**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 the same 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 30 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) 17 years experience in the 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 laboratory, with seven inverted plankton microscopes, two epifluorescence microscopes, all necessary field equipment and a complete series of identification reference keys for all phytoplankton categories; and 5.) a fully equipped $^{14}C$ productivity measurement facility with an experienced plant physiologist who has been conducting these measurements for 11 years in the Virginia Bay Monitoring Program.

A. OBJECTIVES AND SCOPE OF PROJECT.

1. To determine the composition, and abundance of all phytoplankton populations, above and below the pycnocline at stations in the lower Chesapeake Bay and within the upper and lower regions of the water column at stations in the Elizabeth, James, York and Rappahannock Rivers (Fig. 1).

The principal investigator, and all who conduct the microscopic analysis, have established expertise in the systematics of these categories within the phytoplankton community. These include fresh water and marine diatoms, dinoflagellates, cryptomonads, chlorophytes, cyanobacteria, euglenophytes, chrysophytes, prasinophytes, haptophytes, and others. The principal investigator is a phycologist who is a recognized expert in phytoplankton systematics, with documentation of past studies in scientific journals, etc. Since the project includes a mixture of marine, estuarine and fresh water species, taxonomic expertise experience in each of these areas is essential and is provided by the PI.

2. To determine concentrations of the autotrophic picoplankton, above and below the pycnocline at 14 stations located in the lower

Chesapeake Bay and in the tributaries mentioned above.

3. To determine the $^{14}C$ productivity rates from samples taken of the phytoplankton community, from composite water collections at 14 stations located in the lower Chesapeake Bay and the tributaries mentioned above.

4. To provide the base line data that may be used for data interpretation and statistical analysis of the phytoplankton, picoplankton and productivity studies mentioned above. This data may be used to identify relationships between phytoplankton and picoplankton populations, and their seasonal spatial and temporal distributions in the lower Chesapeake Bay and in each of these tributaries, and to specific water quality conditions, 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 these four tributaries, and relate this presence to the occurrence of these and other toxic species that have occurred historically in the Chesapeake Bay and its rivers (Marshall, 1996).

6. To provide data that can be used to determine relationships over the collection period between the major phytoplankton components (and dominant species), and picoplankton abundance in the lower Chesapeake Bay and tributaries.

7. To identify bloom events and bloom forming species in the lower Chesapeake Bay and these tributaries that occurred during the collection period and evaluate these blooms to historical data, regarding frequency, duration, composition and location.

8. To provide data on the composition of the phytoplankton populations to be used to characterize the health status of regional areas in the lower Chesapeake Bay and in the tributaries.

9. To establish a consistent, long term historical data-base, that may be used in the study of local and regional eutrophication patterns in the study area, and in future trend analyses.

B. COORDINATION ACTIVITIES WITH CBP COMMITTEES

A major value of this study is that it will be conducted at the same time as the water quality collections. This protocol provides a more meaningful basis to examine the relationships that exists between these complete data sets, and to make evaluations to other data sets in the Chesapeake Bay Program. Results obtained from phytoplankton and picoplankton monitoring will also 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 (significant) trends of population

growth, productivity, and eutrophic status. Additional information will be available on the presence and location of toxic and bloom producing phytoplankters, in addition to the abundance of picoplankton. This information has direct relevance to the objectives of several CBP committees and proposed tasks.

## C. STUDY DESIGN

### 1. Project Dates:

The time period for this study is from January 1, 2003 through June 30, 2004. Field collections for phytoplankton, picoplankton and productivity measurements will be from January 2003 through December 2003. Quarterly progress reports and a final report will be delivered in accordance to the dates stipulated in the contract.

### 2. Relationship to Background Information of this Project

The continuation of this project by the present investigator of the lower Chesapeake Bay phytoplankton monitoring study assures consistency and high levels of continual accuracy in the identification of the phytoplankton populations (with over 700 species recognized in the lower Bay, Marshall, 1994). A massive sampling 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 assures 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 the most accurate and 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 monitoring. To be able to associate relationships between the living resources and the water quality variables more fully,

water samples to be used in these analyses are coordinated regarding collection dates for each station at Bay and river stations.

Long-term trend analysis of this data set will provide information regarding trends that may have direct relationships to management decisions concerning nutrient entry into this region. In addition, this material may be used to determine specific relationships between the major producers (phytoplankton and picoplankton) in Chesapeake Bay to specific food web constituents and trophic exchanges in the system. Additional relationships may be sought between the phytoplankton, and benthic populations. Justification for this design is based on long term monitoring plans concerned with the ecological status and health of the Chesapeake Bay system. The station sites have been pre-selected by the Virginia Dept. of Environmental Quality and have been part of the previous monitoring program to date. Figure 1 gives the location of these stations. Refer to Section IV on Sampling Procedures for specific details on Sampling Design, etc.

5. Sampling Locations.

The RFP identifies for 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 the salinity regions in the Virginia rivers, and includes tidalfresh, oligohaline, and mesohaline regions. The Bay stations were located throughout the Bay to provide representative sites along both the eastern and western Bay regions, in addition to main stem locations, and a station at the Bay entrance. They are as follows:

| Station | Description | Latitude | Longitude |
|---|---|---|---|
| TF5.5 | James R. | 37 18 46 | 77 13 59 |
| RET5.2 | James R. | 37 12 24 | 76 47 36 |
| SBE5 | Elizabeth R. | 36 46 03 | 76 17 10 |
| TF4.2 | Pumunkey R. | 37 34 47 | 77 01 19 |
| RET4.3 | York R. | 37 30 24 | 76 47 18 |
| TF3.3 | Rappahan. R. | 38 01 07 | 76 54 30 |
| RET3.1 | Rappahan. R. | 37 55 12 | 76 49 48 |
| CB7.4 | Bay Mouth | 36 59 36 | 76 00 38 |
| CB7.3E | Eastern Shore | 37 13 43 | 76 03 15 |
| CB6.4 | Central Bay | 37 14 11 | 76 12 30 |
| CB6.1 | Main Channel | 37 35 18 | 76 09 45 |
| LE5.5 | Bay at Mouth James R. | 36 59 48 | 76 18 12 |
| WE4.2 | Bay at Mouth York R. | 37 14 30 | 76 23 12 |
| LE3.6 | Bay at Mouth Rapp. R. | 37 35 48 | 76 17 06 |

6. Co-ordinated sampling:

At Bay stations and the Elizabeth River station, the plankton,

water samples are ODU water quality personnel from an ODU vessel. At the remaining 6 tributary stations, personnel from Virginia DEQ will collect all the phytoplankton tributary water samples that will be analyzed in this program. (See IV. B, for details).

7. Parameters to be Measured 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 the diatoms, dinoflagellates, cyanobacteria, chlorophyceans, haptophyceans, silicoflagellates, euglenophyceans, cryptomonads, chrysophyceans, prasinophyceans 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, 1994).

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). The phytoplankton and picoplankton analysis are to be taken from two sets of composite water collections, obtained on station. Productivity samples will be taken from the same upper strata composite water carboy. Physical parameters of the water column (e.g. salinity, secchi depth, temperature) are measured at each station by members of the boat crews taking the plankton samples. Sections IV and VII on Sampling Procedures and Analytical Procedures.

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, 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). The address for all correspondence regarding this project

would be addressed to the PI, at Department of Biological Sciences, Old Dominion University, Norfolk, Va. 23529-0266. Phone: office 757-683-4204, lab 757-683-4994, FAX 757-683-5283, 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 responsibilities of the Laboratory Supervisor and designated Laboratory Experts. 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 all 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 preparing the quarterly progress and final reports concerning this project. He will also meet at regular time intervals, with the other 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 different stages of this project and the reports that have been submitted.

Harold G. Marshall is a phycologist and marine ecologist, with over 30 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 35 years, publishing over 100 articles and over 186 abstracts on phytoplankton, which includes regions from the Chesapeake Bay, its rivers and from regional marine shelf waters. He is a recognized expert in phytoplankton systematics and ecology, and has also published a phytoplankton identification manual (Marshall, 1986). His publications include 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 includes investigations of the on various toxic and bloom producing species within the Chesapeake Bay, with specific studies on the toxic dinoflagellate *Pfiesteria*.

B. QUALITY ASSURANCE OFFICER.

The Quality Assurance Officer will meet periodically with the principal investigator to discuss: 1. operation, sampling and analysis procedures, 2. data entry, 3. timeliness and availability of the monthly data product, and 4. 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 effect the sampling, or data analysis.

C. PHYTOPLANKTON FIELD/LABORATORY SUPERVISOR.

This position is held by Mr. Todd Stem. Mr. Stem has a B.S. in Biology and is in the Ph.D. degree program in Biological Sciences at ODU, with 4 years of experience as a phytoplankton investigator He is responsible for sampling operation, scheduling personnel to collection cruises; and supervising the maintenance of collection gear, 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 persons for this position are Mr. Egerton, Jeremy Hicks, and H. Marshall. Laboratory phone: 757-683-4994.

D. PHYTOPLANKTON LABORATORY EXPERTS IN ADDITION TO H.G. MARSHALL

1. Dr.David Seaborn (B.S., M.S., Ph.D.) is a trained phytoplankton expert, with experience in phytoplankton sampling and analysis. He is a faculty member and has projects in the Phytoplankton laboratory, in addition to assisting in this program.

2. Mr. Todd Stem (B.S.) is a trained phytoplankton specialist and a graduate student at ODU in the Ph.D. program.

3. Ms. Heather Green (B.S.)is a trained phytoplankton specialist and a graduate student at ODU in the M.S. program.

4. Mr. Jeremy Hicks (B.S.) is a trained phytoplankton specialist and a graduate student at ODU in the M.S. program.

5. Mr. Todd Edgerton is a trained phytoplankton specialist and a graduate student at ODU in the M.S. program.

6.Ms. Kelly Alperin,(B.S.) is trained in productivity sampling analysis and is 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.

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 for the lower Bay in 1989.

G. SUB-CONTRACTS

No sub-contracts are included in this project. The use of sub-contractors for analysis is not practical with the high caliber of expertise on phytoplankton systematics of the Chesapeake Bay already in this laboratory, and the experience and an extensive historical record on the capability in analyzing large quantities of samples monthly. In addition, the unique voucher collection of Bay species in this lab assures there will be continuity in year to year identifications over a long time period.

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 audits will be made in sampling procedures by the field chief and the PI on station Routine species checks will also be made of the species identified in the laboratory by the PI and laboratory supervisor. Extensive use of the Chesapeake Bay reference (voucher) collection in the Phytoplankton Laboratory will be carried out to assure consistency in species identification.

## III. QUALITY ASSURANCE OBJECTIVES AND CRITERIA

A. OBJECTIVES AND DATA USAGE

The objectives of the QA standards are to provide an accurate estimate and characterization of phytoplankton and picoplankton populations, and provide the productivity rates for these autotrophic communities in lower Chesapeake Bay and the four rivers. By maintaining the consistent and established protocol, data from these analyses will be used directly to meet the objectives stated for this study. In addition, this information will be useful in management decisions concerning the Bay and these tributaries, by distinguishing between inter-annual seasonal

variability and long-term significant trends that may be taking place. The broad scope of the monthly, seasonal, and annual patterns of composition and abundance for these plankton populations provides valuable information regarding the health status of the region. There is here an unbroken record of the seasonal dynamics of these populations, without any major gaps in the data set. Monthly Bay analysis is especially important because seasonal growth patterns vary among the different constituents is necessary to distinguish these cycles.

The objectives established for and laboratory analysis will meet QA standards given in the RFP and Table 1. The standards of comparability and the representativeness of the data collected during this study will be maintained by the adherence to the sampling and analysis procedures. Precision goals and QA/QC procedures will be enhanced through procedures that include a composite sampling base for collections in the field, in the replicate sample analysis in the picoplankton and productivity measurements, strict following of the protocols, and having the phytoplankton identifications made by trained specialists. The precision, or data reproducibility is enhanced by the use of these experienced specialists in Chesapeake Bay phytoplankton analysis. Mean values of cell concentrations and productivity rates will be the reporting units given for data analysis. Protocol is given in regard to quantitative discrepancies in the Section XIII on Corrective Action. 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. Accuracy goals (80-120 %) for each parameter measured will be achieved through this experience and the internal laboratory checks that are standard practice in this laboratory, in addition to the use of voucher records in the laboratory. Completeness of sample collections and analysis is expected to be greater than 90%. However, cruises may be canceled mainly due to weather, or other reasons.

In the laboratory program, internal quality control comparisons will be made on 10% of the samples (See Section VIII B1.b). A record of cell counts made will be kept, and maintained for recording results of the QA/QC sample analysis. Upon completion of all sample analyses, the raw data sheets are reviewed for possible code, or mathematical errors before data entry takes place. These data sheets are filed in the 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 is 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 organisms, the other is to obtain, as accurate an estimate as possible 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 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 1000 species of phytoplankters, have been collected from this region and are used within the laboratory for reference to assure consistency and provide verification of identifications.

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 (cells 0.2 to 2.0 microns in size) analysis. Samples are taken at the same time as the phytoplankton collections. Standard epifluorescence microscopy procedures are followed to identify and 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. These procedures meet accepted standards for meeting QA/QC standards.

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 are licensed to work with radio-active materials.

## 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. No other unauthorized deviations from this plan are allowed.

### B. DEQ TRIBUTARY SAMPLING

DEQ personnel will collect all plankton and productivity water samples from the 6 river stations (TF3.3, RET3.1, TF4.2, RET4.3, TF5.5, RET5.2). Collections will be made according protocols designated by H. Marshall. DEQ personnel will deliver these samples the same day of collection to personnel in the ODU Phytoplankton Laboratory. Productivity samples must also be provided to the ODU lab personnel in an ice cooler the same day of collection. Prior phone contact by DEQ personnel to ODU Phytoplankton personnel is required to confirm delivery time and the transfer of these samples, and satisfactory status of these samples. DEQ personnel will pick up the collection bottles that will be provided by and at the ODU lab. See Figure 1 for these station locations.

### 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 has made numerous presentations at professional meetings

### 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. In addition, emphasis will be placed on the picoplankton abundance, and the relationships between any component and reduced water quality conditions. Reports will include status and trend patterns. Specific reference to these categories is included among the objectives of this study (section I, Project Description).

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) At each station, two vertical, composite series of five 3 liter water samples are taken at approximate equidistant depths between samples within the upper and lower regions of the water column and placed in two carboys. 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) The depth of the samples taken from the upper layer of the water column represents water within the photic zone calculated from secchi disk readings. If a pycnocline is present, it also is above this region. If a pycnocline is not present alternative method to determine the upper strata for sampling, and typically used used in the tributary collections is to take a Secchi disk reading, multiply this by 3.5 to obtain the photic zone depth. Divide this depth by 5 to determine the sampling depths in this region. Equidistant samples taken from below this depth would be representative of waters below the photic zone, or be represented within the lower third of the depth at the station.

d) This series provides 2 sub-samples 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 pump is housed in a special box for protection and transportation.

A reinforced hose is connected to the pump for the sample collections. The collection and labeling of the samples, and their custody from field site to the phytoplankton laboratory, is the responsibility of the field supervisor from 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 will be flushed after and before each pumping, and rinsed thoroughly after each cruise. These will also be checked routinely for maintenance needs. A backup system for the pump, battery, and hose will be available on each cruise. In total, 336 water samples are taken annually from the Bay stations, and 224 from the tributary stations. Total annually = 560.

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 in an ice cooler until their return to the phytoplankton laboratory. The phytoplankton field supervisor is responsible for the collection of the samples, the labeling of the bottles, their custody, storage and transport to 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. Total annually = 560.

3. Productivity

Water sub-samples for the productivity measurements will be taken from each of the two composite water samples (carboys) taken from the upper water column series and within the photic zone at each station in the lower Bay and the four rivers. From each of the two carboys, 2 - 1 liter water samples (total of 4/station) are placed in labeled bottles and placed in a cooler, until their return to the ODU Phytoplankton Laboratory. In the laboratory their custody will be given to Dr.K. Nesius. A total of 560 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. 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, whether it came from above or below the pycnocline, and from which carboy it came from. Samples will then be boxed and transported directly to the phytoplankton laboratory upon return of the vessel. These samples are then released to 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 have to be kept in an ice cooler and transported 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, Assistant Principal Investigator,

4. Details in sample collection are given in the section (IV) on Sampling Procedures.

## 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 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 later analysis. 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. After analysis, the storage vials will be kept for six months, after which they may be discarded through protocols established by the University (and State of Virginia) and supervised by the university Health and Safety Officer.

## C. FINAL EVIDENCE FILE

A permanent 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 that will contain the chain of custody and 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 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 record keeping, 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. A record of this transfer will be kept on the raw data sheet used for each sample and this sheet is kept on file in the final evidence file in the laboratory. These represent permanent records as to the processing and custody of each sample.

## VI. CALIBRATION PROCEDURES AND FREQUENCY.

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.

Although there only a few instruments used in this project, any maintenance procedures, logs, accepted operational standards, calibration needs, or other requirements currently in use in the Chesapeake Bay program will be followed.

## 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 similar as those presently used consistently since 1985 in the Virginia Chesapeake Bay Monitoring Program. In addition, the same investigating team from this laboratory would continue the on-going monitoring program to guarantee complete continuity and consistency in data acquisition and analysis, and species identification. Results from these analyses will provide compatible data sets that will be essential for long-term statistical data analysis within this region. The methods used have included specific QC objectives addressed in this proposal (section III). References include:

Hobbie et al. (1977), Davis and Seiburth (1982), Marshall (1986, 1994, 1995), 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. A universal Utermöhl method of phytoplankton analysis, using inverted plankton microscopes, is used in this project, and follows the internationally accepted protocol for phytoplankton analysis, and is the same method used since 1985 in Virginia This method is essential to use in order to preserve the representation of species and consistency in the analysis of this community.

2. 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 and placed on a settling table for 72 hours. A series of alternate siphoning and settling steps then follows to obtain a 20-40 ml concentrate from each sample. After the samples are allowed to settle undisturbed for 72 hours, the original 500 ml is reduced by careful siphoning to approximately 200-250 ml. The samples are allowed to stand undisturbed for an additional 48 hours and are again siphoned to the 20-40 ml concentrates. The final 20-40 ml concentrate will be transferred to a previously labeled storage vial, where the label information from the collection bottle has been transferred and verified by the laboratory supervisor. A known volume of the entire concentrate will be placed in an Utermöhl settling chamber for examination with an inverted plankton microscope (e.g. Zeiss) following the Utermöhl method. If the phytoplankton, and/or silt, density is too great in the final concentrate for clear examination, a known volume of the concentrate is drawn off to provide a sub-sample suitable for analysis. Prior to counting, a work sheet is prepared, where information from the sample vial label will be transferred to the data sheet and verified. The microscopic examination will be done at 3 magnifications (Marshall and Alden, 1990). At 312X magnification, a combined random field (10) and minimum cell count (200) procedure will be followed where all taxa are counted to the lowest taxonomic category possible. This examination is repeated at 500x magnification for 10 randomly selected fields. Cells not clearly discernable at the 315x magnification will be counted at 500X. All species will be counted at only one of these magnifications. In addition, the entire chamber will be scanned at 125X for recording previously unrecorded larger species in the chamber. All phytoplankton categories will be included in this analysis. 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 (e.g. Chesapeake Bay) that are maintained in the ODU phytoplankton laboratory. This assures continuity in species identification. New taxa would be verified by a phytoplankton taxonomist (e.g. H. Marshall), and included in the voucher records.

3. In the analysis, all taxa observed and their cell counts are recorded on (raw) data sheets for each station set. The individual doing this analysis will then determine cell concentrations in cells per liter and record this on the raw data sheets, which are initialed and dated and placed in a file until the data is entered in the computer program. All raw data sheets are kept on file and available for later reference.

C. PICOPLANKTON

When brought to the phytoplankton laboratory, samples will be stored in a refrigerator at 4°C and the counting procedures will be completed within 7 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. The autotrophic picoplankton are counted using both a "green" and a blue/violet 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 315x or 500X (e.g. some *Merismopedia* spp.). Mean cell counts of replicate samples are computer entered and cell concentrations determined (cells/liter).

D. BIOMASS

Although not identified as a deliverable the contract, the PI can also make available biomass (e.g. carbon) estimates of the phytoplankton and picoplankton populations.

E. PRODUCTIVITY

Four replicate 1 liter samples will be taken from the composite samples above the pycnocline (or 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.

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.

## F. ANALYTICAL COSTS BASIS

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

## 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. It is one of the most fully equipped

laboratories for phytoplankton analysis in the United States. It contains 6 inverted plankton microscopes, two epifluorescence microscopes, and several compound microscopes. It possesses all necessary collection gear (plus back up systems) for pumping water samples to depths of 60 feet, standard water samplers, and related support gear. 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 will be 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. The Phytoplankton Laboratory has been in operation for over 25 years. Previous phytoplankton studies centered in this laboratory have emphasized the Chesapeake Bay, Virginia lakes and rivers, bloom producing species, toxin producing species, and phytoplankton from the northeastern coastal waters of the United States, the Delaware Bay Basin, the Hudson River, the Caribbean, and the eastern equatorial Pacific. This laboratory has 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 to be made by the collection personnel to assure picoplankton and productivity sample bottles are in the 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 30 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 (monthly) meetings with the entire laboratory staff to discuss the program, data results and current populations in the samples.

2. Verification: Taxa Identification and Abundance

Counts and identifications of the various taxa will be checked by the re-analysis of 10% of the samples collected (See Section X 2). A record is maintained of the results of the QC sample analysis. Upon completion of all sample analyses, the raw data sheets are reviewed for possible code, or mathematical errors before data entry takes place, by the lab supervisor. 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. These steps follow the procedures used to meet and assess precision and accuracy requirements (Table 1).

3. Stock Solution

The stock $^{14}C\text{-}NaHCO_3$ 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 the Academy of Natural Sciences for comparative phytoplankton sample analysis. There will be an annual exchange of split samples from 3 500ml water samples taken in alternate years from Virginia and Maryland stations in the Bay program. Each laboratory will analyze the 3 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 and in appropriate reports.

## X. PERFORMANCE AND SYSTEMS AUDITS.

1. A re-analysis of 10% of the phytoplankton samples will be conducted and checked for errors in taxa identification and/or their cell counts, under the direction of the PI and/or laboratory supervisor. This is done by having the laboratory supervisor, or another technician compare the species identification and their

concentrations from microscopic fields examined in 10% of the total samples examined in the Utermöhl chamber immediately after results are recorded by the first technician. 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.

A re-analysis of 10% of the autotrophic picoplankton samples will also be conducted and will be under the supervision of the PI or laboratory supervisor. The re-analysis will apply to examining a prepared microscopic slide from one of the replicate site samples using epifluorescence microscopy for comparisons of cell counts.

A record of all counts made will be kept for recording QA/QC results. 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. Results of the QA/QC analysis will be included in the quarterly reports and data submission.

2. The laboratory protocol will be under the supervision of the principal investigator and the phytoplankton laboratory supervisor. A review of raw data sheets, indicating custody, 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 the Benedict Laboratory conducting monitoring studies on the phytoplankton of the upper Chesapeake Bay. These interactions include decisions on any new species identification, exchange of data, evaluation of Bay wide status and trends, and other collaborative work. These inter-laboratory exchanges review procedures and assure consistency in sample analysis and species identification.

3. Acceptance criteria for both the field and laboratory analyses will be the adherence to protocols established for these sections. Reports, such as the monthly reports, QA/QC checks, will be kept on file and will be routinely reviewed by the PI, or the field/laboratory supervision, to evaluate performance levels. These audit reviews will be done routinely and will be available for distribution upon request.

**XI. PREVENTIVE MAINTENANCE.**

A. FIELD COLLECTIONS

All collection gear will be routinely cleaned and examined for wear or breakage. Proper maintenance of pumps and all back up gear is the responsibility of the collection crew chief, who reports to the principal investigator regarding the need for any spare parts, or replacements.

Contingency Plan: Back up pumps, hose, batteries, and other

support gear are maintained in the laboratory. Spare parts are available and tools are available in the laboratory. Local dealers are available to satisfy any purchase needs that may arise. Repair sources include a variety of Marine and Supply dealers locally.

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 computer 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. The lab supervisor reports directly to the PI any service, or replacement needs.

## XII. DATA REDUCTION, VALIDATION AND REPORTING

Data transcription, validation and reporting procedures are designed to produce data sets that have met the appropriate criteria for QA/QC and have been verified as exactly reproducing all information from each raw data analysis sheet for the phytoplankton, picoplankton and productivity 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=s AThe 2000 Users guide to Chesapeake Bay Program Biological and Living Resources Monitoring Data@.

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 transferral 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 to magnetic tapes occurs routinely. Monthly backup tapes containing all files are retained for one year and an annual backup tape is retained permanently. In addition, permanent data sets and programs are copied to read-only DC-ROMS on an annual basis. Copies of the DC-ROMS are kept in two separate locations.

B. VALIDATION

The previously defined methodologies will produce a data set for characterizing the phytoplankton/picoplankton populations in the lower Chesapeake Bay and the designated rivers and will meet the validation objectives established for this project. The major criteria in this study is to obtain a representative sample base for the species identification and their concentrations, and productivity rates at the sampling sites. The basic techniques for microscopic analysis follow standard protocols that are internationally accepted and are now used in the program. Precision objectives are enhanced by the composite sampling base and analysis steps. The 33 years of experience in phytoplankton studies from this region by the PI will be a major strength in fulfilling these tasks. Strict adherence to field collection protocol and laboratory analysis protocol will be followed to meet the designated goals of the project.

The precision, accuracy and completeness assessment of the qualitative and quantitative parameters of sample analysis are routinely evaluated by the re-analysis of 10% of all samples analyzed. This procedure promotes precision and accuracy in the sample analysis for improved and valid results. This approach is considered to adequately meet data completeness criteria.

C. REPORTING

1. Raw Data

Data will be submitted to the Chesapeake Bay Data Center via tape or file semi-annually. Data (including associated methodology and QA documentation) will be formatted and verified in a manner consistent with the most recent versions of the Chesapeake Bay Program Data Management Plans.

2. Progress Reports

Quarterly status reports will be submitted to the Virginia Department of Environmental Quality Project Officer. These will include raw data summaries, a brief narrative of progress, any QA/QC problems, cruises completed, 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

Standard measurements for the evaluation of meeting objectives of precision, accuracy and completeness are conducted prior to data entry. These standards are in accord to the objectives established for the project (Table 1). Simple computations will determine if the information on identification of species and concentrations recorded on the raw data sheets evaluated will meet these standards. These data will be available for the evaluation of meeting these requirements.

A set procedure is established to review all data entry. This includes field sampling labeling, the transfer of label information to vials, and to 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.

The PI examines the product of the data analysis and based on his subjective evaluation may call for a re-examination of any part of the data set.

## XIV. CORRECTIVE ACTION

The principal investigator and/or the laboratory supervisor are responsible for evaluating all 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.

1. All work by laboratory personnel will be routinely checked by the PI or the Laboratory Supervisor for identification and total counts. A minimum of 10% of all samples analyzed will be compared for accuracy. If there are inconsistencies, the sample will be re-analyzed.

2. In the productivity measurements of replicate samples, differences greater than 20% among these replicate will result in the measurement of additional samples to be re-checked for accuracy.

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.

In the event occasions occur where corrective action is called for the PI will initiate a specific plan of action to follow to resolve the problem.

## XV. QUALITY ASSURANCE REPORTING TO MANAGEMENT

The principal investigator will be responsible for preparing all reports associated with this project. The PI evaluates quarterly the results of the data analysis for the period. If there are QA problems, the PI is responsible for their correction.

The principal investigator will prepare quarterly/final progress reports available to all management representatives, plus others identified by the grantor that will specifically include data results for that period, and note; 1) progress, 2) problems, 3) results of audits, 4) QA, 5) changes in QA, or 6) major phenomena that took place among the phytoplankters. The PI will submit the reports in accordance to the time specified in the contract.

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**APPENDIX:**

Table 1. Objectives for data quality in sample analysis.

| PARAMETERS | PRECISION | ACCURACY | COMPLETENESS |
| --- | --- | --- | --- |

| | | | |
|---|---|---|---|
| Phytoplankton | <20% | 80-120% | 90% |
| Picoplankton | <20% | 80-120% | 90% |
| Productivity | <20% | 80-120% | 90% |

**ORGANIZATIONAL PLAN**

Harold Marshall
PI

Field Collections
Field Supervisor

Productivity Measurements
K. Nesius, Co-PI

Lab Operations
Lab Supervisor

Data Entry
Graduate Assistants

Figure 2. Organizational Plan

**COLLECTION OF PLANKTON SAMPLES**

Phytoplankton
Replicate carboy series from upper and lower depth strata

Picoplankton
Replicate carboy series from upper and lower depth strata

500 ml samples from each series | 125 ml samples from each series

Samples fixed with Lugol=s solution
Preserved with formalin

Samples fixed with glutaraldehyde
Stored under refrigeration

Samples returned in laboratory
For microscopic analysis

Samples returned to laboratory for
epifluorescence microscopy

Figure 3. Field collections procedures to transfer to laboratory.

**PHYTOPLANKTON LABORATORY PROCEDURES**

1. Samples logged in

2. Formalin preservative added

3. Two 500 ml replicate samples from upper (and lower) water column strata are mixe then one 500 ml sample is obtained off for microscopic analysis

4. A series of settling and siphoning steps are followed to produce a 40 ml concentrate

5. Transfer concentrate to analysis chamber

6. Microscopic analysis takes place

7. Results entered on raw data sheets, with data sheets reviewed for errors

8. QA/QC checks on microscopic analysis

9. Computer data entry

10. Data entry screened for outliers, missing values, duplicate entries, etc.

Figure 4. Phytoplankton sample analysis procedures and data management.

**PHYTOPLANKTON DATA SHEET**

**CRUISE ____________** **500 CONSTANT __________** **VOLUME SAMPLED ___________**
**STATION ___________** **312 CONSTANT __________** **CONCENTRATION ___________**
**DATE ______________** **EPI _____________________** **INVESTIGATOR ______________**

| SPECIES | SPECIES CODE | TOTAL COUNT |
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Figure 8. Raw data sheet for Phytoplankton.

PHYTOPLANKTON QA/QC

CRUISE ____________________ VOLUME SAMPLED ____________________

STATION ____________________ CONCENTRATE ____________________

DATE ____________________

Magnification ______________ Fields _____________

Raw Count

Taxa Investigator Checker

Investigator __________ Checker_________

Number of taxa_________

Counts _________ _________

Approved ________ Not Approved ________ Date __________

Figure 9. Phytoplankton QA/QC data sheet used to record chec

**AUTOTROPHIC PICOPLANKTON DATA SHEET**

Month/Year _______________ Sample Volume (ml) ________________

Station ___________________ Sample Concentrate (ml) ____________

Date of Collection _______________ Investigator __________________

Constant ___________________

I. Upper strata sample Sample Replicate Sample

| | Sample | Replicate Sample |
|---|---|---|
| Total Cells | ____________ | _______________ |
| Total Fields | ____________ | ________________ |
| Mean Values | ____________ | ________________ |

Combined mean value _______________ Mean Abundance ___________________

II. Lower strata sample Sample Replicate Sample

| | Sample | Replicate Sample |
|---|---|---|
| Total Cells | ____________ | _______________ |
| Total Fields | ____________ | ________________ |
| Mean Values | ____________ | ________________ |

Combined mean value _______________ Mean Abundance ___________________

Notes: _________________________________________________________________

_________________________________________________________________

Figure 10. Autotrophic picoplankton work-up sheet.

**AUTOTROPHIC PICOPLANKTON QA/QC DATA SHEET**

Sample Data:

Month/Year ______________ Sample Volume (ml) _______________

Station __________________ Sample Concentrate (ml) ___________

Date of Collection _______________ Investigator __________________

Constant ________________

I. Upper strata sample _______
Original sample analyzed _____

II. Lower strata sample ___________
Replicate sample analyzed ______

| | Original Results | QA/QC Results |
|---|---|---|
| Total Cells | ____________ | _______________ |
| Total Fields | ____________ | ________________ |
| Mean Value | ____________ | ________________ |

Approved: Yes _________ No ___________

If response is No, results of second analysis by checker:

Total Cells _____________ Total Fields __________ Mean Results __________

Results: ______________________________________________________________

Status: Original Analysis Approved: Yes _________ No _______________

Figure 11. Autotrophic picoplankton QA/QC data shee