ATTACHMENT G

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

Prepared by

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Prepared for
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I. PROJECT DESCRIPTION (PHYTOPLANKTON COMPONENT):

This project is responsible for monitoring the composition and abundance of phytoplankton, the concentrations of the autotrophic picoplankton, and the measurement of ^{14}C productivity 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, 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, all necessary field equipment and a complete series of identification reference keys for all phytoplankton categories; cell culture facilities, and 5.) a ^{14}C productivity measurement facility with an experienced plant physiologist who has been conducting these measurements for 18 years in the Virginia Bay Monitoring Program. Additional resources available in the building include molecular genetic analysis and scanning electron microscopy usage in special situations.

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 (including the diatoms, dinoflagellates, cryptomonads, chlorophytes, cyanobacteria, euglenophytes, chrysophytes, prasinophytes, haptophytes, and other components.

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 determine ^{14}C productivity rates from samples taken of the phytoplankton community at stations located in the lower Chesapeake Bay and tributaries mentioned above.

4. To provide base line data that may be used for data interpretation and statistical analysis of the phytoplankton,

picoplankton and productivity studies mentioned above, in addition to the application of the data to long-term trend analysis.

5. 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, etc.).

6. 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 exceeding 50,000 cells/ml) of the cyanobacteria *Microcystis aeruginosa*.

7. 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. 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, productivity, 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, productivity, 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, 2009 through June 30, 2010. Field collections for phytoplankton, picoplankton and productivity measurements will be from January 2009 through December 2009 in the mainstem and from March 2009 – October 2009 in the tributaries of the Bay.

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 the lower Bay, 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 will represent the critical populations needed in studies to determine 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 the 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 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 complete data sets on phytoplankton composition and trends, picoplankton abundance and productivity 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 provide information regarding associations that may have direct relationships to management decisions concerning nutrient entry into this region. 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:

Station	Description	Latitude	Longitude
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 species composition and abundance.
b. Phytoplankton community productivity.
c. Autotrophic picoplankton community 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 are taken at the Bay stations (12 months). Collections in the tributaries are taken monthly March through October (8 months).

9. Types of Samples:

All phytoplankton, productivity and picoplankton data will come from the analysis of water samples collected with a hose, lowered to a specific series of depths, connected to a pump on board a power boat, and delivered to a carboy to form a composite sample. Two sets of composite water samples will be taken on station. 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 and physiology Laboratory at Old Dominion University, under the direction of Dr. Harold G. Marshall (PI) and Dr. Kneeland Nesius (CO-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 hmarshal@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 the 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 40 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 also 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 species 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 7 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:

1. Mr. Todd Egerton (M.S.) is a trained phytoplankton specialist with an M.S. degree from ODU with Dr. Marshall, and is currently a doctoral student at ODU in the Ph.D. program.

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

4. Ms. Kelly Alperin,(M.S.) is trained in productivity analysis and is employed in the departmental phytoplankton analysis lab.

5. Mr. Matthew Muller(B.S.)is a trained phytoplankton specialist, and is a graduate student in the ODU M.S. degree program.

6. Mr. Nathan Bowman (B.S.) is a trained phytoplankton specialist and a graduate student at ODU in the M.S. program.

7. Ms. Charlotte Grizzard (B.S.) is a trained phytoplankton specialist and a graduate student at ODU in the M.S. program.

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. CO-PRINCIPAL INVESTIGATOR:

Dr. Kneeland Nesius, (CO-PI) a plant physiologist, conducts the ^{14}C productivity studies associated with the project. He is responsible for sample preparation, the productivity analysis, preparing productivity reports and assuring the productivity section protocols for the QA/QC procedures are followed. He reports to the principal investigator. Dr. Nesius has been conducting this analysis since these measurements began in 1989 for the Monitoring Program.

G. 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.

H. 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, and productivity rates are provided. By maintaining the consistent and established protocol, 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 two individuals in the laboratory, with an additional replicate sample analyzed in the picoplankton and productivity measurements, with strict following of the protocols, and having and new phytoplankton identifications made by trained specialists. In-lab verifications of identification and cell counts are conducted by the re-examination of 5% of the sample concentrates, and with regular comparisons made in split sample analysis involving the Morgan State Phytoplankton Laboratory. Cell counts and identifications comparisons will also be conducted on in-lab QA/QC results coming from checking and comparing results from 5% of the samples using calculations indicated in Section XII. Values of cell concentrations and productivity rates 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 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 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. All collection bottles are thoroughly washed after usage. All storage vials are used only once. All glassware, 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 a trained and experienced specialist 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 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, replicate 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, the autotrophic picoplankton cells have been reported in this monitoring program.

E. PRODUCTIVITY:

Standard protocol procedures will be followed to guard against errors and maintain accuracy and precision throughout the analysis procedures (Strickland and Parsons, 1972; Marshall and Nesius, 1996). These include first hand instruction to all assistants by the assistant principal (CO-PI) investigator for each step of the protocol, plus periodic re-checks and first hand observations by the CO-PI, and periodic duplicate analyses on samples collected. The ^{14}C work will be performed on four separate replicates taken from each composite sample. Carbonate alkalinity will be determined on four separate replicates. Comparisons between replicates will be constantly monitored. The co-principal investigator and his assistants <u>are</u> licensed and trained to work with radio-active materials as approved by the University's Radiation Safety Officer.

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. DEQ-ODU CO-ORDINATED TRIBUTARY SAMPLING:

DEQ personnel will collect all plankton, picoplankton, and productivity water samples from 6 river stations (TF3.3, RET3.1, TF4.2, RET4.3, TF5.5, and RET5.2). Collections are made according to previous protocols designated by H. Marshall and used to date. DEQ personnel will deliver these samples in a timely manner (without delays) the same day of collection to personnel from the ODU Phytoplankton Analysis Laboratory at designated mutually agreed upon sites. Productivity samples must be kept on ice until delivery to the ODU laboratory the same day of collection. 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.

C. PROJECT OBJECTIVES AND BACKGROUND:

To obtain representative water samples for phytoplankton, picoplankton and productivity 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.

D. 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).

E. 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, 2009In addition, emphasis will also be placed on relationships between these components and the water quality conditions (Marshall et al. 2009.

F. SPECIFIC ELEMENTS TO BE ADDRESSED:

1. Phytoplankton:

a) Two vertical sets of phytoplankton samples for analysis will be taken monthly at each of the previously designated 7 station sites in the lower Chesapeake Bay, and from March through October at the 7 stations in the 4 rivers (see C. Study Design, 5. Sampling locations)

b) In the Bay at each 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 the tributaries at each station two vertical, composite series of five 3-liter water samples are taken above photic zone and below the photic zone regions 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 5 ml of Lugol's solution as a fixative. This process is repeated from the carboys (2) taken from the lower water column series.

c) In the Bay collections vertical conductivity measurements will determine the depth of the pycnocline, whereas, in the tributaries the depth of the photic zone is calculated from secchi disk readings. A secchi disk reading, multiplied by 3.5 is used to

obtain the photic zone depth. The pycnocline is not used in the rivers due to the general mixing nature of the rivers. In the Bay the photic zone is consistently present in waters above the pycnocline.

d) This series 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 returned to the phytoplankton laboratory for processing. 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 stations and 224 from the tributary stations to total 560 annually.

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. The phytoplankton supervisor is responsible for the labeling of the bottles, their custody, and storage in the phytoplankton laboratory, where they are placed under refrigeration. A total of 336 picoplankton samples will be collected annually from the Bay stations and 224 from the tributary stations to total 560 annually.

3. Productivity:

Water sub-samples for productivity measurements will come from each of the two composite water samples (carboys) taken from the upper water column series in the Bay the four rivers. From the two carboys, 4-1 liter water samples (2 from each carboy, total of 4/station) are placed in labeled bottles and placed immediately in a cooler on ice to prevent increased ambient temperature to alter their productivity status and cell production from the time of collection, and are returned directly to the ODU Phytoplankton Laboratory for analysis. In the laboratory their custody will be given to Dr. K. Nesius or his designee. An initial total of 420

samples will be collected for analysis annually.

V. SAMPLE CUSTODY:

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 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 and ^{14}C 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. Custody of the water samples for C^{14} analysis will be transferred immediately to Dr. K. Nesius or his designee for subsequent analysis.

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 will be 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. 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.

B.1. 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) Paximos and Mitchell (2000) recommend 24 hours. Several other references have indicated lesser time periods for settling. With this sinking rate as a guide and a maximum distance of 6.75 cm for settling distance, a settling period at 27 hours would be considered the minimum period for settling in the first step of this process, which the ODU protocol considerably exceeds. 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.

C. 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.

D. 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.

E. 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.

A Beckman Model LS 1701 scintillation counter is used in the productivity measurements. The Department of Biological Sciences

maintains a service contract with Beckman, with the instrument serviced professionally and calibrated generally every six months. A standard sample containing 1uCi C-14 (51,000 dpm) is counted along with the samples to check calibration prior to each collection period.

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, picoplankton, and productivity are mainly similar as those used consistently since 1985 in the Virginia Chesapeake Bay Monitoring Program. For modifications see Section XVI. 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), Strickland and Parsons (1972), 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 shaken 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 on both replicate samples, and averaged. A minimum coverage of 20 fields is the procedure followed 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.PRODUCTIVITY:

Water sub-samples for productivity analysis are taken from each of two composite 15-liter carboys from the photic zone (above the pycnocline) at each station. Water temperatures at time of collections are recorded. From each of the two carboys, two-one liter water samples (total of 4/station) in labeled bottles are immediately placed on ice, and kept in darkness and in an ice cooler prior to delivery to the laboratory.

Placing the collected water samples in an ice cooler has been the procedure followed to reduce further phytoplankton growth and autotrophic metabolic activities if the samples were exposed to light and higher ambient temperatures (especially during extended time on the vessel, land transportation, and time prior to analysis) that were not similar to conditions when the samples were taken. The significance of this procedure has been substantiated by our comparative studies on this issue.

Samples are assayed for C-14 uptake soon after arrival to the laboratory on the collection day. 100 ml samples from each one-liter bottle are placed in 250 ml acid washed milk dilution bottles. The bottles are allowed to incubate for one hour at the temperature they were collected and under saturated light conditions so they will equilibrate to the original conditions. The samples are than inoculated with 5 *u*CiNa$H^{14}CO_3$.

Protocol for alkalinity measurements: The amount of total dissolved inorganic carbon available for productivity measurement is determined by alkalinity measurements using the titration method. The end point of 0.1N sulfuric acid is determined by titration of a standard 0.1 N sulfuric acid against 0.05N sodium carbonate solution. An aliquot of the sample collected is titrated against the standard sulfuric acid to its end point. Using the volume of standard acid used and normality of the acid the amount of sodium carbonate (mgCaCO3/liter) may be determined.

Alkalinity*, mg CaCO3/L = $\frac{A \times N \times 50{,}000}{}$

ml sample

A = ml standard acid; N = Normality of standard acid

*Standard Methods for examination of water. APHA AWWA WPCF, 17th Edition.

The replicate 1-liter samples will be taken from the composite samples photic zone to determine the productivity at each station. One hundred ml samples from each composite sample are placed in separate dilution bottles and transferred to a water bath equipped with a bottle holder that rotates between banks of cool-white fluorescent lights. The light levels will exceed the light saturation point of the phytoplankton. The temperature of the water bath will be the same as the temperature at each station when the samples were taken. After one hour of acclimation the bottles will be inoculated with 2-5 uCi ^{14}C-$NaHCO_3$. The samples will be returned to the water bath for approximately two hours. One of the samples will be analyzed for ^{14}C activity immediately (time of sample). At the end of the incubation period the remaining samples will be filtered through a 25 mm 0.45 pore-size millipore filter under a vacuum less than 5 cm Hg pressure. After the contents of the milk dilution bottle and its rinse are filtered, the millipore filters will be removed and fumed over concentrated HCl for 30 seconds and placed in scintillation vials. Scintillation fluid will be added to each vial and ^{14}C activity will be determined using a Beckman Model LS 1701 scintillation counter. A flow chart for processing the samples is given in the Appendix, Fig. 7.

The amount of ^{14}C in the stock bottle will be determined by placing 20-50 ul of stock solution in scintillation vials containing 0.5 ml phenethylamine. Scintillation fluid will be added to the vials, set in the dark overnight and analyzed for ^{14}C activity.

Precision and accuracy of the data is based on the replication protocol followed in the analysis. Two samples are taken from each of the two bottles (4 total) collected at each station and assayed for C-14 from each station. The goal is for the DPM numbers, indicating uptake of C-14, and based on comparative evaluation for the four samples to be within a 10% to 15% range of comparison.

Acid washed glass dilution bottles, graduates cylinders, pipets, etc. will be used for the phytoplankton productivity studies. All data at the time of collection will be kept in a log. This log will be checked again at the end of each sampling day after the analysis has been completed. Using formulas from Strickland and Parsons (1972) an estimation of the hourly carbon fixation rates will be calculated.

E. MICROCYSTIN AND BLOOM SPECIES ANALYSIS: VIMS will send in overnight or next day delivery preserved and non-preserved water

samples (250 ml) to the ODU Phytoplankton Laboratory during major cyanobacteria blooms of Virginia rivers and streams during the current year analysis of *Microcystis aeruginosa* concentrations. If they exceed 50,000 cells per ml, this laboratory will use ELISA microcystin analysis procedures to analyze for microcystin. During any other bloom event, VIMS will send to this laboratory a 250 ml water sample for species identification for overnight or next day delivery. These results will be reported to DEQ personnel.

F. ANALYTICAL COSTS BASIS:

The project plan stipulates a total of 560 phytoplankton, 560 picoplankton and 560 productivity samples will be collected for subsequent processing procedures over the 12 month period to give a grand total of 1680 samples collected.

G. 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 5 to 6 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 species identification for forms two small to be identified with light optics. It contains both an SEM and TEM. Also located in this building is a physiology laboratory where radio-active carbon productivity studies are conducted and which houses all necessary equipment for that work, in addition to 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 coastal waters of the United States, the Delaware Bay Basin, the Caribbean, and

the eastern equatorial Pacific. This laboratory has over two decades of experience in analyzing large quantities (e.g. over 1000/year) of phytoplankton samples, 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. These procedures measure total error in species identification and abundance counts. After data entry the entered station data is again checked for any entry errors.

2. Stock Solution:

The stock ^{14}C-$NaHCO_3$ will be checked before each laboratory procedure is performed on productivity measurements. It will be checked for proper activity by placing 20-50 ul of stock in a scintillation vial containing 0.5 ml of phenethylamine.

IX. EXTERNAL QUALITY CONTROL CHECK:

The ODU Phytoplankton Analysis Laboratory will exchange water samples with Morgan State University on a predetermined basis. There will be an exchange of split samples from the Bay program. Each laboratory may analyze an agreed upon number of samples and compare phytoplankton species identification and abundance.

Emphasis is placed on species identification. Discrepancies in species identification will be noted and clarified, and any major differences in species abundance will be identified. Results of these comparisons will be reported to the Quality Assurance Officer.

Recommendations on analytical protocols based on split samples results in 2004 were discussed at a Split Sampling session in 2005 and have been followed since 2006. These include a re-classification of centric and pennate diatoms size categories that would be data entered, and a specific list of five phytoplankton categories to be counted at 500-600X magnification by the two laboratories. ODU submitted in June 2005 a new statistical approach, suggestions in writing, plus results of several in house studies comparing the methodology used in comparing data results between the two laboratories.

A review of the Bay species list in 2005 indicated ODU in its sample analysis has in recent years identified over 100 additional taxa in the monitoring program including several previously unreported potentially toxic species. This information supports the value of our protocols in expanding and identifying the Bay's species list. Additional corrections were also noted to address recent name changes attributed to other taxa in the ODU data. As species name changes occur in the future the old name will be reported to CIMS until ITIS updates its list and assigns a new TSN.

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 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 5% of the concentrated samples will be re-analyzed in house for data comparison checks.

2. 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. There will also be close interaction between the PI and personnel from the Old Dominion University Phytoplankton Laboratory with those from Morgan State University conducting monitoring studies on the phytoplankton of the upper Chesapeake Bay. These interactions include decisions on any new species identification, exchange of data, and other collaborative work.

XI. PREVENTIVE MAINTENANCE:

A. FIELD COLLECTIONS:

All collection gear will 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, suggestions for improvement, and data not collected. 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 e 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. In the productivity measurements of replicate samples, differences greater than 20% between the productivity rates of these replicates will result in the re-measurement of the samples to be re-checked for accuracy, and any re-sampling if necessary.

3. 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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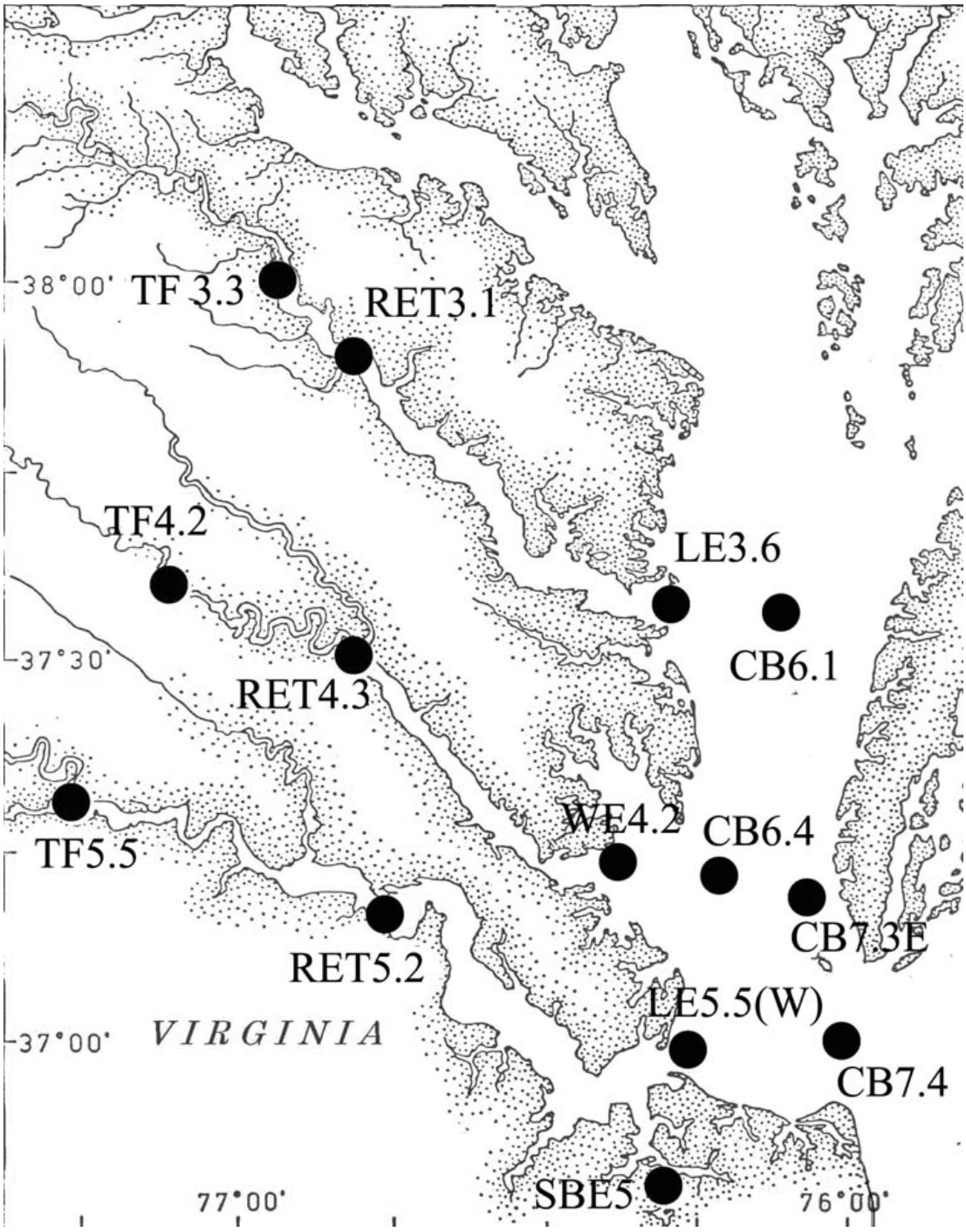

Figure 1. Map of Chesapeake Bay and tributaries, showing station locations.

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 •m, pennate diatoms<10 •m, cryptomonads <10 •m, unidentified green algal spherical cells 3-5•m, and unidentified micro-phytoflagellates <10•m.