ATTACHMENT H

WORK/QUALITY ASSURANCE PROJECT PLAN FOR MONITORING MESOZOOPLANKTON AND MICROZOOPLANKTON IN THE LOWER CHESAPEAKE BAY AND TRIBUTARIES

Prepared by

Kent E. Carpenter, Ph.D
Department of Biological Sciences
Old Dominion University
Norfolk, Virginia 23529-0266

RFP No.: 96-004-BS
For the Period: July 1, 2002 through June 30, 2003

Prepared for
Commonwealth of Virginia
Department of Environmental Quality
PO Box 10009
Richmond, VA 23240-0009

Document Control No.: 842
Revision 5
May 30, 2002
Approvals:

Dr. K. Carpenter, Principal Investigator, ODU BIOL	Date
Mr. Forrest Crock, Quality Assurance Officer, ODU BIOL	Date
Mr. F.A. Hoffman, Project Officer, VA DEQ	Date
Quality Assurance Officer, VA DEQ	Date
Quality Assurance Officer, EPA CBPO	Date

TABLE OF CONTENTS

Figures:

Data Sheets:

Distribution List for WQAPP:	No. Copies
Dr. K. Carpenter, Principal Investigator, ODU BIOL	1
Mr. F.Crock, QAO, ODU AMRL	1
Mr. F.A. Hoffman, Project Officer, VA DEQ	1
Quality Assurance Officer, VA DEQ	1
Quality Assurance Officer, EPA CBPO	1

I. PROJECT DESCRIPTION

This project is responsible for monitoring the composition and abundance of mesozooplankton and microzooplankton at specified sites in Chesapeake Bay and four tributaries. Emphasis is placed on the correct and consistent identification of zooplankton within these waters. To achieve these goals and the consistency required, the Old Dominion University Zooplankton Laboratory will provide the following resources: 1) Proven expertise in the identification of both mesozooplankton and microzooplankton components, with 10 years of past experience of Bay zooplankton monitoring, 2) a reference collection of Bay species to aid in confirmation of species identification, 3) a fully equipped laboratory and several zooplankton taxonomic experts capable of analyzing large numbers of samples per year, and 4) the necessary field gear and boats for the collections.

A. OBJECTIVES AND SCOPE OF THE PROJECT

The primary objectives of the zooplankton monitoring program are:

1. Characterization of the composition and abundance of the mesozooplankton and microzooplankton at specified stations in Chesapeake Bay and four tributaries.

2. Assure continuity in the identification of zooplankton populations within the Chesapeake Bay monitoring program and that these identifications be comparable to those used over the past decade in this program to assure compatibility in the data sets.

3. Provide the continuation of comparable data to that already obtained in the Bay Monitoring Program to be included in a long term data base which will allow for future trend analyses relating spatio-temporal patterns in the plankton to changes in Bay water quality conditions and health status of the Bay.

4. To collect data for the evaluation of trophic interactions.

5. To collect data for baywide indicators.

B. COORDINATION ACTIVITIES WITHIN THE CBP

The rationale for this monitoring sampling program is that the health of the bay ecosystem can best be assessed by measuring a variety of biotic and abiotic variables. While abiotic variables provide a necessary means to detect and evaluate sources

of pollution and assist in the evaluation of control or abatement measures, the ultimate evaluation of the health of any ecosystem must emphasize the living resources. The zooplankton community sampled by the Chesapeake Bay Program is a central component of the estuarine ecosystem. Zooplankton form an essential link in the food web and provide the bulk of the forage prey for most larval and juvenile fishes as well as many other estuarine organisms. A primary objective of the first five years of monitoring has been to establish a baseline of spatial and seasonal values and patterns for the zooplankton community. It is the variations from the normal patterns that provide information through which the health of the ecosystem may be evaluated. This information, plus the relationships to water quality and other Bay living resources will allow a more complete understanding of the dynamic systems and factors operating in Chesapeake Bay.

C. STUDY DESIGN

1. Project Dates

The time period for this study is from January 1, 2002 through December 31, 2002. Field collections for mesozooplankton and microzooplankton will be this same time period. Quarterly progress reports will be delivered in accordance to the dates stipulated in the contract (See Section V.C.4. of the RFP).

2. Relationship to Background Information of this Project

The continuation of this project within this laboratory assures consistency in sample collection and processing, and the high levels of accuracy necessary in the identification of the zooplankton populations. Without this type of continuity, long term trend studies of these populations would be invalid.

3. Data Uses

The sampling and analysis procedures outlined in this proposal provide the essential data to meet the projects objectives. They also represent a continuation of previous methodology that assures consistency in plankton identifications that will be compatible with previous work for subsequent analytical interpretation and application.

4. Sampling Network Design Rationale

Stations selected for this study represent those where phytoplankton, productivity and water quality data are being sampled concurrently with the zooplankton samples. Justification

for this design is based on long term monitoring plans concerned with interpreting the health status of Chesapeake Bay and to evaluate long term trends of the Bay's living resources and health status. These station sites have been pre-selected and have been part of the previous monitoring program to date. Figure 1 provides the location of these stations. Refer to Section IV on Sampling Procedures for specific details on Sampling Design, etc.

5. Sampling Locations

Seven stations are located in the lower Chesapeake Bay and seven stations are in the tributaries (Figure 1). These are listed below:

Station No.	Description	Latitude	Longitude
Tributary			
TF 5.5	James R., Red Buoy 107	37 18 46	77 13 59
RET 5.2	James R., Swann's Pt.	37 12 36	76 47 36
TF 4.2	Pamunkey R., at White House	37 34 47	77 01 19
RET 4.3	York R., Buoy C57	37 20 24	76 47 18
TF 3.3	Rappahannock R., Buoy N 40	38 01 07	76 54 30
RET 3.1	Rappahannock R., N Buoy R10	37 55 12	76 49 42
SBE 5	S. Branch Eliz. R., Va. Pwr.	36 45 26	76 17 32
Mainstem			
CB 7.4	Bay Mouth, Baltimore Channel	36 59 36	76 00 38
CB 7.3E	Eastern Shore Channel	37 13 43	76 03 15
CB 6.4	Central Bay Area	37 14 11	76 12 30
CB 6.1	Main Channel, S. End	37 35 18	76 09 45
LE 5.5	James River Mouth	36 59 48	76 18 12
WE 4.2	York River Mouth	37 14 30	76 23 12
LE 3.6	Rappahannock River Mouth	37 35 48	76 17 06

6. Parameters To Be Measured

The following parameters will be measured:

Mesozooplankton species composition
Mesozooplankton species abundance
Microzooplankton species composition
Microzooplankton species abundance

Identical protocols will be followed as those used in previous years in the Virginia program for the identification and enumeration of the zooplankton components. Special attention is

also directed to the microzooplankton methodology, to assure the smallest components within this group (<40 microns), are included in the process.

7. Frequency Of Collections

Each of the above listed stations sites will be visited monthly for mesozooplankton and microzooplankton collections. In addition, a second mesozooplankton sampling event will occur at stations TF3.3, TF4.2, and TF5.5 during April and May. Details on field and laboratory measurements are given in Sections IV and VII on Sampling and Analytical Procedures.

8. Types Of Samples

The mesozooplankton samples will come from a paired set of bongo nets, taken with a vertical tow at each station. The microzooplankton samples are whole water samples. These are taken from composite water samples taken between the pycnocline and surface. See Section IV on Sampling Procedures.

II. PROJECT ORGANIZATION AND RESPONSIBILITY

The processing and analysis of all samples, plus data computer entry, will be completed in the ODU Zooplankton Laboratory. See Appendix, Fig. 2, for the organizational plan for the zooplankton component of this project.

A. PRINCIPAL INVESTIGATOR

The principal investigator is Dr. Kent Carpenter. Dr. Carpenter is responsible for the technical operation and data interpretation, and management aspects of the zooplankton component. This includes supervision of and supervision of personnel responsible for: 1) zooplankton laboratory personnel, 2) field (boat) collections, 3) operation of the vehicle towing the boat and/or gear, 4) zooplankton sample collections, and 5) the processing the zooplankton samples and data entry. Dr. Carpenter has over 25 years of experience in field and laboratory operations, laboratory analysis, and project management activities, including a minimum of 4 years of direct zooplankton laboratory experience.

B. QUALITY ASSURANCE OFFICER (QAO)

The QAO is Mr. Forrest Crock (B.S., M.S Candidate), who has over 4 years of experience in zooplankton research, including field operations, laboratory processing of samples, analysis of samples, identification of zooplankton, and data handling. He will meet periodically with the principal investigator to discuss: 1) the operation, sampling and analysis procedures, 2) data entry, 3) the timeliness and availability of the monthly data product, and 4) any problems that may arise that would delay any phase of the project. He is responsible for approving the QA/QC protocol used in this project and advises on procedures, in addition to logistics, or other related concerns that may effect the sampling, or data analysis. He is also responsible for QA/QC checks in the laboratory. Office phone: 757-683-5712.

C. ZOOPLANKTON FIELD/LABORATORY SUPERVISOR

The field/laboratory supervisor position is now held be Mr. Forrest Crock (B.S, M.S. candidate), who is a marine zoologist and a Zooplankton Expert in taxonomy. He is responsible for supervision of field operations for sample collection, the preparation of the collected samples for analysis, analysis of the samples, raw data work up, laboratory operations, and data entry. He has 4 years experience in the Chesapeake Bay Monitoring program as a zooplankton expert.

D. ZOOPLANKTON LABORATORY EXPERTS

The following are trained zooplankton laboratory experts in the Old Dominion University laboratory:

1. Ms. Karen Kowalski (B.S, M.S. candidate). Ms. Kowalski has 2 years experience in the Bay program as a zooplankton expert. Her specialty is microzooplankton.

2. Mr. George Mateja (B.S., M.S., Ph.D. candidate) has over 12 years experience in the Bay program as a zooplankton expert.

3. Mr. Forrest Crock (B.S., M.S. candidate) has 4 years experience as a zooplankton expert.

4. Mr Andy Mahon (B.s, MS, MS (second degree) Ph.D canidate) who

has over 1 year experience as a zooplankton expert.

E. ZOOPLANKTON GRADUATE ASSISTANTS

It is the policy in the zooplankton laboratory to have a continuing program of training in zooplankton systematics, with the emphasis on Chesapeake Bay fauna. Mr Mahon has been in this program for a year, has completed his year of zooplankton training and is now a zooplankton expert. In addition, he already has extensive experience in boat operation, sample collection, and data entry. Because of his advanced degree credentials and experience in data handling, he is designated as the data entry expert for the lab.

F. SUB-CONTRACTS

No sub-contracts are included in this project. The use of sub-contractors for analysis is not practical with the high calibre of expertise on Bay zooplankton systematics that is present in this laboratory. This is a demanding area of study, where a broad knowledge of marine, estuarine and freshwater species will be in the samples. This laboratory also has a 11-year history on being capable of collecting and analyzing large quantities of samples monthly and yearly. This laboratory will assure a continuity in year to year identifications from previous to future years of sampling.

G. ADDITIONAL RESPONSIBILITIES

Each step of the laboratory analysis will be routinely reviewed by the QAO and the principal investigator. 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 QAO and the PI on station. Routine species checks will also be made of the species identified in the laboratory by the QAO.

III. QUALITY ASSURANCE OBJECTIVES FOR MEASUREMENT DATA

A. OBJECTIVES AND DATA USAGE

The mesozooplankton component consists of both holoplankton (e.g., copepods, cladocerans, chaetognaths) and meroplankton

subgroups (e.g, fish eggs and larvae, shrimp and crab zoea, barnacle nauplii, polychaete trochophores). The microzooplankton is dominated by a variety of ciliated protozoans, numerous larval stages, and other components. The responsibilities of the zooplankton laboratory personnel are to correctly identify the species (groups) and to accurately determine their densities. These duties require a lengthy training process due to the broad range of taxonomic information involved in the zooplankton identification. This specialized training has been ongoing at the ODU zooplankton laboratory for the past ten years and has involved the training of new graduate students by senior project personnel.

Reference collections and photographs have been made of the major zooplankton species to aid in maintaining accuracy and consistency. The coefficient of variation stabilizing (CVS) method (Alden et al., 1982) has been developed as a QC precaution so that the subsampling error can be controlled. The determination of water volume sampled between replicates help in improving representativeness and accuracy of the zooplankton populations collected. A high level of comparability and precision results from the QC procedures involved in the laboratory analysis and from supervision by the PI and QAO of those procedures during analysis. These Quality Assurance objectives are given in Tables 1, 2, and 3.

Precision is defined as a measure of mutual agreement among individual measurements of the same property, usually under prescribed similar conditions." (QAMS 004/80, U.S. USEPA, 1980). In zooplankton identification per subsample of a sieve size fraction this is given as:

(# of incorrect identifications/total # of species)x100

For zooplankton enumeration per subsample of a sieve size fraction this is given as

(|N-n|/N)X 100

where N is the number counted by the QA/QC officer and n is the number counted by the enumerator being tested.

Accuracy is defined as "the degree of agreement of a measurement (or an average of measurements of the same thing), X, with an accepted reference or true value, T, usually expressed as the difference between the two values, X-T, or the difference as a percentage of the reference or true value, 100(X-T)/T, and sometimes expressed as a ratio, X/T. Accuracy is a measure of the bias in a system." (QAMS 004/80, U.S. USEPA, 1980). In zooplankton analysis the true value T is set by a sample first counted by the QA/QC officer and this is sample is then read by an

enumerator being tested. For species identification, the list of species is set by the QA/QC officer.

The standard Quality Control (QC) for laboratory processing of zooplankton samples consists of complete recounting at least 10% of the samples. Recounts are selected at random and processed by the Quality Assurance Officer using the original dilution volume. After recounting, the relative abundance (#s/m^3) is calculated for the sample using the same formula employed in calculating the relative abundance of the original sample. Then the percent error is calculated using the following formula:

$$\% \text{ Error} = \frac{\text{original RA - QC RA}}{\text{original RA}} \times 100$$

where RA = relative abundance (#s/m^3).

The sample passes if the error is 10% or less.

Next, the original list of species is compared with the QC list to ascertain whether the same species were found in the recount and that the counts within species are similar. New species found in QC are added to original data sheet. If gross differences exist, the sample is re-examined to determine if the species identifications are correct, and the PI notified. The QAO and PI will determine if a problem with taxonomy exists and will schedule retraining of the original technician if necessary.

B. Potential Contamination

It is routine practice to properly rinse all nets and storage containers prior to use. All glassware is cleaned according to standard laboratory procedures. A strict chain of custody from time of collection to sample analysis is maintained to prevent contamination, with records of this transfer made and kept on file.

Table 1. Precision , Accuracy, and Completeness Objectives for Zooplankton Sampling

Parameter	Reference	Condition	Precision	Accuracy	Completion

Sampling	IV.C	sample	<20%	80-120%	90%

Table 2. Precision , Accuracy and Completeness MDL Objectives for Field Measurements

Parameter	Reference	Precision	Accuracy	Compl.	MDL
pH	IV.C	<20%	80-120	90%	0.01 pH
D.O	IV.C	<20%	80-120	90%	0.02 mg/L
Secchi	IV.C	<20%	80-120	90%	0.1 M
Cond.	IV.C	<20%	80-120	90%	1 umho/cm
Salinity	IV.C	<20%	80-120	90%	0.1 ppt
Light Attenuation	IV.C	<20%	80-120	90%	0.05 @ 100% light
Water Temperature	IV.C	<20%	80-120	90%	0.1 C
Depth	IV.C	<20%	80-120	90%	0.02 M

Table 3. Precision, Accuracy and Completeness Objectives for Zooplankton Analysis

Parameter	Reference	Condition	Precision	Accuracy	Completion
Zooplankton Identification	Birdsong, et al., 1983	subsample	< 10%	< 10%	> 90%
Enumeration	Alden, et al., 1982	subsample	< 5%	< 5%	> 95%

IV. SAMPLING PROCEDURES

A. ORGANIZATIONAL PLAN

All project activities are based on established protocols for field and laboratory activities. These represent specific and detailed guidance directions established by the PI and QAO, who meets with the personnel monthly to go over the next months assignments and provides the mechanism for planning and interaction with the staff regarding all aspects of the project. Past protocol and these specific assignments provide for consistent comparability and compatibility, and points for reference, for all tasks associated with field sampling and laboratory analysis. Organization plan given in Appendix, Fig. 2.

B. PROJECT OBJECTIVES AND BACKGROUND

To obtain representative water samples for mesozooplankton and microzooplankton, and to provide accurate identification and abundance data for components of these categories. Background information and detailed objectives statements are given in Section I. Project Description.

C. ANALYSIS OF EXISTING DATA

The PI has analyzed data and reported results from collections of zooplankton in the Chesapeake Bay Monitoring Program since 1996. He is thoroughly familiar with the appropriate protocols used in the program. Data analysis and incorporation into required reports, etc., would continue without interruption and be consistent to previous submissions.

D. ANALYSIS OF INTEREST

Numerous components of this program have distinct ecological importance to understanding the trophic relationships and health status of Chesapeake Bay. These relationships are based on existing populations of zooplankton within the Bay system, and patterns of change that have been noted over time, and associated with specific environmental conditions, or specific regions in the Bay and its tributaries. Any transitional changes within this community would have impact on other plankton components and various economic fisheries in the Bay.

E. SPECIFIC ELEMENTS TO BE ADDRESSED

On field sampling days, the designated field supervisor is responsible for seeing that sampling at all stations is conducted properly and that all chain of custody procedures are followed. Two replicates will be collected at each site visited and the replicates will be combined for enumeration and identifications.

1. Definition

Mesozooplankton are characterized as those animal species within the plankton that are normally collected with a 202 micron mesh net, whereas, microzooplankton are those plankton species that are normally would pass through a 202 micron mesh net.

2. Mesozooplankton Sampling

Prior to each field sampling day, all equipment and supplies are checked for readiness according to the QC protocol. Exact site is determined using a combination of GPS or LORAN, depth recorder and landmarks such as buoys and shore structures. GPS or LORAN coordinates are recorded on the field data sheets prior to sampling for water quality data (relative position does not change appreciably during sampling). The order of tasks at each station is shown in Appendix, Fig 3.

Mesozooplankton sampling occurs concurrently with water quality sampling. For the mainstem and Elizabeth River stations this occurs at the same time and from the same boat as the water quality samples. At the tributary stations this is planned to occur within .5 hours when DEQ samples. During duplicate sampling dates in April, May, July, and August when DEQ does not sample, physical quality data are recorded as per section 4 below.

Samples will be collected with paired 202um mesh , 1/2 m diameter, 2 m long plankton nets (Earnest A. Case, P.O. Box 45, Andover, NJ 07821) each fitted with a calibrated flowmeter (General Oceanics model 2030) attached within the opening to provide an estimation of sampling effort. Flow meter readings are taken prior to setting the net and recorded in the field log. Nets are then towed in a double-oblique pattern from bottom to surface for approximately five minutes. After retrieval of the nets, any problem with the tow is noted and if warranted the tow is repeated after correcting the problem. Upon successful tow completion the final flow readings are recorded in the field log as well as the tow time. Nets are washed down and codends are decanted into prelabeled, one liter field bottles (Nalge Company,

P.O. Box 20375, Rochester, NY., spiked with 7% formaldehyde (Fisher Scientific, 711 Forbes Ave., Pittsburgh, PA.) Tributary collections are also stained with Rose Bengal (Sigma Chemical Company, P.O. Box 14508, St. Louis, MO) to facilitate future identification of planktonic organisms. The bottles are then placed in storage containers for transport to the laboratory. In the event that gelatinous zooplankton is visible in the nets, total volume is determined for the mesoglea after straining from the normal plankton sample. Care will be taken to ensure that no residual plankton remains clinging to either the strainer or to the mesoglea. Percent composition of gelatinous zooplankton groups (ctenophore, moon jelly, stinging nettle) is determined and recorded on the field log. Mesoglea is then discarded.

3. Microzooplankton Sampling

Microzooplankton categories that will be identified and counted are: copepod nauplii, barnacle nauplii, rotifers, tintinnids (loricated ciliates), oligotrichs (non-loricated ciliates, polychaete larvae, cladocerans, sarcodinids, and others. Microzooplankton samples are collected at the same lower Chesapeake Bay and tributary stations as the zooplankton.

Microzooplankton sampling procedures are same as with the phytoplankton collection and occurs at the same time as the mesozooplankton sampling. Basically, two 15 liter carboys are filled on station with pump, taken from a vertical series of 5 depths above the pycnocline at all stations. The carboys are thoroughly mixed when filled, and a one liter subsample is taken from each (see details on phytoplankton sampling procedures, WQAPP, IV, E-F). These two subsamples represent Bottle A and Bottle B.

Bottle A is immediately preserved with 10 ml of Lugol's solution which is already in the bottle and is stored in the storage box at normal temperature.

4. Physical Parameters

A subset of physical data measurements are the responsibility of the zooplankton laboratory during duplicate sampling dates when DEQ does not take water quality parameters in April, May, July, and August. Salinity, temperature, dissolved oxygen, pH and conductivity are measured using a CTD (Conductivity, Temperature, Depth (pressure)) instrument (YSI 600XL: YSI Corp., Yellow Springs, OH., 45387; Hydrolab Surveyor: Hydrolab Corporation, P.O. Box 50116, Austin, TX 78763, or a comparable instrument) designed for this purpose. This unit is calibrated prior to each cruise

(see addendum for calibration methodology). The CTD contains a sonde unit, 25 meters of cable, a display unit, and a data logger. Parameters to be measured using this unit include sea temperature, pH, dissolved oxygen, conductivity, salinity and depth. All data will be recorded both by recording them on the field data sheet and by logging them electronically for transfer into the laboratory computer at the end of the field day. Physical data are taken upon arrival at each site after zooplankton towing but prior to phytoplankton sampling. Zooplankton sampling is initiated upon arrival at station due to the need to utilize the washdown capabilities of the phytoplankton pump that is only functional when stationary. Parameters are recorded from surface to bottom at one meter intervals, for the first 15 meters, and every two meters thereafter, with the last reading taken one meter above the bottom.

Secchi depth is recorded using a 20 cm disk attached to a line marked off in 0.1 meter increments. Sampling is accomplished by slowly lowering the disk until it is no longer visible, and recording the depth using the line markings. The disk is then slowly raised until it just becomes visible, and this "up" reading is also recorded. The mean of the two is recorded as the secchi depth. If the two readings vary by more than 0.5 meters, the entire process will be performed again.

Tidal stage and weather codes are recorded in the field log/data sheet. The weather codes include air temperature, wind direction, wind speed, cloud cover, precipitation type and amount, as well as wave height. (see appendices for an example of the field data sheet and particulars on tidal stage and weather codes). After the field supervisor has determined that all data have been collected and recorded, and all procedures have been followed correctly, the sampling site is departed. The field supervisor is responsible for all data logs and samples until transferred to the custody of the laboratory supervisor of the phytoplankton lab.

F. FLOW CHARTS

Diagram flow charts regarding the field collection procedures are presented mesozooplankton in Figure 3. The microzooplankton pair of samples are collected from the surface sample composites during the phytoplankton collections.

G. SPLIT SAMPLE CHECKS

On a yearly basis, samples will be traded between the Maryland and ODU zooplankton teams to check for taxonomic and

abundance accuracy. The method of this routine split sample check will be determined on a yearly basis depending on budget and quality assurance priority set by discussions between the Virginia, Maryland, and EPA QA/QC officers.

V. SAMPLE CUSTODY

A. FIELD SAMPLING PROCEDURES

The zooplankton field chief is designated field sample custodian and is responsible for the proper collection, labeling and preservation of each sample in the field. The time of collection, exact location of the sampling site, preservative used (see sampling procedures, Section IV. E for source of preservatives and stains) and any problems with sampling are noted on the field sheet. The recording of all field data on the field data sheets/field log and the delivery of samples and field data are his responsibility as well. Examples of the labels and field data sheets are given in the Appendices.

B. LABORATORY PROCEDURES

The field supervisor relinquishes control of the samples and data sheets to the laboratory supervisor who is responsible for overseeing the processing and workup of the samples. This is facilitated by the use two chain of custody forms, one for the processing section of the lab and the other for the analytic section.

The laboratory manager, who acts as sample custodian for the laboratory, immediately places the field data sheets in a secure area to await review. the data logging unit is taken to the laboratory computer and the information is downloaded into the appropriate data file. After the information is received by the computer, the data logger is cleaned and checked for damage and then stored.

The zooplankton samples are then taken by the laboratory manager to a secure location, where they are logged in and await processing. Processing proceeds via a chain of custody whereby dates of splitting, sieving and recondensing, as well as the individual involved, are recorded. The names of individual technicians performing the identification and enumeration, as well as the current date and any problems with the workup of the sample are recorded on the split/count sheets provided for each sample.

C. FINAL EVIDENCE FILE

A permanent record of custody for each sample analyzed will be kept on file in the zooplankton laboratory. this will consist of the original raw data sheets which will contain the chain of custody and will be available for future reference.

D. PRESERVATIVES

Preservatives used in this project will be prepared by the Old Dominion University Support Facility from standard stock supplies provided by supply companies. All materials that are hazardous will have met the acceptable standards established by the University, and federal and state guidelines and will be routinely inspected by the University Health and Safety Officer, as will the laboratory. 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

Sample custody passes from the field supervisor to 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.

The laboratory supervisor is also responsible for reviewing the lab split/count sheets as well as the field data sheets. The originals of the field and lab data sheets are maintained in a secure area in the laboratory. Hardcopy (originals) of the field and laboratory documents will be maintained in a secure location within the plankton laboratory.

VI. CALIBRATION PROCEDURES AND FREQUENCY

A. FIELD SAMPLING

Calibration of field equipment consists of following the manufacturer's recommendations for the calibration of the CTD. Calibration of this unit occurs one day prior to the scheduled sampling date. All solutions used for standards are made up in the ODU Zooplankton laboratory using chemicals obtained from Fisher Scientific and at the Biological Sciences Support Facility located in the Department of Biological Sciences, ODU.

Calibration of the flowmeters is accomplished using a General

Oceanics Calibration Checker Model 2030-F (General Oceanics, Inc., 1295 163rd Street, Miami, Florida, 33169). Flowmeters are calibrated monthly and paired based upon similar calibration figures. If a flowmeter is found to deviate substantially (>10%) from the others, the unit is shipped to General Oceanics for repair or replacement.

B. LABORATORY OPERATIONS

Calibration in the laboratory involves the dissecting microscopes used for plankton identification and the analytical balance. Calibration of the dissecting scopes is accomplished by placing a stage micrometer on the stage of the microscope and comparing it to the incrementation on the ocular micrometer in the microscopes eyepiece. The true location of the 1:1 ratio reading is then marked on the adjusting knob of the scope. This permits more accurate identification of zooplankton species when size is an important factor. These microscopes are also serviced yearly by a qualified technician and no deviation has been found during our calibration checks.

VII. ANALYTICAL PROCEDURES

A. JUSTIFICATION AND COMPATIBILITY OF DATA

Procedures for the field and analysis parameters used in this proposal concerning the measurements associated with the mesozooplankton and microzooplankton are the same as those presently used by this laboratory for the past decade in the 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. Results from these analyses will provide compatible data sets that will be essential for long term statistical data analysis within this region.

B. MESOZOOPLANKTON

Upon arrival at the laboratory, samples are stored in cabinets used exclusively for this purpose and are located in the Zooplankton Processing Room. Depending upon sample backlog, a period of from two to three weeks may elapse before all samples are processed and analyzed.

CVS Method: September 1987 through April 2000

From the inception of the mesozooplankton sampling project (September 1987) until April 2000, the processing and analysis of the zooplankton samples were carried on by the coefficient of variation stabilizing (CVS) method (Alden et al., 1982). This method has numerous advantages over other zooplankton enumeration techniques. The CVS method provides abundance estimates with equitable coefficients of variation for species of interest in the zooplankton subsamples. Specifically, it is particularly useful in increasing the precision of estimates of numbers of large species of relatively low abundance which may be important due to their biomass, trophic position or economic significance. The investigator can be confident that the precision of the abundance estimates is at least at the predetermined level for all species processed by the CVS method. The method also has the advantage of allowing the investigator to set a level of precision which is consistent with cost, manpower or time constraints.

The CVS method involves the size fractionation of the samples into five size classes: 2000, 850, 600, 300, and 200 µm. This method was modified in March of 1998 to include the 75 µm size fraction. This series has been found useful for Chesapeake Bay zooplankton communities. Any size classes that are appropriate for whole counts will be placed in predesignated sample workup areas within the processing room. Those size classes in which the organisms are to numerous to count in their entirety are split with a folsom plankton splitter until an appropriate sample size is reached for statistically valid counts of the dominant species. The chosen level of 35% requires that each species of interest be counted to achieve a range of between 20 and 42 individuals in any given split. During the splitting process, each size class is halved repeatedly and reserve those reserve splits are numbered and placed in order for analysis. Any sample not counted within one day is spiked with 70% isopropanol to prevent degeneration of the sample before analysis. Those species that are observed to be subdominant in the final split are counted until they have achieved the range for the 35% error level. Any additional species encountered in the reserve splits are also counted but not to the same level of precision. The samples are counted in custom-designed micropetri dishes which are divided into quadrants. We have found these to be improved over Borgorov trays because there is a reduced meniscus effect and they allow for background illumination when necessary. Taxomonic identifications are made under American Optical Model 570 dissecting microscopes using Reichert Model 1177 fiber optic

illuminators. Difficulties with identifications or unknowns are resolved using a combination of a Nikon Model SMZ-ZT dissecting scope and a Nikon Optiphot compound scope which can be connected to a Panasonic video camera/monitor/VHS recorder system, as well as the extensive zooplankton reference library.

During the sieving/splitting process a chain of custody form is utilized to track each sample through to recondensing. A similar form is used in the analytic stage where the sample id is recorded upon completion and returned for recondensing. Data sheets are maintained during the analysis to record sample number, date collected, analysis technician, taxon name, size class, raw counts and split number (see Figure 5 for laboratory procedure flow chart).

Henson-Stempel (H-S) Method: April 2000 through the present

Beginning with the second quarter of the 2000 sampling season (April 2000), the processing and analysis of the samples was changed to the Henson-Stempel method employed by the Maryland Chesapeake Bay Mesozooplankton Program as described in the Maryland Chesapeake Bay Mesozooplankton Program Standard Operating Procedures (Versar, Inc., 2000).

Sample enumeration and identification begins with pouring each sample into a 63 um sieve under the hood and rinse off all formalin. Each sample is then sieved through a 500 um sieve which is suspended over a 63 um sieve, especially if grasses, large fish and other detritus are present in the sample. When excessive amounts of grasses are in the sample, the sample is first washed and then detritus material is placed into a large beaker of water (200 ml) and rinsed. The retained water from the washed detritus is then washed through a 63 um sieve.

Following washing the plankton are transferred to the appropriately calibrated beaker for the size of the sample to be counted. The first step is to get the “feel” of the proper dilution volume to use. A good rule of thumb will be to have a dilution volume/subsample volume combination of one hundred organisms in a one or two ml subsample. Common dilution volumes are 100, 200, 400, 500, 1000, and 2000 ml. Next, the samples are mixed thoroughly by constant aeration (by either an aquarium pump or by blowing through a pipette attached to a plastic hose). A 1-ml subsample is immediately removed with a Henson-Stempel Pipette (H-S pipette). Care is maintained to ensure no air bubbles or large lumps of detritus are in the sample chamber. Before transferring the contents of the H-S pipette to the

counting chamber, the outside of the H-S pipette is rinsed into a second container. Then the material in the 1-ml pipette is transferred to the counting chamber. The pipette piston is rinsed into the chamber a second time to remove any remaining organisms. For 2, 5 or 10 ml samples, the H-S Pipette is rinsed into a very small beaker (50 ml) and then the contents are transferred into the counting chamber.

Samples having exceptionally large volumes of zooplankton are first split with the Folsom Plankton Splitter. This initial step expedites sample processing while maintaining reliable estimates of abundance. The splitter is placed on a level surface and the legs adjusted to center the bubble in the leveling gauge. The sample is poured into the chamber and diluted until the chamber is three-quarters full. The sample is stirred thoroughly, the drum tilted gently, and the contents carefully poured into the collection trays. The water levels are checked that the levels in the two collection trays are equal. The chamber is rinsed after each use and wash water poured into collection trays. Next the contents of one of the collection trays is poured (a one half split) back into the chamber; the other half is poured into a labeled beaker and repeat the process until the desired split is obtained. The count is multiplied by the inverse of the split which is the estimated number of organism in the sample.

A hierarchical counting technique is employed to obtain density estimates for all taxa. This procedure consists of first counting at least 60 individuals of the dominant species (e.g. *Acartia tonsa)* in a small subsample (1 or 2 ml), followed by 5- and 10-ml subsamples, from which all species that had counts less than 60 in the previous subsample were counted. Mechanical tallies are used to count species in a subsample. After the 10-ml subsample has been counted, the entire sample is poured through an 850-um sieve. Mesozooplankton that were retained in the 850-micrometer sieve that were not previously identified in the subsamples and/or macrozooplankton are counted and identified. All species counts are recorded on a laboratory count data sheet. All counted zooplankton samples are retained for future reference and archived according to project specifications (five years for the DNR mesozooplankton project).

The relative abundance (#s/m^3) of each species is calculated with the following formula:

$$\text{Relative Abundance } (\#s/m^3) = A \times B/CD$$

where

A = the number of individuals counted in the subsample
B = dilution volume (ml)
C = subsample volume (ml)
D = volume of water filtered (m^3)

C. MICROZOOPLANKTON

All the samples are settled for at least 72 hours in the laboratory before the first siphoning to make a 300 ml concentrate. See Figure 4, microzooplankton sample analysis flow chart. These two concentrated replicates (300 ml each) are mixed (then 600 ml) and settled, to be siphoned again to make a 250 ml concentrate after 48 hours of settling. This 250 ml concentrate is transferred to 300 ml glass jar from one liter sampling bottle and settled for 48 hours. The third siphoning is applied to make 90 ml concentrate. The concentrate is filtered with 73 um mesh to separate relatively large detritus and plankters from the smaller ones. Most of rotifers, copepod nauplii, polychaete larvae, cladocerans and barnacle nauplii are trapped in the mesh and the materials on the screen are washed into a chamber which represents the "Group I" reading. A 2.5 or 5 percent aliquot is taken in the three different depths from the remaining concentrate in a 100 ml graduated cylinder after sieving. This aliquot is transferred to a second chamber, with enough buffered formalin solution added to the chamber to bring to a total of 25 ml volume. After 3-5 minutes, 15 ml of the 25 ml is removed from the surface of the second chamber and placed in a third chamber. Both chambers are brought to 25 ml final volume with 10% formalin and allowed to settle for 24 hours before examination with the inverted plankton microscope. The entire surface of the settling chamber is examined at 100X for chamber I, and at 200X for chamber II and III, respectively (see Table 5 for size ranges). The microzooplankton counts are given as the number of individuals per liter.

Table 5. Microzooplankton size ranges for three groups

Groups	Size Ranges	Plankters
I	over 73 um	Copepod N., Barnacle N., Poly Lar., Rotifers, Clad, etc.
II	30 - 73 um	Large Tintinnids and Oligotrichs, Small Rotifers
III	below 30 um	Small Ciliates

VIII. INTERNAL QUALITY CONTROL CHECKS

A. FIELD CHECKS

Quality control for the field measurements involves the proper maintenance and calibration of all field equipment (see Section VI-Calibration and Section X-Preventative Maintenance. Acceptance limits for the field are found in Table 2 for the physical data collection.

B. LABORATORY CHECKS

For the laboratory analysis, acceptance limits for enumeration and identification are listed in Table 3 of the QA objectives, but is briefly described as follows. Ten percent of the samples will be randomly selected for subsampling and re-identification and re-counting. This subsampling will be stratified by sieve size fraction and technician to ensure that each technician is tested for accuracy of identification at all sieve size fractions over a period of time. If the duplicate counts for the major species identified deviates by more than 10%, or the number counted by more than 5%, another sample is selected and recounted. If the technician fails to pass the second set of precision and accuracy tests, they are either retrained or removed from the project. These procedures are used to promote precision and accuracy in the sample analysis and to adequately meet completeness criteria.

IX. PERFORMANCE AND SYSTEMS AUDITS

In the field activities, the QAO will participate in several cruises each year to assure proper performance practices are followed in the collection process, and to check protocol used by the Zooplankton Field Supervisor. The laboratory protocol will be constantly under the supervision of the Program Manager and the Laboratory Supervisor. This includes the QA/QC protocols that will be followed regarding sample identifications and counts, as described in Section VIII, B. Acceptance criteria for both the field and laboratory analyses will be the adherence to protocols and goals established for these sections. Reports will be kept on file and will be routinely reviewed by the PI and Project Manager to evaluate performance levels. These audit reviews will be available for review and will include QA/QC analysis.

Representatives of the DEQ and EPA have previously made annual visitations to the field and laboratory operation to observe the practices followed. These visitations are expected to continue. Exchanges between the Zooplankton Laboratory and Versar, Inc. allow the exchange of information including agreements on species identification. This allows for the standardization and control of consistency with regard to sample and analytic procedures. Audits for the field and lab procedures are carried out by the laboratory supervisor.

XI. PREVENTATIVE MAINTENANCE PROCEDURES

Normal preventative maintenance would consists of the following practices for field and laboratory equipment:

A. Field Collections

1) Washdown and inspection of the CTD control unit, cables and sonde after each field day.
2) Monthly cleaning of sonde sensing area.
3) Monthly lubrication of all cable connections between sonde and data control unit.
4) Inspection and repair or replacement of plankton nets before and after each cruise.
5) Inspection and repair or replacement of flowmeters before and after each cruise.
6) Cleaning, inspection, and replacement of all field bottles.
7) Flushing and refilling of flowmeters with tap water after each cruise.

8) Wipedown of depth finder, GPS or LORAN, compass and marine radio after each cruise.
9) Monthly lubrication of all electrical connections (depth finder, GPS or LORAN, marine radio, winch and all boat gauges and trailer connections.
10) Monthly wire dressing of winch cables
11) Yearly tuneup of boat engine, and replacement of all worn/damaged components (spark plugs, gaskets, filters).
12) Inspection and operation of the winch and all trailering/running lights prior to each cruise.

B. Laboratory
1) Monthly lubrication of the sieving apparatus.
2) Daily leveling of the Folsom plankton splitter.
3) Quarterly adjustment/ calibration of the ocular micrometers in each microscope.
4) Annual cleaning and adjustment of dissecting scopes.

All general repairs are handled within the laboratory (plankton nets, flowmeters, boat equipment). Those items which can not be repaired within the lab are returned to the vendors for repair or replacement. In the event of unforeseen circumstances resulting in the malfunction of any equipment, alternate equipment is available as backups for all equipment used in the project. The laboratory has at its disposal:
1) Three towing vehicles.
2) Two small research vessels, each outfitted with GPS or LORAN, depth finder, compass, electric winches, and towing booms.
3) Two CTD multiparameter sampling systems with data loggers, display units, battery packs and transmitting cables.
4) Three sets of bongo-rigged, 202 um mesh plankton nets with frames.
5) Two 202 um mesh dropnets with frames.
6) Two field sample boxes with two complete sets of field bottles for all stations.
7) Two 20 cm secchi disks.
8) Three Imhoff volumetric cones.
9) Three Folsom plankton splitters.
10) Two ultra-fine water picks.
11) Six dissecting scopes.
12) Four fiber optic illuminators.
13) Numerous micropetri dishes, microprobes, and other plankton manipulation tools.
14) Two drying ovens.
15) Two analytical balances.

Almost every item listed above is directly controlled by the Zooplankton Laboratory. Those items that are not under our direct

control are readily available from other laboratories in our department, or on campus with short notice. Spare plankton nets, flowmeters, field bottles, Physical data recording equipment, data sheets and other field gear are always taken out into the field.

The Zooplankton Laboratory will be in compliance with all Occupational Health and Safety (OSHA) standards as interpreted by the Old Dominion University Health and Safety Office.

The operators of the research vessels will also be in compliance with state and federal regulations concerning the use of tributyltin, the Resource Conservation Recovery Act, the Clean Water Act, and the Water Quality Act of 1987. Furthermore, the Zooplankton Laboratory will comply with the applicable requirements of the health and safety protocols for EPA vessels of comparable class to those used in this project.

XI. 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 mesozooplankton and microzooplankton analyses.

A. Data Entry

All samples are logged and tracked with the chain of custody procedures described in the QA/QC section. Split/count sheets used to record raw zooplankton data contain information on date of collection, site number, taxon name, species code (internal species codes are cross-referenced with those reported, which consist of a combination of both the NODOC code and a Federal taxon serial number; these are found in the Chesapeake Bay Program document "Comprehensive List of Chesapeake Bay Basin Species"), split number and number counted, as well as technician performing the identification and counts. Data are entered into the computer by a member of the zooplankton technical staff and each entry is checked against the data sheet by a second member of the zooplankton technical staff. After this entry the data are screened for errors and outliers through computerized manipulation. The data are then standardized to number or weight per cubic meter of water filtered in the combined replicates. If the flow meter readings for the two field replicates differ by more than 10% a tracking procedure working back to the original field log sheet is instituted to determine if an error in

recording or gear can be found. Most likely sources of error are: flow meter incorrectly read or recorded; flow meter malfunction; or data incorrectly punched. If the discrepancy is large (> 20% of the maximum value) and the error cannot be found or corrected, the sample is excluded from the data.

B. Data Reduction

After standardizing the data to number per unit volume individual monthly data sets are merged into the analysis data set, and this data set is re-screened for outliers. These screening results are also used to double check results of later analyses. A species list containing all species encountered during monitoring is generated. A multi-criteria species reduction protocol is then performed to focus further analyses on the most abundant and informative taxa. The reduced data set includes no less than 99% of the individual zooplankters sampled.

C. SAS Data Files

After all data for each sampling cruise have been entered and verified as accurate in the raw data files, the data files are sent to the university mainframe computer via a hard line. A series of SAS programs are run, during which species names and abundance are appended to the raw data files (containing species codes and counts).

D. Data Storage

At the Zooplankton Laboratory the project files and raw data files are stored on hard disks with regular network backups. The SAS data files and report files are also stored network hard drives with regular network backups. All original raw data sheets are stored in the Zooplankton Laboratory.

E. Validation

The previously defined methodologies will produce a data set for characterizing the zooplankton populations in the lower Chesapeake Bay and these 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, at the sampling sites. The basic techniques for analysis follow standard protocols. Precision objectives are enhanced by the methodology

protocols, which will be carried out by a trained and experienced staff in Chesapeake Bay mesozooplankton and microzooplankton populations.

F. Reporting

1. Raw Data

Raw 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.

Data deliverables submission due dates:

January-June data	11/15
July-December data	4/15

2. Progress Reports

Quarterly status reports will be submitted to the Department of Environmental Quality Project Officer by the following dates. These will include raw data summaries, a brief narrative of progress, QA/QC problems, cruises completed, suggestions for improvement, and data not collected. Copies of published manuscripts will also be sent to DEQ.

Progress report deliverables submissions due dates:

Quarterly Progress Rept. 1/1 - 3/31	4/15
Quarterly Progress Rept. 4/1 - 6/30	7/15
Quarterly Progress Rept. 7/1 - 9/30	10/15
Quarterly Progress Rept. 10/1 -12/31	2/15

XII. 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. 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 QAO.

Data entered into the computer is screened after each station entry by the laboratory supervisor to check for double entry, species codes, or any other errors. These values are checked against the raw data sheets.

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

XIII. CORRECTIVE ACTION

For field operations, the field chief is responsible for ensuring that equipment to be used in the field is in good working order. This includes the prior calibration of the CTD (Hydrolab Surveyor II) and flowmeters, as well as checking with the technician responsible for vessel and maintenance.

The laboratory has two small research vessels and three towing vehicles at its disposal. Both vessel are equipped with the necessary equipment to perform the assigned tasks in this project (depth finder, GPS or LORAN, compass, winches, and all necessary safety gear). In the event of equipment malfunction, either of the vessels and any of the other tow vehicles can be used as replacements.

With respect to the CTD, a calibration procedure will be performed prior to every cruise, followed by a post-cruise calibration at the end of the sampling day. Deviations of the post -cruise calibration value from the pre-cruise calibration value will be noted in the calibration log book. Deviations of greater than 10% from the accepted calibration values will result in the securing of the apparatus, and readjustment and/or repair can be completed on the instrument or probe involved. Consultation with the manufacturer on the problem and its resolution will also occur in these cases.

Flowmeters will be calibrated monthly (See Section IX, Calibration) by the field chief and are compared for similarity in total counts resulting from the calibration. Flowmeters are then matched by closeness of counts (by letter designation) and paired for use in the field. Any flowmeters that exceed 20% of the

average difference will be set aside for repair or replacement. In the field, after each plankton tow, the pre and post tow flowmeter readings are compared. Where the higher reading exceeds the lower reading by 20% or greater, the flowmeters will be checked for obstructions and damage. If no problems are evident, the tow will be retaken, and the readings checked again. A second out-of-range reading will result in replacing the flowmeters with the back-up flowmeters and a third tow will be taken. These problems and the corrective actions taken will be noted in the filed log. This will be stored in the document area of the zooplankton laboratory.

The laboratory supervisor and QAO will participate in the training of new laboratory personnel. Once the required degree of accuracy and precision is attained by the trainee, the individual is incorporated into the lab analysis group. Only then is the technician evaluated by random QA/QC checks. These checks are performed by the QAO. If a technician fails to pass either the precision tests (having an error greater than 10% in total counts) or the accuracy tests (failing to properly identify over 5% of a dominant taxon,), corrective action will be taken. This will involve either retraining the technician by the laboratory manager or removal from the project at the discretion of the Primary Investigator. A log will be maintained of the these QA/QC analyses.

XIV. QUALITY ASSURANCE REPORTS TO MANAGEMENT

The PI will be responsible for preparing quarterly reports on this project. These reports will include conformance to the scope of work, information detailing each cruise during the quarter, status of the submitted data, quality assurance, and comments detailing any changes in the QA Project Plan. Also included will be an explanation of any important observations concerning the program, such as lost or uncollected data, out-of control analyses, or system problems. Results of quality assurance checks will be kept on file and routinely forwarded to the VA DEQ-CBP.

Hardcopy (originals) of the field and laboratory documents will be maintained in a secure location within the plankton laboratory for a period of five years from the date of delivery to the CBPO. Dates for the submission of quarterly reports are given in Section XI, part F. Information included in these reports will be: 1. progress, 2. problems, 3. results of audits, 4. QA/QC results, 5. Changes in QA, and 6. major phenomena that took place among the zooplankters.

XV. REFERENCES

Alden, R.W. III., R.C. Dahiya and R.J. Young, Jr. 1982. A method for the enumeration of zooplankton samples. J. exp. Mar. Biol. Ecol. 59:185-209.

Gehringer, J.W. and W. Aron. 1968. Field techniques. In: D. J. Tranter (ed.) Zooplankton Sampling. UNESCO, pp. 87-104.

Griffiths, F.B., A. Fleminger, B. Kimor and M. Vannucci. 1976. Shipboard and curating techniques. In: H. F. Steadman (ed.) Zooplankton Fixation and Preservation. UNESCO, pp 17-31.

Tranter, D. J. and P. W. Smith. 1968. Smaller mesozooplankton. Report of Working Party No. 2. In: D. J. Tranter (ed.), Zooplankton Sampling, pp. 153-159.

Williams, W.T. and W. Stephenson. 1973. The analysis of three-dimensional data (sites x species x times) in marine ecology. J. exp. Mar. Biol. Ecol. 11: 207-227.

XVI. APPENDIX: Figure 1

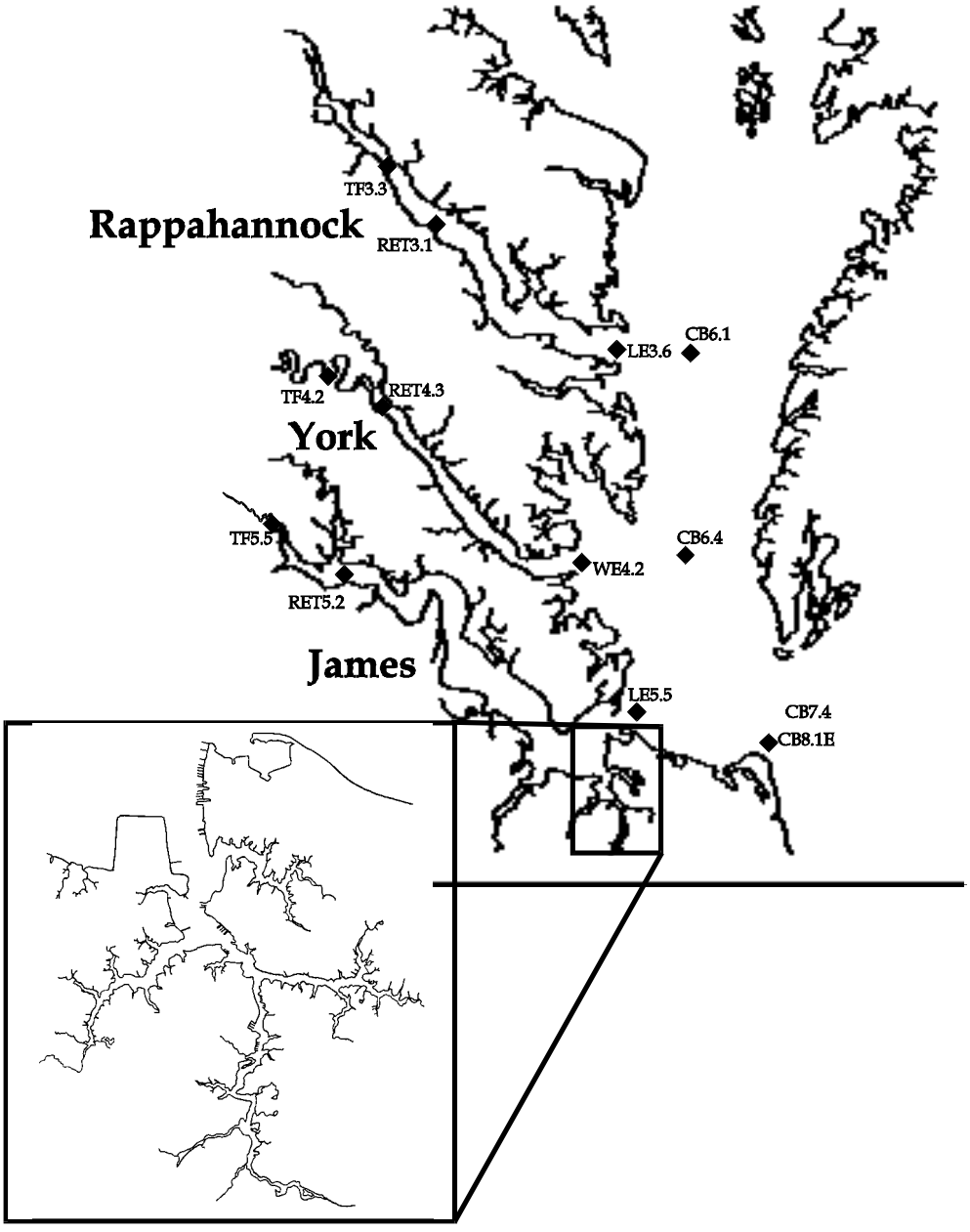

Figure 1. Map of station sites.

XVI. APPENDIX: Figure 2

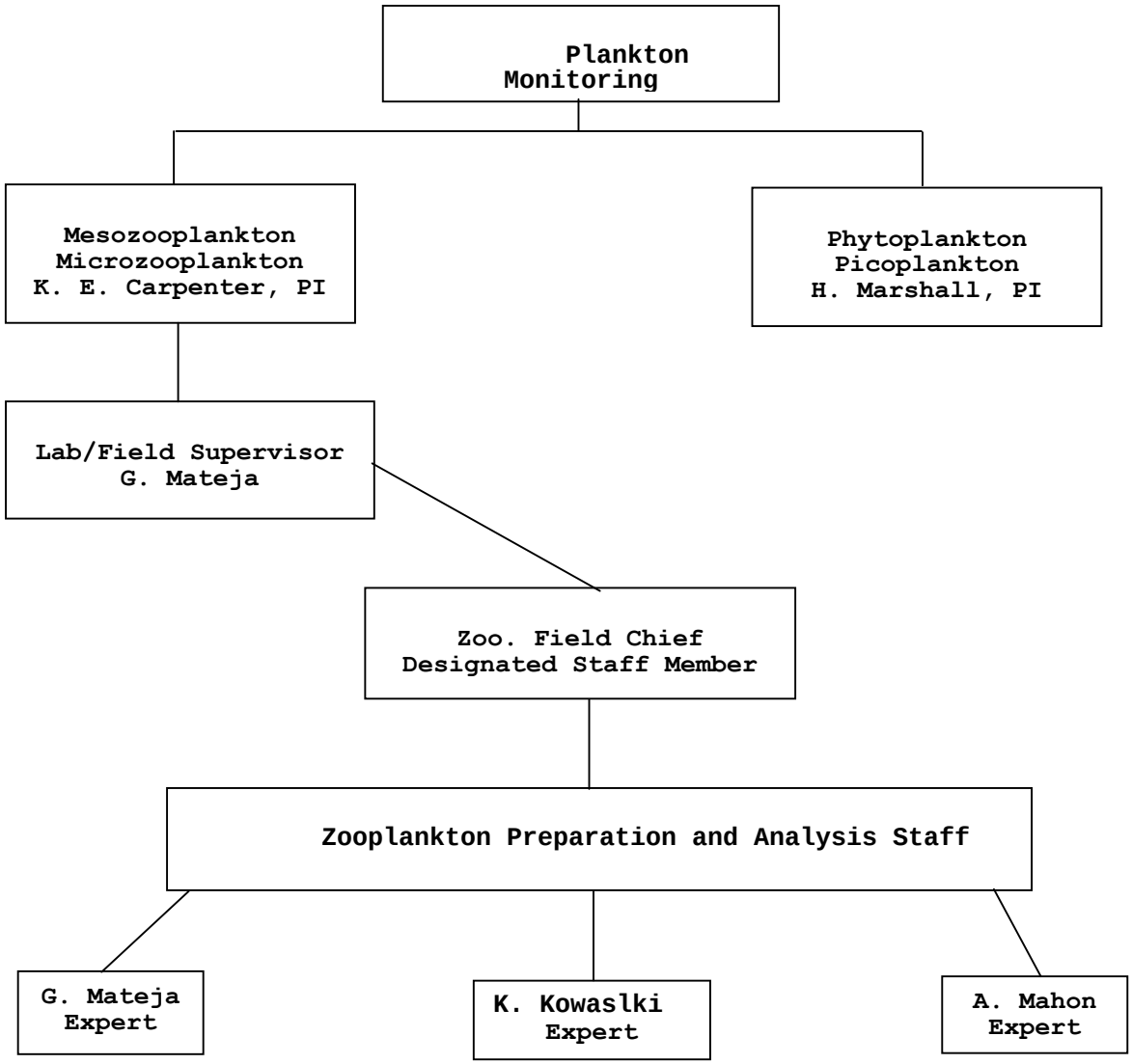

Figure 2. Organizational plan for the zooplankton component.

XVI. APPENDIX: Figure 3

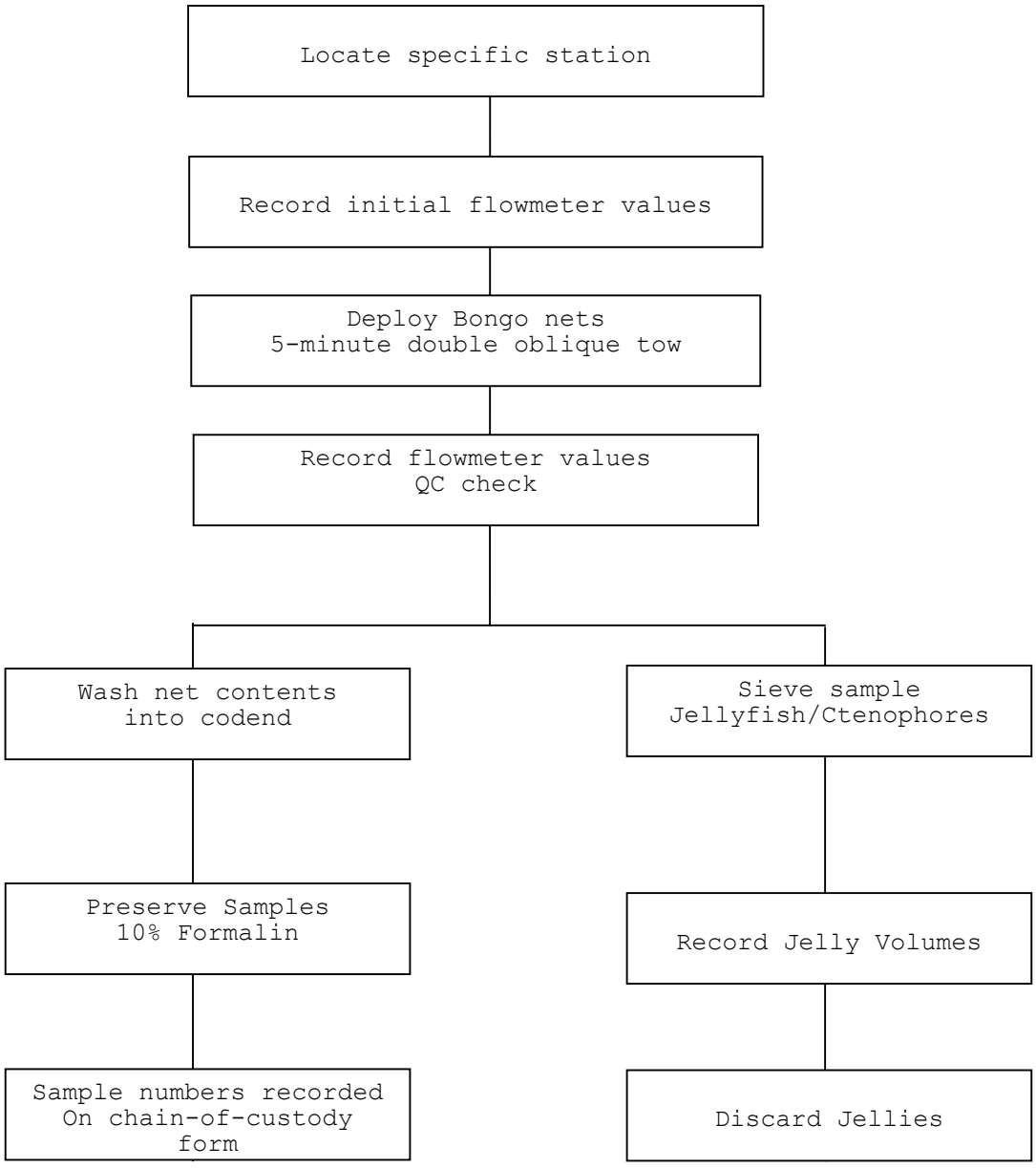

Figure 3. Mesozooplankton collection flow chart.

XVI. APPENDIX: Figure 4

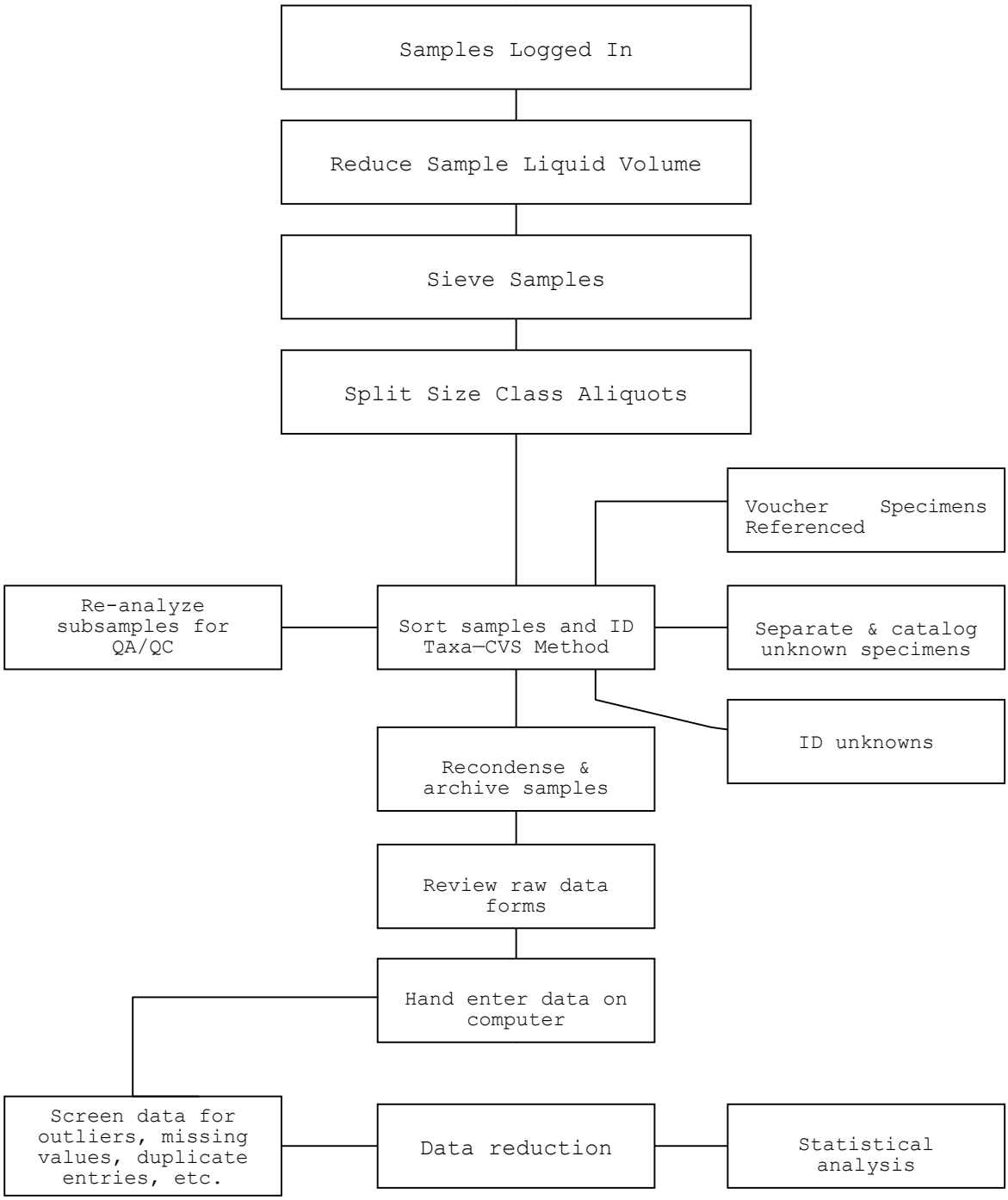

Figure 4. Mesozooplankton sample analysis flow chart.

XVI. APPENDIX: Figure 5

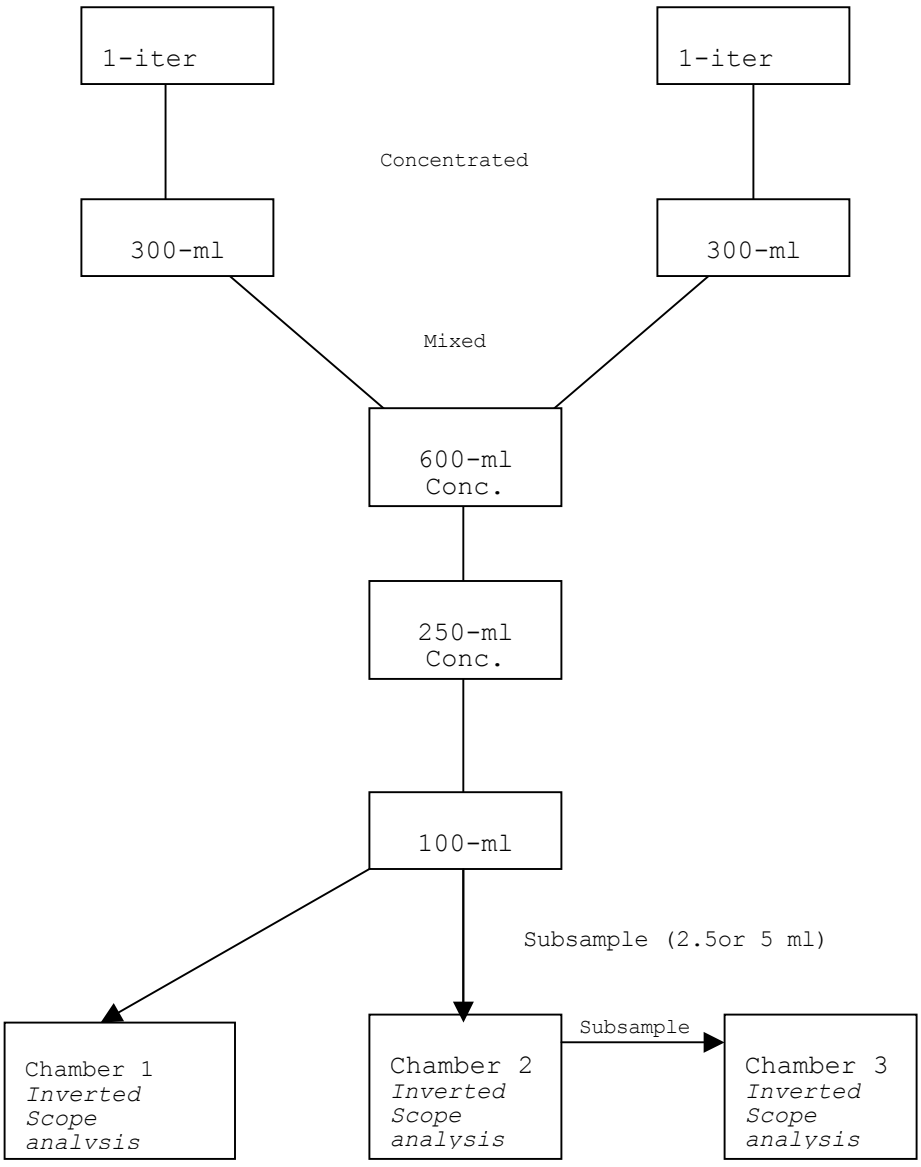

Figure 5. Microzooplankton sample analysis flow chart.

XVI. APPENDIX: Data Sheet 2

MICROZOOPLANKTON DATA SHEET (V6.0)

STATION		CONCENTRATED		INVESTIGATOR	
DATE		SUBSAMPLING VOL		LOCATION	

	TAXONOMIC GROUP (dilution factor)	Group I (×)	GROUP II (X)	GROUP III (X)	NO/L	BIOMASS (mg C/l)
1	Copepod Nauplii					
2	Barnacle Nauplii					
3	Rotifers					
4	Tintinnids					
5	Oligotrichs					
6	Polychaete Larvae					
7	Sarcodinids					
8	Cladocerans					
9	Others					

species name	no	species name	no	species name	no

NOTES AND REMARKS

microzooplankton data sheet (version 6.0) designed by gyung soo park - copy right reserved - aug 12 1994

Data Sheet 2: Example of a Microzooplankton Data Sheet (V6.0) (2ppg)

VI. APPENDIX: Data Sheet 3

Chesapeake Bay Program -- Species Sampling (Hensen - Stempel Method)

Date & Initial: **Sample Read:** _____ **Computer Entry:** _____ **Entry Checked:** _____

Station: **Tributaries:** ☐ TF3.3 ☐ RET3.1 ☐ RET4.3 ☐ TF5.5 ☐ RET5.2 ☐ TF4.2 ☐ SBE5
Mainstem: ☐ LE5.5 ☐ CB7.4 ☐ CB7.3E ☐ CB6.4 ☐ LE3.6 ☐ CB6.1 ☐ WE4.2

Split Sample (sent to MD): Y / N **Side:** _____ of _____

Work Up Difficulties:

Station: / Field Date:	SIZE CLASS						
Volume: / Species Latin Name	Species Code	1 ml Count	2 ml Count	5 ml Count	10 ml Count	850 um Count	Other Split Factor: ☐
1.							
2.							
3.							
4.							
5.							
6.							
7.							
8.							
9.							
10.							
11.							
12.							
13.							
14.							
15.							
16.							
17.							
18.							
19.							
20.							
21.							
22.							
23.							
24.							
25.							
26.							

Data Sheet 3: Example of a Zooplankton Count Benchsheet (2pgs)

XVI. APPENDIX: Data Sheet 4

Chesapeake Bay Monitoring Program
Zooplankton Lab Field Data

Station:	Station No.:	RV:	
		Date:	Time:
Zoo Field Chief:	Crew:	Latitude:	N
		Longitude:	W

Zooplankton Net Data

Net:	A	B	Tow Time:	Depth Data (in Meters)
Meter Stop:				Secchi Depth:
Meter Start:			Observations:	Total Depth:
Total Revs.:				

Weather Data

Cloud Cover Codes	Precipitation Type	Wind Speed	Sea State	Weather Codes
0 - Clear (0-10%)	10 - None	0: 0 - 01 Knots	0: Calm	Cloud Cover:
1 - Part. Cloudy (10-50%)	11 - Drizzle	1: 02 - 10 Knots	1: <1 ft.	Precip. Type:
2 - Part. Cloudy (50-90%)	12 - Rain	3: 21 - 30 Knots	2: <2 ft.	Wind Speed:
3 - Overcast (>90%)	13 - Rain (Heavy)	4: 31 - 40 Knots	3: <3 ft.	Sea State:
4 - Foggy	14 - Squally	5: 41 - 50 Knots	4: <4 ft.	Wind Dir.:
5 - Hazy	15 - Frozen Precip.	Observations:	5: >4 ft.	Tidal Stage: H L F E
6 - Cloudy (No % Given)	16 - Rain Snow			

Mesoglea Data

Mesoglea Vol.	A	B	[redacted]	A	B
TOTAL	ML	ML		ML	ML
Type/Family (Fill Out)	% Composition	% Composition	Genera (Fill Out)	% Composition	% Composition
Ctenophores (Comb-Jellies)			Beroe		
			Mnemopsis		
Scyphozoa (Typical Jellyfish)			Chrysaora		
			Aurelia		

Environmental Data

Depth (M)	Temp (°C)	Salinity (ppt)	Sp. Cond. (µS/CM)	D. O. (PPM)	pH	Other
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						

Data Sheet 4: Example of a Field Data Sheet