

**QUALITY ASSURANCE PROJECT PLAN
FOR SPECIAL STUDY OF
Chlorophyll-a Methods**

**Commonwealth of Virginia
Departmental of Environmental Quality
Water Quality Assessment
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1. PROJECT MANAGEMENT

1.1. Project/Task Organization

1.1.1. Study Manger

The study manager is responsible for task implementation, technical quality control and performing or overseeing data evaluation activities. The study manager is responsible for ensuring the QA/QC procedures described in this QA project plan are followed

1.1.2. Field Team Coordinator (FTC)

The FTC is responsible for monitoring and directing the field effort. The FTC will ensure that the field staff is properly trained and equipped to execute the field sampling methods and procedures, as well as the sampling handling and custody procedures.

1.1.3. Laboratory Quality Assurance Officer:

The Laboratory QA Officer will:

1. Conduct audits of laboratory activities.
2. Validate laboratory data before final certification.
3. Ensure that corrective action, if necessary, is properly implemented and documented.

1.1.4. Field Staff

The field staff is responsible for all field activities including, 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

Under Section 2.2-1105 of the Code of Virginia, Virginia Environmental Laboratory Accreditation Program (VELAP) was established to certify environmental laboratories that perform tests, analyses, measurements or monitoring required pursuant to the Commonwealth's air, waste, and water laws and their associated regulations. On January 1, 2009, 1 VAC 30, Chapter 45 (Certification for Noncommercial Environmental Laboratories), and 1 VAC 30, Chapter 46 (Accreditation for Commercial Environmental Laboratories) became effective.

Specifically, these regulations required certification/accreditation beginning January 1, 2012, for all laboratories that analyze samples and provide data used for purposes of the Virginia Air Pollution Control Law, the Virginia Waste Management Act or the State Water Control Law (§10.1-1300 et seq., §10.1-1400 et seq., and §62.1-44.2 et seq., respectively). VELAP certification/accreditation is administered by the Department of Consolidated Laboratory Services (DCLS). A Memorandum of Understanding between DCLS and DEQ was implemented in 2009.

In early 2012, DEQ contracted with VCU to collect chlorophyll-a data in the tidal freshwater James River in support of the James River Chlorophyll Study; a study whose results may support a potential water quality standards rulemaking process. The VCU Scope of Work included VCU's Environmental Analysis

Laboratory (EAL) applying for accreditation through DCLS in order to meet regulatory requirements outlined above.

As part of the VELAP accreditation process, DCLS has determined that the analytical method for chlorophyll-a (CHLa) currently used by VCU's EAL employs a method similar to EPA approved Method 445.0 but that EAL's method does not include the sample preparation steps for grinding and centrifuging; two steps employed by EPA's Method 445.0. Therefore, EAL has submitted a corrective action plan to DCLS which includes the incorporation of grinding and centrifuging into their CHLa analytical method for future samples.

The objective of this study is to determine whether CHLa results obtained from the EAL lab using VCU's current CHLa analytical methods are comparable to results obtained from a VELAP certified lab which uses EPA's Method 445.0. Results from this study may be used to assess the comparability of data produced using these two methods such as developing a universal correction factor.

1.3. Project/Task Description

1.3.1. Project Tasks

Task 1. The Study Manager develops the Quality Assurance Project Plan. The plan will establish stations and determine sample collection logistics.

Task 2. Collect samples. VCU will collect water samples for CHLa analysis from four sites (2-JMS099.30, 2-JMS075.04, 2-JMS069.08, and 2-JMS055.94) once per month during a five month period from June through October and twice per month in July, August, and September for a total of 32 samples. Samples will be collected as per VCU's Field SOP (appendix 1), returned to VCU's EAL lab and then filtered as per EAL's SOP (appendix 2) in preparation for CHLa analysis. One of the duplicate filters will be analyzed by VCU's EAL and the other by VIMS (a VELAP certified lab which uses EPA Method 445.0 for CHLa analysis; appendix 3). This will provide results from paired samples to use for this comparison study.

Task 3. Laboratory analysis of samples. VCU and VIMS will each analyze one set of filters for CHLa according to their respective SOPs (appendices 2 and 3). VIMS uses EPA Method 445.0 with no deviations. VCU's current method follows EPA method 445.0 with the exception that they do not include the grinding and centrifuging preparation steps as stipulated in EPA Method 445.0. This study will compare VCU results to VIMS results using paired samples collected by VCU. Each lab will provide DEQ with their analytical results which will include values for 1) total CHLa (uncorrected for pheophytin), 2) total pheophytin, and 3) CHLa corrected for pheophytin. DEQ will use these results to evaluate comparability of data.

Task 4. Data validation and analysis. The laboratory analytical results are validated to assess for bias, completeness, representativeness, and acceptable levels of precision and accuracy by the lab QA Officer. The study manager performs data analysis and prepares final report.

1.3.2. Work Schedule

Task	Description	Schedule
1	Develop QAPP	May 2014
2	Sample collection	June - October 2014
3	Lab analysis	June - October 2014
4	Data validation and analysis	October 2014

1.3.3. Station Location

Station	Description	Latitude	Longitude
2-JMS099.30	James River at Buoy 157	37.40294	-77.39194
2-JMS075.04	James River at Red Buoy 107	37.31278	-77.23056
2-JMS069.08	James River Buoy 91 Near Herring Creek	37.30004	-77.125
2-JMS055.94	James River Brandon Point Buoy 74	37.27472	-76.98847

1.3.4. Estimated Project Costs

Project activities	Estimated cost
VCU	\$18,764 (data collection, preparation of duplicates, and analysis)
VIMS	\$2719 (analysis only of one set of filters)

1.4. Quality Objectives and Criteria for Measurement Data

Data produced by this study will be used to evaluate analytical results from the use of two CHLa analytical methods: VCU's current method which is similar to EPA method 445.0 but with modification in that it does not include grinding or centrifuging of samples, and VIMS' method which follows EPA Method 445.0 including the grinding and centrifuging steps stated in the method. Data generated in this study may be used to make an assessment of the comparability of data produced using these two methods such as developing a universal correction factor. 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.

1.5. Special Personnel Training Requirement

This study will be performed by experienced field and laboratory personnel. There is no special training needed not already mentioned in the VCU and VIMS SOP manuals included with this QAPP (appendices 1, 2, and 3).

1.6. Documentation and Records

Documentation of field and laboratory data is to be stored in the Comprehensive Environmental Data System Water Quality Module, CEDSWQM. In addition the QAPP and any final reports or conclusions are to be stored in CEDSWQM. This section identifies the documents and reports to be generated throughout the study and the information to be included in these documents and reports.

1.6.1. Field Documentation

The field team will be responsible for maintaining the following documents:

- (1) Field Data Sheet
- (2) Sample container labels

1.6.2. Laboratory Documentation

Laboratory documentation will include producing the following information:

Certificates of analysis

1.6.3. Data Validation Reports

Only valid and certified data will be transferred to the VADEQ from the laboratory. Data validation flags and descriptions will be applied to those sample results that fall outside of specific limits.

The Laboratory Quality Assurance Officer will identify biases inherent in the data, including assessment of laboratory performance, and overall precision, accuracy, representativeness and completeness. The data validation report will address whether the quality of the flagged data affects the ability to use the data as intended. As needed, the data validation reports will be supplemented by a corrective action plan, to be implemented as soon as feasible, to correct each observation or finding of erroneous procedures.

2. MEASUREMENT/ DATA ACQUISITION

2.1. Experimental Design

Water samples will be collected from four sites (table in section 1.3.3) in the tidal freshwater James River. In order to capture a wide range of CHLa results, the study includes spatial and temporal variation with sites encompassing approximately 54 river miles and with sample dates encompassing multiple seasons from June through October. Each site will be sampled once per month from June through October and twice per month in July, August, and September for a total of 32 samples. Water samples will be collected, stored on board, and returned to VCU's EAL according to EAL's Field SOP (appendix 1). Each sample will be thoroughly mixed and then equal aliquots will be filtered onto two filter pads and stored at -20° C according to protocols outlined in EAL's Laboratory SOP (appendix 2). One randomly selected set of paired filters will be analyzed for CHLa by VCU and the other set of paired filters will be transported on ice to VIMS for CHLa analysis. VCU and VIMS will then follow their own protocols for CHLa analysis (appendix 2 and 3, respectively). Results will be provided to VA DEQ and will include the following: 1) total CHLa (uncorrected for pheophytin), 2) total pheophytin, and 3) CHLa corrected for pheophytin.

VA DEQ will evaluate the laboratory results against method requirements, study requirements, and quality control sample results. DEQ will use appropriate statistical methods such as a Wilcoxon paired T test to determine if there is a direct relationship between VCU and VIMS results.

2.2. Sampling Methods Requirements

The field procedures for sample collection are followed by VCU's EAL Field SOP (appendix 1).

2.2.1. Sample Numbering System

Standard DEQ sample identification procedures will be applied to each sample collected. Each stream station ID, date, and time will be used as sample identification.

2.2.2. Field Forms

The field sampling team will be responsible for maintaining field data sheets.

An entry will be made on VCU's field data sheet for each sample collected. The intent of the field data sheet is to document the place, date, and time 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. Sample Handling and Custody Requirement

Water samples will be collected and stored in 1 liter sample bottles for transport to the VCU EAL lab where they will be immediately filtered for CHLa analysis according to EAL's SOP (appendix 2). Filters will be stored in the dark at -20° C in the VCU EAL lab. One pair of filters will be transported on ice to VIMS by DEQ staff with delivery time minimized to prevent sample thawing. The DEQ staff will randomly select the paired filters to transport to VIMS. Once VIMS receives their set of filters, they will be immediately stored in the dark at -20° C until analysis.

Since this study will not be used for legal purposes, formal chain-of-custody procedures are not required. Samples will be located in a secure facility while being processed and under DEQ staff supervision during transport to the VIMS facility.

2.3.1. Analytical method Requirements

The analytical methods used by the contract laboratories for this study are listed in the following table:

Parameters	Analytical method
Chlorophyll-a (VCU)	VCU EAL SOP – method similar to EPA Method 445.0 (Appendix 2)
Chlorophyll-a (VIMS)	EPA Method 445.0 (Appendix 3)

2.4. Quality Control Requirements

2.4.1. Field Duplicate Samples

As this study consists of duplicate samples to compare two different preparation protocols, additional duplicate samples are not necessary.

2.4.2. Laboratory Quality Control

Quality control (QC) samples will be analyzed in accordance with the methods which the laboratories refer to as outlined in Appendix 2 and 3.

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

The FTC will be responsible for maintaining the equipment followed by their respective SOPs as outlined in Appendix 1, 2 and 3.

2.6. Inspection/Acceptance Requirements for Supplies and Consumables

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

2.7. Data Management

Study data will include computer and handwritten entries. Sample collection time will be entered on the field data sheet. Data analyzed in the laboratory will be entered into an Excel spreadsheet and following validation and approval, data is shipped through e-mail electronically to DEQ.

3. Data Validation and Usability

3.1. Data Review, Validation, and Verification

The laboratory activities will be reviewed to assess whether these activities are performed in a manner that is appropriate for accomplishing the study objectives. Data verification is confirmation by examination and provision of objective evidence that specified requirements have been fulfilled. Data verification concerns the process of examining a result of a given activity to determine conformance to the stated requirements for that activity. Data validation concerns the process of examining a product or result to determine conformance to the user needs.

Each of following areas will be reviewed:

- (1) Sample collection procedures
- (2) Sample handling
- (3) Analytical procedures
- (4) Quality control samples

3.2. Validation and Verification Methods

Data verification will be performed, reviewed and interpreted by the DEQ Study Manager and designated DEQ staff. The analytical laboratory report will be reviewed for compliance with the applicable method and for the quality of the data reported. The data verification procedures are designed to 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, in the form of Remark Codes, to those sample results that fall outside of the QC acceptance criteria.

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

After the project is complete the Study Manager is responsible for collecting all the data, analyzing the data and preparing a written final interpretation to be included in the CEDS special study as the Final Report.

4. REFERENCES

1997 EPA Method 445.0 In Vitro Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence. www.epa.gov/microbes/documents/m445_0.pdf

1997 EPA Guidance for Quality Assurance Project Plans, EPA QA/G-5. Office of Research and Development. EPA/600/R-96/055

2001. EPA Requirements for Quality Assurance Project Plans. Office of Environmental Information. EPA QA/R-5. <http://www.epa.gov/quality/qs-docs/r5-final.pdf>.

VCU EAL Field SOP manual (Appendix 1)

VCU EAL Chlorophyll a SOP manual (Appendix 2)

VIMS Chlorophyll a SOP manual (Appendix 3)

Appendix 1

VCU's 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

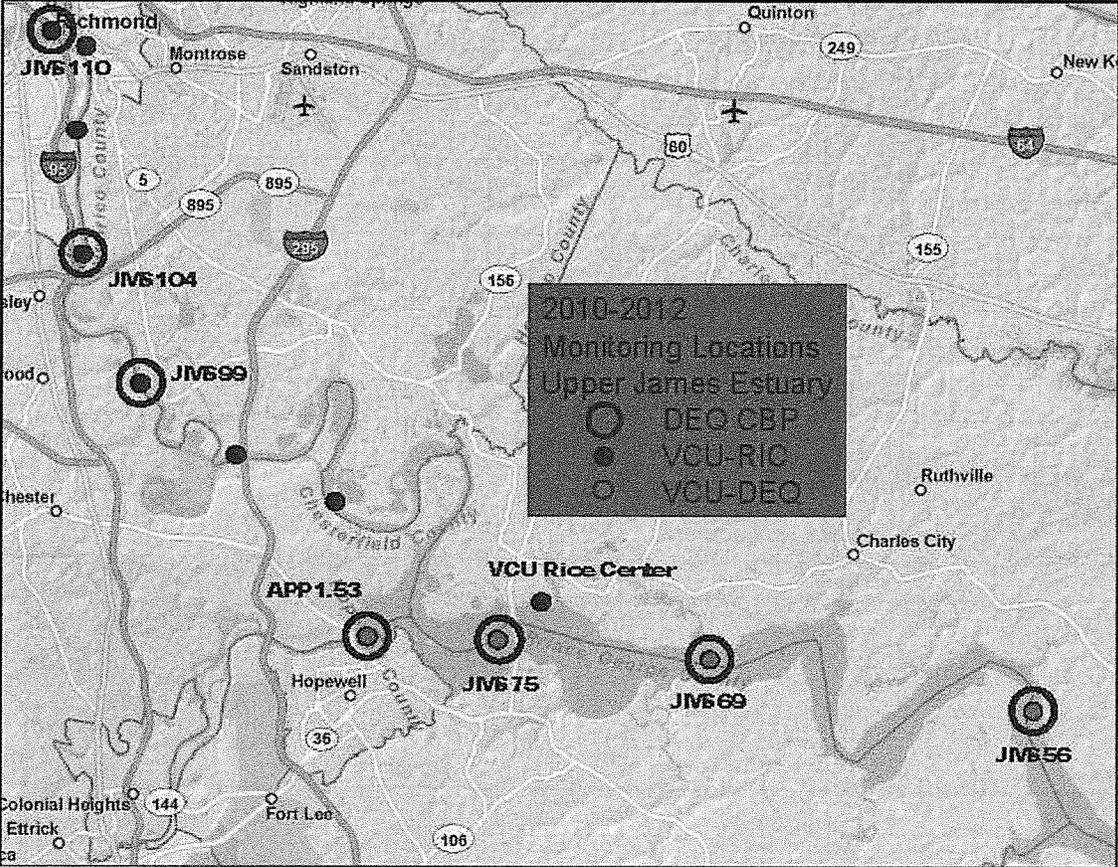
Upon return to the laboratory, samples for TSS, CHLa and nutrient analyses are transferred to the Environmental Analyses Lab (EAL) and samples for fecal coliforms and *E. coli* are delivered to the Microbiology Lab. Phytoplankton samples are retained by EAL for subsequent transport to ODU (Dr. Harold 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's 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

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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.

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- 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:

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

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

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:

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

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

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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
VIMS Analytical Standard Operating Procedures

**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
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NOTE: The following pages do not provide an inclusive description of all protocols provided in the Quality Assurance Project Plan submitted by VIMS as a part of the James River Chlorophyll Study. The pages included herein are extracted from the VIMS Quality Assurance Project Plan and provide only those protocols specific to laboratory analysis of Chlorophyll and pheophytin. These specific protocols are extracted for the purposes of inclusion in VA DEQ's Quality Assurance Project Plan for Special Study of Chlorophyll-a Methods.

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.
- 2.7.1.5 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 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.

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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