STATE OF MARYLAND

Quality Assurance/Quality Control Work Plan

MONITORING PROGRAM

PHYTOPLANKTON MONITORING PROGRAM

DEPARTMENT OF NATURAL RESOURCES

RESOURCE ASSESSMENT ADMINISTRATION

MONITORING AND NON-TIDAL ASSESSMENT DIVISION

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Project Officer
and
Quality Assurance Officer

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WORK QUALITY ASSURANCE PLAN FOR
PHYTOPLANKTON MONITORING PROGRAM

1) Project Name: Basic Water Quality Monitoring for Phytoplankton.
2) Project Requested By: EPA-Region III
3) Date of Request: July, 1978
4) Date of Project Initiation: January 1979
5) Project Officer: Walter L. Butler
6) Quality Assurance Officer: Walter L. Butler
7) Project Description:

A. Objective and Scope Statement

This project is designed to monitor the aquatic environment by sampling the community of phytoplankton to characterize the community and identify dominant species in the Maryland portion of the Chesapeake Bay Basin. It allows for the tracking of algae blooms in terms of temporal and spatial distribution which in turn provides some predictability. It allows for the identification of possible toxic algal forms which may impact molluscan shell fisheries such as Diarrhetic Shellfish Poisoning(DSP) and Paralytic Shellfish Poisoning(PSP) and it also allows for detection of Pfiesteria-like cells. The project also reflects existing nutrient levels, since overloading of nutrients generally results in dense algae blooms which affect both light penetration and oxygen levels in the water column. Phytoplankton samples are collected at 48 stations on a monthly basis January through December. A total of 576 samples per year are collected through this program.

B. Data Usage

The data collected in this study will be utilized to determine long-term trends in general water quality as reflected by the nature and diversity of the phytoplankton community. It will also be used in the short-term to predict or describe existing bloom conditions or the presence of nuisance algal blooms or possible toxic forms.

C. Monitoring Network Design and Rationale

1. Phytoplankton Monitoring(General)

The sampling station network for phytoplankton monitoring utilizes the basic water monitoring program stations also known as "CORE" stations which were selected in 1976. There are eight "CORE" stations including three in the bay mainstem, two in the Potomac, and one each in the Choptank, Chester, and Patapsco rivers. In addition, eight other stations on the Potomac and four stations on the Patuxent River are sampled for phytoplankton. The Potomac stations are part of an earlier EPA sampling network(1977) which were picked up by the State(MDE and predecessors)eventually becoming part of the Potomac Regional Monitoring Program(COG) and finally part of the Bay tributary sampling network. The four Patuxent River phytoplankton sampling stations are part of the Bay tributary sampling network where sampling was initiated in March 1983.

The overall design of the monitoring network selected was established with the aid of recommended station siting criteria(EPA 440/9-76-025) which included paired

configuration, i.e., upstream and downstream of representative land use areas such as municipal/industrial and agricultural/rural and potential areas of development. Selected stations include both problem areas and clean water areas of concern. Stations were sited when possible where historical data was available. Many single stations were located in small and homogenous sub-basins which include surface water intakes and recreational areas. Stations were cited within the bay as well as within major rivers and significant tributaries of the state's drainage areas. In 1998 Pfiesteria probable tributaries were sampled for phytoplankton. Eight tributaries were identified for sampling based on nutrient loading. The tributaries were Pocomoke River, Big Annemessex, Manokin River, Chicamicomico River, Nanticoke River, Wicomico River, Trappe Creek, and St. Martin's River. In 2000 Middle River was added. This project increased yearly samples collected by 266 for a total of 691 phytoplankton samples. In 2001 Coastal Bays phytoplankton sampling was added to support Harmful algal problems in that area. This project is yearly sampling and adds an additional 72 samples for a total of 763 samples per year. In 2001 this lab started receiving Rapid Response samples that added 90 samples for a total of 853 plus QA/QC adds an additional 85 samples making the total 938 for the year. In 2002 the laboratory received 1,035 phytoplankton samples for identification. Most of the additional samples were from bloom reports associated with blue-greens(Microcystis) and Dinoflagellates(Dinophysis). In 2003 the laboratory received 1,166 samples for identification. The additional samples were from blooms of blue-greens, dinoflagellates and a contract with MES for samples from Poplar and Hart & Miller Island. In 2005 and 2006 the laboratory received 1,250 and 1,244 samples respectively. In 2007 and 2008 the lab received 1,200 and 1,112 samples respectively. In 2009 and 2010 the lab received 1,189 and 1,064 samples respectively.

D. Monitoring Parameters and Frequency of Collection

Phytoplankton samples are collected on a monthly basis from January through December once a month. Grab samples are taken at the surface except at the Cedar Point and Sandy Point stations where surface,mid-depth and bottom samples are taken(mid-depth,bottom by pump), and at the Morgantown station on the Potomac River where sampling is done at the surface and at 30.0 feet. Samples are not preserved.

E. Parameter Table

1) Identification to lowest possible taxa(usually genus or species)
Abundance(cell densities)
Percent composition by major groups(Diatoms, Greens, Pigmented flagellates, Blue greens)
Sketch and measurements of unidentifiable forms.

2)

Parameters	No. of samples	Sample Matrix	Analytical Method Reference	Sample Preservation	Holding Time
Grab sample	1,244	Phytoplankton	EPA 670-73-	None	1-3 days

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8) Project Fiscal Information

A. Analytical (processing, identification and counting organisms) and data management	$64,000
B. Supplies	100
C. Millage - piggybacked on Bay & Bay tributary sampling	0
D. Salaries - covered under (A)	0
E. Major Equipment Items Utilized:	
Microscope - Compound-Phrase contrast inverted	32,000
Sedgewick Rafter plankton counting cell	200
Stage Micrometer	100
Total	$96,400

9) Schedule of Task and Products

	O	N	D	J	F	M	A	M	J	J	A	S
a. Project Request	-											
b. Project Plan Review	-											
c. Project Plan Finalized	-											
d. Field Reconnaisance- Not applicable												
e. Sample collection	----------											
f. Lab Analysis Completed & submitted to Project Officer	----------											
g. Data Entry into Computer	----------											
h. Interim Project Report	----------											
i. Final Project Report	-											

10) Project Organization and Responsibility

The following are a list of key project personnel and their corresponding responsibilities:

Walter Butler	Sampling operations
Walter Butler	Sampling QC
Walter Butler	Laboratory analysis
Walter Butler	Laboratory QC
Walter Butler	Data processing activities
Walter Butler	Data processing QC
Walter Butler	Data Quality review
Walter Butler	Performance auditing
Walter Butler	Overall QA
Walter Butler	Overall project coordination

John Griffin

Chesapeake Bay and Watershed Program
Frank Dawson

Resource Assessment Services
Bruce Michael

Monitoring and Non-Tidal Assessment Division
Ron Klauda

Monitoring Program
Sally Bowen

Phytoplankton Monitoring
Walter Butler

11). Data Quality Requirements and Assessments

Data Limits and Quality Assurance Objectives

Parameter Sample

Phytoplankton grab sample - 1 liter approximately three quarters filled from which 1 milliliter is pipetted after thorough mixing (45seconds) into a Sedgewick-Rafter Plankton Counting cell which has been calibrated. The counting chamber is allowed to settle for 15 minutes before counting and ID is started. A one-strip count is made which represents 130 one-fourth square milliliter fields and the total strip count is then multiplied by a derived enumeration factor(53.0) representing the portion of the S-R cell counted to determine the number of phytoplankton cells per milliliter. After the initial strip count is made the entire perimeter of the cell is scanned along with two diagonal cell scans representing an additional 521 one-fourth square milliliter fields to examine for any additional plankters not encountered in the initial strip count. These are documented as being present (P) and represented by 1.0 in database.

The number of plankton in the S-R cell are derived from the following:

$$\text{No./ml} = \frac{C \times 1000\ mm3}{L \times D \times W \times S}$$

Where:

C= number of organisms counted
L= length of each strip(S-R cell length),mm,
D= depth of a strip(S-R cell depth),mm,
W= width of a strip(Whipple grid image width),mm,
S= number of strips counted

If cell densities are high less fields may be counted and appropriate multiplication correction factors applied. Five percent of the samples are re-identified for Quality Assurances and Quality Control and is computerized along with the regular data. This data is identified by adding QAQC to the station code. Bloom samples and samples containing harmful algal specie will be archived in Lugol♣s preservative.

Detection Limit - 0 organisms

Accuracy - not calculated

Precision - Utilizing Standard Methods for the Examination of Water and Wastewater(latest edition) assuming the distribution of organisms in the counting cell is random the counting error may be estimated. The approximate 95% confidence limits, as a percentage of the number of units counted (N) equals:

$$\frac{2}{N}(100\%)$$

Generally 100 or more units are counted whereby the 95% confidence limits would approximate +20% of the mean. Work by Joseph H. Kutkuhn(Limn.&Ocy,V3, No.1, pp 9-83, 1958) on the precision of numerical plankton estimates utilizing a Sedgewick-Rafter plankton counting cell examined the validity of estimates from field counts in a single Sedgewick-Rafter cell mount. The investigation dealt with the number of fields(one-fourth square milliliter) in any given cell mount required to attain a specified precision. Twenty fields as a sub-sample size was found to yield individual cell estimates in error by about 10 percent on the average. Utilizing 100 fields as a sub-sample size reduced the error to about 4 percent. Although estimates from single cell mounts were shown to be fairly reliable the investigators results showed large amounts of variation resulting from differences between counts in successive cell mounts which often tended to nullify the most precise estimates from individual cell mounts. To increase precision a subsampling technique was recommended using 10 field counts in each of four cell mounts resulting in precision to within 10-12 percent of the mean at the 95% confidence probability level. Due to the time and personnel restraints the one strip count is utilized.

Alternate Counting Procedure

This procedure is used when counting single specie with low numbers or when a definite count and efficiency is warranted. The phytoplankton grab sample is a 1 liter container approximately three quarters filled fixed with 2.0 milliliters of Lugol♣s from which 1.0 milliliter is pipetted after thorough mixing. This 1.0 ml. sample is pipette into a Sedgewick-Rafter plankton counting cell. The total cell is counted using 100 power. Starting at one corner and moving across the cell until you reach the opposite corner. Then using a fixed point in the upper or lower field(Whipple Grid, overall area), move up or down to that point and continue to move across in the opposite direction. Keep doing this until you scan the total cell. Results can be expressed as number per milliliter. Bloom samples are also fixed with Lugol♣s but only after live identification of the sample and bloom specie has been identified

In October of 2005 a new microscope was received. The scope is a Zeiss Axiovert 200 that is inverted. This microscope will allow for higher magnification during routine counting and ID. The same basic procedure will be used with this scope except the Sedgewick-Rafter cell will be settled upside down to allow the cells to settle on the coverslip. The routine magnification will be 640X as opposed to 200X with the older scope. The conversion factor to convert to cells per milliliter will be change from 53 to 127. The data up to 2006 was identified with the older scope. Starting in 2006 the new microscope will be used for routine ID and counting. The long-term dataset will have a new field that will indicate which microscope was used. The field is SCOPE. The field is coded so 1 represents the old scope and 2 represents the new scope.
To determine what the difference is from switching from old microscope to new would be. Random samples were identified with both microscopes to determine what the differences were.

These samples were identified by placing a “D” at the end of the station code. To determine the differences between live and preserved identification would be. Random samples were chosen to be identified both live and preserved with Lugol’s. To determine what samples were identified this way a “P” was added to the station code.

Sampling Procedure

Reference - Biological Methods Manual - DNR-RAS-MANTA.
Field and Laboratory Methods(EPA-670/4-73-100, July 1973)
Standard Methods For The Examination of Water and Wastewater, 17th Edition APHA-AWWA-WPCF, 1989.

13) Sampling Custody Procedure

Not applicable

14) Calibration Procedures and Preventative Maintenance

Calibration sheets for Microscopes and plankton counting cells.
Periodic Microscope servicing

15) Documentation

Reference - Laboratory Notebook
Laboratory Bench Sheets (filed by month by river basin)

16) Data Reduction and Reporting

Data collected manually is entered on specialized bench sheets that fully describe the orgin and nature of the sample. Entries are maintained in a file. Step-wise procedures for computer entry have been completed with procedures for routine data transfer.

17) Data Validation

The validation of data is the prime responsibility of utilizing methods documented in the Procedure Manuals referenced in Section 15.B. Final validation is the responsibility of the Quality Assurance Officer.

18) Performance and Systems Audits

The Agency will be participating in external performance evaluation studies as they become available. In-house reference specimen collections including microphotographs have been developed and are used along with the use of outside taxonomic expertise to confirm or provide identifications for problem organisms.

19) Corrective Action

The corrective action mechanism is defined in the Procedure Manuals cited in Section 12 and 15.

20) Reports

Reports utilizing the phytoplankton data include Maryland Water Quality Reports (305b) for 1985, 1987 and 1989. The phytoplankton data is also analyzed and used in terms of the priority of the need for specific problem areas or for specific types of phytoplankton organisms which might be deemed harmful or bothersome including heavy bloom phenomena which can affect dissolved oxygen levels and the pH of the water column. Phytoplankton trend data is also used to predict bloom arrivals, and to aid in preparation of

educational documents or media releases. It is also provided at request both inter-and intra departmentally within the state and to other state and Federal agencies as well as private consultants.

Phytoplankton data is currently being entered in the computer and made available for Bay program use and to allow phytoplankton trend reports to be produced on a more predictable and prompt schedule including utilization of quality assurance applications. The data-set is updated each year and is maintained as a SAS data-set at the field office and backed up on Mantadc server within the SAS folder. In 2000 a variable was added to the dataset to accommodate cell count. That variable was called CELL_CNT. Prior to 2000 cell count was not recorded. In 2006 several new variables were added to the dataset. They were Scope, NEWSTATION and LAYER. Scope defines which microscope was used for counting and identification. Number 1 in that variable represents the older microscope and 2 represents the new microscope. NEWSTATION variable is a station code used by the Chesapeake Bay program for station location. The LAYER variable defines the depth at which the sample was collected. Surface is S, mid-depth is M and bottom is B. The following variables make up the long term dataset as of 2007;

AGENCY DATE STATION DEPTH TIME NODC COUNT YEAR MONTH CELL_CNT COMM1 COMM2 ID Scope NEWSTATION LAYER.