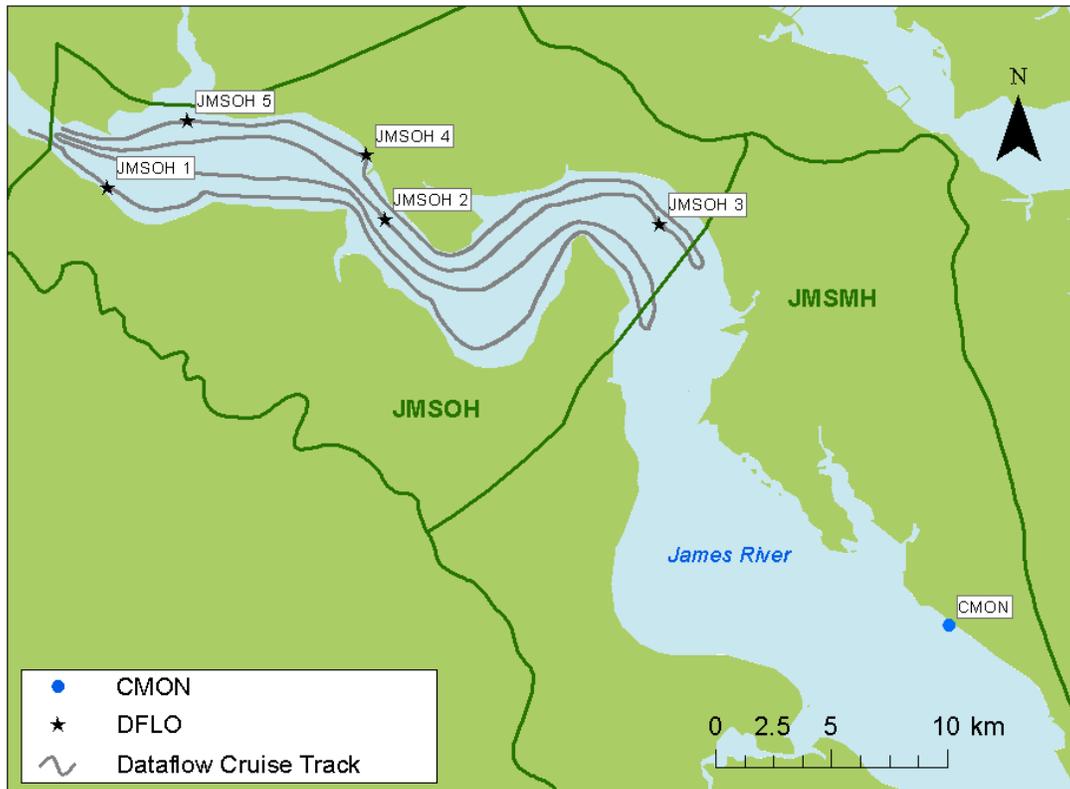
4.4.7. Data Submission

Data will be formatted according to EPA standards and protocols. As QAQC on the mapping and fixed station data is completed it will be uploaded to a database and made available for download by VaDEQ. All data will be provided to VaDEQ as they request at the end of the grant period.

4.5. Schedule of Deliverables

PROGRESS REPORTS: Quarterly status reports will be submitted at every third month commencing with actual award date (May 1, 2012). These reports will contain the following sections: Description of tasks; Accomplishments this quarter; Issues or problems this quarter and impact on future deliverables; Objectives/deliverables for next quarter. VIMS is an institution of higher education located in Virginia performing analyses for the purpose of providing environmental research data to DEQ. Therefore, we are not subject to the requirements of either 1VAC30-45 or 1VAC30-46 (<http://www.dgs.state.va.us/LinkClick.aspx?fileticket=sE5V%2fqtrNfE%3d&tabid=1059>). During the study outlined above, we will meet or exceed the QA/QC requirements specified by DEQ and the EPA CBPO.

Appendix A: Map of Study Site Locations



Locations of DATAFLOW (DFLO) cruise track (grey lines), DATAFLOW verification/calibration sampling stations (stars) and fixed continuous monitoring (CMON) station (filled circle).

Appendix B: Examples of calibration worksheets, field checklist, and field datasheets.

Calibration Work Sheet – Dataflow

Date of Calibration: _____ Technician: _____

DO membrane changed? Y N Note: Wait 6-8 hours before final DO calibration.

Turbidity wiper changed? Y N Wiper Parks ≈ 180° from optics? Y N

Chlorophyll wiper changed? Y N Wiper Parks ≈ 180° from optics? Y N

Record Battery: _____

DO Warmup 60sec Y N Wiper Interval 15 Y N RS232 Sleep Disabled Y N

Record Calibration Values

Record diagnostics after calibration

	Actual	Sonde after cal		
Conductivity	_____	_____	Conductivity constant	_____ Range 5.0 ±.5
pH (mid)	_____	_____	pH MV mid buffer	_____ Range +180 ±50
pH (high)	_____	_____	pH MV High buffer	_____ Range -180 ±50
			Span between the 7 and 10 MV numbers should be	
Turbidity (0)	_____	_____	≈ 170 – 180 MV	
Turbidity (123)	_____	_____	(if post cal # for 0 turbidity is > +/- 2.0 see manual!)	
Chlorophyll (0)	_____	_____		
Chlorophyll (100)	_____	_____	DO Charge	_____ Range 50 ±25
Dissolved Oxygen	_____	_____	DO Gain	_____ Range 1.0 -.3 to +.4
Barometric Pressure	_____	_____	Low charge: low electrolyte or tear	
			High charge: Oxidation of probe	

DO Calibration Hi/Lo Transmission Test _____ Accept _____ Reject

Morning of Deployment

Dissolved Oxygen _____ DO Charge _____ Range 50 ±25
 Barometric Pressure _____ DO Gain _____ Range 1.0 -.3 to +.4

Proper parameters selected in Report Menu?? Y N

Time, Temp (C), SpCond (mS/cm), Sal (ppt), DO (%), DO (mg/l), pH, Turbidity (NTU), Chlorophyll (mg/l), Battery (v)

Post Calibration:

Conductivity (24.82): _____ pH (6.86): _____ Turbidity (0): _____
 Chlorophyll (0): _____ Battery: _____
 DO (precal): _____ DO (postcal): _____ Barometer: _____

If any parameter has drifted can do a more indepth post cal (eg high and low standard for Chl/Turb.)

Notes:

JMSOH Cruise Check List

Cruise Date: _____
Dataflow Operator: _____

Dissolved Oxygen/LaMotte Kit

Sample Bottles
 Lamotte Kit
 DI Water Rinse Bottle

Filtration Kit

Whirlpak Bags - Chlorophyll
 100 ml. Graduated Cylinder
 5ml Pipette
 Pipette Tips
 Vacuum Hand Pump
 Receptacle Flask
 Chlorophyll Chimney
 Chlorophyll Frit
 Chlorophyll Clamp
 Filter Forceps (2)
 Foil for Chlorophyll Filters
 Chlorophyll Filters
 DI Rinse Squirt Bottle
 Kim-wipes
 Sharpie
 Extra Whirlpaks
 Trash Bin

Sample Coolers

Large Sample Cooler
 Small Sample Cooler
 Large Nalgene Sample Bottles
 Small Nalgene Sample Bottles
 Large Nalgene with DI Water
 Ice

Light Attenuation Kit

Datalogger
 Deck Sensor
 Underwater Pole/Sensor
 Secchi Disk

Clipboard

Laminated Station Map
 Sample Protocols
 Field Data Sheets
 DO Profile Data Sheets
 Kestrel Handheld
 Pencils
 Sharpies
 Extra clipboard

 Computer Equipment
 Toughbook Computer (TB2 and TB3)
 TB2 and TB3 Container
 Powercord
 Dual Serial Cord
 GPS to Computer Cable
 Wireless Modem (optional)
 Computer Cig Lighter Adapter

Dataflow Equipment
 YSI to Computer Cable
 YSI 6600
 YSI 6600 Backup
 Intake Hose
 Effluent Hose
 Hose Clamps (3)
 Bungee Cords
 Ram Intake with Pump
 Spare Pump
 Bucket
 Spare PVC Intakes

Other Essentials

Spare Batteries
 Spare DO Membranes
 Spare DO Electrolyte
 Spare fuses
 Toolbox
 Towels

***Insure correct datalogger matched with sensors

***Insure correct computer/power cord combination

***Take both computers if possible

***Double check GPS & Appropriate data card on board before leaving VIMS

Water Monitoring – Dataflow Field Sheet

Date: _____ Cruise Location: James River (Day 1)
Personnel: _____
Sample Location: James River Station (JM5OH1)
Time: _____ DF 6600 ID: _____ Sample Bottle #: 1
Computer File Name (s): _____

DF 6600 Data (on station):

Time (on DF): _____
Dissolved Oxygen (%): _____
Dissolved Oxygen (mg/l): _____
Water Temperature: _____
Salinity: _____
pH: _____
Turbidity _____
Chlorophyll _____

YSI-600 ID # _____ for Verification:

Dissolved Oxygen (%): _____
Dissolved Oxygen (mg/l): _____
Water Temperature: _____
Salinity: _____
pH: _____

Station Conditions:

Station Depth: _____
Air Temp: _____
Wind Speed (m/s): _____
Wind Direction: _____
Cloud Cover (0-6): _____
Wave Condition (0-5): _____
Precipitation Type (10-16): _____
Tide Stage (E, F, H, L): _____

Other Data:

Secchi Depth: _____
Light Attenuation Time: _____
Chl Amt Filter: _____

Make Note of Agreement btw 6600/600:

D.O. should be within 0.5 mg/l
Salinity should be within 0.4 ppt
Temperature should be within 0.25 degrees

Comments:

DF 6600 (averages - lab use only):

Latitude: _____
Longitude: _____
Depth: _____
Water Temp: _____
Salinity: _____
pH: _____
Dissolved Oxygen (%): _____
Dissolved Oxygen (mg/l): _____
Turbidity: _____
Chlorophyll: _____

Data entered/edited by _____

WQ Initiative YSI 6600-V2 ROX CALIBRATION, DEPLOYMENT+RETRIEVAL LOG

DataLogger ID: _____ RIVER SYSTEM: _____

Date (MM/DD/YY): _____ Time correct (EST): YES NO

Version: _____ Sonde Serial Number: _____ Calibrated By: _____

Conductivity Probe ID: _____ pH probe ID: _____ Optical DO Probe ID: _____

Turb. Probe ID: _____ Chlorophyll probe ID: _____ OTHER ID: _____

Battery Volts: _____ Changed batteries?: YES NO

Turbidity Wiper Changed: Y N ; Wiper parks ~180o from optics: Y N

Chlorophyll Wiper Changed: Y N ; Wiper parks ~180o from optics: Y N

Optical DO Wiper Changed: Y N ; Wiper Parks ~180° from optics: Y N

Calibration: (enter standard)

Spec. Cond. : _____ error message: _____

pH 7 : _____ error message: _____

pH 10 : _____ error message: _____

pH 4 : _____ error message: _____

Turbidity : (0)____(123) _____ error message: _____

Depth : _____ (Depth Offset: (mmHg): _____ = _____ (m)) cal'd to: _____

*ROX DO%: _____ / _____ **Temperature stabilized? Yes No

Barometric press: (mm Hg) _____ = _____ (millibars)

Date and time recorded: _____ (____%)

Record the following diagnostic numbers after calibration:

Conductivity Cell Constant _____ (range 4.6 to 5.45)

DO Gain _____ (range 0.8 to 1.7)

pH MV Buffer 7 _____ (range 0 mv + 50 mv) Slope \square mV(7) - mV(10)= _____

pH MV Buffer 10 _____ (range -180 mv + 50 mv) (should be 165-180)

pH MV Buffer 4 _____ (range +180 mv + 50 mv)

Notes: _____

Programming:

Deleted files (circle one): YES NO Disk Free Space: _____(bytes)

Parameters: date, time, temp (C), SpCond (mS/cm), Sal (ppt), DOSAT%, DO (mg/L), Depth (m), pH, Turb (NTU), Batt

Datalogger FILENAME (SSyymmdd): _____

Free memory - _____ days

Battery life - _____ days

Sampling interval: 15 - minute

Sampling duration: "365 days"

Start Date (mm/dd/yy): __/__/__ Checked Logging :
Start Time (HH:MM): __:__:__ (:14 :29 :44 :59) Sample Hold (CO, __) :

Angled wiper on ROX for long pH

Datalogger Deployment: SITE: _____

Date (mm/dd/yy): __/__/__ Time (HH:MM, LST): __:__:__

Weather: _____ TIDE: Flood, Ebb, Slack_High, Slack_Low

Notes: __current wind spd _____ m/s Rel Humidity _____%

Datalogger Retrieval:

Date (mm/dd/yy): __/__/__ Time (HH:MM, LST): __:__:__

Weather: _____ TIDE: Flood, Ebb, Slack_High, Slack_Low

Notes: __current wind spd _____ m/s Rel Humidity _____%

Post Deployment Calibration Check: SONDE ID: _____

Post Calibrated by: _____

Date : _____ Time correct: _____

Stopped Logging : _____ @ _____ Battery volts: _____

Downloaded files: _____ Disabled SAMPLE HOLD:

FILE NAME: _____ .dat _____ .txt

Sp Cond: _____ Cond Cell Constant: _____ (range 4.6 to 5.45)

pH 7 : _____ pH mv _____ (range 0 mv + 50 mv) *Clean Sonde Filtrate:

pH 10: _____ pH mv _____ (range -180 mv + 50 mv) CHL : _____ □ g/L

pH 4 : _____ pH mv _____ (range +180 mv + 50 mv) RFU/FS: _____ %

Turbidity [0]: _____ Turbidity (123) : _____ CHL [0] : _____

(Chl temp @ _____ C = _____ ug/L) CHL [_____] : reading _____ g/L

% FS/ RFU: [0] _____

DEPTH : _____ [Depth Offset: (mmHg) _____ = _____ (m)]

ROX % DO : _____ % DO GAIN: _____

DO NOTES: _____

Barometric Pressure (mmHg) _____ = _____ (millibars)

Time/Date recorded: _____ [mmHg / 7.6 = ~ _____ theoretical sat'n DO]

Wipers park: _____ Turb _____ Chla _____ ODO _____ other

Wipers changed : _____ Turb _____ Chla _____ ODO _____ other

Cleaned unit: _____ Changed Batteries: _____

SONDE LAB NOTES: _____

FIELD NOTES: _____

Verification Water sampling using MINISONDE @ 0.25 m from bottom:

_____ C _____ ppt _____ pH _____ DO % _____ DO mg/L

Please circle Minisonde used today: #1 #2 #3

COMMENTS:

Appendix C: YSI 6-series Multiparameter Water Quality Monitoring Standard Operating Procedure

INTRODUCTION

This document outlines the standard operating procedures (SOP) for the collection of data using YSI 6600 series Multi-Parameter Water Quality Monitors (sondes). The CONMON and vertical profiling stations utilize the ROX optical dissolved oxygen probes (YSI 6600EDS V2) while the DATAFLOW mapping system utilizes the Rapid Pulse dissolved oxygen probes (YSI 6600), therefore different calibration procedures are used. Calibration procedures for all other parameters are identical.

I. PREDEPLOYMENT

Before the instrument can be deployed it must be calibrated and several maintenance steps performed. The instrument should be visually inspected for any abnormalities, such as a cracked pH probe or cracked bulkhead. A **YSI 6-Series Lab Calibration, Deployment, and Retrieval Log** must be completed for each instrument calibration, deployment, and retrieval procedures (Appendix B).

I.A: Probe Calibration Procedures:

During the calibration of the probes **NEVER** accept any calibrations that you have been given a warning message on. You must determine the cause of the problem, correct the problem, and recalibrate the probe before deploying the instrument. Standards must be active (check expiration date) and fresh for all calibrations. Previously used standards may be used to rinse probes but must not be used to calibrate. Discard and replace all expired standards.

Calibrations should only be done with a PC (e.g., desktop, laptop, palmtop). The DM610 will automatically accept the turbidity calibration which can lead to a calibration corruption (see section I.A.6). Therefore the DM610 should only be used to transfer or collect data.

Most system diagnostic parameters (e.g., DO charge, ISE charge, conductivity cell constant) are found in the “Cal-Constant” menu which you get to by selecting “Advanced” on the “Main” menu.

I.A.1.a: Dissolved Oxygen Calibration for YSI 6600 Rapid Pulse type sensor (DATAFLOW)

The dissolved oxygen membranes are to be replaced before each deployment. The oxygen probe needs to rest for a minimum of 6 hours (12 hours is optimal) after the membrane is changed to allow the probe to stabilize electro-chemically. Calibration must be done in the following order:

1. Replace DO membrane;
2. Place probe (or sonde) in calibration cup with a wet sponge and let rest for 6-12 hours;
3. After 6-12 hours, run instrument in discrete sample mode until the DO % reading stabilizes. If the DO probe is working correctly, the past 24 numbers (one screen width) should be the same indicating the probe has stabilized (do not worry about the actual number, you have not calibrated the probe yet);
4. Calibrate the DO sensor following YSI’s instructions for 100% air saturation calibrations.
5. Check the DO charge and gain. The DO gain is in the “Cal-constants” screen; the DO charge must be added to the report menu (remember to remove this from the report menu prior to programming). DO charge should be in the range of 25-75 counts. Counts below this range indicate low electrolyte or a tear in the membrane and counts above may be due to oxidation of the electrodes.
6. Perform a high/low transmission test. Start another discrete sampling. If the %saturation is at or above 100% and then drifts down (or slightly up) to 100%, the probe is functioning properly. If the %saturation is very low or negative and then climbs to 100%, the probe is failing due to reverse polarity of the

electrodes. If this happens, the probe is either in dire need of a reconditioning or will need to be replaced. Record either pass/fail on tracking sheet. Do not deploy the probe if it fails this test. The data will not be valid.

I.A.1.b.: YSI Alternate method

The above protocol (I.A.1.a.) is sufficient for collecting data at the accuracy we are striving for and will also identify a faulty probe. The benefits of this alternate method are to: 1) provide additional time to identify a faulty probe prior to deployment; 2) allow a greater stability to the DO probe; and 3) provide additional data prior to deployment (6 to 12 hours of data) which can aid in the determination of the cause of invalid data.

Calibration procedures must be done in the following order:

1. Replace the DO membrane;
2. Place the sonde in the calibration cup with a wet sponge and wait five minutes. Calibrate the DO sensor. After calibration confirm that the charge, gain, and high/low transition tests pass (see section I.A.1.a). Record diagnostic numbers on the tracking sheet. NOTE: this is only a DO calibration check, the final probe calibration will be completed after the probe rests with the new membrane for 6 to 12 hours;
3. Calibrate pH (I.A.4);
4. Calibrate depth (I.A.2);
5. Calibrate conductivity (I.A.5);
6. Calibrate turbidity (I.A.6);
7. Calibrate chlorophyll (I.A.7);
8. After calibration is completed, begin the unattended sampling for 6 to 12 hours before deployment. This will allow the DO probe to stabilize. Just prior to deployment, calibrate the DO probe one last time (this can be done with the sonde still in unattended mode). Do not run the sonde in discrete mode for 12 hours as an alternative. The pre-deployment data that will now be in the data record will be a valuable tool to identify problems encountered during deployment.

I.A.1.c.: Dissolved Oxygen Calibration for YSI 6600EDS V2 ROX Optical type sensor (COMMON and Vertical Profilers)

1. Aerate a 5 gallon bucket $\frac{3}{4}$ filled with tap water for at least 1 hour prior to calibrating the Optical D.O. probe. This will create an air-saturated water environment (hereafter referred to as a 100% air-saturated water bath).
2. Place the sonde in the bucket (with pump still running) for at least 15-20 minutes in order to achieve temperature stabilization. Ensure that air bubbles are not streaming directly towards the Optical D.O. probe face. Warning: The membrane on the ROX DO probe MUST be kept hydrated in either water

saturated air or immersed in water when not in use. If the membrane is allowed to dry out it will need to be hydrated per the instructions in the YSI manual.

3. Check the sonde (in discrete mode) to ensure temperature stabilization has occurred. Note: The ROX DO does not have a burn-in period and can be used in Discrete or Unattended applications with the same calibration. No DO warm-up or Auto-Sleep settings are required.

4. Begin the Optical D.O. Probe calibration

5. Choose “1-point calibration”.

6. Enter the barometric pressure when prompted and continue calibrating. Record the DO gain just as you would with the rapid pulse probe. NOTE: There is no DO charge option for this probe.

7. Return the sonde to the 100% air-saturated water bath upon completion of all calibrations.

8. Place the sonde in unattended sampling mode at the normal 15 minute interval.

9. Leave the sonde in the air-saturated water bath until deployment.

10. At deployment time, check the D.O. value (DO %) to ensure the probe is reading accurately. The true reading that the probe should be showing is calculated using the following equation:

$$\text{BP}/7.6 = \% \text{ Saturation Value}$$

(BP = current barometric pressure in mmHG)

11. The calculated and measured DO readings must agree within 1% before deployment. If they do not, the probe can be recalibrated while still in Unattended mode. Some drift can occur after the initial calibration if the membrane was not fully hydrated.

12. The probe's membrane requires annual replacement and is performed by unfastening the flat head screws located on the probe face. Please follow all directions that came with the probe provided by YSI.

I.A.2: Depth/Pressure

The depth sensor is calibrated to sea level or level of the laboratory. CAUTION: When calibrating the 6600 with vented depth you must use a vented cable during the calibration procedure. Failure to do so will result in a 3-4 meter shift in the water depth.

I.A.3: Temperature

The temperature probe does not require calibration.

I.A.4: pH

Check the ISE charge (pH mV) before calibration:

pH mV must be added to the report menu then run discrete sampling. Remember to remove pH mV from report before programming.

The ISE charge (pH mV) in pH 4 is 180 +40mV, in pH 7 is 0 +40mV and pH 10 is -180 +40mV. Analog voltages above or below may indicate a problem with the sensor or the sonde. Note the diagnostic milli-volt numbers on tracking sheet. Note the span between the 7 and 10 pH milli-volt numbers and confirm that all meet specifications.

Buffer solutions of pH 7, 10, (or 4 depending on anticipated range of pH measurements) for the two point pH calibration are purchased pre-made from a scientific supply house. Old solutions can be used for rinsing probes, but guard against cross contamination of solutions. The pH calibration solutions are certified at a temperature of 25°C (see bottle label), and calibrations far outside of this range should be avoided.

I.A.5: Salinity and/or Conductivity

The salinity and conductivity standards are obtained from filtered seawater taken from the local area and analyzed using an osmometer (or counter top meter) OR should be purchased from a chemical supply company. After a correct calibration, the conductivity cell constants should be 4.6 - 5.45 as seen in the "Diagnostics" or "Cal-Constants" screen. Also note any change between the last calibration and the cell constant of the most recent. A probe that suddenly goes out of range usually means that either the standard used was contaminated, the probe had a trapped air bubble, or not enough standard was used to cover the vent hole on the side of the sensor.

I.A.6: Turbidity

Do not use plastic beakers or opaque plastics for this calibration. Best results are obtained using **glass beakers** where you can see into the standard and watch for air bubbles near the optics and also check to make sure the wiper parks 180° from the optics. The probe face must be kept a minimum of 1 inch from the container bottom. Calibrations should also only be done over a **flat black surface since glare can interfere with the probe reading.**

Carefully check the set screw on the turbidity wiper block for tightness and inspect wiper sponge for wear and color (the wiper should be white). It is recommended that the wiper be changed before all deployments. For turbidity, the calibration software setting should be 1 wipe (the default) every five minutes, but may need to be increased depending on fouling. The averaging interval for turbidity should be the default of 8. For DATAFLOW a wipe should occur every fifteen minutes. The turbidity calibration solutions are purchased at 100, 200, 800, or 1000 NTU concentrations (other NTU standards can be special ordered). Two-point turbidity calibrations may not be needed at every deployment and all should only be performed in the lab. YSI recommends that the two-point calibration only be done once a month (or when drift is evident). Low end (0.0 NTU) calibrations are the most critical and must be done for every deployment.

CAUTION: It is possible to corrupt the internal turbidity calibration constant of the instrument. Once this is done, **slightly negative or positive values will be recorded for 0.0 NTU (greater than +/- 2.0 NTU).** This corruption occurs when either the probe is not clean, an air bubble intercepts the light beam, or the 0.0 NTU standard is contaminated when you accept the calibration for 0.0 NTU. Care is therefore essential to insure the standard is clean and nothing is interfering with the probe (hence the need to use clear glass when calibrating turbidity). Once the calibration constant has been corrupted, you will need to contact YSI to reset (the YSI technician can walk you through the procedure). Until this is done, all future calibrations will be inaccurate and all turbidity data collected will be erroneous. In the PC environment you are given a choice to accept the calibration, however, using the DM610 you are not

given this acceptance step so it is easier to cause this internal corruption problem when calibrating turbidity with the DM610.

CAUTION: Never accept a calibration when the message “high probe off-set” appears. This indicates that either the sensor is malfunctioning or the standard is contaminated.

I.A.7: Chlorophyll

Carefully check the set screw on the chlorophyll wiper block for tightness and inspect wiper sponge for wear and color (the wiper should be white). It is recommended that the wiper be changed before all deployments. For chlorophyll, the calibration software setting should be 1 wipe (the default) every five minutes, but may need to be increased depending on fouling. For DATAFLOW a wipe should occur every fifteen minutes. The averaging interval for chlorophyll should be the default of 8. Chlorophyll calibration solutions are prepared using either an acridine orange standard solution or a rhodamine B standard solution (refer to YSI 6-Series Environmental Monitoring Systems Operations Manual). Two-point chlorophyll calibrations may not be needed at every deployment and all should only be performed in the lab. YSI recommends that the two-point calibration only be done once a month (or when drift is evident). Low end (0.0□g) calibrations are the most critical and must be done for every deployment.

II. DEPLOYMENT

II.A.: Pre-Deployment Settings

When programming for unattended sampling, specify the site description to include information such as location, month, day, and year. The sample interval is to be set for readings to be taken every 30 minutes or less. Time convention used must be Local Standard Time and sampling must start on the half hour. Parameters measured must include: temperature, specific conductance, salinity, dissolved oxygen saturation, dissolved oxygen concentration, depth, pH, chlorophyll, and turbidity. It is also recommended you collect battery voltage during the deployment since these data can help identify an electrical short. After entering the program, check the battery voltage, battery life, and memory in the "set-up information" screen. If the battery life or memory is not sufficient for the length of the deployment, take appropriate action such as changing batteries or deleting files (format flash disk). The YSI 6-Series operates best with single use alkaline batteries.

II.B.: Deployment Methods

All sondes will be deployed so that the probes stay submerged at low tides and are at 0.5 meters off the bottom to allow for tidal and flow amplitude measurements. The stations will be located at 1.5 meters depth MLW.

III. RETRIEVAL

Retrieve the sonde from the water and visually examine the probes for fouling and damage. Gently clean the sonde of debris and place it in a secure container that will prevent any severe vibrations to the unit during transportation.

IV. POST DEPLOYMENT

IV.A.: Post Deployment Calibration Check

In the laboratory a calibration check must be performed on oxygen, conductivity, and pH probes before cleaning and on the turbidity probe after cleaning (to prevent contamination of the 0.0 NTU standard). Rinse each probe in the calibration standard (previously used is acceptable) and then compare the readings in fresh calibration standard with the instrument in discrete sample mode. Also, if previously used standards (that have NOT been contaminated with even DI water) are used to check calibrations, ensure these standards are never used for the primary calibrations (section I.A.). To check if the DO saturation calibration strayed, place the sonde in a calibration cup with a wet sponge, allow adequate time for the air to become saturated and temperature to stabilize (15 minutes to 2 hours depending on sonde temperature), and record the percent saturation. Post calibration checks should be done for DO %, depth, specific conductance/salinity, turbidity, chlorophyll, and pH.

IV.B.: Probe Cleaning

Prior to or after the sonde is cleaned the data may be uploaded to a Personal Computer (PC) or YSI 610DM and then uploaded into a PC.

Once the post deployment calibration check is completed the sonde body and probes should be completely cleaned. Remove the sonde guard and clean all of the sensors according to the instructions in the YSI 6-Series Operations Manual. You need to pay extra attention to the instructions below.

IV.B.1: pH Probe

This probe should be removed from the guard to insure adequate cleaning. If fouling is not a problem, you may not need to do this for every deployment. These probes are also easily cracked so care must be taken during the cleaning process. Although cleaning with DI water is acceptable, YSI recommends cleaning this probe with tap water to ensure a full probe life.

IV.B.2.: DO/Cond/Temp

If significant solid material has built up near the threads of the probe, the probe should be removed from the bulkhead and cleaned. Remove the old DO membrane and inspect the probe surface. If silver electrodes show significant darkening, follow the instructions to resurface the probe face with a fine sandpaper disk found in the 6035 DO reconditioning kit. Alternatively, you can use 2400 grit sandpaper. When you change the dissolved oxygen membrane, be careful not to touch the surface of the membrane that covers the probe. Clean the conductivity sensor with the brush provided in the maintenance kit. Wet the cavities and brush, then brush both cavities several times and rinse thoroughly with water (you can also brush the cavities under running water).

IV.B.3.: Turbidity and Chlorophyll

Inspect the turbidity and chlorophyll wipers and replace if worn. Under normal use, the wiper will last up to 30 days of deployment time but excess fouling will shorten the life of the wiper. A dirty or disintegrating wiper is likely to cause incorrect parking of the wiper assembly during deployment. Also, a disintegrated wiper sponge may come dislodged during deployment.

V. YSI 6-Series DEPLOYMENT and RETRIEVAL LOG

A deployment and retrieval log must be filled out with every instrument deployment/retrieval (Appendix B). This sheet will aid in data QA/QC procedures and help identify faulty equipment. After completion,

this sheet, along with the Ecowatch graph should be reviewed to identify anomalous data and faulty sondes/probes.

VI. VIEWING and PRINTING the ECOWatch GRAPHS

The final step in data collection is to view and print the PC6000/ECOWatch graph of all data collected during the deployment and look for patterns and outliers that can identify faulty probes and instruments. This graph must be kept with the YSI 6-Series Lab Calibration, Deployment, and Retrieval Log Sheet (tracking sheet). Both the tracking sheets and these graphs will be your best tools during the QA/QC processing of the data.

CAUTION: Always note the scales which are automatic by default in these programs and can mask a problem with the data set if you assume a linear scale from 0 (zero) to the probes acceptable maximum measurement.

VII. PROBE CARE AND STORAGE

Most of the probes, especially pH and DO, have a limited life span. The pH probes have a maximum life of 2 years (rarely longer), so any pH probes over 1 year old are near needing replacement. If probes will not accept calibrations or are slow to respond to standards, suspect aging. DO and pH probes also have a limited shelf life, so do not purchase replacements too far in advance. Dissolved oxygen probes have a life expectancy of 2-3 years.

VII.A.: Storage

The procedure for storage is different for short-term (1 month or less) or long-term (greater than 1 month).

VII.A.1.: Short-term Storage

For short term storage, it is important to keep the probes moist but not immersed in water. Keep probes attached to the sonde and place the sonde in approx. 0.5in of water (not distilled) in the calibration cup. A moist sponge can be used in place of the 0.5in of water.

VII.A.2.: Long-term Storage

Dissolved oxygen/conductivity/temperature and dissolved oxygen probes should be stored with membrane and electrolyte in place and place sonde in a water filled transport or calibration cup (it is important to ensure that water completely covers the DO sensor). If the probe is removed from the sonde, it should be stored with membrane and electrolyte in place and submerged in water (the conductivity cells should also be submerged). The pH probe should be removed from the sonde and stored with the pH storage cap containing 2-4 molar KCL or buffer (check manufacturers specifications). No special precautions are necessary for the chlorophyll and turbidity probes or depth/level probes.

Appendix D: Laboratory and Sampling Protocols

Sample Collection, Preservation, and Processing for the Continuous Surface Water Quality Mapping

1.0 Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS):

1.1 Water samples are collected from the effluent tubing of the DATAFLOW System in pre-rinsed (w/ambient water) darkened 1L Nalgene bottles. Exact time of collection is noted to facilitate comparison with data generated by the YSI 6600. Samples are placed in darkened bottles and packed in ice and upon return to the laboratory are processed immediately (Sections 4.0, 9.0).

2.0 Chlorophyll and Pheophytin:

2.1 Water samples are collected from the effluent tubing of the DATAFLOW System in pre-rinsed (w/ambient water) darkened 1L Nalgene bottles. Exact time of collection is noted to facilitate comparison with data generated by the YSI 6600. Immediately upon collection, known volumes of samples are filtered through 25 mm GFF filters. Filters are folded in half and placed in plastic petri dishes covered with aluminum foil. Samples are then placed in ice and upon return to the laboratory are stored at -20°C until analyses can be conducted (Section 10.0).

Verification Sample Procedures for the James River Monitoring Program

1.0 Total Suspended Solids and Volatile Suspended Solids

1.1 Summary of Method:

1.1.1 A well-mixed sample of known volume is filtered through a glass-fiber filter, and the residue retained on the filter is dried to constant weight at 103 - 105oC.

1.1.2 The residue obtained from the determination of total suspended solids is ignited at 550oC for 2 hours in a muffle furnace. The remaining solids after ignition are reported as mg volatile suspended solids/L.

1.2 Interferences

1.2.1 The principal source of error in the determination is failure to obtain a representative sample.

1.2.2 The test is subject to many errors due to loss of water from crystallization, loss of volatile organic matter prior to combustion, incomplete oxidation of certain complex organics, and decomposition of mineral salts during combustion.

1.3 Apparatus and Materials

1.3.1 47 mm glass fiber filters (GFF, 0.7 µm pore size).

1.3.2 Filtering Apparatus with 47mm filter holders.

1.3.3 Drying Oven: Capable of maintaining a temperature of 103 +2oC.

1.3.4 Analytical balance: Capable of weighing to 0.1 mg.

1.3.5 Muffle oven: Capable of heating to 550oC.

1.4 Sample Handling

1.4.1 Preservation of the unfiltered sample is not practical; analysis should begin as soon as possible. Refrigeration or icing minimizes the microbiological decomposition. After filtering, the filter can be stored frozen at -20 oC for 28 days.

1.5 Procedure:

1.5.1 Preparation of filters for Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS):

1.5.1.1 Pre-number aluminum weigh boats (i.e. 1, 2, etc.).

1.5.1.2 Place filters wrinkled side up on clean filtering apparatus.

- 1.5.1.3 Run three successive 30 mL washes of ASTM Type I water through each filter while applying suction. Do not exceed a vacuum of 10 psi.
- 1.5.1.4 Allow suction pump to run until the filters are "dry".
- 1.5.1.5 Carefully transfer filters to pre-numbered weigh boats using forceps.
- 1.5.1.6 Ignite blank filters in the muffle furnace at 550oC for approximately 15 to 20 minutes.
- 1.5.1.7 Place filters in a desiccator until cool.
- 1.5.1.8 After cooling (approximately 15 minutes), weigh filters on an analytical balance capable of measuring to 0.1 mg. Record weights and return filters to weigh boats.
- 1.5.1.9 Return filters to the oven for at least 1 hour. Reweigh filters a second time. Filter weights must not be more than + 0.5 mg. If greater, return filters to oven for at least 1 more hour.
- 1.5.1.10 After dry weights have been determined, filters are stored in their aluminum weigh boats in an airtight container until needed.

1.5.2 Determination of Suspended Solids:

- 1.5.2.1 Place pre-weighed filter on a clean filter holder with wrinkled side up. Turn on vacuum pump. Do not exceed a vacuum of 10 psi.
- 1.5.2.2 Vigorously shake sample.
- 1.5.2.3 Rinse 250 mL graduated cylinder with a small quantity of sample.
- 1.5.2.4 Measure 250 mL of sample into cylinder and pour into the filtering funnel. Repeat for a total volume of 500 mL. Smaller volumes may be used if sample has a great deal of suspended matter or larger volumes may be used if sample has low levels of suspended matter.
- 1.5.2.5 Record volume filtered (mL).
- 1.5.2.6 Rinse filter with DDI water to remove salts.
- 1.5.2.7 Allow the vacuum pump to run until filter is "dry".
- 1.5.2.8 Run one duplicate (500 mL sample, 500 mL duplicate) for every set of ten samples. If less than ten samples are run, then one duplicate should be made.
- 1.5.2.9 Remove filter with forceps.
- 1.5.2.10 Place filter in numbered weigh boat. Dry to a constant weight in a 103-105oC oven at least 4 hours (preferably overnight).

1.5.2.11 Place filters in desiccator until cool.

1.5.2.12 Repeat weighing procedure

1.5.2.13 Return filters to the oven for at least one hour.

1.5.2.14 Repeat weighing procedure. If filters do not weigh within +0.5mg of their first weight, the process of drying and weighing should be repeated as many times as needed to achieve this constant weight.

1.5.2.15 For VSS muffle filters for 15 minutes at 550oC. Repeat weighing procedure.

1.6 Calculation

$$\text{mg volatile suspended solids/L} = \frac{(A-B) \times 1000}{\text{sample volume, mL}}$$

$$\text{mg total suspended solids/L} = \frac{(A-C) \times 1000}{\text{sample volume, mL}}$$

where:

A = weight of residue + filter before ignition, mg

B = weight of residue + filter after ignition, mg

C = weight of filter, mg

1.7 Reference(s):

U. S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes, Method 160.2.

Standard Methods, 18th edition, pp. 2-54 to 2-57.

2. Chlorophyll and Pheophytin

2.1 Scope and Application:

- 2.1.1 Chlorophyll data may be used to determine long-term trends in water quality and the trophic status of surface waters, to detect adverse effects of pollutants on plankton, and to provide estimates of studies attempting to estimate algal biomass and productivity.

2.2 Summary of Methods:

- 2.2.1 The method for determining Chlorophyll a given here is with a Turner Design fluorometer. The method used requires filtering a known quantity of water through a glass fiber filter. This filter is later ground with a tissue grinder made of teflon/glass. Approximately 1-3mLs of 90% acetone are added to the filter before grinding. Acetone is also used to wash the filter into 17 x 150 test tube with tight fitting cap. The sample is steeped at least 2 hours and not exceeding 24 hours at 4°C, in the dark. The samples are centrifuged and read on a fluorometer. If the samples cannot be read within that time period, storage in the freezer at -20°C for a few days is acceptable. If pheophytin measurements are desired, the sample is acidified and read again.

2.3 Reagents:

NOTE: Use fresh, distilled, DDI water. Label, date, and initial all reagents.

- 2.3.1 Aqueous Acetone solution (90%) - Mix 90 parts acetone (Optima grade) with 10 parts DDI water. Add 4 drops of 1N NaOH/L.

Note: Mix the reagents in the appropriately marked bottle in the following order: 1800 mL Acetone, 200 mL DDI water, 8 drops 1N NaOH. This is to be stored in the yellow FLAMMABLE cabinet.

2.4 Equipment:

- 2.4.1 The Turner Designs TD-700 Laboratory Fluorometer is now used in this laboratory to make chlorophyll A and pheophytin determinations. The Fluorometer is equipped as follows:

- 2.4.1.1 Daylight White Lamp P/N 10-045

- 2.4.1.2 Filters (For Chlorophyll A and Pheophytin measurements)
Excitation: 340-500 nm P/N 10-050R
Emission: >665 nm P/N 10-015R

- Filters (For Chlorophyll A only)

P/N 10-113
Emission: 680 nm P/N 10-115

- 2.4.2 Centrifuge - capable of holding the 17 x 150mm tubes.
- 2.4.3 Tissue grinder and equipment - Teflon/glass type pestles, electric motor, stand and glass grinding vessels.
- 2.4.4 Centrifuge culture tubes - 17x 150 mm culture tubes with tight fitting caps.
- 2.4.5 Filtration equipment - Vacuum pump (vacuum should not exceed 1/2 atm or 15psi, filter holder for 47mm GF/F filters, 47mm GF/F filters, 2X4 ziplock bags, foil or a dark storage container.

2.5 Standards

- 2.5.1 Fluorometer BLANK: (Used as a "Filter Blank"). An unfiltered blank filter will be analyzed as if it were a sample, extracted as described in Volume V, Section 5.
- 2.5.2 Calibration Standard: Standards are obtained from Turner Instruments. Calibration standards consist of a minimum of one high standard and one low standard maintained in 90% acetone. An intermediate standard may be made by diluting the high standard appropriately with 90% acetone. This solution should be determined to be a high purity blank. Calibration Standards should be frozen at -20 o C prior to use.

2.6 Calibration of Fluorometer

- 2.6.1 Calibration Standards and a Calibration Blank (90% optima grade acetone) are required for this step
- 2.6.2 Proceed to Calibration set-up menu on the TD-700 fluorometer. A minimum of two standards should be used for the calibration. Three standards and a blank are optimal.
- 2.6.3 Use A multi-optional@ mode with / Direct Concentration/ug/L units
- 2.6.4 Calibration
 - 2.6.4.1 Enter Highest Standard first. Key in the high concentration value. Insert test tube with high standard in fluorometer. Press <*> when stable. The sensitivity setting will be automatically set. The fluorometer will read the high standard then ask for subsequent standards.
 - 2.6.4.2 When all standards have been read, insert the blank.
 - 2.6.4.3 Press <0> when the value is stable.
 - 2.6.4.4 Calibration will be completed
- 2.6.5 Acid Ratio Determination:

2.6.5.1 The acid ratio (the ratio of the fluorescence of any extract containing only chlorophyll, before and after the addition of acid) should be determined for each fluorometer run.

2.6.5.2 Calibrate the fluorometer as described in Section 5.5.

2.6.5.3 Reread intermediate calibration standard (R_b)

2.6.5.4 Acidify the standard with 2 drops of 0.1N HCL. Mix. Wait 90 seconds.

2.6.5.5 Read sample as R_a.

2.6.5.6 Acid ratio

$$R = R_b/R_a$$

2.7 Procedure:

2.7.1 Grinding procedure

NOTE: Before grinding, set up samples by listing the sample ID and volume on the bench sheets. Label small pieces of white tape with the ID volume, apply these to the culture tubes.

2.7.1.1 Close half-curtain around grinding area. This blocks out fluorescent light which destroys chlorophyll. Turn on the incandescent light and the hood fan.

2.7.1.2 Place filter in grinding vessel and add 1-3mL of the 90% acetone.

2.7.1.3 Insert pestle in grinding tube, and turn on grinder by using switch on post of apparatus. NEVER turn on the grinding motor without having the pestle in the vessel.

2.7.1.4 Thoroughly grind filter for approximately 2 minutes. Be sure there are no discernable pieces left. Pull pestle to the top of the vessel and rinse lightly with the 90% acetone.

2.7.1.5 Rinse pestle with 20 ml of 90% acetone into the culture tube.

2.7.1.6 Cap tube and shake lightly.

2.7.1.7 Store tubes in racks in a closed box and place in refrigerator for 2-24 hours. The sample tubes may be stored for a couple of days at -20°C if necessary.

2.7.2 Centrifuging samples

2.7.2.1 Before removing samples from closed box, turn off lights. Fluorescent light destroys chlorophyll!

2.7.2.2 Shake samples to ensure thorough mixing.

2.7.2.3 Place samples in centrifuge in an order that can be remembered (tubes must be kept in order).

2.7.2.4 Close cover until it clicks. Adjust setting to approximate 675g.

2.7.2.5 Turn TIME/MIN knob to 15 minutes. This starts the centrifuge spinning.

2.7.2.6 After centrifuge has stopped spinning, open top (pull up firmly on lever on top of cover), remove tubes and replace in rack. Check the order against sheets.

2.7.3 Reading on Fluorometer

2.7.3.1 Pipette samples into fluorometric cuvettes.

2.7.3.2 Read sample in fluorometer. Results are read in direct concentration.

2.7.3.3 Read sample; record as RB.

2.7.3.4 Add 2 drops of 0.1 N HCL and shake well.

2.7.3.5 Read sample record as Ra.

2.8 Calculations:

2.8.1 For uncorrected Chlorophyll A using Method 445.0 with acidification: (Instrument must be equipped with Excitation: 340-500 nm P/N 10-050R
Emission: >665 nm P/N 10-015R)

$$2.8.1.1 C_{E,u} = R_b \times F_s$$

Where: $C_{E,u}$ = uncorrected chlorophyll A concentration (ug/L) in the extract solution analyzed

R_b = fluorescence response of sample extract before acidification, and
 F_s = fluorescence response factor for sensitivity setting S (which =1 for the TD-700 fluorometer)

2.8.1.2 Calculate the “uncorrected” concentration of chlorophyll A in the whole water sample as follows:

$$C_{S,u} = \frac{C_{E,u} \times \text{extract volume (L)} \times DF}{\text{Sample volume (L)}}$$

Where: $C_{S,u}$ = uncorrected chlorophyll A concentration (ug/L) in the whole water sample

Extract volume = volume (L) of 90% acetone used for extraction of the filter

DF = Dilution factor

Sample volume = volume (L) of whole water filtered through filter

2.8.2 For corrected Chlorophyll A using Method 445.0 with acidification:

$$2.8.2.1 \quad CE,C = F_s (r/r-1) (R_b - R_a)$$

Where: CE,C = corrected chlorophyll A concentration (ug/L) in the extract solution analyzed

F_s = response factor for sensitivity setting S,

r = the before to after acidification ratio of the pure chlorophyll standard

R_b = fluorescence of sample extract before acidification, and

R_a = fluorescence of sample extract after acidification

2.8.2.2 Calculate the @corrected@ concentration of chlorophyll A in the whole water sample as follows:

$$CS,C = \frac{CE,U \times \text{extract volume (L)} \times DF}{\text{Sample volume (L)}}$$

Where: CS,C = corrected chlorophyll A concentration (ug/L) in the whole water sample

Extract volume = volume (L) of 90% acetone used for extraction of the filter

DF = Dilution factor

Sample volume = volume (L) of whole water filtered through filter

2.8.2.3 For corrected pheophytin using Method 445.0 with acidification:

$$PE = F_s (r/r-1) (rR_b - R_a)$$

$$PS = \frac{PE \times \text{extract volume (L)} \times DF}{\text{Sample volume (L)}}$$

Where: PE = pheophytin concentration (ug/L) in the sample extract, and

PS = pheophytin concentration (ug/L) in the whole water sample

2.8.3 For Corrected Chlorophyll A using Method 445.0 without acidification: (Instrument must be equipped with Excitation: 436 nm P/N 10-113 Emission: 680 nm P/N 10-115)

2.8.3.1 Calculate the correct concentration of chlorophyll A in the whole water sample as follows:

$$C = \frac{CC \times \text{extract volume (L)} \times DF}{\text{Sample volume (L)}}$$

Where: C = corrected chlorophyll A concentration (ug/L) in the whole water sample

CC = corrected chlorophyll A concentration (ug/L) in the extract solution analyzed

Extract volume = volume (L) of 90% acetone used for extraction of the filter

DF = Dilution factor

Sample volume = volume (L) of whole water filtered through filter

2.9 Reference(s):

Strickland, J.D.H., and Parson, T.R. 1972. A Practical Handbook of Seawater Analysis. Fish. Res. Bd. Canada 167:310.

TD-700 Laboratory Fluorometer Operating Manual. Version 1.8. July 7, 1999. Turner Designs, 845 West Maude Avenue, Sunnyvale, CA 94086.

EPA /600/ R-97/072 - Method 445.0. In Vitro Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence. Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices Revision 1.2. September 1997.

Using the Turner Designs Model 10 Analog, The 10AU Digital, Or the TD-700 Fluorometer with EPA Method 445.0. January 19, 1999. Turner Designs, 845 West Maude Avenue, Sunnyvale, CA 94086

4.0 Secchi Depth

4.1 Scope and Application

4.1.1 The turbidity of natural water is a critical factor in sustaining submerged aquatic vegetation as well as epiphyte and phytoplankton growth.

4.2 Summary of Method

4.2.1 A black and white Secchi disk that is attached to a weighted, ruled line is lowered slowly into the water until it disappears and then raised. The depth at which the disk disappears is averaged with the depth at which it reappears; this measurement (in meters) is the secchi depth.

4.3 Procedure

4.3.1 A round, weighted Secchi disk measuring 20cm in diameter is used. The upper surface is divided into four equal quadrants that are colored so that the two quadrants opposite each other are black and the intervening ones are white. It is attached to a ruler line that is marked in 0.1m intervals.

4.3.2 Readings with the Secchi disk are made in situ without the aid of sunglasses on the shady side of the vessel to reduce possible sun glint on the water surface.

4.3.3 The disk is slowly lowered into the water until it disappears and then raised. The depth at which the disk disappears is averaged with the depth at which it reappears; this measurement (rounded to the nearest 0.1m increment) is the secchi depth.

4.3.4 The Secchi disk is not permitted to touch the sediment surface. If the secchi depth exceeds the water depth in shallow water no measurement is recorded and the profile is repositioned in slightly deeper water.

4.3.5 The time of the secchi measurement is recorded. For continuous surface water quality verification stations and fixed stations this facilitates matching of secchi depth readings with transmissometer (NTU) data.

4.4 Quality Control

4.4.1 Reported Units

4.4.1.1 (0.1m)

4.5.1 Detection Limits

4.5.1.1 Upper Limit - N/A

4.5.1.2 Lower Limit – 0.1m

4.5 References

Tyler, John. 1968. The secchi disk. *Limnol. Oceanogr.* 13 (1): 1-6.

5.0 Attenuation of Underwater Photosynthetically Available Radiation (PAR 400- 700nm)

5.1 Scope and Application

5.1.1 Light attenuation of PAR through the water column is an important apparent optical property of natural water that is a critical factor in sustaining SAV and well as regulating epiphyte and phytoplankton growth. The degree of this attenuation is related largely to the concentration of suspended and dissolved substances in the water. PAR is that component of the solar radiation spectrum that is typically defined as visible light and within this range of wavelengths are those predominantly used by plants for photosynthesis.

5.2 Summary of Method

5.2.1 Downwelling light penetrating the water column (PAR) is measured underwater at several depths to calculate the light attenuation coefficient, K_d . This procedure may also be used to estimate the depth of the photic zone. Concurrent incident light readings are taken on deck with each depth measurement. Simultaneous deck and submersed measurement are necessary because of variability in incident surface irradiance due to changing atmospheric conditions (i.e. cloud cover).

5.2.2 K_d is calculated as the negative exponential decay function of underwater light with depth. Light is typically measured as the quantum flux of downwelling irradiance using cosine-corrected downwelling quantum sensors.

5.2.3 Equipment: manufactured by LI-COR, Inc.

5.2.3.1 LI-192SA, Underwater Quantum Sensor

5.2.3.2 LI-190SA, Quantum Sensor (deck)

5.2.3.3 Li-1000 or LI-1400 Datalogger

5.3 Procedure

5.3.1 PAR is determined from the sunny side of a vessel or pier during daylight hours. Both deck and submersed sensors must be away from the shadow of the vessel or objects on the vessel.

5.3.2 Check the sensor operation by connecting the deck sensor and underwater sensor to corresponding ports of the Li-Cor data logger, and turn the power on. Check battery level. Cover each sensor and check to see if the output is very near zero. Place the sensor under a light source to assure that it has a positive reading. A negative reading indicates that the polarity of the sensor is reversed (it is plugged into the cable backwards). A very positive or negative reading under dark indicates a possible short in the cable or some other electrical short.

5.3.3 In the field, mount the deck sensor in a location that is level and unobstructed by shadows. Ensure that sensors are positioned properly on the deck sensor mounting and the lowering frame. Connect deck sensor and underwater sensor to corresponding ports of the meter, and turn the power on.

5.3.4 Set instrument averaging constant to display results from previous 10 or 15 seconds.

5.3.5 Lower the frame until the sensor is just below the surface of the water. At a depth of 0.1 meter (~ 4 inches), simultaneously record the deck sensor and the underwater readings. If wave action prevents this,

take the initial reading at 0.5 meters. Depth of the underwater sensor diffuser below the surface of the water is determined with a calibrated pole or non-stretching rope.

5.3.6 Lower the frame and take subsequent measurements at depths appropriate to the monitoring location and purpose of the project (see below). Allow the instrument output to stabilize, at least for the averaging period, and then record both deck and underwater readings at each depth.

5.3.7 The simultaneous deck and submersed readings are stored by pressing “Enter”. Each record will be stored with a time stamp which will flash on the data logger readout. Record this time. All readings can be subsequently retrieved at the end of the cruise.

5.3.8 Mainstem and Tidal Tributary Photic Zone. Record initial PAR at a depth of 0.1 meter below the surface. At mainstem stations, record subsequent measurements at 1 meter intervals, and in the tributary stations, at 0.5 meter intervals when total depth is greater than 2 meters. If depth to bottom is less than 2 meters, take readings at 0.25 meter increments.

5.3.9 Measurements are recorded until the meter reads <1% of the initial subsurface value, or if the bottom is reached. If water is clear and deep, the increments may be increased to 2 meters. If water is turbid or colored, (low secchi depth), 0.25 meter increments may be appropriate.

5.3.10 Data Flow Mapping and High Frequency Meter Regressions - Record initial PAR at a depth of 0.1 meter below the surface. When depth to bottom is less than 2 meters, record 3 to 4 additional PAR measurements at 0.25, 0.5, 0.75 and 1.0 meters. When depth to bottom exceeds 2 meters, record PAR at 5 depths (e.g., 0.1, 0.5, 1.0, 1.5 and 2.0 meters).

5.3.11 Replicate PAR profile at each location to make sure readings are correct.

5.4 Quality Control

5.4.1 The deck cell should have the highest PAR value, and the underwater sensor output should decrease as the sensor is lowered.

5.4.2 Periodically check that sensors are linear and not out of calibration with respect to each other.

5.4.3 Calibration of the sensors by LICOR, Inc. is recommended every year, and required every two years.(AMQAW, 2002)

5.5 Kd Calculations

5.5.1 Light measurements are taken simultaneously of both surface (I_0) and submarine PAR intensity (I_{z+n}). Submarine PAR is measured at the surface (0.1 meters), and successive intervals thereafter (I_{z+1} , I_{z+2} ,... I_{z+n}).

5.5.2 Each deck reading is normalized to an arbitrary deck reading of 2000 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ and the simultaneously recorded submarine reading is multiplied by this ratio to correct the individual submersed readings of each profile for any difference in solar insolation intensity during the profile. The attenuation coefficient (Kd) can then be calculated between any two depths on one profile as:

$$K_{z,z+n} = \frac{\ln(I_{z+n}/ I_{z+(n-1)})}{\Delta z}$$

where:

$K_{z,z+n}$ = attenuation coefficient (m^{-1}) over the depth interval $z+n, z+(n-1)$

I_{z+n} = normalized PAR intensity at depth $z+n$ ($\mu\text{mol } m^{-2}s^{-1}$), (i.e, deeper value)

$I_{z+(n-1)}$ = normalized PAR intensity $z+(n-1)$ depth ($\mu\text{mol } m^{-2}s^{-1}$)

Δz = difference in depth (m) between $z+n, z+(n-1)$

5.5.3 For more than two depths on one profile plot the natural log of each normalized PAR value on the x-axis vs. depth. The slope of the line times (-1.0) is K_d .

5.6 Reporting

5.6.1 The CIMS data base stores PAR values from the deck sensor and each underwater measurement under the following method codes:

5.6.1.1 EPAR_D F01: Flat Cosine Quantum Sensor LI-192SA

5.6.1.2 EPAR_S F01: Deck (reference) Quantum Sensor LI-190SA

5.6.1.3 SDEPTH: Sample depth at which PAR reading is recorded, in meters

5.7 References

Kirk, J.T.O. 1994. Light and photosynthesis in aquatic ecosystems. 2nd ed. University Press, Cambridge. 509 pp.

Appendix E: DATAFLOW QA/QC Protocol

1. Data Processing for EPA/DEQ Dataflow

2. For each tributary a folder is created with the year (ex JMSOH 2010)

2.1. In this folder each cruise date gets a folder (ex Mar 23)

2.2. In this folder are a series of files

2.2.1. The raw text file (ex 230802.txt)

2.2.2. The raw Excel file (ex JMSOH03-23-12RAW)

2.2.3. The first cut Excel QAQC File (ex JMSOH03-23-12)

2.2.4. A Minitab file w/ station averages and graphs (ex JMSOH03-23-12)

2.2.5. A file with the EPA format and error codes (ex JMSOH03-23-12EPA)

2.2.6. There is also a Tributary Metadata File (ex JMSOH 2012 Metadata)

2.2.6.1. YSI Post Cal for all parameters

2.2.6.2. Date and Location of cruise

2.2.6.3. Time stamp of all deleted data due to electronic interference

2.2.6.4. Time stamp of data line with no depth information

2.2.6.5. Time stamp with any questionable data and action taken

2.2.6.6. Track the process of QA-ing the file

(see example Metadata file)

3. Download data from field computer

3.1. Original file is left on field computer

3.2. Data file is backed up in "Raw Downloads" folder

3.2.1. Data is also placed into the Tributary file under Date of cruise (see above formatting)

4. The macro (ex. moore_df_EPA) is opened in Excel

4.1. The raw text file is opened in Notepad

4.2. Entire file is selected and pasted into Excel

4.3. Macro is run on the raw file (text to columns, headers, convert lat lon to correct form, etc)

4.4. The file is then saved as CBPSegmentmm-dd-yyRAW.xls (ex JMSOH03-23-12RAW.xls)

5. First round of QAQC

- 5.1. Save file as CBPsegmentmm-dd-yy.xls (JM SOH03-23-12.xls)
- 5.2. Delete all data up to the recorded cruise track start time, record in metadata
- 5.3. Delete the following fields

5.3.1. GPS Time

5.3.2. GPS Quality

5.3.3. Lat Dir

5.3.4. Lon Dir

5.3.5. Heading

5.3.6. Date

5.3.7. Unknown

5.4. Delete all incomplete data caused by electronic interference, record in metadata

5.5. Look for gaps in data due to running aground, pulling intake, GPS malfunction, computer malfunction, and depth sounder malfunction

5.6. Clear all cells containing “*57” depth errors and record in metadata

5.7. Examine all field notes noting events such as running aground, blooms, etc

5.7.1. Examine the data for effects of these events

5.7.2. Highlight any questionable data and note in metadata with time stamp

5.7.3. If an event did not affect the data note this also

5.7.4. Save file as CBPSegmm-dd-yy.xls (ex. JM SOH03-23-12.xls)

6. YSI Post Calibration information

6.1. Looking at the post cal notes see that all parameters post cal within limits

6.1.1. Acceptable limits listed in table below

6.2. Enter all info into the metadata for that cruise date

6.3. Highlight any data that falls outside the limits and record in metadata

6.4. If made any changes to CBPSegmm-dd-yy.xls resave

7. Second Round QAQC – Station Averages

7.1. Copy all data from Excel file (ex. JM5OH03-23-12.xls) into Minitab program

7.2. Run code written to find the average of the 10 observations before and after the verification sampling time. Graphs are generated by this code as well.

7.3. Record Lat and Lon at the time of verification sampling on the field data sheet in appropriate location

7.4. Record the averages calculated by Minitab of the following parameters on field data sheet in appropriate place: depth, water temperature, salinity, pH, dissolved oxygen (%), dissolved oxygen (mg/l), turbidity, and chlorophyll

7.5. Copy the Lat and Lon and averages of the above parameters from the Minitab file into an Excel file for later use (ex 2012 JM5OH Minitab Verification Data.xls).

8. Second Round QAQC – Graphs

8.1. Using the Minitab file generated above, examine the graph for each parameter carefully for unusual trends in data

8.1.1. Unexplained plumes in data

Look at the surrounding data and the depth. If there is no indication of probe failure or stirring up the bottom, leave data in the data set, a comment if it is questionable can always be added to the comments column later

8.1.2. Single spikes or drops in values

To determine if a single spike should be flagged, take the Standard Deviation of the 10 numbers before and after the questionable value and if the value is $<> 3$ times the Stdev highlight it

8.1.3. Negative CHL and NTU data

8.1.4. Strange patterns in DO data

8.1.5. Any indications of probe issues

8.1.6. Record any abnormalities on metadata and highlight in spreadsheet

9. Second Round QAQC – Visual Check

9.1. Using the same file take a closer look at patterns of data

9.2. Record any issues on metadata with time stamp and highlight in spreadsheet

9.2.1. Examine DO data

9.2.2. Examine CHL data

9.2.3. Examine NTU data

9.2.4. Remember to use verification data to verify highs in data

10. Final QAQC

10.1. Using all the appropriate information decide what data is unacceptable and assign error codes accordingly (record in metadata)

10.2. If data is questionable but there is not a reason to actually mask it with error codes put information into COMMENT column (record in metadata)

11. Create EPA file

11.1. Open Excel and DFtoEPAFormat macro

11.1.1. Open original file CBPSegmm-dd-yy.xls

11.1.2. Select the whole file; copy and paste it into the macro sheet

11.1.3. Add a column after the CHL column

11.1.4. Run the DFtoEPAFormat macro

11.1.5. Save as CBPSegmm-dd-yyEPA.xls

12. Create EPA_Final file using ACCESS

12.1. Open ACCESS file "dataflowQA.mdb" file and browse to open proper Excel file (CBPSegmm-dd-yyEPA.xls) and import the following:

12.1.1. SAMPLE DATE (ex mmddyyyy)

12.1.2. CRUISE_TRACK = xxxmmddyyyy (xxx – 3 letter Trib code)

12.1.3. WATER_BODY = River Name (ex JAMES)

12.1.4. PRI_SEG = Primary Segment covered by cruise (ex JM SOH)

12.1.5. STATION = PRI_SEG (ex JM SOH)

12.1.6. SONDE = the serial number of YSI used

12.2. Export data to create the EPA_Final Excel file (ex MOBPH03-23-10EPA_FINAL.xls). This file is now populated with all other necessary units, identifications, depths, layers, etc.

13. Create official EPA file

13.1. Using the metadata add the appropriate error codes (see attached list of error codes and meanings as well as comments).

13.2. Change the file name to the official EPA file CBPSegmmddy.xls (ex MOBPH032310)

Acceptable Limits of YSI Post Calibration

Parameter Units

Parameter Units Method Code

TOTAL_DEPTH M -

BATT - -

BOAT_SPEED KNOTS -

WTEMP DEG C F01

SPCOND UMHOS/CM F01

SALINITY PPT F01

DO_SAT PCT F01

DO MG/L F01

PH SU F01

TURB_NTU NTU F01 for 6136 or F02 for 6026

FLUOR %FS NA

TCHL_PRE_CAL UG/L F01

CHLA UG/L NND

DATAFLOW & Continuous Monitoring Specific Error Codes

Category	Code	Description
Specific Probe Code	PSW	Salinity Calibrated to Incorrect Level
Specific Probe Code	PDP	DO Poisoning (anoxia)
General Probe Code	GBO	Blocked Optic
General Probe Code	GWM	Wiper Malfunction
General Probe Code	GWL	Wiper Lost
General Probe Code	GSC	Seal Compromise
General Probe Code	GPF	Probe Failure
General Probe Code	GPC	Post Calibration Out of Range
General Probe Code	GNV	Negative Value
Non-Probe Codes	NPF	Power Failure
Non-Probe Codes	NOW	Instrument Out of Water
Non-Probe Codes	NNF	Ram Clogged/No Flow
Non-Probe Codes	NND	No Data
Non-Probe Codes	NIS	Invalid Data Due to Incorrect YSI Setup
Non-Probe Codes	NIR	Instrument Removed
Non-Probe Codes	NQR	Data Rejected Due to QA
Comments	CTS	Time Skip
Comments	CBF	Biofouling
Comments	CWD	Instrument at Wrong Depth
Comments	CTF	Temperature Probe Failure
Comments	CSW	Salinity Level Calibrated Incorrectly
Comments	CLF	Flow Low
Comments	CDB	Disturbed Bottom
Comments	CTC	Time Change
Comments	CTW	Turbid Water
Comments	CFK	Fish Kill
Comments	CSC	Site Location Change
Comments	CAS	Algal Sample Taken
Comments	CAB	Algal Bloom – No Sample Taken

See http://www.chesapeakebay.net/pubs/cbwqdb2004_RB.PDF for any questions parameter units, other error codes, method codes, etc

Appendix F: DATAFLOW GIS Protocol for Interpolations & Cruise Tracks

Water Clarity Acres Determination Methodology

1. Create analysis grids

a. Create segment analysis grid (analysis\yp).

i. The Chesapeake Bay segments, (James tidal fresh split, 92 segments) coverage is used for the polygon input (baselayers\cbsegsplit -cov).

ii. Import coverage into blank ArcMap document.

iii. Select target segment

iv. Right click the layer and select Data Export Data

v. Save the file as baselayers\yp.shp and add the newly created file to the map

vi. Open Feature to Raster Tool

1. Input features = features created in 1av.

2. Output Raster = baselayers\yp_tmp

3. Field = cbpseg

4. Set extent to include at least entire available bottom (bathymetry = 0-2m) for the segment.

5. Round extent to nearest 25 meters (for yrkph segment, we use top: 4138875, left: 352575, right: 376775, bottom: 4117725).

6. Cell Size = 25m.

7. Projection = NAD 1983 UTM Zone 18N.

vii. Open Multi Output Map Algebra tool

1. expression: $\text{segmask} = \text{con}(\text{isnull}(\text{setnull}(\text{cbseg_tmp} = 0, \text{cbseg_tmp})), 0, 1)$

2. where, $\text{segmask} = \text{analysis_grids}\backslash\text{yp}$

3. $\text{cbseg_tmp} = \text{analysis_grids}\backslash\text{yp_tmp}$

viii. Delete analysis_grids\yp_tmp in ArcCatalog

b. Use Feature to Raster Tool to create bathymetry grid (analysis\ypbathy).

i. Use .5 meter Chesapeake Bay bathymetry (baselayers\bathy0502 – cov)

ii. Field = Tier

- iii. Use same extent and cell size as in step 1a.
- c. Use Feature to Raster Tool to create current SAV grid (“analysis\yp_06”).
 - i. Use VIMS SAV coverage for most current year (“baselayers\beds06” – cov)
 - ii. Field = Density
 - iii. Use same extent and cell size as in step 1a.
- d. Use Feature to Raster Tool to create exclusion zone grid (“analysis\ypxz”).
 - i. Use Chesapeake Bay no grow zones (“baselayers\nogrow_zones” – cov)
 - ii. Field = Exclude
 - iii. Use same extent and cell size as in step 1a.

2. Query VECOS database

- a. Query dataflow data for single date and segment from VECOS. Note: The query is designed to take each unique combination of SAMPLE_DATETIME

(combination of date and time for each point sampled by dataflow), LATITUDE, & LONGITUDE. This is done because sometimes there is duplicate data for the same time or same location. In these instances, the query is designed to take the mean for each duplicate measurement of SALINITY, CHLA, AND TURBIDITY. The query also assigns a value of -999 where data has an associated error code.

- b. Save the result of the query as a table in an access database (db = “wq_data.mdb”, table = “yp052907”). Note: Example table has more parameters than salinity, chla, and turbidity. However, they are not necessary for this analysis. In table: salinity = sa_mean, chla = ch_mean, and turbidity = tu_mean.

- 3. Copy table into ArcGIS geodatabase (gdb = wq_data.gdb, table = yp052907).

4. Create point feature from table

- a. Open New ArcMap document. Add table (wq_data.gdb \yp052907)
- b. Right click on table and select “Display XY Data”
 - i. X field = Longitude
 - ii. Y field = Latitude

iii. Projection = GCS North American Datum 1983

c. Use Project tool to project XY event layer

i. Input = event layer

ii. Projection = NAD 1983 UTM Zone 18N

iii. Output = geodatabase (gdb = wq_data.gdb, table = "yp052907p")

5. Check for any outlying spatial points (rarely, Lat/Long is wrong in the table as in the example). Delete any outlying points from dataset in an ArcMap edit session. Save edits. May need to repeat step 4c on edited feature if zoom-layer does not focus on remaining points. Output: gdb = wq_data.gdb, table = "yp052907p")

6. Add resulting point feature to blank ArcMap document

7. Select where $ch_mean > -999$. Note how much data may be invalid. Decide if too much data has an associated error code to proceed with interpolation for this dataset. This decision is made with a concern for the amount and spatial distribution of data that will be excluded. In the future, we would like to move toward a less-subjective approach though the use of an associated standard error grid. If the interpolation is not made, that particular date is ultimately thrown out of any annual mean water clarity acreage calculations. If there is sufficient data to continue, clear the selection before continuing with the interpolation.

8. Create the Ordinary Kriging Prediction Map (**For segments where interpolations will be executed without the use of barriers. For segments where barriers are used, see alternative steps 8 and 9 at the end of this document).

a. From the Geostatistical Analyst toolbar, choose Geostatistical Wizard

b. In the Choose Input Data and Method dialog box

i. Input Data: current feature (should default to the correct file)

ii. Attribute: choose the parameter for interpolation (ch_mean).

iii. Check the Use NODATA value: box and enter -999 as the NODATA value

iv. Choose Kriging in the Methods box (or allow to default)

v. Click Next

c. In the Handling Coincidental Samples popup, choose Use Mean and click OK (should not matter, because all coincident points were removed in the initial query).

d. In the Step 1 of 4 – Geostatistical Method Selection dialog box, accept the defaults and click Next

e. In the Step 2 of 4 – Semivariogram/Covariance Modeling dialog box, check the Anisotropy box and click Next

f. In the Step 3 of 4 – Searching Neighborhood dialog box, change Neighbors to Include: to 25, set Shape Type: to the second type from the left (immediately to the right of One Sector) and click Next

g. In the Step 4 of 4 – Cross Validation dialog box, click Finish

h. Click OK in method summary window.

9. Use GA Layer to Grid Tool to create output grid

a. Input = GA Layer result from step 8

b. Output surface grid = grids\ + segcode + \YY\ MMDDYY\ + 2-letter parameter designation + MMDDYY (for example, grids\yp\07\040904\ch052907)

c. Set cell size and Extent to the same as 1a.

10. Repeat steps 7, 8, and 9 for salinity(sa_mean) and turbidity(tu_mean) for the current date.

11. Use Multi Output Map Algebra Tool to calculate Kd grid from salinity, turbidity, and chlorophyll grids.

a. Use appropriate equation in expression to calculate Kd(Perry, 2006).

Group 1a: MPNOH, MPNTF

$Kd = 1.192674757 + 0.295620722 (TU ^ 1 / 1.5) - 0.056160407 (SA) + 0.000274598 (CH)$

Group 1b: CHKOH, JMSPH, JMSOH, JMSMH, JMSTF1, JMSTF2, APPTF:

$Kd = 0.859551 + 0.292803 (TU ^ 1 / 1.5) - 0.069875 (SA) + 0.028297 (CH)$

Group 2: LYNPH, PMKOH, PMKTF, YRKPH, YRKMH, PIAMH:

Group 3: POTMH, POTTF, POTOH

$$Kd = 0.350711 + 0.298464 (TU ^ 1 / 1.5) + .004668 (SA) + .011035 (CH)$$

Group 4: RPPMH, RPPOH, RPPTF, CRRMH

$$Kd = 0.591062 + 0.302307 (TU ^ 1 / 1.5) - .021831 (SA) + .023192 (CH)$$

Group 5: (preliminary): MOBPH, CB6PH

$$Kd = -0.169897 + 0.333521 (TU ^ 1 / 1.5) + .019517 (SA) + .017814 (CH)$$

b. Example expression for yrkph(yp):

$$kdgrid = 0.5275793536 + 0.3193475331 * pow(tugrid, 1 / 1.5) + 0.0176700982 * sagrid + 0.0271723238 * chgrid$$

where, kdgrid = location and name of output grid (grids\yp\07\052907\kd0052907)

tugrid = location of turbidity grid from steps 7-9 (grids\yp\07\052907\tu052907)

sagrid= location of salinity grid from steps 7-9 (grids\yp\07\052907\sa052907)

chgrid= location of chlorophyll grid from steps 7-9 (grids\yp\07\052907\ch052907)

12. Determine if each cell in kdgrid meets appropriate Kd threshold using Multi Output Map Algebra Tool

Table 1. Kd Thresholds

Expression for yrkph(yp) 0-1m:

$$kdgridt1 = con(kdgrid > 1.51, 0, 1)$$

where kdgridt1 = attainment grid for 0-1 meters (grids\yp\07\052907\t1052907)

kdgrid = result from step 11 (grids\yp\07\052907\kd052907)

Expression for yrkph(yp) 1-2m:

$$kdgridt2 = con(kdgrid > .76, 0, 1)$$

where kdgridt2 = attainment grid for 1-2 meters (grids\yp\07\052907\t2052907)

kdgrid = result from step 11 (grids\yp\07\052907\kd052907)

13. Merge results from step 12 based on depth using Multi Output Map Algebra Tool:

Expression for yrkph(yp):

kdgrida = con(seg bath == 4, kdgridt2,(con(seg bath == 2 or seg bath == 3, kdgridt1, 0)))

where, kdgridt1 = result from step 12 for 0-1m (grids\yp\07\052907\t1052907)

kdgridt2 = result from step 12 for 1-2m (grids\yp\07\052907\t2052907)

seg bath = result from step 1b (baselayers\ypbathy)

kdgrida = attainment grid (grids\yp\07\052907\ax052907)

14. Use the Extract by Mask Tool to remove the exclusion zones from the analysis.

a. Input Grid = Result of step 13 (grids\yp\07\052907\ax052907)

b. Mask Raster = result of step 1c (analysis\ypxz)

c. Output Raster = (grids\yp\07\052907\ax052907)

15. Combine grids for assessment using Combine Tool

a. Input Rasters = cbseg; cusav_grd; seg bath; kdgridax

Where cbseg = result of 1a

cusav_grid = result of 1c

seg bath = result of 1b

kdgridax = result of step 14

b. Output grid = combine result (grids\yp\07\052907\wc052907)

16. Export results table to Access

a. Add the result from step 15 (grids\yp\07\052907\wc052907) to an ArcMap document

b. Right click on the grid and select Open Attribute Table

c. Click on Option in the lower right hand corner and select export

i. Export: all records

ii. Save the output table to the access database where water clarity acres will be calculated (gdb = wca.gdb, table = yp052907)

17. Calculate water clarity acres

a. Open access database where result of step 16 was saved

- b. Open result from step 16 in design view
- c. Rename the 4 fields after Count(order of columns dependent on order columns were combined in step 15. If entered in order designated in the this guide:
 - i. 1st Column after Count_ (YP) should be renamed SEG_BNDRY
 - ii. 2nd Column after Count_ (YP_CU06) should be renamed CU_SAV
 - iii. 3rd Column after Count_ (YPBATH) should be renamed BATHY
 - iv. 4th Column after Count_ (WC052907) should be renamed ATTAINMENT

18. Append Tables

- a. Open wca.gdb
- b. Create copy of first table (yp052907) and rename it “summary” (This step can also be done in ArcCatalog)
- c. Close Access
- d. Open Append Tool
 - i. Input datasets are all other tables other than the one that was copied in 18a in wca.mdb (yp041907, yp052907, 061307, 070207, 082707, 091307, 101007, 111207)
 - ii. Target dataset is “wca.mdb\summary”

19. Calculate water clarity acres

- a. Open wca.gdb
- b. Run Query 1
- c. Run Query 2
- d. Run Query 3
- e. Monthly and mean Annual water clarity acres are now located in the table called monthly_wca and annual_wca, respectively.

Note: These queries are used to calculate water clarity acres by initially converting cell counts of attainment into acreage of attainment inside and outside of current SAV areas for each dataflow cruise. Water clarity acres for the segment are then calculated by the taking the annual mean of the monthly acreage. Total acreage of SAV within the segment’s watershed boundary is also calculated.

Key for Access Tables (annual_wca & monthly_wca)

WCA = Water Clarity Acres

NSWCA = Water Clarity Acres outside of current SAV

CUSAV + NSWCA = Current SAV + Water Clarity Acres outside of Current SAV

In VA, a segment is in attainment if at least one of three conditions is met.

1. Current SAV \geq Water Clarity Acreage Goal
2. Total Acreage of Attainment \geq Water Clarity Acreage Goal
3. Current SAV + Acreage of Attainment Outside of Current SAV \geq Water Clarity Acreage Goal

Segment goals are defined in DEQ document 9 VAC 25-260 Virginia Water Quality Standards (2005).

These are alternative Steps following 8 and 9 for segments where the use of barriers is appropriate. Follow steps 8 and 9 to create interpolation without barriers. Follow the steps below to interpolate with barriers and then merged to interpolation without barrier to ensure coverage for the all available bottom in the segment:

1. Create barrier layer for segment:

a. Use Select Tool (Analysis Tools -> Extract -> Select) to create segment polygon (baselayers/potmh_coa_seg.shp)

i. Input = baselayers/cbsegsplit

ii. Output = baselayers/potmh_coa_seg.shp

iii. Expression = "CBSEG" = "POTMH_COA"

b. Use Clip Tool (Analysis Tools -> Extract -> Clip) to create segment barrier

i. Input = baselayers/potmh_coa_seg.shp

ii. Output = baselayers/potmh_coa_barrier.shp

2. Create shapefile where cruise points with error codes are selected out.

a. Add dataflow point feature (wq_data.gdb/pc041907p) to blank map document

b. Select where ch_mean > -999. Note how much data may be invalid. Decide if too much data has an associated error code to proceed with interpolation for this dataset. This decision is made with a concern for the amount and spatial distribution of data that will be excluded. In the future, we would like to move toward a less-subjective approach though the use of an associated standard error grid. If the interpolation is not made, that particular date is ultimately thrown out of any annual mean water clarity acreage

calculations. If there is sufficient data to continue, clear the selection before continuing with the interpolation.

- c. With selection still in place, export selected features to shapefile
 - i. Right click on feature in TOC and choose Data > Export data
 - ii. Export: selected features
 - iii. Use same coordinate system as layer's source data
 - iv. Output shapefile: analysis_shapefile\pc041907p.shp
3. Use aml Kriging function to create interpolation incorporating new barrier file
 - a. Open Single Output Map Algebra tool (Spatial Analyst Tools > Map Algebra > Single Output Map Algebra)
 - b. Expression (make sure to replace '~' with location of file on local machine):

Kriging(~\analysis_shapefile\pc041907p.shp , ch_mean, ~\baselayers\potmh_coa_barrier.shp , GRID, #, SPHERICAL, SAMPLE, 25, 1500, 25)

- c. Output Grid: ~\grids\pc\041907\tch041907
- d. Set cell size and Extent to the same as analysis_grids/pc
4. Open Con tool (Spatial Analyst Tools > Conditional > Con) to limit extent of interpolated barrier grid
 - a. Conditional grid: ~analysis grids/pc_bath
 - b. Expression: "VALUE" > 1
 - c. Input True Raster: ~\grids\pc\041907\tch041907
 - d. Input False Raster: ~\grids\pc\041907\ch041907
 - e. Output raster: ~\grids\pc\041907\bch041907
5. Merge barrier grid with interpolated grid without barriers.
 - a. Open Single Output Map Algebra tool (Spatial Analyst Tools > Map Algebra > Single Output Map Algebra)
 - b. Expression (make sure to replace '~' with location of file on local machine):

Merge(~\grids\pc\041907\bch041907, ~\grids\pc\041907\bch041907)

c. Output Grid: ~\grids\pc\041907\mch041907

6. Repeat this procedure for all parameters

7. To Proceed: delete ~\grids\pc\041907\ch041907 and rename ~\grids\pc\041907\mch041907 to ~\grids\pc\041907\ch041907. Use this renamed merged grid in all future steps (go to step 10 above to continue with water clarity analysis)

Sources

Perry, E. (2006). Notes on Lumping vs Splitting $K_d = f(\text{turbidity})$ calibration. Unpublished Report.

9 VAC 25-260 Virginia Water Quality Standards. (2005). § 62.1-44.15 3a of the Code of Virginia. Retrieved February 21, 2007, from http://www.epa.gov/waterscience/standards/wqslibrary/va/va_3_wqs.pdf.

Appendix G: CMON Station QA/QC Protocols in Brief

Continuous Station Coarse QA/QC Protocol

Step 1 Insert appropriate parameter qualifiers

Step 2 Identify negative “-“ values

All negative values are flagged with a “GNV” in the appropriate parameter_a column

Step 3 Identify time periods when the datalogger or certain probes were not deployed.

In the appropriate parameter_a column, flag all non-deployments with a “NND”.

Step 4 Identify and flag single spike NTU values

In the NTU_a column, flag all single spike NTU values >300 with “NQR”.

Step 5 Identify and flag single spike Chl values

In the Chl_a column, flag all single spike Chl values >400 with “NQR”.

Step 6 Identify and flag single spike %Fluor values

In the Fluor_a column, flag all single spike Fluor values >100 with “NQR”.

Step 7 Identify and flag probe failures

In the appropriate parameter_a column, flag time intervals with “GPF”.

Note: This would apply to DO membrane punctures, pH bulb breakage etc.

Step 8 Identify and flag time periods in which specific probes failed to meet acceptable post calibration criteria

In the appropriate parameter_a column, flag time intervals with “GPC”.

Note: This would apply to DO drift and similar issues. If you can not clearly identify where the problem began, flag the entire deployment interval.

Step 10 Identify and flag time periods in which entire instrument or specific probes were out of water.

In the appropriate parameter_a column, flag affected time intervals with “NOW”.

Note: If entire instrument is out of water, all parameter_a columns must be flagged.

Step 12 Identify and flag time periods affected by a time skip.

In the comment column, flag effected time period with “CTS”.

Step 13 Identify and flag time periods affected by station maintenance.

In the appropriate parameter_a column, flag affected time interval with “NQR”

Note: This was seen in some of our turbidity and depth data.

Step 14 Link deployments and look for probe drifts, significant mismatch, etc

In the appropriate parameter_a column, flag affected data with the appropriate error code.

**Quality Assurance Project Plan for the Project:
Fulfilling Data Needs for Assessing Numeric CHLa Criteria of the Lower James
River Estuary, *Subtask 1.1- Expand Monitoring Network***

(For the Period: May 1, 2013 through April 30, 2014)

Virginia Institute of Marine Science
School of Marine Science
College of William and Mary
Gloucester Point, Virginia 23062

Kenneth A. Moore

Dr. Kenneth A. Moore, Project Manager, VIMS

9/16/2013

Date

Betty B. Neikirk

Mrs. Betty B. Neikirk, Quality Assurance Officer, VIMS

9/16/2013

Date

Dr. Arthur Butt, Program Officer, Va. DEQ

Date

1. PROJECT DESCRIPTION

1.1 Project Definition and Background

The VA DEQ is undertaking a comprehensive review of the existing Site-Specific Numeric Chlorophyll-*a* (CHL*a*) criteria and associated modeling framework for the tidal James River. This effort will provide the scientific basis for a potential water quality standards rulemaking process, which may result in revisions to nutrient allocations contained in the Chesapeake Bay TMDL. A Science Advisory Panel was established by DEQ to provide recommendations on data and modeling needs for assessing the existing CHL*a* standard. The Panel reviewed existing data resources and modeling capacity to identify knowledge gaps in characterizing the occurrence of algal blooms in the tidal James River and associated impairments to designated uses. The Panel's recommendations provided an overall framework for addressing these needs as well as specific tasks for data collection and model development.

The Lower James River Estuary (inclusive of the oligo-, meso- and poly- haline regions) experiences algal blooms that are more ephemeral in time and place. Given the large spatial area of the Lower James, and the sporadic incidence of algal blooms, a greater proportion of data collection activities must be allocated to characterizing the frequency and extent of blooms. Advanced technologies including continuous, fixed-station monitoring and continuous on-board monitoring will be needed to map their spatial extent and identify zones of bloom initiation. Assessing impairments in the Lower James is also challenging because the blooms are typically comprised of dinoflagellates which are known to cause harmful effects, though these may not be linked to the occurrence of specific toxins.

Algal blooms occurring in the Lower James River Estuary are ephemeral in nature and unpredictable in their timing, location and duration. Algae have the capacity to bloom quickly and to be transported by currents. As a result, sites of bloom initiation may be geographically distinct from areas where blooms develop and cause detrimental effects on water quality and living resources. The distinction between sites of initiation and impact is important because mitigation actions designed to prevent blooms would need to be focused at sites of bloom initiation whereas actions aimed at mitigating bloom impacts would need to focus on sites where blooms accumulate. Fixed station monitoring, such as the program carried out by DEQ for the CBP, is not designed to locate, map and track these events. Thus, alternative monitoring strategies are needed to characterize the occurrence of algal blooms in the Lower James. A method of on-board and underway monitoring (DATAFLOW) of CHL*a* can be used in conjunction with GPS navigation to provide real-time mapping of algal blooms. Presently this technology is employed by the Hampton Roads Sanitation District (HRSD) scientists to map spatial variation in CHL*a* for the meso- and poly- haline segments of the James, Elizabeth and Lafayette Rivers on a weekly basis. This method provides the most effective means for determining the size, intensity and location of algal blooms. The Panel recommended that these efforts should be expanded to include the oligohaline segment of the James (Appendix 3).

There is also a need to complement CHL_a mapping efforts with fixed-station, continuous monitoring (CMON) to enhance temporal coverage and bloom detection capabilities. Specifically, CHL_a sensors should be deployed in potential “hot spots” for bloom initiation that are identified from previous mapping efforts and/or their proximity to nutrient inputs. One site in the JMSMH segment will be sampled using this protocol (Figure 4). The JMSMH location represents a region where algal blooms are often first observed either by initiation and/or hydrodynamic transport. One Chesapeake Bay Program (CBP) segment, the James River Oligohaline (JMSOH) will be sampled by this program in 2013-14 using water quality mapping (DATAFLOW) sampling to complement other DATAFLOW sample currently conducted by HRSD.

1.2. Objectives and Scope of Project (Fulfilling Data Needs for Assessing Numeric CHL_a Criteria of the Lower James River Estuary, 2013. Subtask 1.1 – Expand Monitoring Network)

- 1) Collect data to be used in assessing numeric water quality standards for Chlorophyll.
- 2) Collect data for diagnosing reasons for any non-attainment of these water quality criteria.
- 3) Collect data to improve overall understanding and modeling of processes influencing these water quality criteria.
- 4) Provide calibration data for refined James River Model simulations of water clarity, and phytoplankton that will be completed over the next three years.
- 5) Provide continuous water quality data from a site in the JMSMH in conjunction with in situ plantings of oysters to assess biological impairments as they relate to the duration and intensity of exposure to bloom events.

DATAFLOW sampling in 2013 will be conducted in one Chesapeake Bay Program segment, the James River Oligohaline (JMSOH). Collection of data from 0.25-0.5m below the surface will be performed once a month from April through July in parallel to cruises conducted by the Hampton Roads Sanitary District. During the most intensive bloom period of July-September the cruises will be conducted twice monthly if needed and again once monthly in October. The DATAFLOW system allows the continuous measurement of dissolved oxygen, chlorophyll, turbidity, salinity, specific conductivity, temperature, and pH while underway in a small boat. The data collected in any one day can then be interpolated to provide a complete surface “map” of water quality conditions throughout the segment that can then be compared against water quality criteria.

The specific goal of the fixed station continuous monitoring system is to assess water quality standards as well as quantify short-term variability and long-term changes in water quality constituents in specific shallow water areas. One fixed continuous monitoring (CONMON) station for 2013 will be located in the lower James River at or near the James River Country

Club (Appendix A). The station will consist of a YSI extended deployment datasonde, which will sample dissolved oxygen, chlorophyll, turbidity, salinity, specific conductivity, temperature, and pH. The ConMon station in the JMSMH segment established in 2013 in conjunction with the *in situ* oyster monitoring station (K. Reece, VIMS) will run continuously from February to October.

Concomitant data on chlorophyll and suspended sediment conditions are collected at single point sites during the dataflow mapping cruises and when the fixed stations sondes are exchanged. These data, for dataflow verification stations, will include: chlorophyll and pheophytin, total suspended solids (TSS), volatile suspended solids (VSS), dissolved oxygen using a YSI 600 XLM sonde, secchi depth, and vertical profiles of photosynthetically available radiation (PAR) and temperature, dissolved oxygen, salinity, specific conductivity, and pH. For the fixed station these data will include: chlorophyll and pheophytin, total suspended solids (TSS), volatile suspended solids (VSS), dissolved oxygen by a YSI 6920 sonde, secchi depth, as well as a vertical profile for temperature, dissolved oxygen, salinity and pH and vertical profiles of PAR. These data will be used to help assess the ecological conditions affecting the water quality criteria and provide enhanced monitoring data for refined James River model simulations of chlorophyll and HAB concentrations.

1.3. Project Location Descriptions

The James River Oligohaline segment begins at the boundary of the mesohaline segment near Carter's Grove Home, about 1.25 miles southeast of Grove Creek on the north shoreline to 0.7 miles north of Hunnicut Creek, south of Hog Island on the southern shoreline. It then extends upriver to adjoin the mouth of the Chickahominy River and upward to Tettington on the north shore and 0.3 miles downstream of Sloop Point on the southern shore. The segment includes Hog Island, Surry Nuclear Power Plant, and Jamestown Island.

1.4. Project Dates

The James River Mesohaline segment begins at the boundary of the polyhaline segment extending from Newport News Point just south of Lincoln Park on the north shore to the US Army Disposal Area at Craney Island on the south shore. It extends upriver to near Carter's Grove Home, about 1.25 miles southeast of Grove Creek on the north shoreline to 0.7 miles north of Hunnicut Creek, south of Hog Island on the southern shoreline. This segment includes the Warwick, Pagan, and Nanesmond Rivers as well as Mulberry Island.

The time period for this study is from February 1, 2013 through April 2014. Status reports will be delivered in accordance with the dates stipulated under deliverables in the contract.

1.5. Data Quality Objectives

The main objective of this program is to collect data of sufficient quantity and quality to assess James River standards for chlorophyll. This data must be representative and comparable across all the monitored tributaries. The greater spatial and temporal density of data which can be used to assess surface water quality criteria and standards is an important component of this monitoring program. Another strength of this study is the comparability of data with that collected by HRSD for lower segments in the James River, as well as ongoing data collections by other Chesapeake Bay Monitoring Programs. Through the use of the same Chesapeake Bay Program approved protocols, instrumentation, quality control checks, and communication, an integrated net of data is generated for this system.

2. PROJECT ORGANIZATION AND RESPONSIBILITIES

2.1. Project Organization

The collection and preparation of samples, plus data entry and management will be completed at the Virginia Institute of Marine Science under the direction of Dr. Kenneth A. Moore (PI). All water quality analyses (Chl *a* and suspended solids) are performed by VIMS Analytical Services Center (ASC).

2.2. Principal Investigator

The principal investigator (PI), Dr. Kenneth Moore (moore@vims.edu, 804-684-7384), will supervise all activities associated with this project. This includes fieldwork, data management and report writing. He will be responsible for all stages of the analysis of samples, resolving problems that may arise, and assure the satisfactory completion of the study. He is responsible for data review and oversight and submission of data. The PI will review the results of the analyses and approve the quality assurance/quality control protocols to insure the validity of the results. The PI will administer the financial and technical requirements of the project and be responsible for preparing the data and progress report and the final report to be submitted at the end of the project. He will also meet, at regular time intervals, with the other members of staff to discuss and review their responsibilities in relation to the project. The PI will respond to questions by the contracting agencies regarding the completion of different stages of the project and the reports that have to be submitted as part of the deliverables outlined in the project contract. Dr. Kenneth Moore holds a B.S. degree in Biology from The Pennsylvania State University, a M.S. degree in Marine Science from the University of Virginia and a Ph.D. degree in Marine, Environmental and Estuarine Science from the University of Maryland.

2.3. Personnel Qualification Summary

Mrs. Betty Neikirk (betty@vims.edu, 804-684-7400) is a Marine Scientist Supervisor at VIMS and holds a B.S in Biology from Randolph-Macon College and a M.S. in Marine Science from the College of William and Mary. Mrs. Neikirk is the Quality Assurance Officer for the Shallow Water Monitoring Program and Field Manager. She has been involved with water quality research and monitoring at VIMS for 27 years and oversees the DATAFLOW and CMON (Continuous Monitoring) field programs, has responsibility for data analysis, coordinates and schedules all field operations, as well as participates in the field operations. She reports to the principal investigator.

Mr. David Parrish (parrishd@vims.edu, 804-684-7835) is a GIS Developer/Scientist II at the Virginia Institute of Marine Science and holds a B.S. in Integrated Science and Technology from James Madison University and an M.S. in Natural Resource Management from Central Washington University. He will serve as Data Manager. He conducts spatial and statistical analysis of water quality data, provides technical support to the program, and manages the VECOS (www.vecos.org) database and website.

Mrs. Joy D. Austin (justjoy@vims.edu, 804-684-7307) is a Laboratory & Research Specialist II/Laboratory Supervisor at the Virginia Institute of Marine Science. She is currently in charge of the day-to-day activities of the water quality and fixed station monitoring at Chesapeake Bay National Estuarine Research Reserve in Virginia (CBNERRVA) and is familiar with all aspects of CBNERRVA monitoring. She will be responsible for overseeing the continuous, fixed station monitoring activities including activities such as sonde calibration, QA/QC of data and data management, sampling coordination, purchases of equipment and supplies. She will coordinate fixed station activities with Mrs. Betty Neikirk.

Ms. Alynda Miller (alynda@vims.edu, 804-684-7576) is a Laboratory and Research Specialist II at VIMS. She has a B.S. in Biology from the Millersville University of Pennsylvania. She will aid in the fixed monitoring program including activities such as sonde calibration, QA/QC of data and data management, sampling coordination, purchases of equipment and supplies.

Ms. Lisa Ott (lott@vims.edu, 804-684-7576) is Laboratory and Research Specialist II at VIMS. She has a B.S. in Biology from Christopher Newport University?. She will aid in the fixed monitoring program including activities such as sonde calibration, sample filtration, and maintaining equipment and preparation of field supplies.

Mr. Jim Goins (goins@vims.edu, 804-684-7559) is a Laboratory & Research Specialist II /Field Manager at VIMS. He will help conduct the field sampling activities associated with this project including; Dataflow mapping, water quality verification sampling and fixed station sampling and participates in other monitoring programs as needed. He has extensive

small vessel handling experience, and holds US Coast Guard Basic Seamanship and Safety and VIMS small vessel certification

Mr. Steve Snyder (snyder@vims.edu, 804-684-7442) is a Laboratory and Research Specialist II at VIMS who holds an Associates Degree in Applied Science from Thomas Nelson Community College. He assists in the field and laboratory work related to this project including the dataflow mapping, water quality verification sampling and the calibration of the instruments. He also has extensive small vessel handling experience and holds US Coast Guard Basic Seamanship and Safety and VIMS small vessel certification.

Mr. Voight Hogge (voight@vims.edu, 804-684-7087) is a Field Supervisor at VIMS. He has extensive boating experience as well as knowledge of local water ways. He will be working as a boat captain assisting with the field effort associated with the DATAFLOW and Continuous Monitoring Programs. Mr. Hogge will also be doing routine boat maintenance. He holds a US Coast Guard 50 Tons Master Captain License, US Coast Guard Basic Seamanship & Safety certification, and VIMS small vessel certification.

Mrs. Carol Pollard (pollard@vims.edu, 804-684-7213) is a Laboratory Director/Scientist II (Supervisor). She has a B.S. in Environmental Science from Florida Institute of Technology and a M.S. in Marine Science from the College of William and Mary. Mrs. Pollard is the Director of the Analytical Services Center at the Virginia Institute of Marine Science. Her responsibilities include lab goals, budget, coordinating personal and maintaining safety and quality control. Mrs. Pollard also coordinates with the Virginia Department of Environmental Quality and the U.S. Environmental Protection Agency in blind audits, cross laboratory calibration samples and the Chesapeake Bay Program's Coordinated Split Sample Program (CSSP).

All of the above personnel are located at VIMS, P.O. Box 1346, Gloucester Point, VA 23062.

2.4. Organizational Chart

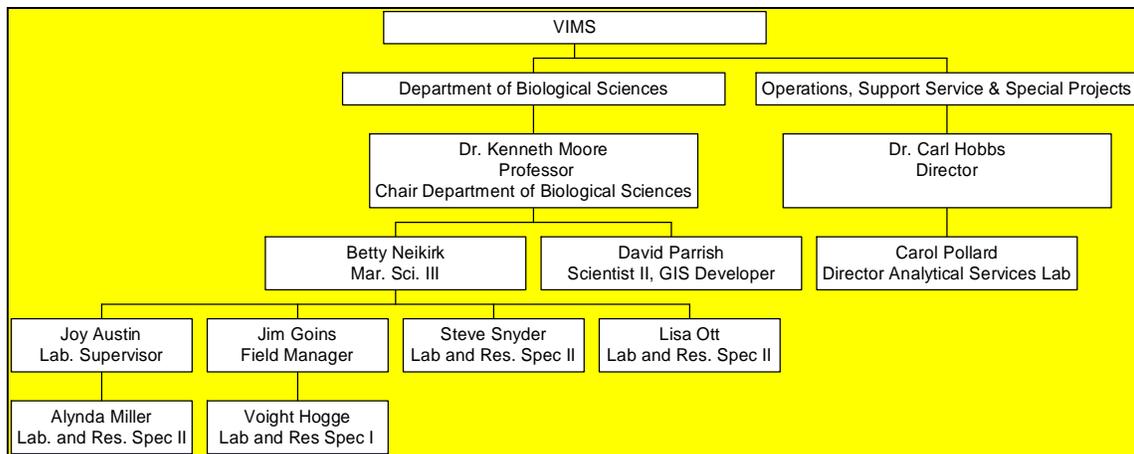


Table 1. Organizational Chart

2.5. Special Training and Certification

Vessel certification is required for use of all small boats at VIMS. This includes successful completion of US Coast Guard approved small vessel navigation and safety course and a yearly renewable, vessel-specific certification by the VIMS Vessels Center. A current list of approved small vessel operators can be found at the web site: <http://www.vims.edu/admin/vessels>.

3. SAMPLING PROCEDURES

3.1. Continuous Surface Water Quality Mapping in the James River Oligohaline

3.1.1. DATAFLOW Mapping system

DATAFLOW is a compact, self-contained surface water quality mapping system, suitable for use in a small boat operating at speeds of about 25 KT. The system collects water through a pipe ("ram") deployed on the transom of the vessel, pumps it through an array of water quality sensors, and then discharges the water overboard. The entire system from intake ram tube to the return hose are shielded from light to negate any effect high intensity surface light might have on phytoplankton in the flow-through water that is being sampled. A blackened sample chamber is also used to minimize any effect of light on measurements by the fluorescence probe.

3.1.2. Area of Operations, Cruise Tracking, and Sample Frequency

The area of operations will include the James River Oligohaline area (JMSOH). This area includes of the EPA Chesapeake Bay Program Office's designated Chesapeake Bay segments (see www.chesapeakebay.net/pubs/segmentscheme.pdf for description of CBP segments).

2012 JMSOH cruises (Appendix A) are scheduled to take place twice a month in May, June and October during one spring and one neap tidal period. Cruises will be conducted weekly from July through September. All cruise dates are dependent on weather conditions optimum for the safety of the field crew, tidal stage, and ability to attain quality data. Cruises which have to be cancelled due to weather conditions will be rescheduled immediately as weather permits. Cruises will take place during the mid day, over an approximate four to five hour interval beginning at approximately 0900 to 1000.

Operations follow different cruise tracks depending on the morphology of the area segment being monitored and the amount of navigable shallow water. In the lower segment of the river, where the width of the river is normally wide, a series of tracks running parallel to the shoreline along fixed depth contours is followed. For example, the track may follow the shoreline down river along the ≤ 2 meter depth contour, then up river along a mid depth contour (approximately 5 meters), then down river along the channel (>10 meters depth), then finish up along the other shoreline in the shallows (Appendix A).

The DATAFLOW mapping system collects a sample once every 3-4 seconds. The resulting distance between samples is therefore a function of vessel speed. Vessel speeds vary throughout the cruise depending on depth of water, navigational hazards, weather conditions and the slowing of the vessel approaching or leaving verification stations. Average speed underway is approximately 20 knots, which results in an observation collected every 30 meters. As speeds decrease this means samples will occur closer together, but for the most part when underway between the speeds of 10-20 knots samples will occur every 15-30 meters. A total of five stations for verification samples will also be sampled at intervals along the cruise track in JMSOH (Appendix A; Table 3).

3.1.3. Water Quality Instrumentation

The DATAFLOW system has a YSI 6600 sonde equipped with a flow-through chamber. The sensors include a Clark-type 6562 DO probe, a 6561 pH probe, a 6560 conductivity/temperature probe, a 6136 turbidity probe, and a 6025 chlorophyll probe. The sonde transmits data collected from the sensors directly to a Panasonic Toughbook, ruggedized laptop computer using a data acquisition system created with LabView software (National Instruments, Inc.). Custom software written in the Labview environment provides for data acquisition, display, control, and storage. Real-time graphs and indicators provide feedback to the operator in the field, ensuring quality data is being collected. All calibrations

and maintenance on the YSI 6600 sondes are completed in accordance with the YSI, Inc. operating manual methods (YSI 6-series Environmental Monitoring Systems Manual; YSI, Inc. Yellow Springs, OH).

Table 2. Quality of YSI Data (6600)

PARAMETER	UNITS	PRECISION	ACCURACY	MDL
DO	% Saturation	0.1%	± 2%	0 %
DO	mg/L	0.01mg/L	±0.2mg/L	0 mg/L
Salinity	ppt	0.01ppt	0.1ppt	0 ppt
Temperature	°C	0.01°C	±0.15°C	-5°C
pH	unit	0.01units	±0.2units	0 units
Turbidity	NTU	0.1NTU	2 NTU	0 NTU
Chlorophyll	µg/L Chl	0.1µg/L Chl	-	0 µg/L Chl

3.1.4. Positioning, Depth Information and Data Acquisition

The DATAFLOW system is equipped with a Garmin GPSMAP 540s with sounder. This unit serves several functions including chart plotting, position information, and depth. The unit is WAAS (Wide Area Augmentation System) enabled providing a position accuracy of better than three meters 95 percent of the time. The NEMA 0183 data sentence containing all pertinent position and depth information is output to the SBC data acquisition system.

The DATAFLOW system utilizes a SBC data acquisition system for data collection and storage. The system is based on a Panasonic Toughbook, ruggedized laptop computer. Custom software written in a Labview environment provides for data acquisition, display, control, and storage. All data is collected simultaneously in one file, removing the chore (and possible errors) of merging separate files into one.

3.1.5. Cruise Scheduling

Three cruises per month will be scheduled in February and March, one per month in May through October 2013. During the most intensive bloom period of July-September 2013 extra cruises will be conducted, if necessary, to quantify the bloom events. As stated previously cruise dates are subject to change due to inclement weather conditions or mechanical problems with the research vessel. A schedule for activities for each day of the individual cruises is submitted to the PI and other members of staff. Cruises that are cancelled due to weather or mechanical problems with the research vessel are rescheduled.

3.1.6. Sampling Procedures

3.1.6.1. Real-Time Field Verification

The DATAFLOW system provides field personnel with a real time display of parameters as the data is being collected. The field crew, who are knowledgeable and experienced in estuarine water quality monitoring, accesses this data in real time. The crew has a working knowledge of the normal water quality conditions and how weather patterns, diurnal patterns, seasons, and events such as algal blooms might affect data. When unusual results are observed, action is taken to investigate the cause of the unusual data and either note the cause or take action to correct any equipment issues that may be contributing factors. There are various factors which can lead to erroneous data including; fouling of the ramjet intake, wiper malfunction on the optical probes, probe failure, etc. When these conditions are observed the affected part of the data record is noted in the field notes. During the data review the Quality Assurance Officer takes these notes into account and flags the data accordingly. At verification stations, dissolved oxygen, pH, salinity and temperature are verified using a YSI 600 XLM . If there is a greater than 0.5mg/l difference in dissolved oxygen, 0.2 SU difference in pH, or greater than 1 degree Celsius difference between instruments this is investigated and corrective action is taken. This usually involves switching the YSI 6600 for the spare that is carried on board the research vessel. Close attention is also given to YSI 6600 chlorophyll and turbidity and if readings are questionable the YSI 6600 is switched out with the spare on board.

3.1.6.2. Verification Sample Collection

A total of five verification stations (Appendix A and Table 3) will be sampled along the cruise track in JMSOH. Stations are selected to maximize the range of values that are seen along a track (e.g. when moving up a tributary with a salinity gradient, samples are taken to get a high, medium, and low salinity value). Within each segment, the fixed monitoring stations from this program as well as the stations from the VA Department of Environmental Quality's tributary monitoring program will be utilized as verification stations. Extra sampling supplies will be available to sample more stations under special conditions such as in areas of large blooms. At each station the boat is stopped and water samples are collected from the effluent tubing of the DATAFLOW System (sampling water depth of approximately 0.25 - 0.50 m) for total suspended solids (TSS), volatile suspended (VSS), chlorophyll, and pheophytin. At these stations secchi depth, a vertical profile of photosynthetically available radiation (PAR), as well as a vertical profile for temperature, dissolved oxygen, salinity and pH are also done (See Appendix D for further details). Samples for TSS, VSS, chlorophyll and pheophytin are collected in darkened bottles, which are rinsed three times with ambient water before filling. Samples for chlorophyll and pheophytin are filtered on board the vessel. (See Appendix D for further details). These are then packed on ice in a darkened cooler, and returned to the laboratory where they are stored at -20°C. Samples for TSS and VSS are

packed on ice, in a darkened cooler, and returned to the laboratory where they are filtered immediately upon return and frozen (Appendix D). Samples are then delivered to the VIMS Analytical Service Center for further processing.

3.1.6.3. Vertical Profile of Dissolved Oxygen, Water Temperature, and Salinity

A vertical profile of the water column is accomplished using a YSI 600 XLM which includes a Clark-type YSI 6562 DO probe, a YSI 6560 conductivity/temperature probe, and a 6561 pH probe. This profile includes water temperature, specific conductivity, salinity, dissolved oxygen and pH. At the shallow water verification stations (water depth less than 2 meters), this is accomplished by placing the sensor at a 0.1 meter depth, letting it equilibrate and recording the measurements. The same procedure is then followed at successive 0.25 m intervals thereafter until the final reading is taken approximately 5 cm above the bottom. At deep-water verification stations the YSI is lowered until the bottom is reached. This depth will be rounded to the nearest meter and then the sonde will be pulled to 1 meter above the bottom and allowed to equilibrate. Measurements will be recorded and then the sonde will be raised through the water column stopping at each 1 meter interval to allow equilibration and recording of readings until a depth of 1 meter below the surface has been reached. All calibrations and maintenance on the YSI 600 XLM sonde is completed in accordance with the YSI, Inc. operating manual methods (YSI 6-series Environmental Monitoring Systems Manual; YSI, Inc. Yellow Springs, OH).

3.1.6.4. Field Duplicates

At one verification station, during each individual sampling event, a duplicate sample for all sample types and measurements will be taken. The duplicate information will be used to assess the precision of the sample results and measurements. Field duplicates are taken in quick succession with the initial sample from the outflow of the DATAFLOW system, in separate sample bottles. The precision of field duplicate pairs will be assessed by a combination of relative percent difference (RPD) based on the previous year's average RPD, and absolute difference. If a duplicate's RPD falls outside of two (2) standard deviations of the average it will be considered a warning, outside of three (3) standard deviations is considered failure. Under both circumstances, investigation into the possible source of the issue will ensue.

3.1.6.5. Field Blanks

Artificial sample contamination in the field represents an issue of concern for chlorophyll sampling equipment and bottles. To address these concerns the sample bottles and equipment are prepared and cleaned according to the procedures indicated in Section 4.1.2. Field blanks are collected by processing DI water through the filtration apparatus (including filters) while on location at one verification station per individual field event. Field results will be used to determine if contamination has occurred in the sampling process. Decisions

to invalidate associated project data on the basis of artificial sample contamination will be made based on a comparison of the field blank with the lowest analytical standard in the calibration curve and the detection limit for each parameter. If the concentration of a field blank exceeds the lowest analytical standard in the calibration curve field and/or laboratory contamination is suspected, corrective action will be initiated. This will include investigation of possible contamination sources (both in the field and in the laboratory) as well as potential procedural modifications to alleviate the problem.

3.1.6.6. Field Documentation and Records

All field data are recorded on specially prepared field data sheets. The raw data sheets are reviewed for possible missing data values due to sample collection problems prior to data entry. These sheets are initialed and filed by the Quality Assurance Officer/Data Manager.

3.2. Fixed Station Water Quality Monitoring in the Lower James River

3.2.1. Fixed Monitoring Stations Description

One continuous fixed monitoring station is located at along the JMSPH north shore littoral zone (Appendix A; Figure 3). The fixed station is used to continuously monitor water quality parameters including depth, water temperature, salinity, pH, chlorophyll, turbidity, and dissolved oxygen using YSI 6600EDS V2 data sondes. These stations will provide long term measurements that can be supplemented with shorter term monitoring, such as the DATAFLOW cruises, and other process-oriented studies.

The fixed station is designed to be deployed off of an existing pier or other structure within a locked 4 inch PVC housing. This housing is perforated to allow flow of water freely to the instrument while at the same time protecting the instrument from large pieces of floating debris. The stations are placed at a depth of 1.0 to 1.5 meters MLW. Within the PVC housing, an YSI 6600EDS V2 data logging sonde is secured 0.25-0.5 meters above the bottom. The PVC pipe structure is cleaned with a chimney sweep type tool at each switch out to reduce fouling and the collection of sediment on the inside of the pipe.

3.2.2. Locations of Monitoring Station and Sample Frequency

There is one continuous monitoring station located along the north shoreline of segment JMSMH (Appendix A, Table 3).

Table 3. Locations of All Sampling Stations within James River Oligohaline and Mesohaline Segments

Station	Type	CBP Segment	Latitude	Longitude
JMSOH1	DATAFLOW Verification	JMSOH	37.21335	-76.91730
JMSOH2	DATAFLOW Verification	JMSOH	37.20294	-76.78219
JMSOH3	DATAFLOW Verification	JMSOH	37.20297	-76.64833
JMSOH4	DATAFLOW Verification	JMSOH	37.22775	-76.79147
JMSOH5	DATAFLOW Verification	JMSOH	37.23980	-76.87915
CMON	FIXED Continuous	JMSMH	37.04883	-76.50450

An individual YSI 6600EDS V2 sonde will be deployed for a maximum of 14 days on permanently established stations, where they will log data at 15-minute intervals. Deployment length is adjusted for areas and periods of high fouling. At the end of the deployment period the YSI sonde is returned to the lab for downloading of data, post calibration, cleaning, membrane replacement, and re-calibration. A second YSI 6600EDS V2 is deployed following retrieval of the original sonde in order to maintain a continuous record of data. The two sondes are placed adjacent to one another for 20 minutes to allow for post comparison of the individual units and to allow for no loss of monitoring data.

3.2.3. Water Quality Instrumentation

The fixed station utilizes the YSI 6600EDS V2 equipped with the Clean Sweep Extended Deployment System (EDS) and with sensors including a ROX 6150 Optical DO probe, a YSI 6560 conductivity/temperature probe, a 6561 pH probe, a 6136 turbidity probe, and a 6025 chlorophyll probe. The EDS is comprised of a brush that at set intervals sweeps across the sensors to dislodge any fouling organisms or material that has settled on the sensors. This feature ensures better quality data over longer deployment periods in areas with high fouling rates. The new YSI ROX DO probe utilizes the luminescence-lifetime technique to provide DO measurements which are less likely to be affected by fouling or low DO environments.

Table 4. Quality of YSI Data (YSI 6600EDS V2)

PARAMETER	UNITS	PRECISION	ACCURACY	MDL
ODO	% Saturation	0.1%	±1%	0 %
ODO	mg/L	0.01mg/L	±0.1mg/L	0 mg/L
Salinity	ppt	0.01ppt	0.1ppt	0 ppt
Temperature	°C	0.01°C	±0.15°C	-5°C
pH	unit	0.01units	±0.2units	0 units
Turbidity	NTU	0.1NTU	2 NTU	0 NTU
Chlorophyll	µg/L Chl	0.1µg/L Chl	-	0 µg/L Chl

3.2.4. Schedule of Maintenance and Sonde Exchange

Sondes are deployed for a maximum of 14 days. Upon return to the laboratory the sondes are post calibrated, cleaned, and routine maintenance is performed as needed. The sondes are then stored until they are calibrated just prior to their next deployment. All calibrations and maintenance are completed in accordance with the YSI, Inc. operating manual methods (YSI 6-series Environmental Monitoring Systems Manual; YSI, Inc. Yellow Springs, OH). See Appendix C for more detailed information.

3.2.5. Sampling Procedures

3.2.5.1. Verification Samples

Field verification of pH, salinity, dissolved oxygen and temperature are taken during the deployment/retrieval procedure with a YSI 6920 sonde. Water samples for total suspended solids (TSS), volatile suspended (VSS), chlorophyll-a, and pheophytin are taken when the YSIs are switched out at the depth of the instrumentation. Chlorophyll and pheophytin water samples are immediately filtered and the filter stored in sterile Whirlpak bags (See Appendix D for further details). These are then packed on ice and returned to the laboratory where they are stored at -20°C. Samples for TSS and VSS are packed on ice and returned to the laboratory where they are filtered immediately upon return and frozen (Appendix D). Samples are then delivered to the VIMS Analytical Service Center for further processing. At these stations secchi depth, a vertical profile of photosynthetically available radiation (PAR), as well as a vertical profile for temperature, dissolved oxygen, salinity and pH are also done (See Appendix D for further details).

The data being gathered by the original YSI 6600EDS V2 is also verified by placing the new YSI 6600EDS V2 into the water beside it for a 20 minute time period at the end of its deployment. The two data sets can then be compared to determine that the YSIs are functioning correctly.

33.2.5.2. Vertical Profile of Dissolved Oxygen, Water Temperature, and Salinity

A vertical profile of the water column is accomplished using a YSI 6920 which includes a ROX 6150 Optical DO probe, a YSI 6560 conductivity/temperature probe, and a 6561 pH probe. This profile includes water temperature, specific conductivity, salinity, and dissolved oxygen and pH. This is accomplished by placing the sensor at a 0.1 meter depth, letting it equilibrate and recording the measurements. The same procedure is then followed at successive 0.25 m intervals thereafter until the final reading is taken approximately 5 cm above the bottom. All calibrations and maintenance on the YSI 6920 sonde is completed in accordance with the YSI, Inc. operating manual methods (YSI 6-series Environmental Monitoring Systems Manual; YSI, Inc. Yellow Springs, OH).

3.2.5.3. Summary Table of Monitoring Parameters

Table 5.

PARAMETER	COLLECTION PROCEDURE	PRESER-VATION	PERFORMS ANALYSIS	DETECTION LIMITS	CBP (CIMS) METHOD (unless noted otherwise)
Temperature	YSI 6600V2/6920		Field		F01
pH	YSI 6600 V2/6920		Field		F01
Dissolved Oxygen	YSI 6600 V2/6920		Field		F01
Specific Conductivity	YSI 6600 V2/6920		Field		F01
Salinity	YSI 6600 V2/6920		Field		F01
Fluorescence	YSI6600 V2		Field		NA
Chlorophyll	YSI6600 V2		Field		F01
Secchi Depth	Secchi Disk		Field		F01
PAR Light Attenuation	Li-Cor		Field		F01
Total Suspended Solids	Whole water	ICE	ASC	1.4 mg/l	L01
Fixed Suspended Solids	Whole water	ICE	ASC	2.5 mg/l	L01
Volatile Suspended Solids	Whole water	ICE	ASC	2.5 mg/l	L01
Chlorophyll a	GF/F Filter 47 mm diameter 0.7 um pore size	ICE	ASC	0.50 ug/L	L03
Phaeophytin a	GF/F Filter 47 mm diameter 0.7 um pore size	ICE	ASC	0.50 ug/L	L03

3.2.5.4. Field Documents and Records

All field data are recorded on specially prepared field data sheets. The initials of the person recording the data are recorded on each data sheet. The raw data sheets are reviewed for possible missing data values due to sample collection problems prior to data entry. These sheets are filed with the Laboratory Supervisor.

4. DATA MANAGEMENT PROCEDURES

4.1. QA/QC Field Checks

The objectives of QA/QC standards are to provide accurate measurement of water quality relative to phytoplankton levels and composition. Over the 15 years that the VIMS water quality research group has been making similar measurements, consistent protocols have been developed in the field. Together with laboratory procedures already in place and used by the Analytical Services Center (ASC) and data management procedures, the data obtained can be analyzed and interpreted so that the final report submitted will meet the objectives stated for this study. Information derived from the report will be useful to managers making decisions concerning these areas, as well as comparable to water quality data being generated in other areas and by other partners such as the Hampton roads Sanitary District (HRSD)..

4.1.1. Preparation of Field Gear

During the few days prior to initiating a DATAFLOW or sonde deployment/retrieval cruise all the necessary equipment involved in the collection of water samples and of physical water quality data are inventoried according to "checklists." All equipment is checked to insure that it is fully operational and has been properly cleaned. The equipment is packed into containers that provide for easy transport and loaded aboard the research vessel. The "checklist" is then re-examined to verify the presence of all necessary gear. Standards and reagents used in the calibration of instrumentation are made according to a schedule of shelf life or if the supply is exhausted. All chemicals are handled, prepared, and stored in accordance with standard laboratory practices.

4.1.2. Potential Contamination

During the course of a research cruise different steps are taken to insure that the chances for contamination are minimized. All containers used to collect bulk raw water are rinsed three times with sample (station) water before they are filled and are thoroughly cleaned with an acid wash and rinsed with deionized water and dried at the end of the cruise. All glassware

associated with the preparation of standards and reagents is cleaned with deionized water and acid washed when appropriate.

Another area of potential contamination is the DATAFLOW system itself. Though we have never seen fouling or growth in the tubing, we do take measures to prevent any contamination. During each use, large volumes of water are pumped at a very high velocity through the system which prevents growth. After each use the intake, hoses and flow through cells are taken apart and allowed to dry completely and stored in the laboratory until they are reassembled for the next use. Periodically the components are rinsed with freshwater. The system is examined before each use for any fouling or growth.

4.1.3. Calibration Procedures and Frequency

All instruments (YSI 6600, YSI 6600 V2, YSI 600, and YSI 6920) are maintained in accordance with manufacturer's specifications. Sensors involved in the collection of water quality data (temperature, conductivity, salinity, pH, dissolved oxygen, turbidity, and chlorophyll) are calibrated just prior to each deployment. Standards and reagents involved in the calibration of instrumentation are made according to a schedule of shelf life (i.e. daily, weekly or seasonally) or if the supply is exhausted. All chemicals are handled, prepared and stored in accordance with standard laboratory practices. If any apparent problems arise the instrument is removed from use until the malfunction can be diagnosed and remedied.

The manufacturer calibrates temperature when the instrument is returned for service. The thermistor is also checked yearly against a NIST certified thermometer. For the Continuous Monitoring Program the Conductivity standard has a Specific Conductance of 10 mS/cm and is purchased from Fischer Scientific. For the Dataflow program a 0.2 molar standard solution of potassium chloride is made using DI water and Mallinckrodt Granular Potassium Chloride (FW 47.56) purchased from VWR. The solution is made by weighing out 29.82g of KCl (which has been dried and desiccated) using an analytical balance and adding it to a volumetric flask. The level of DI water is then brought up to 1000mL resulting in a standard of 24.82mS/cm. A 3-point calibration is used to calibrate the pH sensor, this includes a pH of 4.0, 7.0, and 10.0 (VWR Buffer Solutions). Dissolved oxygen calibration incorporates a standard air calibration based on air temperature and barometric pressure or for the Optical DO probe an air saturated water calibration technique. A 2-point calibration is used for calibration of the transmissometer (turbidity sensor): deionized water (0 NTU) and a YSI NTU turbidity standard at a level of 123 NTU. A 2-point calibration is also used for the fluorometer: deionized water (0 ug/l) and an upper standard made with Rhodamine WT and deionized water (See Appendix C for more detailed information).

For transmittance and fluorescence, the manufacturer also recommends that the instrument be verified against in-situ properties measured in the field. This involves collecting verification samples during deployment that are analyzed for total and active chlorophyll-a and total

suspended solids concentrations. These field standards are related to sensor readings via multiple regression procedures.

At all verification stations light attenuation measurements are taken using a Li-COR LI192SA sensor to relate to turbidity measurements gathered from YSI 6600 turbidity sensor as well as to the total suspended solid numbers.

Table 6. Quality of Verification Data

PARAMETER	UNITS	PRECISION	MDL	Method
PAR	μ mol/sec/m ²	-	1 μ mol/sec/m ²	LI-COR 190SA
Light Attenuation	-	-	0.5% @ 100% light	Li-COR 192SA
Secchi Disk	m	-	0.1m	
TSS	mg/L	6 mg/L	2 mg/L	Gravimetric
Chlorophyll	μ g/L	3.29 μ g/L	0.95 μ g/L	Fluorescence

(See appendix D for more information on laboratory SOPs and methodologies)

4.1.3.1. Continuous Surface Water Quality Mapping (DATAFLOW)

4.1.3.1.1. Documents and Records

A cruise checklist is filled out prior to each cruise to ensure all field equipment is in order. A calibration sheet is filled out for both the YSI 600 and 6600 sondes. Data generated from the DATAFLOW System is continuously written to an ASCII text file on the hard disk of the unit. After each cruise the data is copied to backup disk as well as stored on the centrally managed VIMS shared file space that is backed up daily. Calibration station data are first recorded on data sheets, which are copied and stored with the Quality Assurance Officer, then transferred to electronic files, which are stored in an Access database. All field data and observations are recorded on specially prepared field data sheets. The initials of the person recording the data are recorded on each sheet. These sheets are filed with the Quality Assurance Officer. (See Appendix B for copies of all documents for field and lab data).

4.1.3.2. Fixed Station Water Quality Monitoring

4.1.3.2.1. Documents and Records

A calibration sheet will be filled out for each YSI 6600 V2 as it is prepared to be placed out into the field. Verification samples and any information gathered during the retrieval/deployment process, including vertical profiles of dissolved oxygen, salinity and

water temperature, are recorded on data sheets. These are initialed by the field personnel and filed by the Laboratory Supervisor.

4.2. Description of Individual Data Sets

4.2.1. Continuous Surface Water Quality Mapping (DATAFLOW) in the James River Oligohaline

Two data sets contain the continuous surface water quality measurements.

-The sampling station field verification data set contains: date, time, latitude, longitude, total depth; concurrent instrument readings for temperature, specific conductivity, salinity, dissolved oxygen, pH, fluorescence, and turbidity; laboratory analyses for active chlorophyll-a, total chlorophyll-a, TSS, VSS, secchi depth, light attenuation and dissolved oxygen, water temperature, pH, and salinity for the vertical profile.

-The continuously measured DATAFLOW screened data set contains: date, time, depth, latitude, longitude for each record of water temperature, specific conductivity, salinity, dissolved oxygen, pH, chlorophyll and turbidity.

4.2.2. Fixed Station Water Quality Monitoring in the James River Mesohaline

Two data sets are generated at the fixed stations.

-The sampling station field verification data set contains: date, time, latitude, longitude, total depth; concurrent instrument readings for temperature, salinity, dissolved oxygen, pH, fluorescence, and turbidity; laboratory analyses for active chlorophyll-a, total chlorophyll-a, TSS, VSS, secchi depth, light attenuation, and dissolved oxygen, water temperature, pH, and salinity for the vertical profile.

-The continuously measured data set contains: date, time, water temperature, salinity, dissolved oxygen, pH, chlorophyll, turbidity and depth values.

4.3. General Information Related to Data Sets

4.3.1. Continuous Surface Water Quality Mapping (DATAFLOW)

4.3.1.1. Raw Data Set Retrieval

The data are continuously transferred electronically from the YSI 6600 to the computer hard drive during each day of sampling. The data are stored as an ASCII text file. This file is retained indefinitely as the original record of the data collection. Upon completion of the cruise, data is retrieved from the Panasonic Toughbook laptop using a USB drive.

4.3.1.2. Initial QA/QC of Data Set

Very little post-processing is required before the data can be used. A macro has been developed that takes the raw downloaded dataflow text file and breaks it into columns, puts headers on the columns, changes the latitude and longitude to decimal degrees and highlights cells that have incorrectly formatted data (e.g. if there is text where there should be numbers). The data manager then looks over the data file, first focusing on the cells the macro has highlighted. The next step is comparing field notes to the data and highlighting areas of “bad” data (e.g. increased turbidity due to being too shallow while coming onto station or time spans of a clogged intake etc). Each parameter is then graphed to look for things such as single high spikes in the optical data that are questionable, strange trends in DO data (e.g. steadily decreasing levels that could indicate degradation in the electrolyte solution), or any other issues with probes that might appear in the data. These are all highlighted so that the associated error codes can be attributed to the data. Another step when dealing with both sets of data is to examine the YSI post calibration data and be sure that any drifts in the probes are within acceptable ranges. These ranges are listed in the table below. Once these checks have been accomplished, the data can be put into the final form for submission and the appropriate error codes are used to tag erroneous observations. (See Appendix E for a more detailed protocol for the processing of DATAFLOW data.)

Parameter	Value
Chlorophyll	±5 % of true value
Dissolved Oxygen	±0.5 mg/L
pH	±0.2 pH units
Specific Conductance	±5 % of true value
Turbidity	±5 % of true value
Water Temperature	±0.2 °C

Table 7. Allowable drift in YSI sensor readings

4.3.1.3. Further Data Review

As indicated in section 3.1.6.1. of this document, project data is routinely monitored on a real-time basis for potential problems. Additional data reviews are conducted subsequent to the completion of the monitoring events as follows: As soon as the cruise is completed the Quality Assurance Officer/Data Manager downloads the raw data file and reviews the field documentation for legibility, accuracy, and completeness. Any issues are discussed with the field crew and the field sheets are initialed as being complete. Once reviewed the verification station information contained in the field sheets are transcribed onto an electronic version (Excel or ACCESS) and these files are subsequently examined for accuracy and completeness. The information contained on the electronic versions of the field sheets is then incorporated into the verification data database. Subsequent QA/QC of this dataset, as well as data analysis of the data by the Quality Assurance Officer (QAO) offers yet another opportunity to detect and correct any remaining errors which may have been over looked in the reviews and/or audits.

4.3.1.4 Comparisons between DATAFLOW and Laboratory Derived Results

As previously described, the total chlorophyll values as determined by the YSI 6600 sondes can be corrected by results obtained in the laboratory as well as with turbidity values. The goodness of fit (measured as a regression R^2 value) between laboratory derived chlorophyll a and corrected YSI will be statistically evaluated. Light attenuation (K_d) will be predicted by determining statistical relationships between YSI turbidity (NTU) and measured LiCor results (K_d) determined at the verification stations. The goodness of fit (measured as a regression R^2 value) between LiCor measurements and YSI turbidity will be statistically evaluated.

4.3.1.5. Processing of Data

After the data is examined and coded, a final version is stored on the centrally managed VECOS database that is stored in VIMS shared file space and is backed up daily. The data is then imported into ArcMap and maps of the surface water quality conditions are produced using GIS software (ESRI ArcInfo). (See Appendix F for a more detailed GIS protocol to generate interpolations).

4.3.2. Fixed Station Water Quality Monitoring

4.3.2.1. Raw Data Set Retrieval

The data from the YSI 6600EDS V2 are uploaded to a Personal Computer (IBM compatible) upon return to the lab. Files are uploaded to a PC in a comma-delimited format. These are then backed up.

4.3.2.2. Initial QA/QC of Data Set

For the continuous monitoring stations, the data is downloaded into Ecowatch. In this program, graphs are created of each parameter and outliers are examined as well as any data trends which indicate trouble with individual probes. Depth data is compared with the rest of the data so that data can be tagged if the sonde was out of water and optical data is closely examined for periods where wipers may have seated over optics, etc. The same issues with the data/probes that are listed in the dataflow portion are examined in the continuous monitoring dataset. With the continuous monitoring stations, the dataset must also be joined to form a seamless file for the year's deployment. The times associated with any missing data are inserted and the appropriate error codes are placed in the cells to explain the absence of data. As with the Dataflow, YSI post calibration data is checked to be sure that any drifts in the probes are within acceptable ranges. Once these checks have been accomplished, the data can be put into the final form for submission and the appropriate error codes are used to tag erroneous observations. (For acceptable drifts see table above in section 4.3.1.2.) (See Appendix G for summary of QA for the Fixed Station data)

4.3.2.3. Processing of Data

Missing data due to YSI maintenance (down time) are inserted into the spreadsheet and are denoted by time stamps with no records. Edited files are merged to contain one full year of data for each station. Microsoft Excel macros provided by the NERR Centralized Data Management Office are used to discover data set outliers (values which fall outside the range that the instrument is designed to measure) and other erroneous data. Suspect data are evaluated, edited, and documented in each site's metadata document (data documentation)

4.4. Analytical Methods QA/QC

4.4.1 Analytical Services Center (ASC)

The Analytical Services Center (ASC) at VIMS provides water quality analyses to University, State and Federal agencies. As part of the laboratory's QA/QC program, ASC will participate in cross calibration exercises with other institutions and agencies whenever possible. Some examples include:

- Quarterly cross calibration exercises with the Chesapeake Biological Laboratory (CBL) and Old Dominion University (ODU) in conjunction with the Chesapeake Bay Program's Coordinated Split Sample Program (CSSP).
- Environmental Protection Agency (EPA) unknown audits for various nutrients have been conducted.

-EPA audits of known nutrients were analyzed using samples in different salinity water while looking for possible matrix effects.

-The Chesapeake Bay blind audit sample program which includes dissolved nutrients, particulate carbon, nitrogen and phosphorus, and chlorophyll.

As part of the Chesapeake Bay Tributary Monitoring Program, the laboratory analyzed approximately ten percent of the total sample load for QA/QC checks. These samples included laboratory duplicates and spike analyses.

4.4.2 “Bottle Blanks”

“Bottle Blanks” are necessary for the Whirlpak bags, which are used to collect sample filtrate in the field. Whirlpak bags are tested for contamination by selecting a number of bags (5) from each lot, filling them with deionized water, freezing them, and then analyzing the water for ammonium, nitrate, nitrite, and orthophosphate.

4.4.3. Sample Custody

Upon arrival at the laboratory, samples are counted, observed for potential problems (melting, broken containers, etc.), processed, and placed in a freezer until analysis. Sample information and date of arrival, times of filtering and storage by freezing are recorded on a log sheet.

4.4.4. Instrument Maintenance

Analytical instruments are maintained on a regular basis and records are kept of hours of operation, scheduled maintenance, pump tube changes, etc. A critical spare parts inventory is maintained for each instrument. Instrument down time is minimized by troubleshooting instrument problems telephonically with manufacturers and service representatives. Spare parts can be received within 24 hours via next-day air service. Backup YSI 6600EDS V2 sondes are also maintained as backup instruments.

4.4.5. Data Handling

Data are entered in a pre-determined format in an Access Database on a computer. Any necessary corrections are noted and made. Any data errors are given specific error codes. All data are backed up on tape on VIMS server. Specific data handling procedures are provided above and will be provided in the data management protocol.

4.4.6. Data Analysis

Preliminary data analysis is performed in which data from the DATAFLOW system is interpolated over 25m cells for the given study sites using kriging techniques with the software package Geostatistical Analyst (ArcGIS). This data is served, as interim data, on the program website www.vecos.org.

4.4.7. Data Submission

Data will be formatted according to EPA standards and protocols. As QA/QC on the mapping and fixed station data is completed it will be uploaded to a database and made available for download by VaDEQ. All data will be provided to VaDEQ as they request at the end of the grant period.

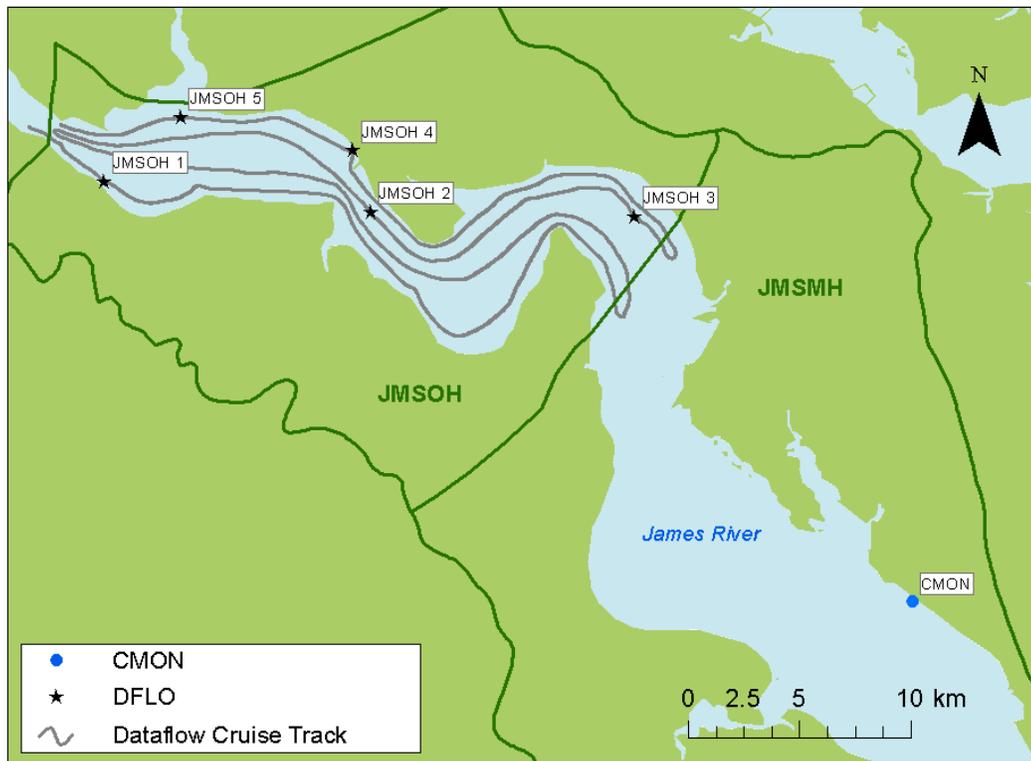
4.5. Schedule of Deliverables

PROGRESS REPORTS: Quarterly status reports will be submitted at every third month commencing with actual award date April 1, 2013 (July 15, Oct 15, Jan 15, 2014). These reports will contain the following sections: Description of tasks; Accomplishments this quarter; Issues or problems this quarter and impact on future deliverables; Objectives/deliverables for next quarter. VIMS is an institution of higher education located in Virginia performing analyses for the purpose of providing environmental research data to DEQ. Therefore, we are not subject to the requirements of either 1VAC30-45 or 1VAC30-46

(<http://www.dgs.state.va.us/LinkClick.aspx?fileticket=sE5V%2fqtrNfE%3d&tabid=1059>).

During the study outlined above we will meet or exceed the QA/QC requirements specified by DEQ and the EPA CBPO. A final Report of findings is due on April 15, 2014.

Appendix A: Map of Study Site Locations



Locations of DATAFLOW (DFLO) cruise track (grey lines), DATAFLOW verification/calibration sampling stations (stars) and fixed continuous monitoring (CMON) station (filled circle).

Appendix B: Examples of calibration worksheets, field checklist, and field datasheets.

Calibration Work Sheet – Dataflow

Date of Calibration: _____ Technician: _____

DO membrane changed? Y N Note: Wait 6-8 hours before final DO calibration.

Turbidity wiper changed? Y N Wiper Parks ≈ 180° from optics? Y N

Chlorophyll wiper changed? Y N Wiper Parks ≈ 180° from optics? Y N

Record Battery: _____

DO Warmup 60sec Y N Wiper Interval 15 Y N RS232 Sleep Disabled Y N

Record Calibration Values			Record diagnostics <u>after</u> calibration.	
	Actual	Sonde after cal		
Conductivity	_____	_____	Conductivity constant _____	Range 5.0 ±.5
pH (mid)	_____	_____	pH MV mid buffer _____	Range +180 ±50
pH (high)	_____	_____	pH MV High buffer _____	Range -180 ±50
			Span between the 7 and 10 MV numbers should be	
			≈ 170 – 180 MV.	
Turbidity (0)	_____	_____	(if post cal # for 0 turbidity is > +/- 2.0 see manual!)	
Turbidity (123)	_____	_____		
Chlorophyll (0)	_____	_____		
Chlorophyll (100)	_____	_____	DO Charge _____	Range 50 ±25
Dissolved Oxygen	_____	_____	DO Gain _____	Range 1.0 -.3 to +.4
Barometric Pressure	_____		Low charge: low electrolyte or tear High charge: Oxidation of probe	

DO Calibration Hi/Lo Transmission Test _____ Accept _____ Reject

Morning of Deployment

Dissolved Oxygen _____ DO Charge _____ Range 50 ±25
 Barometric Pressure _____ DO Gain _____ Range 1.0 -.3 to +.4

Proper parameters selected in Report Menu?? Y N
 Time, Temp (C), SpCond (mS/cm), Sal (ppt), DO (%), DO (mg/l), pH, Turbidity (NTU), Chlorophyll (mg/l),
 Battery (v)

Post Calibration:

Conductivity (24.82): _____ pH (6.86): _____ Turbidity (0): _____
 Chlorophyll (0): _____ Battery: _____
 DO (precal): _____ DO (postcal): _____ Barometer: _____
 If any parameter has drifted can do a more indepth post cal (eg high and low standard for Chl/Turb.)

Notes:

JMSOH Cruise Check List

Cruise Date: _____
Dataflow Operator: _____

2013 VIMS QAPP

Dissolved Oxygen/LaMotte Kit

- Sample Bottles
- Lamotte Kit
- DI Water Rinse Bottle

Filtration Kit

- Whirlpak Bags - Chlorophyll
- 100 ml. Graduated Cylinder
- 5ml Pipette
- Pipette Tips
- Vacuum Hand Pump
- Receptacle Flask
- Chlorophyll Chimney
- Chlorophyll Frit
- Chlorophyll Clamp
- Filter Forceps (2)
- Foil for Chlorophyll Filters
- Chlorophyll Filters
- DI Rinse Squirt Bottle
- Kim-wipes
- Sharpie
- Extra Whirlpaks
- Trash Bin
- Ziploc Bags for Samples

Sample Coolers

- Large Sample Cooler
- Small Sample Cooler
- Large Nalgene Sample Bottles
- Small Nalgene Sample Bottles
- Large Nalgene with DI Water
- Ice

Light Attenuation Kit

- Datalogger
- Deck Sensor
- Underwater Pole/Sensor
- Secchi Disk

Clipboard

- Laminated Station Map
- Sample Protocols
- Field Data Sheets
- DO Profile Data Sheets
- Kestrel Handheld
- Pencils
- Sharpies
- Extra clipboard

Computer Equipment

- Toughbook Computer (TB2 and TB3)
- TB2 and TB3 Container
- Powercord
- Dual Serial Cord
- GPS to Computer Cable
- Wireless Modem (optional)
- Computer Cig Lighter Adapter

Dataflow Equipment

- YSI to Computer Cable
- YSI 6600
- YSI 6600 Backup
- YSI 600 w/handheld (long cable)
- Flow through Cell
- Intake Hose
- Effluent Hose
- Hose Clamps (3)
- Bungee Cords
- Ram Intake with Pump
- Spare Pump
- Bucket
- Spare PVC Intakes

Other Essentials

- Spare Batteries
- Spare DO Membranes
- Spare DO Electrolyte
- Spare fuses
- Toolbox
- Towels

***Insure correct datalogger matched with sensors
 ***Insure correct computer/power cord combination
 ***Take both computers if possible

***Double check GPS & Appropriate data card on board before leaving VIMS

Water Monitoring – Dataflow Field Sheet

Date: _____ Cruise Location: James River (Day 1)
 Personnel: _____
 Sample Location: James River Station (JMSOH1)
 Time: _____ DF 6600 ID: _____ Sample Bottle #: 1
 Computer File Name (s): _____

DF 6600 Data (on station): YSI-600 ID # _____ for Verification:

Time (on DF): _____
 Dissolved Oxygen (%): _____ Dissolved Oxygen (%): _____
 Dissolved Oxygen (mg/l): _____ Dissolved Oxygen (mg/l): _____
 Water Temperature: _____ Water Temperature: _____
 Salinity: _____ Salinity: _____
 pH: _____ pH: _____
 Turbidity _____
 Chlorophyll _____

Station Conditions:

Station Depth: _____
 Air Temp: _____
 Wind Speed (m/s): _____
 Wind Direction: _____
 Cloud Cover (0-6): _____
 Wave Condition (0-5): _____
 Precipitation Type (10-16): _____
 Tide Stage (E, F, H, L): _____

Other Data:

Secchi Depth: _____
 Light Attenuation Time: _____
 Chl Amt Filter: _____

Make Note of Agreement btw 6600/600:

D.O. should be within 0.5 mg/l
 Salinity should be within 0.4 ppt
 Temperature should be within 0.25 degrees

Comments:

DF 6600 (averages - lab use only):

Latitude: _____
 Longitude: _____
 Depth: _____
 Water Temp: _____
 Salinity: _____
 pH: _____
 Dissolved Oxygen (%): _____
 Dissolved Oxygen (mg/l): _____
 Turbidity: _____
 Chlorophyll: _____

Data entered/edited by _____

Revised 4/26/10

WQ Initiative YSI 6600-V2 ROX CALIBRATION, DEPLOYMENT+RETRIEVAL LOG

DataLogger ID: _____ **RIVER SYSTEM:** _____
Date (MM/DD/YY): _____ **Time correct (EST):** YES NO
Version: _____ **Sonde Serial Number:** _____ **Calibrated By:** _____
 Conductivity Probe ID: _____ pH probe ID: _____ Optical DO Probe ID: _____
 Turb. Probe ID: _____ Chlorophyll probe ID: _____ OTHER ID: _____
Battery Volts: _____ **Changed batteries?:** YES NO
 Turbidity Wiper Changed: Y N ; Wiper parks ~180° from optics: Y N
 Chlorophyll Wiper Changed: Y N ; Wiper parks ~180° from optics: Y N
 Optical DO Wiper Changed: Y N ; Wiper Parks ~180° from optics: Y N
Calibration: (enter standard)
Spec. Cond. : _____ error message: _____
 pH 7 : _____ error message: _____
 pH 10 : _____ error message: _____
 pH 4 : _____ error message: _____
Turbidity : (0) _____ (123) _____ error message: _____
 Depth : _____ (Depth Offset: (mmHg): _____ = _____ (m)) cal'd to: _____
ROX DO%:** _____/_____/_____ *Temperature stabilized? Yes No**
 Barometric press: (mm Hg) _____ = _____ (millibars)
 Date and time recorded: _____ (_____%)
Record the following diagnostic numbers after calibration:
 Conductivity Cell Constant _____ (range 4.6 to 5.45)
 DO Gain _____ (range 0.8 to 1.7)
 pH MV Buffer 7 _____ (range 0 mv ± 50 mv) **Slope →** $mV(7) - mV(10) =$ _____
 pH MV Buffer 10 _____ (range -180 mv ± 50 mv) (should be 165-180)
 pH MV Buffer 4 _____ (range +180 mv ± 50 mv)
 Notes: _____

Programming:
 Deleted files (circle one): YES NO Disk Free Space: _____ (bytes)
 Parameters: date, time, temp (C), SpCond (mS/cm), Sal (ppt), DOSAT%, DO (mg/L), Depth (m), pH, Turb (NTU), Batt

Datalogger FILENAME (SSyymmdd): _____

Chlorophyll calibration 2 pt calibration using Rhodamine WT		
DI water (0)	Temp of solution	µg/L
	_____ °C = _____ µg/L]	

Verification Sample	Deploy DL#	Retrieve DL#
DO mg/L		
Mini %	_____	_____
Mini mg/L		
Temp A		
W		
Turb/secchi	cm	cm
Salinity	ppt	ppt
pH		

Free memory - _____ days
 Battery life - _____ days
 Sampling interval: 15 - minute
 Sampling duration: "365 days"

Start Date (mm/dd/yy): ___/___/___ **Start Time (HH:MM):** ___:___ (:14 :29 :44 :59)
 : Hp gnof rof XOR no repiw delgA

Checked Logging :
Sample Hold (CO, ___):

Datalogger Deployment: SITE: _____
 Date (mm/dd/yy): ___/___/___ Time (HH:MM, LST): ___:___:___
 Weather: _____ **TIDE:** Flood, Ebb, Slack_High, Slack_Low
 Notes: ___current wind spd _____ m/s Rel Humidity _____ %

Datalogger Retrieval:
 Date (mm/dd/yy): ___/___/___ Time (HH:MM, LST): ___:___:___
 Weather: _____ **TIDE:** Flood, Ebb, Slack_High, Slack_Low
 Notes: ___current wind spd _____ m/s Rel Humidity _____ %

Post Deployment Calibration Check: SONDE ID: _____

Post Calibrated by: _____
 Date : _____ Time correct: _____

Stopped Logging : _____ @ _____ Battery volts: _____

Downloaded files: _____ Disabled SAMPLE HOLD:

FILE NAME: _____ .dat _____ .txt

Sp Cond: _____ Cond Cell Constant: _____ (range 4.6 to 5.45)

pH 7 : _____ pH mv _____ (range 0 mv ± 50 mv) *Clean Sonde Filtrate:

pH 10: _____ pH mv _____ (range -180 mv ± 50 mv) CHL : _____ µg/L

pH 4 : _____ pH mv _____ (range +180 mv ± 50 mv) RFU/FS: _____ %

Turbidity [0]: _____ Turbidity (123) : _____

CHL [0] : _____

% FS/ RFU: [0] _____

(Chl temp @ _____ °C = _____ ug/L)
CHL [_____] : reading _____ µg/L

DEPTH : _____ [Depth Offset: (mmHg)
 _____ = _____ (m)]

ROX % DO : _____ % DO GAIN: _____

DO NOTES: _____

Barometric Pressure (mmHg) _____ = _____ (millibars)
 Time/Date recorded: _____ [mmHg / 7.6 = ~ theoretical sat'n DO]

Wipers park: _____ Turb _____ Chla _____ ODO _____ other

Wipers changed : _____ Turb _____ Chla _____ ODO _____ other

Cleaned unit: _____ Changed Batteries: _____

SONDE LAB NOTES: _____

FIELD NOTES: _____

Verification Water sampling using MINISONDE @ 0.25 m from bottom:
 _____ °C _____ ppt _____ pH _____ DO % _____ DO mg/L

WATER QUALITY MONITORING DEPLOYMENT AND RETRIEVAL LOG
 CBNERR-FIELD-FRM-YSI DEPLOYMENT AND RETRIEVAL LOG

	mm	dd	yy
DATE			

Field Location		Crew	
----------------	--	------	--

DATALOGGER INFORMATION		
	YSI ID Number	Time (EST)
Deployment (in)		
Retrieval (out)		

WEATHER INFORMATION						
Weather Conditions measured with Kestrel		Wind Speed			Cloud Cover	
Current Wind Speed (m/s)		0	0-1 (knots)	0-1 (m/s)	0	Clear (0-10%)
		1	>1 - 10	1-5	1	Scatter/partly Cloudy (10-50%)
Air Temp (C)		2	>10 - 20	5-10	2	Partly to Broken (50-90%)
		3	>20 - 30	10-15	3	Overcast (>90%)
Relative Humidity (%)		4	>30 - 40	15-21	4	Foggy
		5	> 40	21-26	5	Hazy
					6	Cloud (no percentages)

Precipitation Type		Wind Direction			
10	None	E	fr East (90 deg)	S	fr South (180 deg)
11	Drizzle	ENE	fr East NE (67.5 deg)	SE	fr SE (135 deg)
12	Light Rain	ESE	fr East SE (112.5 deg)	SSE	fr South SE (157.5 deg)
13	Heavy Rain	N	fr North (0 deg)	SSW	fr South SW (202.5 deg)
14	Squally	NE	fr NE (45 deg)	SW	fr SW (225 deg)
15	Frozen Precipitation	NNE	fr North NE (22.5 deg)	W	fr West (270 deg)
16	Mixed Rain&Snow	NNW	fr North NW (337.5 deg)	WNW	fr West NW (292.5 deg)
		NW	fr NW (315 deg)	WSW	fr West SW (247.5 deg)

WATER INFORMATION						
Water and Secchi Depths		Wave Heights		Tidal Stage		VERIFICATION SAMPLES
Water Depth (m)		0	0 <0.1m	(circle one)		Chla Filter Volume
		1	0.1 <0.3m	E	Ebb Tide	
Secchi Depth (m)		2	0.3 <0.6m	F	Flood Tide	
		3	0.6 <1.0m	H	High Tide	
Circle if secchi can be seen at bottom	SD >= WD	4	1.0 <1.3m	L	Low Tide	
		5	>1.3m			

WATER COLUMN DEPTH PROFILE						
Depth m	Temperature	SpCond	Salinity	DO(%Sat)	DO(mg/l)	pH
0.10						
0.25						
0.50						
0.75						
1.00						
1.25						
1.50						
1.75						
2.00						
2.25						
2.50						

Please circle Minisonde used today: #1 #2 #3

COMMENTS:

Appendix C: YSI 6-series Multiparameter Water Quality Monitoring Standard Operating Procedure

INTRODUCTION

This document outlines the standard operating procedures (SOP) for the collection of data using YSI 6600 series Multi-Parameter Water Quality Monitors (sondes). The COMMON and vertical profiling stations utilize the ROX optical dissolved oxygen probes (YSI 6600EDS V2) while the DATAFLOW mapping system utilizes the Rapid Pulse dissolved oxygen probes (YSI 6600), therefore different calibration procedures are used. Calibration procedures for all other parameters are identical.

I. PREDEPLOYMENT

Before the instrument can be deployed it must be calibrated and several maintenance steps performed. The instrument should be visually inspected for any abnormalities, such as a cracked pH probe or cracked bulkhead. A **YSI 6-Series Lab Calibration, Deployment, and Retrieval Log** must be completed for each instrument calibration, deployment, and retrieval procedures (Appendix B).

I.A: Probe Calibration Procedures:

During the calibration of the probes **NEVER** accept any calibrations that you have been given a warning message on. You must determine the cause of the problem, correct the problem, and recalibrate the probe before deploying the instrument. Standards must be active (check expiration date) and fresh for all calibrations. Previously used standards may be used to rinse probes but must not be used to calibrate. Discard and replace all expired standards.

Calibrations should only be done with a PC (e.g., desktop, laptop, palmtop). The DM610 will automatically accept the turbidity calibration which can lead to a calibration corruption (see section I.A.6). Therefore the DM610 should only be used to transfer or collect data.

Most system diagnostic parameters (e.g., DO charge, ISE charge, conductivity cell constant) are found in the “Cal-Constant” menu which you get to by selecting “Advanced” on the “Main” menu.

I.A.1.a: Dissolved Oxygen Calibration for YSI 6600 Rapid Pulse type sensor (DATAFLOW)

The dissolved oxygen membranes are to be replaced before each deployment. The oxygen probe needs to rest for a minimum of 6 hours (12 hours is optimal) after the membrane is changed to allow the probe to stabilize electro-chemically. Calibration must be done in the following order:

1. Replace DO membrane;
2. Place probe (or sonde) in calibration cup with a wet sponge and let rest for 6-12 hours;
3. After 6-12 hours, run instrument in discrete sample mode until the DO % reading stabilizes. If the DO probe is working correctly, the past 24 numbers (one screen width) should be the same indicating the probe has stabilized (do not

- worry about the actual number, you have not calibrated the probe yet);
4. Calibrate the DO sensor following YSI's instructions for 100% air saturation calibrations.
 5. Check the DO charge and gain. The DO gain is in the "Cal-constants" screen; the DO charge must be added to the report menu (remember to remove this from the report menu prior to programming). DO charge should be in the range of 25-75 counts. Counts below this range indicate low electrolyte or a tear in the membrane and counts above may be due to oxidation of the electrodes.
 6. Perform a high/low transmission test. Start another discrete sampling. If the %saturation is at or above 100% and then drifts down (or slightly up) to 100%, the probe is functioning properly. If the %saturation is very low or negative and then climbs to 100%, the probe is failing due to reverse polarity of the electrodes. If this happens, the probe is either in dire need of a reconditioning or will need to be replaced. Record either pass/fail on tracking sheet. **Do not deploy the probe if it fails this test. The data will not be valid.**

I.A.1.b.: YSI Alternate method

The above protocol (I.A.1.a.) is sufficient for collecting data at the accuracy we are striving for and will also identify a faulty probe. The benefits of this alternate method are to: 1) provide additional time to identify a faulty probe prior to deployment; 2) allow a greater stability to the DO probe; and 3) provide additional data prior to deployment (6 to 12 hours of data) which can aid in the determination of the cause of invalid data.

Calibration procedures must be done in the following order:

1. Replace the DO membrane;
2. Place the sonde in the calibration cup with a wet sponge and wait five minutes. Calibrate the DO sensor. After calibration confirm that the charge, gain, and high/low transition tests pass (see section I.A.1.a). Record diagnostic numbers on the tracking sheet. NOTE: this is only a DO calibration check, the final probe calibration will be completed after the probe rests with the new membrane for 6 to 12 hours;
3. Calibrate pH (I.A.4);
4. Calibrate depth (I.A.2);
5. Calibrate conductivity (I.A.5);
6. Calibrate turbidity (I.A.6);
7. Calibrate chlorophyll (I.A.7);
8. After calibration is completed, begin the unattended sampling for 6 to 12 hours before deployment. This will allow the DO probe to stabilize. Just prior to deployment, calibrate the DO probe one last time (this can be done with the sonde still in unattended mode). Do not run the sonde in discrete mode for 12 hours as an alternative. The pre-deployment data that will now be in the data record will be a valuable tool to identify problems encountered during deployment.

I.A.1.c.: Dissolved Oxygen Calibration for YSI 6600EDS V2 ROX Optical type sensor (COMMON and Vertical Profilers)

1. Aerate a 5 gallon bucket $\frac{3}{4}$ filled with tap water for at least 1 hour prior to calibrating the Optical D.O. probe. This will create an air-saturated water environment (hereafter referred to as a 100% air-saturated water bath).
2. Place the sonde in the bucket (with pump still running) for at least 15-20 minutes in order to achieve temperature stabilization. Ensure that air bubbles are not streaming directly towards the Optical D.O. probe face. Warning: The membrane on the ROX DO probe MUST be kept hydrated in either water saturated air or immersed in water when not in use. If the membrane is allowed to dry out it will need to be hydrated per the instructions in the YSI manual.
3. Check the sonde (in discrete mode) to ensure temperature stabilization has occurred. *Note: The ROX DO does not have a burn-in period and can be used in Discrete or Unattended applications with the same calibration. No DO warm-up or Auto-Sleep settings are required.*
4. Begin the Optical D.O. Probe calibration
5. Choose “1-point calibration”.
6. Enter the barometric pressure when prompted and continue calibrating. Record the DO gain just as you would with the rapid pulse probe. *NOTE: There is no DO charge option for this probe.*
7. Return the sonde to the 100% air-saturated water bath upon completion of all calibrations.
8. Place the sonde in unattended sampling mode at the normal 15 minute interval.
9. Leave the sonde in the air-saturated water bath until deployment.
10. At deployment time, check the D.O. value (DO %) to ensure the probe is reading accurately. The true reading that the probe should be showing is calculated using the following equation:

$$\text{BP}/7.6 = \% \text{ Saturation Value}$$

(BP = current barometric pressure in mmHG)

11. The calculated and measured DO readings must agree within 1% before deployment. If they do not, the probe can be recalibrated while still in Unattended mode. Some drift can occur after the initial calibration if the membrane was not fully hydrated.
12. The probe's membrane requires annual replacement and is performed by unfastening the flat head screws located on the probe face. Please follow all directions that came with the probe provided by YSI.

I.A.2: Depth/Pressure

The depth sensor is calibrated to sea level or level of the laboratory. **CAUTION:** When calibrating the 6600 with vented depth you must use a vented cable during the calibration procedure. Failure to do so will result in a 3-4 meter shift in the water depth.

I.A.3: Temperature

The temperature probe does not require calibration.

I.A.4: pH

Check the ISE charge (pH mV) before calibration:

pH mV must be added to the report menu then run discrete sampling. Remember to remove pH mV from report before programming.

The ISE charge (pH mV) in pH 4 is $180 \pm 40\text{mV}$, in pH 7 is $0 \pm 40\text{mV}$ and pH 10 is $-180 \pm 40\text{mV}$. Analog voltages above or below may indicate a problem with the sensor or the sonde. Note the diagnostic milli-volt numbers on tracking sheet. Note the span between the 7 and 10 pH milli-volt numbers and confirm that all meet specifications.

Buffer solutions of pH 7, 10, (or 4 depending on anticipated range of pH measurements) for the two point pH calibration are purchased pre-made from a scientific supply house. Old solutions can be used for rinsing probes, but guard against cross contamination of solutions. The pH calibration solutions are certified at a temperature of 25°C (see bottle label), and calibrations far outside of this range should be avoided.

I.A.5: Salinity and/or Conductivity

The salinity and conductivity standards are obtained from filtered seawater taken from the local area and analyzed using an osmometer (or counter top meter) OR should be purchased from a

chemical supply company. After a correct calibration, the conductivity cell constants should be 4.6 - 5.45 as seen in the "Diagnostics" or "Cal-Constants" screen. Also note any change between the last calibration and the cell constant of the most recent. A probe that suddenly goes out of range usually means that either the standard used was contaminated, the probe had a trapped air bubble, or not enough standard was used to cover the vent hole on the side of the sensor.

I.A.6: Turbidity

Do not use plastic beakers or opaque plastics for this calibration. Best results are obtained using **glass beakers** where you can see into the standard and watch for air bubbles near the optics and also check to make sure the wiper parks 180° from the optics. The probe face must be kept a minimum of 1 inch from the container bottom. Calibrations should also only be done over a **flat black surface since glare can interfere with the probe reading.**

Carefully check the set screw on the turbidity wiper block for tightness and inspect wiper sponge for wear and color (the wiper should be white). It is recommended that the wiper be changed before all deployments. For turbidity, the calibration software setting should be 1 wipe (the default) every five minutes, but may need to be increased depending on fouling. The averaging interval for turbidity should be the default of 8. For DATAFLOW a wipe should occur every fifteen minutes. The turbidity calibration solutions are purchased at 100, 200, 800, or 1000 NTU concentrations (other NTU standards can be special ordered). Two-point turbidity calibrations may not be needed at every deployment and all should only be performed in the lab. YSI recommends that the two-point calibration only be done once a month (or when drift is evident). Low end (0.0 NTU) calibrations are the most critical and must be done for every deployment.

CAUTION: It is possible to corrupt the internal turbidity calibration constant of the instrument. Once this is done, **slightly negative or positive values will be recorded for 0.0 NTU (greater than +/- 2.0 NTU).** This corruption occurs when either the probe is not clean, an air bubble intercepts the light beam, or the 0.0 NTU standard is contaminated when you accept the calibration for 0.0 NTU. Care is therefore essential to insure the standard is clean and nothing is interfering with the probe (hence the need to use clear glass when calibrating turbidity). Once the calibration constant has been corrupted, you will need to contact YSI to reset (the YSI technician can walk you through the procedure). Until this is done, all future calibrations will be inaccurate and all turbidity data collected will be erroneous. In the PC environment you are given a choice to accept the calibration, however, using the DM610 you are not given this acceptance step so it is easier to cause this internal corruption problem when calibrating turbidity with the DM610.

CAUTION: Never accept a calibration when the message "high probe off-set" appears. This indicates that either the sensor is malfunctioning or the standard is contaminated.

I.A.7: Chlorophyll

Carefully check the set screw on the chlorophyll wiper block for tightness and inspect wiper sponge for wear and color (the wiper should be white). It is recommended that the wiper be changed before all deployments. For chlorophyll, the calibration software setting should be 1 wipe (the default) every five minutes, but may need to be increased depending on fouling. For DATAFLOW a wipe should occur every fifteen minutes. The averaging interval for chlorophyll should be the default of 8. Chlorophyll calibration solutions are prepared using either an acridine orange standard solution or a rhodamine B standard solution (refer to YSI 6-Series Environmental Monitoring Systems Operations Manual). Two-point chlorophyll calibrations may not be needed at every deployment and all should only be performed in the lab. YSI recommends that the two-point calibration only be done once a month (or when drift is evident). Low end (0.0µg) calibrations are the most critical and must be done for every deployment.

II. DEPLOYMENT

II.A.: Pre-Deployment Settings

When programming for unattended sampling, specify the site description to include information such as location, month, day, and year. The sample interval is to be set for readings to be taken every 30 minutes or less. Time convention used must be Local Standard Time and sampling must start on the half hour. Parameters measured must include: temperature, specific conductance, salinity, dissolved oxygen saturation, dissolved oxygen concentration, depth, pH, chlorophyll, and turbidity. It is also recommended you collect battery voltage during the deployment since these data can help identify an electrical short. After entering the program, check the battery voltage, battery life, and memory in the "set-up information" screen. If the battery life or memory is not sufficient for the length of the deployment, take appropriate action such as changing batteries or deleting files (format flash disk). The YSI 6-Series operates best with single use alkaline batteries.

II.B.: Deployment Methods

All sondes will be deployed so that the probes stay submerged at low tides and are at 0.5 meters off the bottom to allow for tidal and flow amplitude measurements. The stations will be located at 1.5 meters depth MLW.

III. RETRIEVAL

Retrieve the sonde from the water and visually examine the probes for fouling and damage. Gently clean the sonde of debris and place it in a secure container that will prevent any severe vibrations to the unit during transportation.

IV. POST DEPLOYMENT

IV.A.: Post Deployment Calibration Check

In the laboratory a calibration check must be performed on **oxygen, conductivity, and pH probes before cleaning** and on the **turbidity probe after cleaning (to prevent contamination of the 0.0 NTU standard)**. Rinse each probe in the calibration standard (previously used is acceptable) and then compare the readings in fresh calibration standard with the instrument in discrete sample mode. Also, if previously used standards (that have **NOT** been contaminated with even DI water) are used to check calibrations, ensure these standards are never used for the primary calibrations (section I.A.). To check if the DO saturation calibration strayed, place the sonde in a calibration cup with a wet sponge, allow adequate time for the air to become saturated and temperature to stabilize (15 minutes to 2 hours depending on sonde temperature), and record the percent saturation. Post calibration checks should be done for DO %, depth, specific conductance/salinity, turbidity, chlorophyll, and pH.

IV.B.: Probe Cleaning

Prior to or after the sonde is cleaned the data may be uploaded to a Personal Computer (PC) or YSI 610DM and then uploaded into a PC.

Once the post deployment calibration check is completed the sonde body and probes should be completely cleaned. Remove the sonde guard and clean all of the sensors according to the instructions in the YSI 6-Series Operations Manual. You need to pay extra attention to the instructions below.

IV.B.1: pH Probe

This probe should be removed from the guard to insure adequate cleaning. If fouling is not a problem, you may not need to do this for every deployment. These probes are also easily cracked so care must be taken during the cleaning process. Although cleaning with DI water is acceptable, YSI recommends cleaning this probe with tap water to ensure a full probe life.

IV.B.2.: DO/Cond/Temp

If significant solid material has built up near the threads of the probe, the probe should be removed from the bulkhead and cleaned. Remove the old DO membrane and inspect the probe surface. If silver electrodes show significant darkening, follow the instructions to resurface the probe face with a fine sandpaper disk found in the 6035 DO reconditioning kit. Alternatively, you can use 2400 grit sandpaper. When you change the dissolved oxygen membrane, be careful not to touch the surface of the membrane that covers the probe. Clean the conductivity sensor with the brush provided in the maintenance kit. Wet the cavities and brush, then brush both cavities several times and rinse thoroughly with water (you can also brush the cavities under

running water).

IV.B.3.: Turbidity and Chlorophyll

Inspect the turbidity and chlorophyll wipers and replace if worn. Under normal use, the wiper will last up to 30 days of deployment time but excess fouling will shorten the life of the wiper. A dirty or disintegrating wiper is likely to cause incorrect parking of the wiper assembly during deployment. Also, a disintegrated wiper sponge may come dislodged during deployment.

V. YSI 6-Series DEPLOYMENT and RETRIEVAL LOG

A deployment and retrieval log must be filled out with every instrument deployment/retrieval (Appendix B). This sheet will aid in data QA/QC procedures and help identify faulty equipment. After completion, this sheet, along with the Ecowatch graph should be reviewed to identify anomalous data and faulty sondes/probes.

VI. VIEWING and PRINTING the ECOWatch GRAPHS

The final step in data collection is to view and print the PC6000/ECOWatch graph of all data collected during the deployment and look for patterns and outliers that can identify faulty probes and instruments. This graph must be kept with the YSI 6-Series Lab Calibration, Deployment, and Retrieval Log Sheet (tracking sheet). Both the tracking sheets and these graphs will be your best tools during the QA/QC processing of the data.

CAUTION: Always note the scales which are automatic by default in these programs and can mask a problem with the data set if you assume a linear scale from 0 (zero) to the probes acceptable maximum measurement.

VII. PROBE CARE AND STORAGE

Most of the probes, especially pH and DO, have a limited life span. The pH probes have a maximum life of 2 years (rarely longer), so any pH probes over 1 year old are near needing replacement. If probes will not accept calibrations or are slow to respond to standards, suspect aging. DO and pH probes also have a limited shelf life, so do not purchase replacements too far in advance. Dissolved oxygen probes have a life expectancy of 2-3 years.

VII.A.: Storage

The procedure for storage is different for short-term (1 month or less) or long-term (greater than 1 month).

VII.A.1.: Short-term Storage

For short term storage, it is important to keep the probes moist but not immersed in water. Keep probes attached to the sonde and place the sonde in approx. 0.5in of water (not distilled) in the calibration cup. A moist sponge can be used in place of the 0.5in of water.

VII.A.2.: Long-term Storage

Dissolved oxygen/conductivity/temperature and dissolved oxygen probes should be stored with membrane and electrolyte in place and place sonde in a water filled transport or calibration cup (it is important to ensure that water completely covers the DO sensor). If the probe is removed from the sonde, it should be stored with membrane and electrolyte in place and submerged in water (the conductivity cells should also be submerged). The pH probe should be removed from the sonde and stored with the pH storage cap containing 2-4 molar KCL or buffer (check manufacturers specifications). No special precautions are necessary for the chlorophyll and turbidity probes or depth/level probes.

Appendix D: Laboratory and Sampling Protocols

Sample Collection, Preservation, and Processing for the Continuous Surface Water Quality Mapping

1.0 Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS):

- 1.1 Water samples are collected from the effluent tubing of the DATAFLOW System in pre-rinsed (w/ambient water) darkened 1L Nalgene bottles. Exact time of collection is noted to facilitate comparison with data generated by the YSI 6600. Samples are placed in darkened bottles and packed in ice and upon return to the laboratory are processed immediately (Sections 4.0, 9.0).

2.0 Chlorophyll and Pheophytin:

- 2.1 Water samples are collected from the effluent tubing of the DATAFLOW System in pre-rinsed (w/ambient water) darkened 1L Nalgene bottles. Exact time of collection is noted to facilitate comparison with data generated by the YSI 6600. Immediately upon collection known volumes of samples are filtered through 25 mm GFF filters. Filters are folded in half and placed in plastic petri dishes covered with aluminum foil. Samples are then placed in ice and upon return to the laboratory are stored at -20°C until analyses can be conducted (Section 10.0).

Verification Sample Procedures for the James River Monitoring Program

1.0 Total Suspended Solids and Volatile Suspended Solids

1.1 Summary of Method:

1.1.1 A well-mixed sample of known volume is filtered through a glass-fiber filter, and the residue retained on the filter is dried to constant weight at 103 - 105°C.

1.1.2 The residue obtained from the determination of total suspended solids is ignited at 550°C for 2 hours in a muffle furnace. The remaining solids after ignition are reported as mg volatile suspended solids/L.

1.2 Interferences

1.2.1 The principal source of error in the determination is failure to obtain a representative sample.

1.2.2 The test is subject to many errors due to loss of water from crystallization, loss of volatile organic matter prior to combustion, incomplete oxidation of certain complex organics, and decomposition of mineral salts during combustion.

1.3 Apparatus and Materials

1.3.1 47 mm glass fiber filters (GFF, 0.7 µm pore size).

1.3.2 Filtering Apparatus with 47mm filter holders.

1.3.3 Drying Oven: Capable of maintaining a temperature of 103 ±2°C.

1.3.4 Analytical balance: Capable of weighing to 0.1 mg.

1.3.5 Muffle oven: Capable of heating to 550°C.

1.4 Sample Handling

1.4.1 Preservation of the unfiltered sample is not practical; analysis should begin as soon as possible. Refrigeration or icing minimizes the microbiological decomposition. After filtering, the filter can be stored frozen at -20 °C for 28 days.

1.5 Procedure:

1.5.1 Preparation of filters for Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS):

- 1.5.1.1 Pre-number aluminum weigh boats (i.e. 1,2, etc.).
- 1.5.1.2 Place filters wrinkled side up on clean filtering apparatus.
- 1.5.1.3 Run three successive 30 mL washes of ASTM Type I water through each filter while applying suction. Do not exceed a vacuum of 10 psi.
- 1.5.1.4 Allow suction pump to run until the filters are "dry".
- 1.5.1.5 Carefully transfer filters to pre-numbered weigh boats using forceps.
- 1.5.1.6 Ignite blank filters in the muffle furnace at 550°C for approximately 15 to 20 minutes.
- 1.5.1.7 Place filters in a desiccator until cool.
- 1.5.1.8 After cooling (approximately 15 minutes), weigh filters on an analytical balance capable of measuring to 0.1 mg. Record weights and return filters to weigh boats.
- 1.5.1.9 Return filters to the oven for at least 1 hour. Reweigh filters a second time. Filter weights must not be more than ± 0.5 mg. If greater, return filters to oven for at least 1 more hour.
- 1.5.1.10 After dry weights have been determined, filters are stored in their aluminum weigh boats in an airtight container until needed.

1.5.2 Determination of Suspended Solids:

- 1.5.2.1 Place pre-weighed filter on a clean filter holder with wrinkled side up. Turn on vacuum pump. Do not exceed a vacuum of 10 psi.
- 1.5.2.2 Vigorously shake sample.
- 1.5.2.3 Rinse 250 mL graduated cylinder with a small quantity of sample.
- 1.5.2.4 Measure 250 mL of sample into cylinder and pour into the filtering funnel. Repeat for a total volume of 500 mL. Smaller volumes may

be used if sample has a great deal of suspended matter or larger volumes may be used if sample has low levels of suspended matter.

- 1.5.2.5 Record volume filtered (mL).
- 1.5.2.6 Rinse filter with DDI water to remove salts.
- 1.5.2.7 Allow the vacuum pump to run until filter is "dry".
- 1.5.2.8 Run one duplicate (500 mL sample, 500 mL duplicate) for every set of ten samples. If less than ten samples are run, then one duplicate should be made.
- 1.5.2.9 Remove filter with forceps.
- 1.5.2.10 Place filter in numbered weigh boat. Dry to a constant weight in a 103-105°C oven at least 4 hours (preferably overnight).
- 1.5.2.11 Place filters in desiccator until cool.
- 1.5.2.12 Repeat weighing procedure
- 1.5.2.13 Return filters to the oven for at least one hour.
- 1.5.2.14 Repeat weighing procedure. If filters do not weigh within ± 0.5 mg of their first weight, the process of drying and weighing should be repeated as many times as needed to achieve this constant weight.
- 1.5.2.15 For VSS muffle filters for 15 minutes at 550°C. Repeat weighing procedure.

1.6 Calculation

$$\text{mg volatile suspended solids/L} = \frac{(A-B) \times 1000}{\text{sample volume, mL}}$$

$$\text{mg total suspended solids/L} = \frac{(A-C) \times 1000}{\text{sample volume, mL}}$$

where:

A = weight of residue + filter before ignition, mg

B = weight of residue + filter after ignition, mg

C = weight of filter, mg

1.7 Reference(s):

U. S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes, Method 160.2.

Standard Methods, 18th edition, pp. 2-54 to 2-57.

2. Chlorophyll and Pheophytin

2.1 Scope and Application:

2.1.1 Chlorophyll data may be used to determine long-term trends in water quality and the trophic status of surface waters, to detect adverse effects of pollutants on plankton, and to provide estimates of studies attempting to estimate algal biomass and productivity.

2.2 Summary of Methods:

2.2.1 The method for determining Chlorophyll *a* given here is with a Turner Design fluorometer. The method used requires filtering a known quantity of water through a glass fiber filter. This filter is later ground with a tissue grinder made of teflon/glass. Approximately 1-3mLs of 90% acetone are added to the filter before grinding. Acetone is also used to wash the filter into 17 x 150 test tube with tight fitting cap. The sample is steeped at least 2 hours and not exceeding 24 hours at 4°C, in the dark. The samples are centrifuged and read on a fluorometer. If the samples cannot be read within that time period, storage in the freezer at -20°C for a few days is acceptable. If pheophytin measurements are desired, the sample is acidified and read again.

2.3 Reagents:

NOTE: Use fresh, distilled, DDI water. Label, date, and initial all reagents.

2.3.1 Aqueous Acetone solution (90%) - Mix 90 parts acetone (Optima grade) with 10 parts DDI water. Add 4 drops of 1N NaOH/L.

Note: Mix the reagents in the appropriately marked bottle in the following order: 1800 mL Acetone, 200 mL DDI water, 8 drops 1N NaOH. This is to be stored in the yellow FLAMMABLE cabinet.

2.4 Equipment:

2.4.1 The Turner Designs TD-700 Laboratory Fluorometer is now used in this laboratory to make chlorophyll A and pheophytin determinations. The Fluorometer is equipped as follows:

2.4.1.1 Daylight White Lamp P/N 10-045

2.4.1.2 Filters (For Chlorophyll A and Pheophytin measurements)

Excitation: 340-500 nm P/N 10-050R

Emission: >665 nm P/N 10-015R

Filters (For Chlorophyll A only)

P/N 10-113

Emission: 680 nm P/N 10-115

2.4.2 Centrifuge - capable of holding the 17 x 150mm tubes.

2.4.3 Tissue grinder and equipment - Teflon/glass type pestles, electric motor, stand and glass grinding vessels.

2.4.4 Centrifuge culture tubes - 17x 150 mm culture tubes with tight fitting caps.

2.4.5 Filtration equipment - Vacuum pump (vacuum should not exceed 1/2 atm or 15psi, filter holder for 47mm GF/F filters, 47mm GF/F filters, 2X4 ziplock bags, foil or a dark storage container.

2.5 Standards

2.5.1 Fluorometer BLANK: (Used as a "Filter Blank"). An unfiltered blank filter will be analyzed as if it were a sample, extracted as described in Volume V, Section 5.

2.5.2 Calibration Standard: Standards are obtained from Turner Instruments. Calibration standards consist of a minimum of one high standard and one low standard maintained in 90% acetone. An intermediate standard may be made by diluting the high standard appropriately with 90% acetone. This solution should be determined to be a high purity blank. Calibration Standards should be frozen at -20 °C prior to use.

2.6 Calibration of Fluorometer

2.6.1 Calibration Standards and a Calibration Blank (90% optima grade acetone) are required for this step

- 2.6.2 Proceed to Calibration set-up menu on the TD-700 fluorometer. A minimum of two standards should be used for the calibration. Three standards and a blank are optimal.
- 2.6.3 Use A multi-optional@ mode with / Direct Concentration/ug/L units
- 2.6.4 Calibration
- 2.6.4.1 Enter Highest Standard first. Key in the high concentration value. Insert test tube with high standard in fluorometer. Press <*> when stable. The sensitivity setting will be automatically set. The fluorometer will read the high standard then ask for subsequent standards.
- 2.6.4.2 When all standards have been read, insert the blank.
- 2.6.4.3 Press <0> when the value is stable.
- 2.6.4.4 Calibration will be completed
- 2.6.5 Acid Ratio Determination:
- 2.6.5.1 The acid ratio (the ratio of the fluorescence of any extract containing only chlorophyll, before and after the addition of acid) should be determined for each fluorometer run.
- 2.6.5.2 Calibrate the fluorometer as described in Section 5.5.
- 2.6.5.3 Reread intermediate calibration standard (R_b)
- 2.6.5.4 Acidify the standard with 2 drops of 0.1N HCL. Mix. Wait 90 seconds.
- 2.6.5.5 Read sample as R_a.
- 2.6.5.6 Acid ratio

$$R = R_b/R_a$$

2.7 Procedure:

2.7.1 Grinding procedure

NOTE: Before grinding set up samples by listing the sample ID and volume on the bench sheets. Label small pieces of white tape with the ID volume, apply these to the culture tubes.

- 2.7.1.1 Close half-curtain around grinding area. This blocks out fluorescent light which destroys chlorophyll. Turn on the incandescent light and the hood fan.
 - 2.7.1.2 Place filter in grinding vessel and add 1-3mL of the 90% acetone.
 - 2.7.1.3 Insert pestle in grinding tube, and turn on grinder by using switch on post of apparatus. NEVER turn on the grinding motor without having the pestle in the vessel.
 - 2.7.1.4 Thoroughly grind filter for approximately 2 minutes. Be sure there are no discernable pieces left. Pull pestle to the top of the vessel and rinse lightly with the 90% acetone.
 - 2.7.15 Rinse pestle with 20 ml of 90% acetone into the culture tube.
 - 2.7.1.6 Cap tube and shake lightly.
 - 2.7.1.7 Store tubes in racks in a closed box and place in refrigerator for 2-24 hours. The sample tubes may be stored for a couple of days at -20°C if necessary.
- 2.7.2 Centrifuging samples
- 2.7.2.1 Before removing samples from closed box, turn off lights. Fluorescent light destroys chlorophyll!
 - 2.7.2.2 Shake samples to ensure thorough mixing.
 - 2.7.2.3 Place samples in centrifuge in an order that can be remembered (tubes must be kept in order).
 - 2.7.2.4 Close cover until it clicks. Adjust setting to approximate 675g.
 - 2.7.2.5 Turn TIME/MIN knob to 15 minutes. This starts the centrifuge spinning.

2.7.2.6 After centrifuge has stopped spinning, open top (pull up firmly on lever on top of cover), remove tubes and replace in rack. Check the order against sheets.

2.7.3 Reading on Fluorometer

2.7.3.1 Pipette samples into fluorometric cuvettes.

2.7.3.2 Read sample in fluorometer. Results are read in direct concentration.

2.7.3.3 Read sample; record as R_B .

2.7.3.4 Add 2 drops of 0.1 N HCL and shake well.

2.7.3.5 Read sample record as R_a .

2.8 Calculations:

2.8.1 For uncorrected Chlorophyll A using Method 445.0 with acidification: (Instrument must be equipped with Excitation: 340-500 nm P/N 10-050R Emission: >665 nm P/N 10-015R)

2.8.1.1 $C_{E,u} = R_b \times F_s$

Where: $C_{E,u}$ = uncorrected chlorophyll A concentration (ug/L) in the extract solution analyzed

R_b = fluorescence response of sample extract before acidification, and

F_s = fluorescence response factor for sensitivity setting S (which =1 for the TD-700 fluorometer)

2.8.1.2 Calculate the “uncorrected” concentration of chlorophyll A in the whole water sample as follows:

$$C_{S,u} = \frac{C_{E,u} \times \text{extract volume (L)} \times DF}{\text{Sample volume (L)}}$$

Where: $C_{S,u}$ = uncorrected chlorophyll A concentration (ug/L) in the whole water sample

Extract volume = volume (L) of 90% acetone used for extraction of the filter

DF = Dilution factor

Sample volume = volume (L) of whole water filtered through filter

2.8.2 For corrected Chlorophyll A using Method 445.0 with acidification:

$$2.8.2.1 \quad C_{E,C} = F_s (r/r-1) (R_b - R_a)$$

Where: $C_{E,C}$ = corrected chlorophyll A concentration (ug/L) in the extract solution analyzed

F_s = response factor for sensitivity setting S,

r = the before to after acidification ratio of the pure chlorophyll standard

R_b = fluorescence of sample extract before acidification, and

R_a = fluorescence of sample extract after acidification

2.8.2.2 Calculate the @corrected@ concentration of chlorophyll A in the whole water sample as follows:

$$C_{S,C} = \frac{C_{E,U} \times \text{extract volume (L)} \times DF}{\text{Sample volume (L)}}$$

Where: $C_{S,C}$ = corrected chlorophyll A concentration (ug/L) in the whole water sample

Extract volume = volume (L) of 90% acetone used for extraction of the filter

DF = Dilution factor

Sample volume = volume (L) of whole water filtered through filter

2.8.2.3 For corrected pheophytin using Method 445.0 with acidification:

$$P_E = F_s (r/r-1) (rR_b - R_a)$$

$$P_S = \frac{P_E \times \text{extract volume (L)} \times DF}{\text{Sample volume (L)}}$$

Where: P_E = pheophytin concentration (ug/L) in the sample extract, and

P_S = pheophytin concentration (ug/L) in the whole water sample

2.8.3 For Corrected Chlorophyll A using Method 445.0 without acidification: (Instrument must be equipped with Excitation: 436 nm P/N 10-113 Emission: 680 nm P/N 10-115)

2.8.3.1 Calculate the correct concentration of chlorophyll A in the whole water sample as follows:

$$C = \frac{C_C \times \text{extract volume (L)} \times DF}{\text{Sample volume (L)}}$$

Where: C = corrected chlorophyll A concentration (ug/L) in the whole water sample

C_C = corrected chlorophyll A concentration (ug/L) in the extract solution analyzed

Extract volume = volume (L) of 90% acetone used for extraction of the filter

DF = Dilution factor

Sample volume = volume (L) of whole water filtered through filter

2.9 Reference(s):

Strickland, J.D.H., and Parson, T.R. 1972. A Practical Handbook of Seawater Analysis. Fish. Res. Bd. Canada 167:310.

TD-700 Laboratory Fluorometer Operating Manual. Version 1.8. July 7, 1999. Turner Designs, 845 West Maude Avenue, Sunnyvale, CA 94086.

EPA /600/ R-97/072 - Method 445.0. *In Vitro* Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence. Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices Revision 1.2. September 1997.

Using the Turner Designs Model 10 Analog, The 10AU Digital, Or the TD-700 Fluorometer with EPA Method 445.0. January 19, 1999. Turner Designs, 845 West Maude Avenue, Sunnyvale, CA 94086

4.0 Secchi Depth

4.1 Scope and Application

4.1.1 The turbidity of natural water is a critical factor in sustaining submerged aquatic vegetation as well as epiphyte and phytoplankton growth.

4.2 Summary of Method

4.2.1 A black and white secchi disk that is attached to a weighted, ruled line is lowered slowly into the water until it disappears and then raised. The depth at which the disk disappears is averaged with the depth at which it reappears; this measurement (in meters) is the secchi depth.

4.3 Procedure

4.3.1 A round, weighted secchi disk measuring 20cm in diameter is used. The upper surface is divided into four equal quadrants that are colored so that the two quadrants opposite each other are black and the intervening ones are white. It is attached to a ruler line that is marked in 0.1m intervals.

4.3.2 Readings with the secchi disk are made in situ without the aid of sunglasses on the shady side of the vessel to reduce possible sun glint on the water surface.

4.3.3 The disk is slowly lowered into the water until it disappears and then raised. The depth at which the disk disappears is averaged with the depth at which it reappears; this measurement (rounded to the nearest 0.1m increment) is the secchi depth.

4.3.4 The secchi disk is not permitted to touch the sediment surface. If the secchi depth exceeds the water depth in shallow water no measurement is recorded and the profile is repositioned in slightly deeper water.

4.3.5 The time of the secchi measurement is recorded. For continuous surface water quality verification stations and fixed stations this facilitates matching of secchi depth readings with transmissometer (NTU) data.

4.4 Quality Control

4.4.1 Reported Units

4.4.1.1 (0.1m)

4.5.1 Detection Limits

4.5.1.1 Upper Limit - N/A

4.5.1.2 Lower Limit – 0.1m

4.5 References

Tyler, John. 1968. The secchi disk. *Limnol. Oceanogr.* 13 (1): 1-6.

5.0 Attenuation of Underwater Photosynthetically Available Radiation (PAR 400-700nm)

5.1 Scope and Application

5.1.1 Light attenuation of PAR through the water column is an important apparent optical property of natural water that is a critical factor in sustaining SAV and well as regulating epiphyte and phytoplankton growth. The degree of this attenuation is related largely to the concentration of suspended and dissolved substances in the water. PAR is that component of the solar radiation spectrum that is typically defined as visible light and within this range of wavelengths are those predominantly used by plants for photosynthesis.

5.2 Summary of Method

5.2.1 Downwelling light penetrating the water column (PAR) is measured underwater at several depths to calculate the light attenuation coefficient, K_d . This procedure may also be used to estimate the depth of the photic zone. Concurrent incident light readings are taken on deck with each depth measurement. Simultaneous deck and submersed measurement are necessary because of variability in incident surface irradiance due to changing atmospheric conditions (i.e. cloud cover).

5.2.2 K_d is calculated as the negative exponential decay function of underwater light with depth. Light is typically measured as the quantum flux of downwelling irradiance using cosine-corrected downwelling quantum sensors.

5.2.3 Equipment: manufactured by LI-COR, Inc.

5.2.3.1 LI-192SA, Underwater Quantum Sensor

5.2.3.2 LI-190SA, Quantum Sensor (deck)

5.2.3.3 Li-1000 or LI-1400 Datalogger

5.3 Procedure

5.3.1 PAR is determined from the sunny side of a vessel or pier during daylight hours. Both deck and submersed sensors must be away from the shadow of the vessel or objects on the vessel.

- 5.3.2 Check the sensor operation by connecting the deck sensor and underwater sensor to corresponding ports of the Li-Cor data logger, and turn the power on. Check battery level. Cover each sensor and check to see if the output is very near zero. Place the sensor under a light source to assure that it has a positive reading. A negative reading indicates that the polarity of the sensor is reversed (it is plugged into the cable backwards). A very positive or negative reading under dark indicates a possible short in the cable or some other electrical short.
- 5.3.3 In the field, mount the deck sensor in a location that is level and unobstructed by shadows. Ensure that sensors are positioned properly on the deck sensor mounting and the lowering frame. Connect deck sensor and underwater sensor to corresponding ports of the meter, and turn the power on.
- 5.3.4 Set instrument averaging constant to display results from previous 10 or 15 seconds.
- 5.3.5 Lower the frame until the sensor is just below the surface of the water. At a depth of 0.1 meter (~ 4 inches), simultaneously record the deck sensor and the underwater readings. If wave action prevents this, take the initial reading at 0.5 meters. Depth of the underwater sensor diffuser below the surface of the water is determined with a calibrated pole or non-stretching rope.
- 5.3.6 Lower the frame and take subsequent measurements at depths appropriate to the monitoring location and purpose of the project (see below). Allow the instrument output to stabilize, at least for the averaging period, and then record both deck and underwater readings at each depth.
- 5.3.7 The simultaneous deck and submersed readings are stored by pressing “Enter”. Each record will be stored with a time stamp which will flash on the data logger readout. Record this time. All readings can be subsequently retrieved at the end of the cruise.
- 5.3.8 Mainstem and Tidal Tributary Photic Zone. Record initial PAR at a depth of 0.1 meter below the surface. At mainstem stations, record subsequent measurements at 1 meter intervals, and in the tributary stations, at 0.5 meter intervals when total depth is greater than 2 meters. If depth to bottom is less than 2 meters, take readings at 0.25 meter increments.

- 5.3.9 Measurements are recorded until the meter reads <1% of the initial subsurface value, or if the bottom is reached. If water is clear and deep, the increments may be increased to 2 meters. If water is turbid or colored, (low secchi depth), 0.25 meter increments may be appropriate.
- 5.3.10 Data Flow Mapping and High Frequency Meter Regressions - Record initial PAR at a depth of 0.1 meter below the surface. When depth to bottom is less than 2 meters, record 3 to 4 additional PAR measurements at 0.25, 0.5, 0.75 and 1.0 meters. When depth to bottom exceeds 2 meters, record PAR at 5 depths (e.g., 0.1, 0.5, 1.0, 1.5 and 2.0 meters).
- 5.3.11 Replicate PAR profile at each location to make sure readings are correct.

5.4 Quality Control

- 5.4.1 The deck cell should have the highest PAR value, and the underwater sensor output should decrease as the sensor is lowered.
- 5.4.2 Periodically check that sensors are linear and not out of calibration with respect to each other.
- 5.4.3 Calibration of the sensors by LICOR, Inc. is recommended every year, and required every two years.(AMQAW, 2002)

5.5 K_d Calculations

- 5.5.1 Light measurements are taken simultaneously of both surface (I_0) and submarine PAR intensity (I_{z+n}). Submarine PAR is measured at the surface (0.1 meters), and successive intervals thereafter (I_{z+1} , I_{z+2} ,... I_{z+n}).
- 5.5.2 Each deck reading is normalized to an arbitrary deck reading of $2000 \mu\text{mol m}^{-2} \text{sec}^{-1}$ and the simultaneously recorded submarine reading is multiplied by this ratio to correct the individual submersed readings of each profile for any difference in solar insolation intensity during the profile. The attenuation coefficient (K_d) can then be calculated between any two depths on one profile as:

$$K_{z,z+n} = \frac{\ln(I_{z+n}/I_{z+(n-1)})}{\Delta z}$$

where:

$K_{z,z+n}$ = attenuation coefficient (m^{-1}) over the depth interval $z+n, z+(n-1)$
 I_{z+n} = normalized PAR intensity at depth $z+n$ ($\mu mol\ m^{-2}s^{-1}$), (i.e, deeper value)

$I_{z+(n-1)}$ = normalized PAR intensity $z+(n-1)$ depth ($\mu mol\ m^{-2}s^{-1}$)

Δz = difference in depth (m) between $z+n, z+(n-1)$

5.5.3 For more than two depths on one profile plot the natural log of each normalized PAR value on the x-axis vs. depth. The slope of the line times (-1.0) is K_d .

5.6 Reporting

5.6.1 The CIMS data base stores PAR values from the deck sensor and each underwater measurement under the following method codes:

5.6.1.1 EPAR_D F01: Flat Cosine Quantum Sensor LI-192SA

5.6.1.2 EPAR_S F01: Deck (reference) Quantum Sensor LI-190SA

5.6.1.3 SDEPTH: Sample depth at which PAR reading is recorded, in meters

5.7 References

Kirk, J.T.O. 1994. Light and photosynthesis in aquatic ecosystems. 2nd ed. University Press, Cambridge. 509 pp.

Appendix E: DATAFLOW QA/QC Protocol

1. Data Processing for EPA/DEQ Dataflow
2. For each tributary a folder is created with the year (ex JMSOH 2010)
 - 2.1. In this folder each cruise date gets a folder (ex Mar 23)
 - 2.2. In this folder are a series of files
 - 2.2.1. The raw text file (ex 230802.txt)
 - 2.2.2. The raw Excel file (ex JMSOH03-23-12RAW)
 - 2.2.3. The first cut Excel QAQC File (ex JMSOH03-23-12)
 - 2.2.4. A Minitab file w/ station averages and graphs (ex JMSOH03-23-12)
 - 2.2.5. A file with the EPA format and error codes (ex JMSOH03-23-12EPA)
 - 2.2.6. There is also a Tributary Metadata File (ex JMSOH 2012 Metadata)
 - 2.2.6.1. YSI Post Cal for all parameters
 - 2.2.6.2. Date and Location of cruise
 - 2.2.6.3. Time stamp of all deleted data due to electronic interference
 - 2.2.6.4. Time stamp of data line with no depth information
 - 2.2.6.5. Time stamp with any questionable data and action taken
 - 2.2.6.6. Track the process of QA-ing the file
(see example Metadata file)
3. Download data from field computer
 - 3.1. Original file is left on field computer
 - 3.2. Data file is backed up in “Raw Downloads” folder
 - 3.2.1. Data is also placed into the Tributary file under Date of cruise (see above formatting)
4. The macro (ex. moore_df_EPA) is opened in Excel
 - 4.1. The raw text file is opened in Notepad
 - 4.2. Entire file is selected and pasted into Excel
 - 4.3. Macro is run on the raw file (text to columns, headers, convert lat lon to correct form, etc)
 - 4.4. The file is then saved as *CBPSegmentmm-dd-yyRAW.xls* (ex JMSOH03-23-12RAW.xls)
5. First round of QAQC
 - 5.1. Save file as *CBPSegmentmm-dd-yy.xls* (JMSOH03-23-12.xls)
 - 5.2. Delete all data up to the recorded cruise track start time, record in metadata
 - 5.3. Delete the following fields
 - 5.3.1. GPS Time
 - 5.3.2. GPS Quality
 - 5.3.3. Lat Dir
 - 5.3.4. Lon Dir
 - 5.3.5. Heading
 - 5.3.6. Date
 - 5.3.7. Unknown
 - 5.4. Delete all incomplete data caused by electronic interference, record in metadata

- 5.5. Look for gaps in data due to running aground, pulling intake, GPS malfunction, computer malfunction, and depth sounder malfunction
- 5.6. Clear all cells containing “*57” depth errors and record in metadata
- 5.7. Examine all field notes noting events such as running aground, blooms, etc
 - 5.7.1. Examine the data for effects of these events
 - 5.7.2. Highlight any questionable data and note in metadata with time stamp
 - 5.7.3. If an event did not affect the data note this also
 - 5.7.4. Save file as *CBPSegmm-dd-yy.xls* (ex. *JMSOH03-23-12.xls*)
6. YSI Post Calibration information
 - 6.1. Looking at the post cal notes see that all parameters post cal within limits
 - 6.1.1. Acceptable limits listed in table below
 - 6.2. Enter all info into the metadata for that cruise date
 - 6.3. Highlight any data that falls outside the limits and record in metadata
 - 6.4. If made any changes to *CBPSegmm-dd-yy.xls* resave
7. Second Round QAQC – Station Averages
 - 7.1. Copy all data from Excel file (ex. *JMSOH03-23-12.xls*) into Minitab program
 - 7.2. Run code written to find the average of the 10 observations before and after the verification sampling time. Graphs are generated by this code as well.
 - 7.3. Record Lat and Lon at the time of verification sampling on the field data sheet in appropriate location
 - 7.4. Record the averages calculated by Minitab of the following parameters on field data sheet in appropriate place: depth, water temperature, salinity, pH, dissolved oxygen (%), dissolved oxygen (mg/l), turbidity, and chlorophyll
 - 7.5. Copy the Lat and Lon and averages of the above parameters from the Minitab file into an Excel file for later use (ex 2012 *JMSOH Minitab Verification Data.xls*).
8. Second Round QAQC – Graphs
 - 8.1. Using the Minitab file generated above, examine the graph for each parameter carefully for unusual trends in data
 - 8.1.1. Unexplained plumes in data

Look at the surrounding data and the depth. If there is no indication of probe failure or stirring up the bottom, leave data in the data set, a comment if it is questionable can always be added to the comments column later
 - 8.1.2. Single spikes or drops in values

To determine if a single spike should be flagged, take the Standard Deviation of the 10 numbers before and after the questionable value and if the value is > 3 times the Stdev highlight it
 - 8.1.3. Negative CHL and NTU data
 - 8.1.4. Strange patterns in DO data
 - 8.1.5. Any indications of probe issues
 - 8.1.6. Record any abnormalities on metadata and highlight in spreadsheet

9. Second Round QAQC – Visual Check
 - 9.1. Using the same file take a closer look at patterns of data
 - 9.2. Record any issues on metadata with time stamp and highlight in spreadsheet
 - 9.2.1. Examine DO data
 - 9.2.2. Examine CHL data
 - 9.2.3. Examine NTU data
 - 9.2.4. Remember to use verification data to verify highs in data

10. Final QAQC
 - 10.1. Using all the appropriate information decide what data is unacceptable and assign error codes accordingly (record in metadata)
 - 10.2. If data is questionable but there is not a reason to actually mask it with error codes put information into COMMENT column (record in metadata)

11. Create EPA file
 - 11.1. Open Excel and DFtoEPAFormat macro
 - 11.1.1. Open original file *CBPSegmm-dd-yy.xls*
 - 11.1.2. Select the whole file; copy and paste it into the macro sheet
 - 11.1.3. Add a column after the CHL column
 - 11.1.4. Run the DFtoEPAFormat macro
 - 11.1.5. Save as *CBPSegmm-dd-yyEPA.xls*

12. Create EPA_Final file using ACCESS
 - 12.1. Open ACCESS file “dataflowQA.mdb” file and browse to open proper Excel file (*CBPSegmm-dd-yyEPA.xls*) and import the following:
 - 12.1.1. SAMPLE DATE (ex mmddyyyy)
 - 12.1.2. CRUISE_TRACK = xxxmmddyyyy (xxx – 3 letter Trib code)
 - 12.1.3. WATER_BODY = River Name (ex JAMES)
 - 12.1.4. PRI_SEG = Primary Segment covered by cruise (ex JMSOH)
 - 12.1.5. STATION = PRI_SEG (ex JMSOH)
 - 12.1.6. SONDE = the serial number of YSI used
 - 12.2. Export data to create the EPA_Final Excel file (ex *MOBPH03-23-10EPA_FINAL.xls*). This file is now populated with all other necessary units, identifications, depths, layers, etc.

13. Create official EPA file
 - 13.1. Using the metadata add the appropriate error codes (see attached list of error codes and meanings as well as comments).
 - 13.2. Change the file name to the official EPA file *CBPSegmmddy.xls* (ex *MOBPH032310*)

Acceptable Limits of YSI Post Calibration

Parameter	Value
Chlorophyll	±5 % of true value
Dissolved Oxygen	±0.5 mg/L
PH	±0.2 pH units
Specific Conductance	±5 % of true value
Turbidity	±5 % of true value
Water Temperature	±0.2 °C

Parameter Units

<u>Parameter</u>	<u>Units</u>	<u>Method Code</u>
TOTAL_DEPTH	M	-
BATT	-	-
BOAT_SPEED	KNOTS	-
WTEMP	DEG C	F01
SPCOND	UMHOS/CM	F01
SALINITY	PPT	F01
DO_SAT	PCT	F01
DO	MG/L	F01
PH	SU	F01
TURB_NTU	NTU	F01 for 6136 or F02 for 6026
FLUOR	%FS	NA
TCHL_PRE_CAL	UG/L	F01
CHLA	UG/L	NND

DATAFLOW & Continuous Monitoring Specific Error Codes

<u>Category</u>	<u>Code</u>	<u>Description</u>
Specific Probe Code	PSW	Salinity Calibrated to Incorrect Level
Specific Probe Code	PDP	DO Poisoning (anoxia)
General Probe Code	GBO	Blocked Optic
General Probe Code	GWM	Wiper Malfunction
General Probe Code	GWL	Wiper Lost
General Probe Code	GSC	Seal Compromise
General Probe Code	GPF	Probe Failure
General Probe Code	GPC	Post Calibration Out of Range
General Probe Code	GNV	Negative Value
Non-Probe Codes	NPF	Power Failure
Non-Probe Codes	NOW	Instrument Out of Water
Non-Probe Codes	NNF	Ram Clogged/No Flow
Non-Probe Codes	NND	No Data
Non-Probe Codes	NIS	Invalid Data Due to Incorrect YSI Setup
Non-Probe Codes	NIR	Instrument Removed
Non-Probe Codes	NQR	Data Rejected Due to QA
Comments	CTS	Time Skip
Comments	CBF	Biofouling
Comments	CWD	Instrument at Wrong Depth
Comments	CTF	Temperature Probe Failure
Comments	CSW	Salinity Level Calibrated Incorrectly
Comments	CLF	Flow Low
Comments	CDB	Disturbed Bottom
Comments	CTC	Time Change
Comments	CTW	Turbid Water
Comments	CFK	Fish Kill
Comments	CSC	Site Location Change
Comments	CAS	Algal Sample Taken
Comments	CAB	Algal Bloom – No Sample Taken

See http://www.chesapeakebay.net/pubs/cbwqdb2004_RB.PDF for any questions parameter units, other error codes, method codes, etc

Appendix F: DATAFLOW GIS Protocol for Interpolations & Cruise Tracks

Water Clarity Acres Determination Methodology

1. Create analysis grids
 - a. Create segment analysis grid (analysis\yp).
 - i. The Chesapeake Bay segments, (James tidal fresh split, 92 segments) coverage is used for the polygon input (baselayers\cbsegsplit -cov).
 - ii. Import coverage into blank ArcMap document.
 - iii. Select target segment
 - iv. Right click the layer and select Data → Export Data
 - v. Save the file as baselayers\yp.shp and add the newly created file to the map
 - vi. Open Feature to Raster Tool
 1. Input features = features created in 1av.
 2. Output Raster = baselayers\yp_tmp
 3. Field = cbpseg
 4. Set extent to include at least entire available bottom (bathymetry = 0-2m) for the segment.
 5. Round extent to nearest 25 meters (for yrkph segment, we use top: 4138875, left: 352575, right: 376775, bottom: 4117725).
 6. Cell Size = 25m.
 7. Projection = NAD 1983 UTM Zone 18N.
 - vii. Open Multi Output Map Algebra tool
 1. expression: segmask = con(isnull(setnull(cbseg_tmp == 0,cbseg_tmp)),0,1)
 2. where, segmask = analysis_grids\yp
 3. cbseg_tmp = analysis_grids\yp_tmp
 - viii. Delete analysis_grids\yp_tmp in ArcCatalog
 - b. Use Feature to Raster Tool to create bathymetry grid (analysis\ypbathy).
 - i. Use .5 meter Chesapeake Bay bathymetry (baselayers\bathy0502 – cov)
 - ii. Field = Tier
 - iii. Use same extent and cell size as in step 1a.
 - c. Use Feature to Raster Tool to create current SAV grid (“analysis\yp_06”).
 - i. Use VIMS SAV coverage for most current year (“baselayers\beds06” – cov)
 - ii. Field = Density
 - iii. Use same extent and cell size as in step 1a.
 - d. Use Feature to Raster Tool to create exclusion zone grid (“analysis\ypxz”).
 - i. Use Chesapeake Bay no grow zones (“baselayers\nogrow_zones” – cov)
 - ii. Field = Exclude
 - iii. Use same extent and cell size as in step 1a.
2. Query VECOS database
 - a. Query dataflow data for single date and segment from VECOS. Note: The query is designed to take each unique combination of SAMPLE_DATETIME

- (combination of date and time for each point sampled by dataflow), LATITUDE, & LONGITUDE. This is done because sometimes there is duplicate data for the same time or same location. In these instances, the query is designed to take the mean for each duplicate measurement of SALINITY, CHLA, AND TURBIDITY. The query also assigns a value of -999 where data has an associated error code.
- b. Save the result of the query as a table in an access database (db = "wq_data.mdb", table = "yp052907"). Note: Example table has more parameters than salinity, chla, and turbidity. However, they are not necessary for this analysis. In table: salinity = sa_mean, chla = ch_mean, and turbidity = tu_mean.
3. Copy table into ArcGIS geodatabase (gdb = wq_data.gdb, table = yp052907).
 4. Create point feature from table
 - a. Open New ArcMap document. Add table (wq_data.gdb \yp052907)
 - b. Right click on table and select "Display XY Data"
 - i. X field = Longitude
 - ii. Y field = Latitude
 - iii. Projection = GCS North American Datum 1983
 - c. Use Project tool to project XY event layer
 - i. Input = event layer
 - ii. Projection = NAD 1983 UTM Zone 18N
 - iii. Output = geodatabase (gdb = wq_data.gdb, table = "yp052907p")
 5. Check for any outlying spatial points (rarely, Lat/Long is wrong in the table as in the example). Delete any outlying points from dataset in an ArcMap edit session. Save edits. May need to repeat step 4c on edited feature if zoom-layer does not focus on remaining points. Output: gdb = wq_data.gdb, table = "yp052907p")
 6. Add resulting point feature to blank ArcMap document
 7. Select where ch_mean > -999. Note how much data may be invalid. Decide if too much data has an associated error code to proceed with interpolation for this dataset. This decision is made with a concern for the amount and spatial distribution of data that will be excluded. In the future, we would like to move toward a less-subjective approach though the use of an associated standard error grid. If the interpolation is not made, that particular date is ultimately thrown out of any annual mean water clarity acreage calculations. If there is sufficient data to continue, clear the selection before continuing with the interpolation.
 8. Create the Ordinary Kriging Prediction Map (**For segments where interpolations will be executed without the use of barriers. For segments where barriers are used, see alternative steps 8 and 9 at the end of this document).
 - a. From the Geostatistical Analyst toolbar, choose Geostatistical Wizard
 - b. In the Choose Input Data and Method dialog box

- i. Input Data: current feature (should default to the correct file)
 - ii. Attribute: choose the parameter for interpolation (ch_mean).
 - iii. Check the Use NODATA value: box and enter -999 as the NODATA value
 - iv. Choose Kriging in the Methods box (or allow to default)
 - v. Click Next
 - c. In the Handling Coincidental Samples popup, choose Use Mean and click OK (should not matter, because all coincident points were removed in the initial query).
 - d. In the Step 1 of 4 – Geostatistical Method Selection dialog box, accept the defaults and click Next
 - e. In the Step 2 of 4 – Semivariogram/Covariance Modeling dialog box, check the Anisotropy box and click Next
 - f. In the Step 3 of 4 – Searching Neighborhood dialog box, change Neighbors to Include: to 25, set Shape Type: to the second type from the left (immediately to the right of One Sector) and click Next
 - g. In the Step 4 of 4 – Cross Validation dialog box, click Finish
 - h. Click OK in method summary window.
9. Use GA Layer to Grid Tool to create output grid
- a. Input = GA Layer result from step 8
 - b. Output surface grid = grids\ + segcode + \YY\ MMDDYY\ + 2-letter parameter designation + MMDDYY (for example, grids\yp\07\040904\ch052907)
 - c. Set cell size and Extent to the same as 1a.
10. Repeat steps 7, 8, and 9 for salinity(sa_mean) and turbidity(tu_mean) for the current date.
11. Use Multi Output Map Algebra Tool to calculate K_d grid from salinity, turbidity, and chlorophyll grids.
- a. Use appropriate equation in expression to calculate K_d (Perry, 2006).

Group 1a: MPNOH, MPNTF

$$K_d = 1.192674757 + 0.295620722 (TU \wedge 1 / 1.5) - 0.056160407 (SA) + 0.000274598 (CH)$$

Group 1b: CHKOH, JMSPH, JMSOH, JMSMH, JMSTF1, JMSTF2, APPTF:

$$K_d = 0.859551 + 0.292803 (TU \wedge 1 / 1.5) - 0.069875 (SA) + 0.028297 (CH)$$

Group 2: LYNPH, PMKOH, PMKTF, YRKPH, YRKMH, PIAMH:

$$K_d = 0.5275793536 + 0.3193475331 \times \sqrt[1.5]{TU} + 0.0176700982 \times SA + 0.0271723238 \times CH$$

Group 3: POTMH, POTTF, POTOH

$$K_d = 0.350711 + 0.298464 (TU \wedge 1 / 1.5) + .004668 (SA) + .011035 (CH)$$

Group 4: RPPMH, RPPOH, RPPTF, CRRMH

$$K_d = 0.591062 + 0.302307 (TU \wedge 1 / 1.5) - .021831 (SA) + .023192 (CH)$$

Group 5: (preliminary): MOBPH, CB6PH

$$K_d = -0.169897 + 0.333521 (TU \wedge 1 / 1.5) + .019517 (SA) + .017814 (CH)$$

b. Example expression for yrkph(yp):

$$kdgrid = 0.5275793536 + 0.3193475331 * pow(tugrid, 1 / 1.5) + 0.0176700982 * sagrid + 0.0271723238 * chgrid$$

where, kdgrid = location and name of output grid (grids\yp\07\052907\kd0052907)
 tugrid = location of turbidity grid from steps 7-9 (grids\yp\07\052907\tu052907)
 sagrid = location of salinity grid from steps 7-9 (grids\yp\07\052907\sa052907)
 chgrid = location of chlorophyll grid from steps 7-9 (grids\yp\07\052907\ch052907)

12. Determine if each cell in kdgrid meets appropriate K_d threshold using Multi Output Map Algebra Tool

Table 1. K_d Thresholds

PLL	Segment	Zones		
		0-1m	1-2m	
0.22	Polyhaline, Mesohaline	1.51	0.76	
0.13	Oligohaline, Tidal Fresh	2.04	1.02	

Expression for yrkph(yp) 0-1m:
 $kdgridt1 = con(kdgrid > 1.51, 0, 1)$

where kdgridt1 = attainment grid for 0-1 meters (grids\yp\07\052907\t1052907)
 kdgrid = result from step 11 (grids\yp\07\052907\kd052907)

Expression for yrkph(yp) 1-2m:
 $kdgridt2 = con(kdgrid > .76, 0, 1)$

where kdgridt2 = attainment grid for 1-2 meters (grids\yp\07\052907\t2052907)
kdgrid = result from step 11 (grids\yp\07\052907\kd052907)

13. Merge results from step 12 based on depth using Multi Output Map Algebra Tool:

Expression for yrkph(yp):

kdgrida = con(segpath == 4, kdgridt2,(con(segpath == 2 or segpath == 3, kdgridt1, 0)))

where, kdgridt1 = result from step 12 for 0-1m (grids\yp\07\052907\t1052907)

kdgridt2 = result from step 12 for 1-2m (grids\yp\07\052907\t2052907)

segpath = result from step 1b (baselayers\ypbathy)

kdgrida = attainment grid (grids\yp\07\052907\kd052907)

14. Use the Extract by Mask Tool to remove the exclusion zones from the analysis.

a. Input Grid = Result of step 13 (grids\yp\07\052907\kd052907)

b. Mask Raster = result of step 1c (analysis\ypxz)

c. Output Raster = (grids\yp\07\052907\ax052907)

15. Combine grids for assessment using Combine Tool

a. Input Rasters = cbseg; cusav_grd; segpath; kdgridax

Where cbseg = result of 1a

cusav_grid = result of 1c

segpath = result of 1b

kdgridax = result of step 14

b. Output grid = combine result (grids\yp\07\052907\wc052907)

16. Export results table to Access

a. Add the result from step 15 (grids\yp\07\052907\wc052907) to an ArcMap document

b. Right click on the grid and select Open Attribute Table

c. Click on Option in the lower right hand corner and select export

i. Export: all records

ii. Save the output table to the access database where water clarity acres will be calculated (gdb = wca.gdb, table = yp052907)

17. Calculate water clarity acres

a. Open access database where result of step 16 was saved

b. Open result from step 16 in design view

c. Rename the 4 fields after Count(order of columns dependent on order columns were combined in step 15. If entered in order designated in the this guide:

i. 1st Column after Count_ (YP) should be renamed SEG_BNDRY

ii. 2nd Column after Count_ (YP_CU06) should be renamed CU_SAV

iii. 3rd Column after Count_ (YPBATH) should be renamed BATHY

iv. 4th Column after Count_ (WC052907) should be renamed ATTAINMENT

18. Append Tables

- a. Open wca.gdb
- b. Create copy of first table (yp052907) and rename it “summary” (This step can also be done in ArcCatalog)
- c. Close Access
- d. Open Append Tool
 - i. Input datasets are all other tables other than the one that was copied in 18a in wca.mdb (yp041907, yp052907, 061307, 070207, 082707, 091307, 101007, 111207)
 - ii. Target dataset is “wca.mdb\summary”

19. Calculate water clarity acres

- a. Open wca.gdb
- b. Run Query 1
- c. Run Query 2
- d. Run Query 3
- e. Monthly and mean Annual water clarity acres are now located in the table called monthly_wca and annual_wca, respectively.

Note: These queries are used to calculate water clarity acres by initially converting cell counts of attainment into acreage of attainment inside and outside of current SAV areas for each dataflow cruise. Water clarity acres for the segment are then calculated by the taking the annual mean of the monthly acreage. Total acreage of SAV within the segment’s watershed boundary is also calculated.

Key for Access Tables (annual_wca & monthly_wca)

WCA = Water Clarity Acres

NSWCA = Water Clarity Acres outside of current SAV

CUSAV + NSWCA = Current SAV + Water Clarity Acres outside of
Current SAV

In VA, a segment is in attainment if at least one of three conditions is met.

1. Current SAV \geq Water Clarity Acreage Goal
2. Total Acreage of Attainment \geq Water Clarity Acreage Goal
3. Current SAV + Acreage of Attainment Outside of Current SAV \geq Water Clarity Acreage Goal

Segment goals are defined in DEQ document 9 VAC 25-260 Virginia Water Quality Standards (2005).

These are alternative Steps following 8 and 9 for segments where the use of barriers is appropriate. Follow steps 8 and 9 to create interpolation without barriers. Follow the steps below to interpolate with barriers and then merged to interpolation without barrier to ensure coverage for the all available bottom in the segment:

1. Create barrier layer for segment:
 - a. Use Select Tool (Analysis Tools -> Extract -> Select) to create segment polygon (baselayers/potmh_coa_seg.shp)
 - i. Input = baselayers/cbsegsplit
 - ii. Output = baselayers/potmh_coa_seg.shp
 - iii. Expression = "CBSEG" = "POTMH_COA"
 - b. Use Clip Tool (Analysis Tools -> Extract -> Clip) to create segment barrier
 - i. Input = baselayers/potmh_coa_seg.shp
 - ii. Output = baselayers/potmh_coa_barrier.shp
2. Create shapefile where cruise points with error codes are selected out.
 - a. Add dataflow point feature (wq_data.gdb/pc041907p) to blank map document
 - b. Select where ch_mean > -999. Note how much data may be invalid. Decide if too much data has an associated error code to proceed with interpolation for this dataset. This decision is made with a concern for the amount and spatial distribution of data that will be excluded. In the future, we would like to move toward a less-subjective approach though the use of an associated standard error grid. If the interpolation is not made, that particular date is ultimately thrown out of any annual mean water clarity acreage calculations. If there is sufficient data to continue, clear the selection before continuing with the interpolation.
 - c. With selection still in place, export selected features to shapefile
 - i. Right click on feature in TOC and choose Data > Export data
 - ii. Export: selected features
 - iii. Use same coordinate system as layer's source data
 - iv. Output shapefile: analysis_shapefile\pc041907p.shp
3. Use aml Kriging function to create interpolation incorporating new barrier file
 - a. Open Single Output Map Algebra tool (Spatial Analyst Tools > Map Algebra > Single Output Map Algebra)
 - b. Expression (make sure to replace '~' with location of file on local machine):


```
Kriging(~\analysis_shapefile\pc041907p.shp , ch_mean,
          ~\baselayers\potmh_coa_barrier.shp , GRID, #, SPHERICAL, SAMPLE, 25,
          1500, 25)
```
 - c. Output Grid: ~\grids\pc\041907\tch041907
 - d. Set cell size and Extent to the same as analysis_grids/pc
4. Open Con tool (Spatial Analyst Tools > Conditional > Con) to limit extent of interpolated barrier grid
 - a. Conditional grid: ~analysis_grids/pc_bath
 - b. Expression: "VALUE" > 1
 - c. Input True Raster: ~\grids\pc\041907\tch041907

- d. Input False Raster: ~\grids\pc\041907\ch041907
- e. Output raster: ~\grids\pc\041907\bch041907
- 5. Merge barrier grid with interpolated grid without barriers.
 - a. Open Single Output Map Algebra tool (Spatial Analyst Tools > Map Algebra > Single Output Map Algebra)
 - b. Expression (make sure to replace '~' with location of file on local machine):

 Merge(~\grids\pc\041907\bch041907, ~\grids\pc\041907\bch041907)
 - c. Output Grid: ~\grids\pc\041907\mch041907
- 6. Repeat this procedure for all parameters
- 7. To Proceed: delete ~\grids\pc\041907\ch041907 and rename ~\grids\pc\041907\mch041907 to ~\grids\pc\041907\ch041907. Use this renamed merged grid in all future steps (go to step 10 above to continue with water clarity analysis)

Sources

- Perry, E. (2006). *Notes on Lumping vs Splitting $K_d = f(\text{turbidity})$ calibration*. Unpublished Report.
- 9 VAC 25-260 Virginia Water Quality Standards. (2005). § 62.1-44.15 3a of the Code of Virginia. Retrieved February 21, 2007, from http://www.epa.gov/waterscience/standards/wqslibrary/va/va_3_wqs.pdf.

Appendix G: CMON Station QA/QC Protocols in Brief

Continuous Station Coarse QA/QC Protocol**Step 1 Insert appropriate parameter qualifiers**

Parameter	<	>
Temp	-5	45
SpCond	0	100
Salinity	0	70
DO sat	0	500
DO mg/l	0	50
Depth	0	9.10
PH	0	14
Turb	0	1000
Chl	0	400
Fluor	0	100

Step 2 Identify negative “-“ values

All negative values are flagged with a “GNV” in the appropriate parameter_a column

Step 3 Identify time periods when the datalogger or certain probes were not deployed.

In the appropriate parameter_a column, flag all non-deployments with a “NND”.

Step 4 Identify and flag single spike NTU values

In the NTU_a column, flag all single spike NTU values >300 with “NQR”.

Step 5 Identify and flag single spike Chl values

In the Chl_a column, flag all single spike Chl values >400 with “NQR”.

Step 6 Identify and flag single spike %Fluor values

In the Fluor_a column, flag all single spike Fluor values >100 with “NQR”.

Step 7 Identify and flag probe failures

In the appropriate parameter_a column, flag time intervals with “GPF”.

Note: This would apply to DO membrane punctures, pH bulb breakage etc.

Step 8 Identify and flag time periods in which specific probes failed to meet acceptable post calibration criteria

In the appropriate parameter_a column, flag time intervals with “GPC”.

Note: This would apply to DO drift and similar issues. If you can not clearly identify where the problem began, flag the entire deployment interval.

Step 10 Identify and flag time periods in which entire instrument or specific probes were out of water.

In the appropriate parameter_a column, flag affected time intervals with “NOW”.

Note: If entire instrument is out of water, all parameter_a columns must be flagged.

Step 12 Identify and flag time periods affected by a time skip.

In the comment column, flag effected time period with “CTS”.

Step 13 Identify and flag time periods affected by station maintenance.

In the appropriate parameter_a column, flag affected time interval with “NQR”

Note: This was seen in some of our turbidity and depth data.

Step 14 Link deployments and look for probe drifts, significant mismatch, etc

In the appropriate parameter_a column, flag affected data with the appropriate error code.

Appendix 7

VIMS Reece Standard Operating Procedures

VIMS
Marine and Aquaculture Molecular Genetics Laboratory – Reece
Histopathology Laboratory – Vogelbein

Collection of water samples:

Disposable plastic coliform 120 ml water collection bottles without preservative (Scientific Specialties #C56-004) are provided to the sampling personnel, which includes Moore laboratory and HRSD staff. Two replicate water samples are collected from each site. An additional 250 mL sample will be collected for toxin analysis whenever the collection is in response to reports of a fish kill or if dying fish are observed. The water samples are kept in a cooler with blue ice, but NOT in direct contact with the ice. The samples can be separated from the ice by an insulating material such as Styrofoam, packing material or newspaper. As soon as possible after collection the water samples should be delivered to the Reece Marine and Aquaculture Molecular Genetics laboratory or picked up by Reece laboratory staff.

Microscopic examination of samples:

One of the replicate water samples received is used for microscopic identification and enumeration of cell types. Visual microscopic identification of dominant dinoflagellate, raphidophyte and diatom species in water samples is done by examination on a Zeiss Axiovert 200 (Carl Zeiss Microscopy, LLC, Thornwood, NY) inverted microscope with a digital camera and Axiovision 3.0 imaging software. Bloom samples containing species of interest (i.e. *Heterocapsa triquetra*, *Heterocapsa rotundata*, *Gymnodinium instriatum*, *Scrippsiella trochoidea*, *Cochlodinium polykrikoides*, *Prorocentrum minimum*, *Prorocentrum micans*, *Karlodinium veneficum*, *Akashiwo sanguinea*, *Alexandrium monilatum*, *Chattonella subsalsa*, *Heterosigma akashiwo* or *Microcystis aeruginosa*) are used to establish *in vitro* cultures of these organisms via micro-manipulation for additional genetic and toxicity characterization and, for a subset of bloom samples, for conducting bioassays (see below).

Molecular genetic analysis:

The replicate water samples are filtered for genetic analysis if A) they contain algal species of interest as listed above, and/or B) they are samples from an algal bloom or fish kill. It is important to filter the water sample as soon as possible because a delay between sample collection and sample processing could lead to a change in the organismal composition of the sample. All samples filtered for genetic analysis are filtered onto both a <1 µm membrane to detect cyanobacteria and a 3 µm membrane for the larger species of interest. A “blank” sample (distilled water) is filtered alongside the environmental samples at least twice a month as a negative filtration control to insure that there is no sample-to-sample contamination or "carry-over" during filtration.

The following supplies are used for sample filtration:

1. Water filtration pump.
2. Analytical Test Filter Funnels with 0.2 µm (or 0.45 µm) cellulose nitrate membranes (Fisher #145-0020 or #145-0045), funnel adapters (included with filter funnels), and (#8) rubber stoppers with 1/2" hole.
3. 3 µm Isopore membrane filter (Fisher #TSTP-047-00).
4. Disposable plastic forceps (Cole Parmer #EW-06443-26).
5. Microcentrifuge tubes containing 180 µl ASL lysis buffer and 20 µl Proteinase K from the QIAamp DNA Stool Mini Kit (Qiagen #51504)
6. Lysing Matrix E microcentrifuge tubes (MP Biomedicals #6914)

Samples are filtered as follows:

1. Microcentrifuge tubes are prepared with 360 μ l ASL lysis buffer and 40 μ l Proteinase K for each sample.
2. Disposable test filter funnel is attached to the funnel adapter and stopper on a filtration flask. When possible, 20 mls of the sample is filtered for detection of cyanobacteria. When the sample is turbid, containing a lot of particulate matter, add 5 mls at a time. If unable to filter the entire 20 mls then record the actual volume filtered.
3. Disposable forceps are used to remove the membrane from the filtration apparatus. The membrane is folded in half and then in half again using the forceps and placed into the Lysing Matrix E tube.
4. The Lysing Matrix E tube is immediately labeled with the collection site, date and volume filtered.
5. A 3 μ m Isopore membrane filter is placed onto the same filtration apparatus using sterile technique.
6. Disposable test filter funnel is again attached to the funnel adapter and stopper on a filtration flask. When possible, 100 mls of the sample is filtered. When the sample is turbid add 20 mls at a time. If unable to filter the entire 100 mls then record the actual volume filtered.
7. Disposable forceps are re-used to remove the 3 μ m membrane from the filtration apparatus. The membrane is folded in half and then in half again using the forceps and placed into the first microcentrifuge tube containing buffer solution.
8. The microcentrifuge tube containing the filter and buffer solution is immediately labeled with the collection site, date and volume filtered.
9. The test filter funnel, collection bottle, and forceps are disposed, however the funnel adapter and stopper are not disposable.
10. The microcentrifuge tube containing the filter and buffer solution is stored upright at room temperature until processed for DNA analysis.
11. The microcentrifuge tube containing the filter and glass beads is stored at -20°C until processed for DNA analysis.

DNA extraction protocol:

Samples being screened for cyanobacteria (Lysing Matrix E tubes containing filter):

Samples are processed immediately upon receipt during a cyanobacterial bloom. At least twice a month, "blank" DNA extractions are performed in the laboratory (buffers only, no filter) alongside the samples as negative controls for the extraction protocol to insure that contaminating DNA is not present in the extraction materials or solutions.

1. Remove tube from -20°C and add 590 μ l of Qiagen AE Buffer.
2. Place the tube in a boiling water bath for 10 minutes and then place on ice.
3. Place the tube in the FastPrep-24 (MP Biologicals) bead beater set for 6.0 m/s for 40 seconds. Place on ice.
4. Centrifuge 1 minute at maximum speed.
5. Transfer 400 μ l of the supernatant to a clean, labeled microcentrifuge tube (this is the crude extraction).
6. Centrifuge for 5 minutes at maximum speed.
7. Transfer 350 μ l of the supernatant to a clean, labeled microcentrifuge tube (this is the purified extract)
8. Prepare a 1:5 dilution by transferring 50 μ l of the purified extract into 200 μ l of Qiagen AE Buffer in a clean, labeled microcentrifuge tube.

9. For long-term storage, keep the purified extracts at -20°C. If PCR is to be done within 24 hrs, the sample can be stored at 4°C.

Samples being screened for eukaryotic HAB organisms (Modified from Qiagen's QIAamp DNA Stool Mini Kit Protocol for Isolation of DNA from Stool for Pathogen Detection):

Samples are processed immediately upon receipt during a bloom or 'fish-kill' event. At least twice a month, "blank" DNA extractions are performed in the laboratory (buffers only, no filter) alongside the samples as negative controls for the extraction protocol to insure that contaminating DNA is not present in the extraction materials or solutions.

1. Add an additional 15 µl of Proteinase K to the microcentrifuge tube containing the filter and incubate overnight at 56°C.
2. Centrifuge briefly to remove droplets from the tube lid. Add 520 µl Buffer ASL to each sample. Vortex continuously for 1 min.
3. Heat the suspension for 5 min at 70°C.
4. Vortex the samples for 15 sec then centrifuge for 1 min at max speed.
5. Pipet the full volume of the supernatant into a new 2ml microcentrifuge tube.
6. Add 1/2 InhibitEX tablet to each sample and vortex immediately and continuously for 1 min or until the tablet is completely suspended. Incubate suspension for 1 min at room temperature to allow inhibitors to adsorb into the InhibitEX matrix.
7. Centrifuge sample at full speed for 3 min to pellet inhibitors bound to InhibitEX.
8. Pipet all the supernatant into a new microcentrifuge tube and discard the pellet. (Transfer of small amounts of pellet material will not affect the procedure).
9. Centrifuge sample at full speed for 3 min again.
10. Pipet 15 µl Proteinase K into a new 1.5 ml microcentrifuge tube.
11. Pipet full volume of the supernatant into the microcentrifuge tube containing the Proteinase K (measure the volume).
12. Add a volume of Buffer AL that is equal to volume of supernatant and vortex for 15 sec.
13. Incubate at 70°C for 10 min and then centrifuge briefly to remove drops from the inside of the tube lid.
14. Add a volume of ethanol (96 – 100%) that is equal to the volume of supernatant, mix by vortexing and then centrifuge briefly to remove drops from the inside of the tube lid.
15. Place a new, labeled QIAamp spin column in a 2 ml collection tube. Carefully apply the complete lysate (600 µl at a time) to the spin column without moistening the rim. Close the cap and centrifuge at full speed for 1 min. Place the spin column in a new 2 ml collection, and discard the tube containing the filtrate.
16. Carefully open the spin column and add 500 µl Buffer AW1. Centrifuge at full speed for 1 min. Place the spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.
17. Carefully open the spin column and add 500 µl Buffer AW2. Centrifuge at full speed for 1 min.
18. Pour off filtrate from collection tube, replace spin column and centrifuge at full speed for 3 min. Discard the collection tube containing the filtrate.
19. Transfer the spin column into a new, labeled microcentrifuge tube and pipet 100 µl Buffer AE directly onto the membrane. Incubate 1 minute at room temperature then centrifuge at full speed for 1 minute to elute DNA .
20. Apply the eluate to the spin column, incubate 1 minute at room temperature and then centrifuge at full speed for 1 minute for a second elution.
21. Repeat step 20 for a third elution.
22. For long-term storage, keep the eluate at -20°C. If PCR is to be done within 24 hrs, the

sample can be stored at 4°C.

PCR protocols:

VIMS Marine and Aquaculture Molecular genetics laboratory has developed and optimized standard and quantitative real-time PCR assays for *Alexandrium monilatum*, *Karlodinium veneficum*, *Cochlodinium polykrikoides*, *Pfiesteria piscicida*, *Psuedopfiesteria shumwayae*, *Luciella* spp. and *Microcystis aeruginosa*. We also are using previously published assays for *Chattonella subsalsa* and *Heterosigma akashiwo* (Bowers et al. 2006), but are doing sequencing of amplicons in order to develop our own assays optimized for strains in this region. In addition, we are developing and optimizing molecular detection assays for *Heterocapsa triquetra*, *H. rotundata*, *Scrippsiella trochoidea*, *Akashiwo sanguinea*, *Gymnodinium instriatum*, *Prorocentrum minimum* and *P. micans*.

Water samples are screened as follows:

1. Cell counts for bloom samples are immediately determined using the specific quantitative real-time PCR assay.
2. All samples are screened with standard PCR assays for the following organisms:
 - a. *Alexandrium monilatum*
 - b. *Cochlodinium polykrikoides*
 - c. *Pfiesteria piscicida*
 - d. *Psuedopfiesteria shumwayae*
3. *Luciella* spp. All samples with positive standard PCR results for the species under bullet point #2 are screened with the corresponding quantitative real-time PCR assay.
4. All samples are screened with quantitative real-time PCR assays for *Karlodinium veneficum* and for *Chattonella subsalsa* and *H. akashiwo* if raphidophytes are observed in the sample microscopically.
5. All samples from oligo- and mesohaline sites are screened with the quantitative real-time PCR assay for *Microcystis aeruginosa*

PCR Primers (Operon):

Organism	Standard PCR or qPCR	Primer/Probe	Sequence (5'-3')
<i>A. monilatum</i>	Standard PCR	Am-SSU-174F AmSSU-649R	CAATCAAACCTGACTCTTGTGGG CCTCAACAGAAATCCAACACTACGAGC
	qPCR	Am-SSU-174F Am-SSU-409R	CAATCAAACCTGACTCTTGTGGG CACAGGACCTTACCTTTC
<i>C. polykrikoides</i>	Both	YK_CpITS_116F Yk_CpITS_496R	TCATTACACGCATTCCATCC CGCAGGAACAAAGGCATAGACAC
<i>C. subsalsa</i> (Bowers et al. 2006)	qPCR	SubsalsaFor SubsalsaRev SubsalsaProbe	TTGGATTCCGACGGGC ATATGCTTAAATTACAGCGGGTTTT TTCGGCCAAGCACACATCCTC

<i>K. veneficum</i>	qPCR	KvITS_242F KvITS_328R Kv_266probe	TTCGTTGTGTAGTTGTTGACTCG TGCTGACCTAACTTCATGTCTTG AGCCTGCTCCAGCTCACGACTCCT
<i>M. aeruginosa</i>	Both	Micro-4f Micro-182r	
<i>P. piscicida</i>	Both	PCO_160_LSU Pp_450_LSU	CATCAGTGAGGGTGAGAATC CACAGCAACCCTTGCGGTGG
<i>P. shumwayae</i>	Both	PfBSSU PfBITSR201L	CCAGCTTCTGGATTTTGTTCGC GCGATGAGGAAGAGAAAAATGACG
<i>Luciella</i> spp.	Both	LucyF85ITS LucyR590ITS	GGTGTGAGGCTCTGTGTARG CCAGGGATMTCTCCATGTC

Maintenance of control material cultures for generating standard curves for qPCR and biotoxicity assays:

Control stocks are maintained in L1.5 medium supplemented with vitamins at ~25° C, 14:10-h light:dark cycle, approximately 100 $\mu\text{mol photons}\cdot\text{m}^2\cdot\text{s}^{-1}$. L1.5 media is prepared with sterile Wachapreague, Virginia seawater (~35 psu) or York River water (~20 psu) with the addition of either MilliQ water or Marine SeaSalt to reach the desired salinity. Stock cultures are split every 25-30 days by transferring a portion of the culture to fresh media. Cultures are enumerated by cell counts using one of the following methods depending on culture density and cell size.

- 10 μM or less (eg. *K. veneficum*) – a Bright-line hemocytometer is used.
 - 10 μl volume is applied to the slide
 - For low density cultures all 9 blocks on the grid are counted and cells/ml = (the total number of cells counted/9)* 10^4
 - For high density cultures where the cells are evenly distributed over the grid 3 blocks on the grid are counted and cells/ml = (the total number of cells counted /3)* 10^4
- Greater than 10 μM (eg. *C. polykrikoides* and *G. instriatum*) – a Nannoplankton Counting chamber is used
 - Each counting chamber is calibrated individually. A specific volume and multiplication factor is labeled on the slide. PhycoTech ID #478, for example, specifies that you add 64 μl to the chamber and multiply the total number of cells counted by 15.625
 - Cells must be counted in three dimensions as there is a depth to the chamber
- Very low density cultures – a Sedgewick Rafter cell counting slide is used.
 - A 1 ml volume is applied to the slide and the total number of cells/ml is counted.
 - Cells must be counted in three dimensions as there is a depth to the chamber
- Chain forming cells (eg. *A. monilatum*) or colony forming cells (eg. *M. aeruginosa*) – a microscope slide is used
 - Place three drops of 10 μl each on the slide and count all cells. The cells/ml = (the total number of cells counted/3)*100

DNA is extracted from a known number of cells and used as positive control material for both standard and real-time PCR assays. Real-time PCR standard curves are generated by using in vitro cell cultures of

the various species.

The following cultures are currently maintained at VIMS:

Table 2: HAB clonal cultures currently maintained at VIMS.

Organism	Origin	Identifier
<i>Alexandrium monilatum</i>	York River isolate	YK-Am
<i>Alexandrium monilatum</i>	Mississippi isolate	AM01
<i>Chattonella subsalsa</i>	Delaware isolate	CCMP 2191
<i>Cochlodinium polykrikoides</i>	Lafayette River isolate	Laf-Cp
<i>Cochlodinium polykrikoides</i>	York River isolate	YK-Cp
<i>Cochlodinium polykrikoides</i>	Florida isolate	FL-Cp
<i>Gymnodinium aureolum</i>	James River isolate	JR-Ga
<i>Heterosigma akashiwo</i>	Delaware isolate	CCMP 2393
<i>Heterosigma akashiwo</i>	Lafayette River isolate	Laf-Ha
<i>Karlodinium veneficum</i>	Chesapeake Bay isolate	CCMP 1974
<i>Karlodinium veneficum</i>	James River isolate	JR-Kv
<i>Karlodinium veneficum</i>	Poquoson River isolate	VIMS 2006
<i>Microcystis aeruginosa</i>	Cypress Creek, VA isolate	CC-Ma
<i>Microcystis aeruginosa</i>	Lake Rooty, VA isolate	LR-Ma
<i>Prorocentrum minimum</i>	Choptank River isolate	JA98.10
<i>Prorocentrum minimum</i>	Colonial Beach isolate	CB-Pm
<i>Prorocentrum minimum</i>	James River isolate	JR-Pm
<i>Scrippsiella trochoidea</i>	Lafayette River isolate	Laf-St-1
<i>Scrippsiella trochoidea</i>	Lafayette River isolate	Laf-St-2

Standard PCR Reagents:

AmpliTaq DNA Polymerase with Buffer I (Applied Biosystems #N8080152)
 AmpliTaq DNA Polymerase with Buffer II (Applied Biosystems #N8080153)
 GENEAMP dNTPs (Applied Biosystems #N8080007)
 Molecular Biology Grade BSA (10 mg/ml working stock)
 Agarose (ISC Bioexpress #E-3120-500)
 Low-Melt Agarose (Fisher #BP1360)

Standard PCR Reaction Conditions:

Amplifications are performed with 0.5 µl of DNA in 25 µl reactions. A negative control (no DNA) and a positive control are included for each of the primer sets. Products are electrophoresed on a 3.0% gel (1.5% low-melt and 1.5 % standard agarose), then stained with ethidium bromide and digitally recorded. The reagent concentrations and PCR cycling parameters are as follows:

1) *A. monilatum* (final concentration of MgCl₂ is 1.0 mM)

Reagents:	Final conc.	
BSA (1 mg/ml)	0.4	
PCR Buffer II (10 X)	1	
MgCl ₂ (25 mM)	1	
Nucleotides (each, 10 mM)	0.8	
Primers (each, 100 µM)	1	
Taq (5 U/µl)	0.024	

Profile:	Temp (°C)	Time
Initial denaturation	94	4 min
# Cycles		40
Denaturation	94	30 sec
Annealing	64	30 sec
Extension	72	1 min 30 sec
Final Extension	72	5 min

2) *C. polykrikoides* (Buffer I contains 1.5 mM MgCl₂)

Reagents:	Final conc.	
BSA (1 mg/ml)	0.4	
PCR Buffer I (10 X)	1	
Nucleotides (each, 10 mM)	0.8	
Primers (each, 100 µM)	1	
Taq (5 U/µl)	0.024	

Profile:	Temp (°C)	Time
Initial denaturation	94	4 min
# Cycles		40
Denaturation	94	30 sec

Annealing	56	30 sec
Extension	72	1 min 30 sec
Final Extension	72	5 min

3) *P. piscicida* and *P. shumwayae* and *M. aeruginosa* (final concentration of MgCl₂ is 1.0 mM)

Reagents:	Final conc.
BSA (1 mg/ml)	0.4
PCR Buffer II (10 X)	1
MgCl ₂ (25 mM)	1
Nucleotides (each, 10 mM)	0.8
Primers (each, 100 μM)	1
Taq (5 U/μl)	0.024

Profile:	Temp (°C)	Time
Initial denaturation	94	4 min
# Cycles		40
Denaturation	94	30 sec
Annealing	56	30 sec
Extension	72	1 min 30 sec
Final Extension	72	5 min

4) *Luciella* spp. (Buffer I contains 1.5 mM MgCl₂)

Reagents:	Final conc.
BSA (1 mg/ml)	0.4
PCR Buffer I (10 X)	1
Nucleotides (each, 10 mM)	0.8
Primers (each, 100 μM)	1
Taq (5 U/μl)	0.024

Profile:	Temp (°C)	Time
Initial denaturation	94	4 min
# Cycles		40
Denaturation	94	30 sec
Annealing	56	30 sec
Extension	72	1 min 30 sec
Final Extension	72	5 min

Real-time PCR:

All samples are run in duplicate. A standard curve containing 5 points or more along with negative and positive controls are included with each assay run. Reactions are prepared with 1 µl of DNA in 10 µl total reaction volumes. The samples are run on a 7500 Fast Real-Time PCR system using the Fast 7500 mode with the default cycling parameters. Quantification is determined using the automatic Ct and baseline settings. Dissociation curve analysis is performed for all SYBR® Green assays and the melting temperature of all positive samples are compared to the control for verification of identity.

Real-time PCR Reagents:

Desalted primers (as listed above from Operon)
Molecular Biology Grade BSA (1 mg/ml working stock)
TaqMan® Fast Advanced Master Mix (Applied Biosystems #4444963)
Fast SYBR® Green Master Mix (Applied Biosystems #4385616)

Real-time PCR reaction conditions:

Reagents:	Initial conc.	Final conc. <i>C. polykrikoides</i> , <i>P. piscicida</i> <i>P. shumwayae</i>	Final conc. <i>A. monilatum</i> , <i>M. aeruginosa</i> , <i>Luciella spp.</i>	Final conc. <i>K. veneficum</i> , <i>C. subsalsa</i>
Forward Primer (µM)	10	1.0	0.9	0.9
Reverse Primer (µM)	10	1.0	0.9	0.9
Probe (µM)	1	0	0	0.1
Master mix (X)	2	1	1	1

Initial Denaturation:	Temp (°C)	Time (sec)	Acquisition
	95	20	none
Amplification:	Temp (°C)	Time	Acquisition
40 Cycles	95	3	none
	60	30	single
Melting analysis:	Temp (°C)	Time (sec)	Acquisition
Step 1	95	15	none
Step 2	60	60	none
Step 3	95	15	none
Step 4	60	15	continuous

Toxin analysis:

Toxin analysis is performed whenever an animal kill occurs in association with a bloom of a HAB species that has been reported under some conditions to produce saxitoxins, brevetoxins or microcystins. These include some of the *Alexandrium* species (note: this does not include *A. monilatum*, which produces a different toxin), *Karenia brevis*, *C. subsalsa*, *H. akashiwo*, *Microcystis aeruginosa* and several other cyanobacteria. The appropriate ELISA kit [i.e. Brevetoxin (NSP) #520026, Saxitoxin #52255B, Microcystins/Nodularins (ADDA) #520011ES] from Abraxis LLC (Warminster, PA) is used according to

the manufacturer's protocol. Toxin analysis is also done on the *M. aeruginosa* cultures used for laboratory biotoxicity assays (see below).

Laboratory biotoxicity assays:

Biotoxicity assays are conducted using bloom samples and clonal cultures (see above) established from bloom samples. We are currently working on the development of the toxicity bioassays for *M. aeruginosa*, which likely will employ *Ceriodaphnia dubia* as the invertebrate test species because the assays will be conducted under very low salinities < 3ppt. Larval sheepshead minnow, *Cyprinodon variegatus*, the vertebrate test species, must be acclimated to low salinities for several days prior to bioassays with *M. aeruginosa*. For bioassays with HAB species from higher salinities, oyster veligers (*Crassostrea virginica*) and *C. variegatus* are used as the test animals. Typically bioassays are done using bloom samples or cultures with cell densities ranging from ~100 cells/ml to ~10,000 cells/ml at salinities dependent upon the typical field conditions where the HAB species is collected. A dilution series of the bloom samples or bloom-derived culture may be performed to achieve the desired concentration for exposure. Brine shrimp nauplii, oyster veligers, (each at 10 animals per well with three replicates), or 7-day old sheepshead minnows (one animal per well with 10 replicates) are placed in 24-well plastic tissue culture plates with 2mL of bloom or culture material per well. Controls consist of test animals in media alone or in media combined with a control food source (*Isochrysis galbana*, *Pavlova pinguis* or *Chaetoceros muelleri* depending on the species of test animal; fish larvae are either not fed or are fed *Artemia* nauplii). Assays using minnow larvae are run at an ambient temperature of ~24 - 28° C with a 14:10 light:dark cycle at a light intensity of approximately $150 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$ for 96 hours with observations and data collection every 12 hours. Assays using veligers are run under the same conditions for 120 hours with data collection every 24 hours. A subsample of 5 ml of culture material will be filtered at the start of each assay and stored at -20C until analyzed by the VIMS Analytical Services Center.

Observations are performed on an Axiovert 200 inverted microscope. Activity is noted in order to determine the condition of the test animals during the exposures. Mortality is determined based on the failure of animals to remain swimming in the water column and the lack of any movement of appendages in the case of brine shrimp nauplii or the lack of heartbeat and movement within the shell of the oyster veligers. Dose response curves are generated from these assays by plotting the percent mortality over time for the different treatments.

Field exposure studies:

175 small market-sized oysters (~75 mm) are deployed in a cage near the ConMon stations in James and Lafayette Rivers. A sample of 5 oysters is processed for histology prior to deployment. During bloom events 15-30 oysters are collected from the cage, mortalities are recorded and live animals are processed for histological analyses. Briefly, the oysters are shucked and the tissue is fixed in Davidson's Fixative and then processed using standard methods for paraffin histology. Six-micron sections are cut and mounted on slides. These slides are stained with hemotoxilyn and eosin for pathological evaluation or used for fluorescent *in situ* hybridization (FISH) assays with species-specific DNA probes.

Whole fish histopathology:

Whole fish histopathology will be implemented during 2013 in select bioassays of field samples where mortality may not be a suitable endpoint (e.g., *M. aeruginosa*) or where more information on the mechanisms of toxicity may be highly desirable (*C. polykrikoides*).

Fluorescent *in situ* hybridization (FISH):

FISH Reagents:

Xylene

Ethanol

P buffer (50 mM Tris-HCL pH 7.5, 5 mM EDTA)

Pronase (12.5 mg/ml in water)

Acetic Anhydride Solution (0.1 M triethanolamine-HCL, 5% v/v acetic anhydride)

5X SET (750 mM NaCl, 6.4 mM EDTA, 100 mM Tris-HCL)

Prehybridization buffer (0.2 mg/ml BSA, 0.025% v/v SDS) at 42° C

PBS (137 mM NaCl, 2.7 mM KCl, 100 mM Na₂HPO₄, 2mM KH₂PO₄ at pH 7.4)

Fluoromount (Sigma F4680)

FISH Protocol:

Species-specific DNA probes are being developed and optimized for bloom organisms of interest. Paraffin-embedded tissue sections are dewaxed in Xylene and rehydrated in dilutions of ethanol. Cells are permeabilized using Pronase (125 µg/ml in P buffer) for 30 minutes at 37° C. The slides are washed in P buffer twice for 5 minutes each time to remove the Pronase. The slides are then slowly stirred in acetic anhydride solution for 10 minutes to reduce background. After washing the slides in PBS for 5 minutes they are washed in 5X SET for 5 minutes. The remaining steps are done in the dark. The probe is added to pre-warmed prehybridization buffer to a concentration of 12 ng/µl (exact concentration is determined during the optimization procedure). 100 µl is applied to the tissue on each slide, the slides are covered with plastic coverslips and incubated at 42°C overnight. 0.2X SET is prepared and pre-warmed at 42°C overnight. The next day, the slides are washed with pre-warmed 0.2X SET three times for 1 minute each time. After the slides are air-dried for 15-30 minutes they are covered with Fluoromount and glass coverslips. The Fluoromount is allowed to set for 30 minutes and then fingernail polish is applied to the edges of the coverslip to prevent evaporation.

Literature Cited

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Appendix 8

VIMS Anderson Standard Operating Procedures

**Laboratory and Field Protocols for
Sediment Oxygen and Nutrient Exchange (SONE) Study in the James River Estuary
Nutrient Cycling**

VIMS Nutrient Cycling Laboratory – Dr. Iris Anderson

I. Water Quality Sample Collection and Filtration for Nutrients Protocol

Supplies/Equipment:

Collection: 250 mL brown nalgene bottles, cooler with ice

Filtration: gloves, 60 mL BD syringe with luerlok tip, Whatman 25 mm syringe filters with 0.45 μm polyethersulfone membrane, 2 oz. Whirlpak bags, 20 mL pre-combusted scintillation vials (borosilicate glass; combust vial by covering with small square piece of aluminum foil and combusting in muffle oven for 5 hours at 500°C) and screw caps (Polypropylene; foil or Teflon lined).

Water quality sample collection:

1. Deploy bilge pump intake to midway in the water column. Allow to run for 2 minutes to rinse tubing.
2. Rinse bottles three times with sample water.
3. Fill bottles
4. Place in cooler with ice.
5. When return to lab, filter immediately.

Filtration for dissolved nutrients:

1. Wear gloves.
2. Shake bottle. Fill 60 mL syringe to max capacity with sample and place Acrodisc syringe filter (0.45 μm) on end, being careful to not touch tip of syringe and filter directly.
3. Filter and discard 5 mL of sample.
4. Filter and collect 25 mL of water for dissolved inorganic nitrogen and phosphorus (2oz whirlpak bag), 14 mL for dissolved organic nitrogen (2oz whirlpak bag), 16 mL for pre-combusted scintillation vial (remove aluminum foil on top, add filtered water, and replace foil on top before capping scintillation vial).
5. Refill syringe with 20 mL of sample water and filter 20 mL of water for dissolved silica (2oz whirlpak bag)
6. Put samples on ice immediately.
7. Repeat with remaining bottles, using new syringe and filter for each water sample.
8. Freeze samples, analyze within 1 month.

II. Water Column Chlorophyll Collection and Filtration Protocol

Supplies/Equipment:

Collection and filtration: 250 mL brown nalgene bottles, 20mL BD syringe, Whatman Glass Microfiber Filters (GF/F) filter (2.5cm, 0.7 μ m), 250 mL beaker of DI water, 25mm glass filter holders (chimneys, stoppered filter holders, clamps), 2 filter forceps, foil envelopes, wash bottle filled with DI water, waste beaker, filter rig set-up (filter funnel manifold, vacuum pump, tubing, Erlenmeyer filter flask, waste bottle)

Water sample collection (note: bottles are also used to collect nutrient samples):

1. Deploy bilge pump intake to mid depth of the water column. Allow to run for 2 minutes to rinse tubing.
2. Rinse bottles three times with sample water.
3. Fill bottles
4. Place in cooler with ice.
5. When return to lab, filter immediately.

Filtration:

1. Set up filtration manifold or Erlenmeyer filter flask (if in field) with stoppered glass filter holder (25 mm). Place small Whatman GF/F filter (2.5cm), graph side up, on glass filter holder and secure chimney tower to holder with metal clamp. Make sure filter is centered on filter holder.
2. Shake bottle. With 20 mL syringe, draw up sample and expel into waste beaker (this cleans the pipette between each sample). Carefully draw up a second 20 mL sample and filter. Filter additional water until there is color observed on filter (e.g., not white; this can be assessed when the glass chimney is removed and there is a color variation between the filtered portion and the outside edge of the filter). Record total volume filtered on data sheet.
3. Draw up 5mL of DI water into syringe to rinse syringe and filter.
4. Rinse the glass chimney tower with approximately 5 mL of DI water from wash bottle and filter again.
5. Unclamp and remove glass chimney. Use forceps to fold filter in half twice lengthwise and place into foil envelope. While folding, be careful to not touch the filtered material by handling only the edges of the filter. Do not touch the filters with gloved or ungloved hands due to oils or contaminants on hands.
6. Repeat for each sample.
7. After finishing filtration, rinse glass filter chimneys and holders 5 times with DI water and air-dry. Wash other glassware and bottles according to Cleaning Protocol.
8. Place samples in foil envelopes in freezer. (Max holding time is 1 month)
9. Send samples to VIMS Analytical Service Center.

III. Photosynthetically Active Radiation (PAR)/ Light Attenuation Measurement Protocol

Equipment:

Li-Cor LI-1400 Datalogger, Li-Cor LI-192SA Underwater quantum sensor, Li-Cor underwater cable, Li-Cor lowering frame attached to PVC pole (marked at depth intervals of 10 cm), Li-Cor LI-190 Quantum Sensor.

PAR Measurements

1. Connect cables for deck and submerged sensors to light meter.
2. Ensure that deck sensor is on a flat surface with no shadows due to equipment on the boat or people and level so that bubble is in the middle.
3. PAR measurements should be made on the sunny side of the boat to avoid the shadow of the boat or person making the measurement.
4. Turn on light meter and measure PAR values from both deck sensor (incident irradiance) and underwater sensor at multiple depths, including just below water surface, mid-way in water column, and above sediment surface at each station. Record depths.
 - a. For the 1 m sites, measure at just below the water surface, 0.3m, 0.6m, and above sediment surface (determined when pole, on which the underwater PAR sensor is mounted, touches the bottom)
 - b. For the 2 m sites, measured at just below the water surface, 0.5m, 1.0m, and above sediment surface.
5. Calculate percent (%) incident irradiance (I_0) at each water depth.
6. Calculate light attenuation (K_d) using the Beer-Lambert law, based on the slope of the % I_0 measured at each water depth.

IV. Sediment Characterization Protocol

Determinations of Sediment Bulk Density, Percent Organic Matter, and Extractable Nutrients

Supplies/Equipment:

Collection: clear acrylic cores, 5.7 cm inner diameter by 20 cm tall (depends on total depth of interest), rubber stoppers, 5.7 cm diameter red lids, cooler with ice.

Processing/extraction: gloves, silicone tubing, 30 mL BD syringe with luerlok tip, rubber plunger (of slightly smaller diameter than acrylic core) on free-standing pole, metal plate, metal spatula, 5 gallon bucket, clear acrylic cores cut to similar heights as sediment sub-section depths (e.g., 2cm core for a 0-2 cm sub-section), de-ionized (DI) water for cleaning.

Bulk density/Percent Organic Matter determination: pre-weighed/numbered aluminum foil envelopes (use pencil to number envelope), analytical balance capable of 3 decimal places, aluminum baking pans, drying oven set at 50°C, muffle furnace capable of maintaining 500°C.

Extractable Nutrients determination: potassium chloride (KCl; granular, certified ACS), Whirlpak bags large enough for both KCl solution and sediment (4 oz to 24 oz, depends on sediment sub-section depths), analytical balance capable of 3 decimal places, container of suitable volume to make 2M KCl (e.g., carboy with spigot, large beaker), DI water, graduated cylinders, shaker table, 50 mL centrifuge tubes, centrifuge, 30 mL BD syringe with luerlok tip, Acrodisc 25 mm Whatman syringe filters with 0.45 µm polyethersulfone membrane, 2 oz. Whirlpak bags.

2M KCl preparation for extractable nutrients:

1. Prepare 2M KCl solution by dissolving KCl in DI water. To do this, mix 149.1 g of KCl (FW=74.55) with DI water to a total volume of 1 L. For example, for a 2.5 kg bottle of KCl, add entire bottle of KCl to a large carboy and use DI water to help dissolve and break-up KCl in the bottle. After adding all 2.5kg of KCl, add DI water to carboy until total volume of solution is 16.77 L.
2. To extract nutrients from the sediment, the volume of 2M KCl used is based on 2:1 KCl to sediment volume ratio. Thus, determine the total volume of sediment for each sediment depth interval to be collected and multiply by 2 for the volume of 2M KCl required.

Note: 2 M KCl volume should be a minimum of 30 mL to provide sufficient volume for nutrient analysis. If more KCl is needed, a larger volume of sediment should be collected (e.g., 2 quarter sections of the sub-core).

3. Pre-label Whirlpak bags with appropriate depth sections, core #, and site name and fill with the pre-determined volume of KCl for the specific sediment depth interval using graduated cylinders. The Whirlpak bag should have sufficient size to hold both the KCl and sediment that will be added during sampling.
 - a. 25 ml KCl in 4oz Whirlpak bag for 0-1cm depth section and 55 mL KCl in 7 oz Whirlpak bag for 1-5cm depth section.
4. Weigh each Whirlpak bag of KCl solution.

Collection:

1. At each location where a sediment flux core is collect, push 2 cores into sediment to at least 10cm or more in depth.
2. Put stopper on top, pull core out of sediment with overlying water, and place another stopper on bottom. Ensure that top and bottom stoppers are secure, and hold bottom in place when moving core. After collection, red lids may be used instead of the rubber stoppers on top of the cores for transporting them. Place core in cooler with ice.

Sample processing and nutrient extraction:

1. Wear gloves to reduce nutrient contamination while processing the sediment.
2. Return to lab and remove overlying water from core by vacuum suction using silicon tubing and 30 mL syringe.
3. Place plunger in 5 gallon bucket. With stopper on top of core, remove bottom stopper. Place core on top of plunger, remove top stopper, and push sediment up inside core with plunger until sediment surface is pushed to top of core.
4. To sub-core, place core marked with 1 cm marked on side and push sediment to 1cm mark of sub-core. Insert metal plate between cores to separate it. This sub-section represents the 0-1cm surface sediment section.
5. Repeat step #4, with the 4cm sub-core to represent the 1-5cm depth horizon.
6. Repeat with 2nd core from sample site/location only for the 0-1cm depth horizon.
7. For one of the 0-1cm sub-core, cut section in ½ using metal plate. For the other, leave whole.
8. For the 1-5 cm subcore, cut in ½ first. Cut one of the halves in half again (thus 2 quarter sections) using metal plate.
9. *For grain size determination:*
 - a. Place subsection into 7 oz whirlpak bag:
 - i. For 0-1cm subsection, use whole section.
 - ii. For 1-5cm subsection, use 1/2 of core section.
 - b. Put Whirlpak bags in labeled ziplock bag and place in freezer.
10. *For bulk density/% organic matter determination:*
 - a. Put one section into pre-weighed, numbered foil envelope using DI-rinsed metal spatula. Weigh and record weight, sediment core # or site from where it was collected, depth section on datasheet.
 - i. For 0-1cm subsection put ½ of core section in foil envelope.
 - ii. For all the (1-5cm) put ¼ of core section in foil envelope.

- iii. See “Bulk Density and Percent Organic Matter Processing” below.
11. *For extractable nutrients determination:*
- a. Put one section into pre-weighed, pre-labeled Whirlpak bag with 2M KCl, weigh, and place on shaker table for 1 hour. Pour sample into centrifuge tube and centrifuge for 6 minutes at 4000 RPM. Filter (Whatman 0.45 um polyethersulfone syringe filter) 25 mL into pre-labeled 2 oz Whirlpak bag (later to be analyzed for NH_4^+ , NO_x , and PO_4^{3-} on nutrient autoanalyzer).
 - i. For 0-1cm subsection put $\frac{1}{2}$ of core section in 4oz Whirlpak bag.
 - ii. For the 1-5cm subsection put $\frac{1}{4}$ of core section in 7oz Whirlpak bag.
 - b. Put Whirlpak bags in labeled ziplock bag and place in freezer and analyze within 1 month.
12. Discard remaining sediment sections in 5 gallon bucket.
13. Rinse acrylic sub-cores, metal plates and metal spatulas with DI water between core sampling.

Bulk Density and Percent Organic Matter Processing:

1. Place foil envelopes with sediment in drying oven (50°C). Place in aluminum baking pan standing up, slightly open to allow sufficient drying. Do not pack foil envelopes tightly in aluminum baking pan because it may cause the foil envelopes to form holes and leak.
 - i. Fold over top of envelope for transport.
2. Dry sediment at 50°C until constant weight (about 2-3 weeks depending on sediment type and size of depth section; muddy sediment requires more time)
3. Place in desiccator to cool before weighing for 1st time; record weight. Return to drying oven for 1-2 days. Place in desiccator to cool. Repeat weighing procedure on same balance. If sediment does not weigh within ± 0.01 g of their first weight (e.g., weight appears to be decreasing), the process of drying and weighing should be repeated as many times as needed to achieve constant weight. Record final dry weight.
4. Because the sediment will also be analyzed for particulate organic carbon, total nitrogen, and inorganic and organic phosphorus contents, the sediment will need to be ground in a mortar and pestle to homogenize, and a sub-sample (~2 grams) placed in a pre-combusted scintillation vial for separate preparation and analysis. Place remaining sediment back into foil envelope or into a pre-weighed aluminum pan. Re-weigh sample in envelope or pan and record. This sample may then be used for % organic matter determination.
5. Combust sediment in muffle oven for 5 hours at 500°C. Cool in desiccator. Weigh again.

Calculations:

1. Bulk Density

$$\text{BD (g dry weight} \cdot \text{mL}^{-1}) = \frac{[\text{Weight of dry sediment \& envelope (g) - Weight of envelope (g)}]}{\text{sediment volume (mL)}}$$

2. % Organic Matter

Weight of dry sediment (g) = Weight of dry sediment & envelope (g) – Weight of envelope (g)

Weight of combusted sediment (g) = Weight of combusted sediment & envelope (g) – Weight of envelope (g)

$$\text{organic matter (\%)} = 100 \times \frac{[\text{Weight of dry sediment (g) - Weight of combusted sediment (g)}]}{\text{Weight of dry sediment (g)}}$$

3. % Water

Weight of wet sediment (g) = Weight of wet sediment & envelope (g) – Weight of envelope (g)

$$\text{water (\%)} = 100 \times \frac{[\text{Weight of wet sediment (g) - Weight of dry sediment (g)}]}{\text{Weight of wet sediment (g)}}$$

V. CHN Sediment Preparation Protocol

Gentle Reminders:

- Double check that your samples in the crimped capsules are 1) round and have no sharp edges (see last page for example pictures) and 2) not leaking any sediment (check the well that it is stored in for any sediment).
- Anything that comes out of a drying (not muffle) oven goes right into desiccators.
- Wear gloves to avoid contamination.

Supply Preparation

1. Tin capsules
 - Soak tin capsules (5mm x 9 mm, Costech) in beaker with acetone for 4 hours.
 - Drain off acetone and let capsules dry off in fume hood for 1 hour
 - Place in oven to dry overnight
2. Muffle aluminum tray holder used for acidification step (along with extra foil to cover it with) at 500°C for 5 hrs. Do not muffle the 3-position stainless steel holder and the holder used to crimp/package the capsules. Just rinse with DI water and wipe with kimwipes.
3. Wipe small sediment spatula and forceps with kimwipes between samples, but do not muffle
4. If sediment collected in aluminum foil envelope: muffle scintillation vials covered with aluminum foil squares at 500°C for 5 hrs – can muffle at same time as aluminum plates.

Sediment Preparation

1. Dry sediment at 50°C in scintillation vials with foil still on top, but cap removed. *If sediment is collected as part of the bulk density/% organic matter determination, see 3a. below.*
2. Dry until constant weight: Place in desiccator to cool before weighing for 1st time. Return to drying oven for 1-2 days. Place in desiccator to cool. Repeat weighing procedure on same balance. If sediment does not weigh within ± 0.01 g of their first weight (weight appears to be decreasing), the process of drying and weighing should be repeated as many times as needed to achieve this constant weight. Record final dry weight.
3. Grind sediment until homogeneous using mortar and pestle, return sediment to container and store desiccated until ready to weigh. Wipe mortar and pestle with kimwipes between samples and use brush to remove additional sediment dust; do not wash with DI water.
 - a. *If sediment was collected as part of the bulk density and % organic matter determinations:* Follow steps 1 and 2 above with the aluminum foil envelope. After final dry weight is measured, grind sediment in mortar and pestle to homogenize. Remove a sub-sample (~2 grams) and place in a pre-combusted scintillation vial, replace aluminum foil, and cap. Place remaining sediment back into foil envelope (if no holes in the envelope) or into a pre-weighed aluminum pan. Re-weigh sample in envelope or pan and record. This sample may then be used for % organic matter determination.
4. Dry sediment at 50°C prior to weighing. (see sediment acidification note first before next step)

5. Place aluminum foil on table before weighing capsules. Only use forceps to hold and touch the capsules.
6. Tare a tin cup on the microbalance– before using, hold each cup up to the light to verify that there isn't a hole in the bottom
7. Remove cup from balance and place it in the 3-position stainless steel holder; put a small amount of sediment in the cup and return it to the balance:
 - a. Sandy sediments requires about 40-50mg, but not more because it is difficult to close cups without splitting
 - b. Muddy/high organic sediments requires approx. 18-22mg sediment
8. Brush bottom of the capsule for loose sediment and record weight of sediment sample
9. After weighing, place samples in aluminum plates with holder
10. Run duplicates on every 10th sample

Sediment Acidification

Note: this method only works on sediment with very low carbonate content, e.g., they fizz little to not at all when acidified. If it has high carbonate content, samples should be acidified directly in the scintillation vials (the scintillation vial with sediment should be weighed before and after acidification, then subtract out avg. weight of scint vials).

1. Add 1 drop 10% HCl to each cup using glass pipette (see Environmental Science and Tech., vol 31, no. 1, pp. 203-209, 1997) and return to aluminum tray, covered with muffled foil.
2. Acidify empty tin cups to use for blanks – you will need 2 acidified cups to use to create a standard curve and then one for every 7-10 samples to verify that the baseline is still acceptable
3. Dry acidified sediment and empty cups until they look dry on hot plate in fume hood, approx. 30 minutes (set at 2 in the hood – warm to touch).
4. If any of the sediments bubble when exposed to the acid, repeat this step until there are no bubbles (the sandier soils with shell fragments are the most likely to need multiple acidification steps). Try not to add more than 3 drops because capsule may become too brittle. If more than 3 drops are required, place capsule in another tin capsule before adding more drops.
5. After final acidification, dry for several hours (overnight) in the oven ~50°C, covered with muffled aluminum foil. **It is not good for the cups to sit for more than 1 night after being acidified (i.e. a weekend). The cup will most likely get too brittle.**
6. Place aluminum foil down on table before crimping capsules. Only use forceps to hold and touch the capsules. The 3-position stainless steel holder or the “crimper” may be used for crimping the samples.
7. Place sample capsule into another tin capsule and crimp closed. If it splits, put into another cup and crimp and make a note of it – acid makes the cups brittle.
8. Make sure that the crimped capsule has no sharp edges and is round. See pictures below.
9. The crimped capsules do not need to be weighed after acidification.
10. Store crimped capsules in well plate in desiccator.
11. Run samples on CHN elemental analyzer within 1 month. Samples that are more acidified should be run sooner or the capsules may break and lose sample material.

VI. Particulate Phosphorus Determination on Plankton & Sediments Protocol

(modified from ASPILA, K. I., H. AGEMIAN, and A. S. CHAU. 1976. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. Analyst 101: 187-197.)

Equipment/supplies:

Analytical Balance, Muffle furnace (500°C), Drying oven (60°C), Acid washed 15 mL glass centrifuge tubes with glass ground stoppers, Aluminum foil, Standard Reference Material (Tomato leaves); 10 ml or 5 ml pipette, Hydrochloric acid 1.2 N, nutrient autoanalyzer

Procedure

1. Weigh out ~ 0.02-0.03 g dried/ground sediments or plankton into duplicate combusted and acid cleaned 15 ml glass centrifuge tubes (e.g., 2 tubes per sample).
2. Also weigh out reference material in duplicate (e.g., 4 tubes total).
3. Set aside 2 sets of 3 empty vials and process as samples; these will be your blanks
4. Particulate inorganic phosphorus vial: Cover 1st set of duplicate vials with screw caps and set aside until step 5
5. Total particulate phosphorus vial: Cover the 2nd set of duplicate vials with aluminum foil, load into glass beakers and combust samples in muffle furnace at 500°C for 4.5 hours.
6. After cooling down to room temperature, add 10 ml 1.2 N HCl, cover with screw caps, and shake vigorously (or vortex), suspending all the particulates into the acid.
7. Leach at 60°C for 1 hour in drying oven.
8. Spin samples at 1000xg (2100 RPM) for 15 minutes.
9. Dilute 500µl of each supernatant to a total volume of 5 ml with DIW in a new glass 10mL test tube.
10. Measure orthophosphate concentrations in duplicate per sample on Lachat nutrient autoanalyzer.

VII. Benthic Chlorophyll Collection and Extraction Protocol

Supplies/Equipment:

Collection: 5 mL NormJect syringes (tips cut off flat), small metal spatula, metric ruler, 15 mL centrifuge tubes, test tube racks (capable of holding 15 mL centrifuge tubes)

Extraction and filtration: 90% acetone extractant (90% acetone by volume, 10% DI water by volume), repipetter, vortexer, sonicator (Fisher Scientific 60 Sonic Dismembrator), centrifuge, 10 mL BD syringe with luerlok tip, 13 gauge stainless steel reusable hypodermic needle with Luer-tip connector, several 250 mL beakers for clean extractant and waste, Acrodisc filters (HPLC Gelman 0.45µm CR-PTFE), 10mL disposable culture tubes, test tube racks.

Spectrophotometric analysis: parafilm, Spectrophotometer, 10% HCl acid (10% HCl by volume, 90% DI water by volume), 40-200 uL volume pipetter and disposable tips, 90% acetone extractant.

NOTE: Exposure of cores or sediment sections to direct light (sunlight or indoor) may cause changes in photosynthetic pigment concentrations and should be avoided. **Keep samples covered and lights dim throughout whole process.**

Safety Precautions: While sonicating, please wear eye and ear protection and close the door to protect the hearing of others. Avoid inhalation of extractant. Please wear gloves during entire analysis process.

Collection:

1. Using 5 mL NormJect syringes (tip cut off evenly, flat syringe plunger), sample sediments to desired depth, either from a collected sediment core or directly from the sediment.
2. Cut sediment into desired section, 0-1cm, and store in clean, non-acid washed 15 mL centrifuge tube.
3. Store in cooler with ice until samples can be frozen.

Samples must be stored in freezer for at least 24h, but < 1 month before analysis.

Analysis

1. Add 10 mL of extractant (90% acetone, 10% DI water by volume [CBH DI tap is okay] note this volume is suitable for sediment taken with 5 mL Norm-ject syringe, \leq 1cm section) to each sample with repipetter in **fume hood**. Wear eye protection.
2. Vortex each sample for 30-sec on full power to mix.
3. Sonicate each sample for 30-sec with Fisher Scientific 60 Sonic Dismembrator
 - a. Turn power on. Output watts should read '0'.
 - b. Place tip of probe in sample vial, put end of probe a couple mm above bottom of vial.
 - i. *Probe tip should not touch any solid surface while power is on.*
 - c. Use dial on top of sonicator to increase output watts to 4-5 (reading of 3 on dial).
 - i. *Do not turn dial (i.e., increase output power above '0' watts) unless probe tip is immersed in liquid. Doing so can damage the probe tip.*
 - d. Sonicate each sample for 30sec, and then turn dial so output reads '0' watts.
 - e. Remove probe from sample, recap sample vial, and return to rack.
 - f. Rinse probe tip with extractant between samples, and with DI water after last sample.
4. Store vortexed, sonicated samples in freezer for **24 hours** before continuing. **Samples should not sit for any additional time before filtering and reading on the spectrophotometer because chl a will degrade.**
 - a. Allows particles to settle out.
5. When the extraction is complete, clarify fluid using filtration.
 - a. Centrifuge at 3500rpm for 6-min. Check setting of centrifuge rotor (*note: if particulates in sample have settled, this step may be skipped*)
 - b. Fit an Acrodisc (HPLC Gelman 0.45 μ m CR-PTFE) with stainless steel 13 gauge needle onto a 10-mL syringe.
 - c. Pull a 1 mL sample across the filter and discard.
 - d. Then pull a **8-mL sample** (without pulling mud through the filter) across the filter, invert the syringe, and remove the filter.
 - e. Dispense the sample into a 10-mL disposable culture tube. ***Be sure to put same amount of extractant into every tube. Note on datasheet if smaller volume.***
 - f. Fill syringe with 1-2 mL extractant and rinse the syringe with extractant. Replace the Acrodisc on the syringe, and rinse the filter by dispensing the extractant.
 - i. Process can be repeated for 30-50 samples per Acrodisc filter. This part of the procedure increases precision and decreases variability associated with particulate material interference in the spectrophotometer.
6. Filter all samples into culture tubes and allow them to equilibrate to room temperature. **Try to keep the culture tubes in the dark as much as possible.**
7. Spectrophotometric analysis (*Shimadzu UV-1800 UV Spectrophotometer*)
 - a. Determine absorbance at 665, 750, 664, 647, 630 nm for each sample.
 - b. Run a blank (extractant) every 5 – 8 samples.
 - c. Acidification step after reading all samples: Add 150 μ L of 10% HCl to each tube, invert sample with parafilm to mix well, wait 2 min, and reread absorbance at the same wavelengths as above. Again, run a blank (extractant) every 5 – 8 samples.

8. Calculations:

- a. Drift correction: correct for linear drift by adding (or subtracting) changes in blank samples to the actual samples they bracket. Drift should be ≤ 0.010 within a run.
- b. Background correction: for each sample (both pre- and post-acidification), subtract absorbance at 750nm from the respective absorbance at each wavelength (630, 647, 664, and 665 nm) to account for potential background absorbance from materials not removed by filtration.
- c. Chlorophyll_a and Phaeophytin: equations provided by Lorenzen (1967); chl_a is corrected for phaeophytin.

$$\text{Chl}_a \left(\text{mg} \cdot \text{m}^{-2} \right) = \frac{26.7 \times (\lambda_{665} - \lambda_{665_{\text{acid}}})}{(L)} \times \frac{V}{SA} \times \frac{1 \text{ mg}}{1000 \mu\text{g}} \times \frac{10000 \text{ cm}^2}{1 \text{ m}^2}$$

$$\text{Phaeo} \left(\text{mg} \cdot \text{m}^{-2} \right) = \frac{26.7 \times [(1.7 \times \lambda_{665_{\text{acid}}}) - \lambda_{665}]}{(L)} \times \frac{V}{SA} \times \frac{1 \text{ mg}}{1000 \mu\text{g}} \times \frac{10000 \text{ cm}^2}{1 \text{ m}^2}$$

V = volume of extractant in mL

SA = core area in cm²

L = light path length or cuvette width in cm

(L = 1 for Milton Roy SPEC 1001 and UV-1601 Shimadzu)

References:

Lorenzen, C. (1967). Determination of chlorophyll and phaeopigments: spectrophotometric equations. *Limnol. Oceanogr.* 12:343-346.

Jeffrey SW, and NA Welschmeyer (1997). Spectrophotometric and fluorometric equations in common use in oceanography. p. 597–615. *In:* [Jeffrey SW, RFC Mantoura, and SW Wright, eds.] Phytoplankton pigments in oceanography: Guidelines to modern methods. UNESCO, Paris, France.

Method developed by personal communication of Dr. Iris Anderson with Dr. Jay Pinckney, University of South Carolina (general procedures and type of extractant) and Dr. Carolyn Currin, NOAA (use of sonicator).

VIII. Sediment Oxygen and Nutrient Flux Experiment Protocol

SEDIMENT FLUX CORE COLLECTION AND EXPERIMENT SET-UP

Day 1

Field collections

1. Collect 3 Sediment+water cores (~20cm sediment depth) from each site (cores were collected from 2 sites in each SONE experiment; 3 experiments total per season). Each site had 3 randomly selected stations, at which each core is collected. Ensure sediment is collected at appropriate water depth goal (1m or 2m MSL), taking into account tidal stage and range.
2. Collect ambient water at each site from midway in the water column) for core incubations, replacement water during flux measurements, and water blank cores.
3. Collect profiles through water column (surface, mid, and bottom water) of temperature, DO, salinity, chlorophyll, and turbidity at each site using YSI 6600.

Setting up cores overnight

1. Measure water column heights of each sediment core to determine water volume within each core (at 4 equidistant locations around the core)
2. Wear gloves to reduce contamination of water.
3. Set-up cores (clear cast acrylic, 40cm height, 13.33cm ID) in a temperature & light controlled environmental chamber
 - a. Set temperature to in-situ water temperature at the sites (measured with YSI 6600)
4. Fill fiberglass chamber with water from its respective ambient site water.
5. Replacement water: for each of the site, pour 5-gal carboy of ambient water into a 5-gal carboy with spigot. Cover with black trash bags.
6. Submerge cores (without lids) in the water in their respective chambers & gently bubble with air using a 'T' made of PVC, silicon tubing, & small aerators. Connect 6 cores per pump (pump has 2 connectors on it).
 - a. Allow cores to sit over night for temperature & DO equilibration
 - b. Setup 3 Water only cores for each site and treat similarly to Sediment+water cores
 - c. Total of 6 cores per site and tank.
7. Place minijet pump inside of chamber to help circulate the water.

Adjust light field for cores

1. Measure PAR levels inside the chambers under water at the same depth as the sediment surface.
2. Determine target PAR levels for each site based on measured field % light and incident irradiance of $1600 \text{ uE m}^{-2} \text{ s}^{-1}$ to simulate mid-day light levels.
3. Use screen material and shade cloth to attain the approx. PAR level for each set of cores from the site by covering the fiberglass chamber including the sides.

- a. Record the # of layers of shade cloth and type for each site and the PAR levels available under the shade cloth inside each of the chambers.
4. Set the lights to turn off at 10pm this first day.

Day 2

Setting up cores for experiment (in the dark—under ‘red light’)

1. Put lids with stirring apparatus on each core under water.
 - a. Each lid has two ports (1-inflow from reservoir, 2-outflow for sampling) with luer lock fittings; stirring apparatus fitted with magnets.
 - b. Minimize bubbles within the cores—if bubbles are visible after the lid is put on a core, remove the lid and bubbles and replace the lid on the core.
2. Set up a central battery powered magnet apparatus in each tank
 - a. Central apparatus causes magnets to spin in each core within a tank
3. Place cores around central magnet stirrer in order from same site: reps 1-3 and Water blank (WB)1-3 (6 total)
 - a. If needed, secure with bungee cords to keep cores from tipping over.
4. Connect reservoirs with site-specific ambient water to each core via a manifold & norprene tubing—water flows out of a core due to the gravity head caused by the reservoir—as water flows out of a core, it is replaced by reservoir (covered)
 - a. Attach the black tubing (Norprene, 1.5m length; 1/8” ID x 1/4” OD) from the manifold to the “in” center port, which does **not** have a tube underneath.
 - b. **Before attaching**, fill the tube up with the replacement water by opening the stopcock (make sure other stopcocks are closed except the carboy port) and pull water in with syringe. Close stopcock on manifold before detaching syringe.
5. Repeat for the other cores.
 - a. When finished, Close carboy stopcock (off ▲)
6. Fill yellow tubing with 3-way stopcock (“sampling tube”) with site water (Tygon F4040-A, 1.5m length; 1/8” ID x 1/4” OD) using syringe and connect to the other “sampling” port on the core.
7. Attach central magnet stirrer to battery.
8. Turn lights on to begin SONE experiment.

Calibrate and check DO meters

1. Prior to experiment, conduct calibrations of each HACH LDO probe following HACH protocol (see HACH LDO manual)
2. **Set the salinity value to in situ site-specific values for each HACH DO meter based on YSI readings.**
3. Set the HACH DO meter to log DO readings (30 sec averaging interval, slow response/increased accuracy setting) every 30 minutes.
4. At the end of SONE experiment for each set of cores (e.g., after 24 hours), check calibration of the HACH DO probes in the air-saturated water in the lab to make sure it is measuring close to published values

FLUX EXPERIMENT SAMPLE COLLECTION

1. Record DO readings and collect water samples at approx. 8am, 2pm and 8pm on first day, 8am 2nd day:
 - a. Turn off central magnet stirrer during sampling to reduce dilution of flux core water with replacement water.
 - b. Start logging DO at 8am.
 - c. Before collecting each water sample for a core, record the time, DO concentration, and water temperature.

A. Flush Core Site #1 Rep 1 line

1. Open carboy stopcock and Site#1 Rep 1 stopcock on manifold.
2. Find **Site #1 Rep 1** “sampling” tube coming from core and connect respective 30mL syringe to stopcock.
3. Open stopcock on tube and pull 15 mL of water to flush line.
4. Close stopcock on tube and empty syringe.

B. Take DIC and nutrient sample

1. Open stopcock, pull water slowly in to syringe to volume of 30 mL.
2. Close stopcock and remove syringe.
3. Make sure no air space in syringe. Dispense DIC sample into DIC hungate tube.
 - DIC collection: make sure that there is a meniscus on the DIC vials before capping them. Double-check no bubbles in the hungate tubes. If so, add more water sample and re-cap.
 - Put in plastic cup and invert under water.
 - Place in refrigerator in lab
4. Use remaining sample water for silicate. Save syringe for filtering and put in cooler on ice.
5. Connect 60mL syringe, open stopcock, pull water slowly in to syringe to volume of 60 mL. Save syringe for filtering nutrient samples and put in cooler on ice.

C. Repeat with other cores

1. Start over again at Flush Site **#1 Rep 2**.

D. At end when finished collecting samples

2. Close carboy stopcock (off ▲)
3. Filter samples in 30mL and 60mL syringes, following the “Water Quality Sample Collection and Filtration for Nutrients Protocol”
4. Rinse syringes with MQ water before next time step.
5. Turn on central magnet stirrers.

6. If dawn sampling, turn the lights on. If dusk sampling, turn the lights off.

Total water removed from flux core = 115 mL per timestep:

- 15 mL to flush core line (1.5 meter of tubing; 1 m of tubing holds 8 mL of water)
- 65 mL for nutrients (5 mL pre-filter, 25 mL DIN/DIP, 14 mL DON, 16mL DOC, 5mL extra for pre-filter if need 2nd filter disk)
- 10 mL for DIC
- 25 mL for silicate (5 mL pre-filter, 20 mL silica)

E. Calculations:

Benthic DIC, DO, and nutrients fluxes were corrected for DIC, DO, and nutrient uptake or release measured in the water blanks. Sediment oxygen demand, benthic metabolism (DIC based) and daily nutrient fluxes were calculated as shown in Equations 1 through 5 as follows:

$$\text{Sediment oxygen demand} = F_d * 24 \text{ hours} \quad (\text{Eq. 1})$$

$$\text{Respiration (R)} = F_d * 24 \text{ hours} \quad (\text{Eq. 2})$$

$$\text{Gross Primary Production (GPP)} = h_l * (F_d - F_l) \quad (\text{Eq. 3})$$

$$\text{Net Community Production (NCP)} = - (GPP - R) \quad (\text{Eq. 4})$$

$$\text{Daily nutrient flux} = (F_l * h_l) + (F_d * h_d) \quad (\text{Eq. 5})$$

F_d = Hourly flux in the dark

F_l = Hourly flux in the light

h_l = Hours of light

h_d = Hours of dark

NCP (Equation 4) is represented as a negative number when GPP exceeds R because it was measured as uptake of C. When NCP is negative it represents net autotrophy and net uptake of C; when positive it represents net heterotrophy and net release of C.

IX. Summary of analytical methods

Analyses	Methods/Instrument	References
Nutrient		
Nitrate, Nitrite	Cadmium reduction/diazotization; Lachat ¹	Smith and Bogren, 2001
Ammonium	Phenol Hypochlorite method; Lachat ¹	Liao, 2001
Dissolved inorganic phosphorus (phosphate)	Molybdate method; Lachat ¹	Knepel and Bogren, 2001
Total dissolved nitrogen (TDN) / dissolved organic nitrogen (DON)	Alkaline persulfate digestion; Lachat ¹	Koroleff, 1983
Dissolved inorganic carbon (DIC)	Acidification to CO ₂ ; LI-6252 CO ₂ analyzer	Neubauer and Anderson, 2003
Dissolved organic carbon (DOC)	680°C catalytically-aided combustion oxidation/non-dispersive infrared detection; Shimadzu TOC-V analyzer	
Silicate	Molybdate in acidic solution method; Lachat ¹	Wolters, 2002
Temperature, salinity, dissolved oxygen, turbidity, chlorophyll <i>a</i> (in vivo) (field measurements)	YSI 6600 multiparameter sonde	
Dissolved oxygen (metabolism experiments)	Hach Luminescence DO sensor	Hach Method 10360
Chlorophyll <i>a</i> (extracted; phytoplankton biomass)	Chl <i>a</i> – Acetone – DMSO Extract/ fluorometry; Turner Designs Flurometer, Model 10-AU	Shoaf and Lium, 1976, Arar and Collins, 1997.
Photosynthetically active radiation (PAR)	LiCor LI-192SA Underwater and LI-190SA quantum sensors	
Sediment characterization		
Sediment organic content	Loss on ignition (500°C)	
Benthic chlorophyll <i>a</i> and phaeophytin (microalgae biomass)	Chl <i>a</i> – Acetone Extract/ spectrophotometry; Beckman Coulter DU800 Spectrophotometer	Neubauer et al., 2000; Lorenzen, 1967
Sediment nutrients (dissolved inorganic N and P)	Potassium chloride-extraction	Kenney and Nelson, 1982
Sediment grain size	sieving method (>63μ); pipette method (<63μ)	Plumb, 1981
Total N and organic C content	Fision Model EA 1108 Elemental Analyzer	
Organic and inorganic P content	HCl extraction; Molybdate method; Lachat ¹	Aspila et al., 1976

¹ The Lachat auto analyzer (QuikChem 8000 Automated Ion Analyzer, Lachat Instruments, Loveland, CO) is a continuous flow automated analytical system that complies with US Environmental Protection Agency (EPA) standards.

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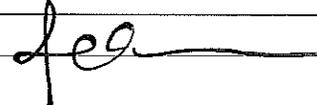
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Appendix 9

**Signatures from Project Leaders: Drs. Iris Anderson, Paul
Bukaveckas, Todd Egerton, Ken Moore, Margie
Mulholland, and Kim Reece**

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TITLE	NAME	DATE	APPROVAL
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VADEQ Water Quality Monitoring Quality Assurance Coordinator	James Beckley		
ODU Project Leaders	Todd Egerton and Margie Mulholland		
VCU Project Leader	Paul Bukaveckas		
VIMS Project Leaders	Kim Reece and Ken Moore		
VIMS Project Leader (SONE Study)	Iris Anderson	8/28/14	

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