MARYLAND CHESAPEAKE BAY
MESOZOOPLANKTON PROGRAM
STANDARD OPERATING PROCEDURES

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STANDARD OPERATING PROCEDURES

ZOOPLANKTON

This document provides standard operating procedures for the field and laboratory elements of the Department of Natural Resources (DNR) mesozooplankton component of the Chesapeake Bay Monitoring program. The procedures discussed are tailored to the specifications of this program, but the general procedures can be used for any zooplankton sampling effort.

Chesapeake bay mesozooplankton samples are collected once a month at 12 regular stations from March through October, and once in the month of December within the main stem and its tributaries (Fig. 1). An additional station is sampled in the main stem, Patuxent River, Choptank River, and Potomac River during the spring spawning season to collect additional data from striped bass spawning habitats during the months of April, May, and June. In addition, a second series of collections are taken at six of the regular stations located in anadromous fish spawning habitats as well as the four additional striped bass spawning stations during the months of April and May. Collections are performed in conjunction with DNR water quality and the Academy of Natural Sciences (ANS) phytoplankton/ microzooplankton monitoring programs.

Field preparation and sampling methods are specific to vessels used for the sampling. The main bay and the Patuxent stations are sampled from large research vessels, 48 to 62 feet. The tributary and the lower Potomac cruises are sampled from smaller research vessels including a 25 foot Sea Hawk and smaller whalers. Procedures vary according to the vessel from which sampling is performed, but the objective is consistent.

1.0 FIELD SAMPLING

1.1 Field Preparation

1. Log sheets: Fill in dates (yymmdd format) and sample serial numbers on the master zooplankton log data spread sheet (Fig. 2). Refer to the field schedule from the DNR for dates. Sample serial numbers are unique combinations of letters

Sampling locations and station designations of the mesozooplankton monitoring

Figure 1.
program.

Figure 2. Zooplankton log sheet

and numbers representing one individual sample. Example, CZM00PX000A: CZM stands for Chesapeake Zooplankton Monitoring; 00 is the year the sample was taken; a two-letter code for the region sampled (PX indicates the Patuxent River); a three digit number of the sample; and an "A" or "B" indicating the replicate.

2. Field data sheets (Fig. 2): To create the field data sheets follow these steps:

    1. Edit previous month lotus.wk4 file (ex. April1.wk3)
    2. Open WP file LABEL2.DAT
    3. Select Insert, Spreadsheet, Edit Link - select new month lotus file (range A8..I23)
    4. CLICK on Save (same name)
    5. Click Goto form, select LABEL.FRM
    6. Merge from LABEL.FRM
    7. Save new labels as "April2.lab
    8. Print labels
    9. Close
    10. Open Field data sheets (field.w51)
    11. Merge from field.w51 (this takes a while)
    12. Print

    Copy field data sheets (Fig. 3) onto water proof paper and put them into the three-ring binders that correspond to each field trip: tributary, Lower Potomac, main bay, and Patuxent.

3. Sample container preparation: Place a label on one 1000 ml sample bottle for each station. Two tows are performed to serve as replicates. Each preprinted label should show river, station number, replicate number, date, and serial number.

4. Put sample bottles with corresponding field notebooks into milk crates according to trip.

ZOOPLANKTON MONITORING FIELD DATA SHEET

LOCATION: **Patuxent** COLLECTORS _____

STATION DESIGNATION **TF1.5 (pxt0402)** REPLICATE **1**

DATE (MONTH DAY YEAR) **07/01/99** SERIAL **CZM99PX022A** GEAR BON202

DEPTH (M) **10** TOW DEPTH (M) **12** SECCHI DEPTH____(CM)

WIND DIR ____ (SO= SOUTH, NO= NORTH, WE= WEST, EA= EAST, SW= SOUTHWEST, SE= SOUTHEAST, NW= NORTHWEST, NE= NORTHEAST) WIND VEL____ (knots) TIDE ____ (1= FLOOD, 2= EBB, 3= HIGH SLACK, 4= LOW SLACK)

WEATHER____ (1= SUNNY 2= CLOUDY 3= PARTLY CLOUDY 4= RAIN)

STEPPED OBLIQUE TOW

PYCNOCLINE ☐ YES ☐ NO DEPTH _____

TIME IN _____ FLOW METER SERIAL # _____

INITIAL ☐☐☐☐☐☐

MNEMIOPSIS SP. _____ # JELLYFISH _____ FINAL ☐☐☐☐☐☐

VOL DIS. _____ VOL DIS. _____ INITIAL ☐☐☐☐☐☐

BEROE SP. _____ HYDRA MEDUSA _____ FINAL ☐☐☐☐☐☐

VOL DIS. _____ VOL DIS. _____ TOTAL REVS ☐☐☐☐☐☐

SAL. CAT. ____ VOL. FIL_____ 2 x REVS X .0008442 = VOL FIL

DEPTH (M)	TEMP (C)	SAL (PPT)	D.O. (PPM)	**COMMENTS**
0.5				TOW MINUTES
1.0				2.5 or 5 or ____
2.0				
3.0				DEPTHS
4.0				0
5.0				
6.0				
7.0				
8.0				
9.0				
10.0				
11.0				
12.0				
13.0				
14.0				
15.0				
16.0				
17.0 18.0				Remember to combine all four halves of the tows and to count all the jellies
19.0 20.0				
21.0 22.0				
23.0				
24.0				
25.0				

Figure 3. Zooplankton monitoring field data sheet

5. Mesh inspection: Inspect plankton nets, collection cups and field sieves for holes in the 202 µm mesh. Make repairs with silicon sealer if necessary while prepping so that the silicon has time to dry before the equipment is used in the field.

6. Flow meters: Refill with water, if necessary, and see that the numbers turn while the propeller spins smoothly. Extra flow meters are taken in the field in the event that one fails. (Flow meters should be calibrated once a year in a flume to insure accuracy.)

7. Field equipment list (Fig. 4): Use to distribute equipment according to trip.

8. Preservative: Formalin is used in 10% solution to preserve samples. Determine amount needed according to number of stations to be sampled.

9. Bongo nets: Provide two bongos when possible in case one is damaged in the field.

10. Fresh water: Fresh water for rinsing samples is needed on the small boats. The large research vessels have fresh water on board. Bay/river water filtered through a 110 µm sieve may be used in place of fresh water.

11. Watches: Provide watches for field personnel to time zooplankton tows.

12. Recheck equipment: Use the field equipment list as a checklist to make sure nothing is forgotten.

ZOOPLANKTON FIELD CHECK LIST

202u mesh sieve
large mesh metal sieve
sorting tray
funnel
bongs w/nets
gallon water jugs (small boats only)
buckets
flow meters (filled with water, and spinning easily)
squirt bottles
milk crate
collection cups
labeled sample bottles w/whirl pacs
field book
beakers
formalin
ditty bag -
- screwdriver
- scissors
- ex-whirl pacs
- ex-bottles
- ex-nets
- collection permit
- cable ties
- rubber bands
- lab tape
- Sharpies
- pencils
- graduated cylinder
- small beaker
- silicon
- field watch

50-cm bong net 505 (m msh)

On Small Boat - alone
Washdown pump
Extra battery (for pump)
Meter wheel
Depressor
Extra shackles

Figure 4. Zooplankton field check list

13. Personal gear: Notify all field personnel that they are responsible for their own rain gear, waterproof boots, and insulated overalls (mustang style) suits in cold weather. Personnel sampling on the Department of Natural Resources Discovery and the Chesapeake Bay Laboratory's vessels, the Aquarius and the Orion, must bring a survival suit in the ambient temperature is below 40 degrees Fahrenheit.

14. Travel: Sign out company vehicles if possible and contact DNR and ANS to coordinate meeting times for field personnel.

1.2 Sampling Procedures - Large Research Vessels

1. Equipment: Load all equipment onto research vessel and store out of the way. Store the ditty bag (with flow meters) and field data notebook in the boat's cabin to keep dry and to prevent flow meters from freezing in the cold months.

2. Pre-check: Check nets and sieves for holes prior to sampling and periodically during field use. Make repairs on small holes with silicon sealer. (Note - always store mesh sieves with the mesh up to prevent contact with any surface that could rip it.)

3. Organization: Assemble necessary collection gear in the work area to make sampling efficient. This includes bongo nets with flow meter attached, station bottles with biomass bags inside, buckets, sieves, full squirt bottles, a funnel, graduated beakers for measuring ctenophores, and the field notebook.

4. Type of tows: Samples are obtained by towing a 20-cm bongo net (202 µm mesh net) in a stepped oblique fashion. Two complete tows are made and combined for zooplankton. The entire water column was sampled by first deploying the gear just above the bottom and raising the net in timed progressive steps, usually 0.5 to 1.5 minutes/step. An additional tow for jellyfish and ctenophores using a 50-cm bongo net (505 µm mesh net) will be deployed in the same manner as the 20-cm bongo net. The additional tow for jellyfish and ctenophores will be conducted during the months of July, August, and September at the following high salinity stations; LE1.1, LE2.2, CB5.2, CB4.3C, CB3.3C, ET5.2, and WT5.1.

5. Sampling depths: Check to see that actual bottom depth is the same as the bottom depth on the field data sheet. If not, note correct bottom depth on data sheet and adjust tow depth by multiplying bottom depth by 1.4 meters for large boats and 1.2 for small boats. Stepped increments may also be adjusted to maintain an even sampling distribution within the water column. Write sampling depths on a piece of lab tape for the mate, who operates the winch, for reference while towing.

6. Mud bags: If the bongo net touches the bottom, mud or sand probably got into the net (known as a mud bag). This sample cannot be used. The net must be brought up and washed out, and the tow started again.

7. Tow times: Evaluate the ANS microzooplankton samples for clarity and quantity of detritus to determine length of tows needed to get an adequate sample. A standard tow is 2.5 or 5 minutes. (Rule of thumb: when zooplankton are easily seen in these samples, 5-minute tows are sufficient. If only a few individuals can be seen with the naked eye, a 10-minute tow is standard.) Large amounts of ctenophores, jellyfish, detritus or zooplankton may clog the nets. This interferes with consistent flow and indicates the need for a reduced tow time. Experienced field personnel from DNR or ANS may assist in determining tow times. Notify the boat captain of the length of tows to be performed.

8. Before deployment: Attach bongo net and depressor to the winch cable and record the initial flow meter reading on the data sheet.

9. Deployment: Deploy the bongo net making sure the gear goes out untangled and depressor is hanging free. The tow angle of the cable should be approximately 45° with the nets just below the surface of the water.

10. Timing the tow: Lower the bongo net to the bottom depth and begin timing.

11. Retrieving bongo net: When the nets are brought up, bring the depressor on board to stabilized the bongo net and record the final flow meter reading. Check the data sheet to see if the difference between the flow meter readings (final -

initial = the number of revolutions of the tow) is within the acceptable ranges. A table of acceptable ranges is located in each field notebook.

12. Retrieving samples: Rinse nets thoroughly from top to bottom at least twice to collect all organisms in the cup at the cod end. Remove collection cup over a bucket. Rinse the end of the net and the collection cup into the bucket spraying outside the mesh holes and rinsing inside the cup three times. Repeat this procedure for the second net in a separate bucket. (Rinsing mostly from the outside of the net prevents damaging organisms and reduces the number of zooplankton that may be added to the sample. No cross-contamination occurs as this water comes from the same sample area, and the number of organisms introduced to the sample is insignificant.)

13. Second replicate: Repeat the tow procedure for a second replicate and combine with the first tow.

14. Sieving: Pour the sample from the bucket through the mesh sieve (If there are jellyfish or ctenophores place a metal sieve over, but not touching, the mesh sieve.) Perform the sieving process over a bucket. Any large fish, pieces of detritus, or vegetation can be rinsed off into the mesh sieve and discarded.

15. Bottled sample: Using the squirt bottle of fresh water, rinse all zooplankton to one side of the sieve and wash through a funnel into the sample bottle. Rinse the sieve thoroughly (at least three times) and examine to make sure no organisms are left in the sieve.

16. Preserving the bottled sample: Add formalin to make a 10% formalin solution. Use a funnel to prevent splashing.

17. Jellyfish and ctenophores: Jellyfish and ctenophore measurements are taken by combining both 20-cm bongo net tows. In addition, process the 50-cm bongo net individually during the summer months. Collect these organisms in the metal sieve, pour them into a beaker, and set aside for measuring after samples are bottled and bagged. Use a bucket, if they are abundant, and record a total

volume. Subsample (one half, one quarter, etc.) using the graduated cylinder or measured beakers and count the subsample. Multiply by the inverse of the split to find the total estimated number of organisms. The

18. Storage: Store the bottled preserved samples in the milk crate.

19. Station information: Copy relevant DNR station data into the field notebook; required information is listed on the data sheets. Note any additional significant information in the comment section.

1.3 Sampling Procedures - Small Research Vessels

Sampling procedures on small research vessels are similar to those on large research vessels with the following changes:

1. Winches: The winches on the smaller boats are hand operated. The boat captain will demonstrate use of the winch and field personnel are responsible for deploying, "zeroing", and bringing up their own tows. (Note - the winch for the lower Potomac station is incremented in feet, and the data sheets for this station are in corresponding units.)

2. Depressor: Attach the depressor securely to the tow cable.

3. Tow speed: Boat speed should be maintained at approximately 1100 rpm for towing. Adjust the boat speed so that the cable has a 45° angle when the nets are being towed at the surface.

4. Tow direction: Perform both tows in the same direction (the current affects the amount of water filtered through the nets) to ensure replicate similarity.

5. Tow times: Generally, a 5-minute tow is sufficient in the river stations; they tend to have higher zooplankton densities and more detritus than the main stem stations. When detritus, zooplankton, or jellyfish volumes are extremely high, a two-and-a-half-minute tow may be performed to reduce clogging of the nets and inaccurate flow meter readings.

6. Performing tows on anchor: If the current is very strong samples may be collected while the boat is anchored. In this situation, when the nets are deployed, the force of the water should establish a 45° cable angle. If a tow is attempted in strong currents on a moving vessel, the organisms may be crushed against the mesh or the amount of detritus collected may clog the nets.

1.4 Post-trip Equipment and Sample Storage

1. Checking in samples: Check samples collected in the field with information registered in the log book. Store the bottles on the designated shelf by month.

2. Formalin storage: Formalin jugs are stored under the hood in the plankton lab for proper ventilation. DO NOT leave formalin in the equipment cage. Fumes are harmful to health.

3. Equipment: Store sampling equipment neatly in the zooplankton section of the equipment cages. Place like equipment together. Turn buckets upside down to drain, take flow meters out of nets, and report any broken equipment or problems to zooplankton lab technicians.

4. Field data: The zooplankton technician checks the field data for errors, calculates the volumes filtered, and photocopies the field data sheets. To calculate the volume of water filtered use the following formula:

$$M^3 = 2 \times 3.14 \times (\text{net radius m})^2 \times \left(\frac{\text{Revolutions} \times 26{,}873}{999999} \right)$$

* 26,873 is the rotor constant for the General Oceanics standard rotor

2.0 LABORATORY PROCESSING

2.1 Enumeration and Identification

1. Begin sample processing: Sign out sample in the zooplankton laboratory log book with initials and date (yymmdd). Be sure it is labeled correctly and is scheduled for processing.

2. Sieving: Pour sample into 110 µm sieve under the hood and rinse off all formalin.

3. If grasses and other detritus are in the sample, wash each piece of material in a 5 mm sieve which is suspended over the 110 µm sieve to retain the organisms.

4. When excessive amounts of grasses are in the sample, first follow step 3, then place the washed material into a large beaker of water (200 mls) and rinse. Pour the retained water with the organisms through the 110 µm sieve.

5. Diluting sample: Transfer plankton to the appropriately calibrated beaker for the size of the sample to be counted. Your supervisor will work closely with you at first to get the "feel" of the proper dilution volume to use. A good rule of thumb is 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.

6. Taking subsamples: Mix the sample thoroughly by constant aeration (blowing through a pipette attached to a plastic hose) and remove a 1 ml subsample immediately with a Hensen-Stempel Pipette. Make sure no air bubbles or large lumps of detritus are in the sample chamber. Rinse the pipette into a second container.

7. Transfer to counting chamber: For the 1-ml sample, open the Hensen-Stempel Pipette over the counting chamber; rinse any remaining organisms into chamber. For 2, 5 or 10-ml samples, open Henson Stemple Pipette over a very small beaker (50 ml) and rinse organisms into it.

8. Splitting samples: Split samples with excessive amounts of zooplankton using the Folsom Plankton Splitter. This expedites sample processing while maintaining reliable estimates of abundance. Place the splitter on a level surface and adjust the legs to center the bubble in the leveling gauge. Pour the sample into the chamber and dilute until the chamber is three-quarters full. Stir the sample thoroughly, gently tilt the drum, and carefully pour the contents into the collection trays. Check that the water levels in the two collection trays are equal. Rinse the chamber after each use and pour wash water into collection trays. Next pour the contents of one of the collection trays (a one half split) back into the chamber; pour the other half into a labeled beaker and repeat the process until the desired split is obtained. The count multiplied by the inverse of the split is the estimated number of organism in the sample.

9. Counting chamber: Use the circular counting chamber when processing with a dissecting microscope. This chamber eliminates the possibility of counting any individual twice.

10. The count: Use a hierarchial counting technique 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. Use mechanical tallies to count species in a subsample. After the 10-ml subsample has been counted pour the entire sample through an 850 micrometer sieve. Mesozooplankton that were retained in the 850 micrometer sieve that were not previously identified in the subsamples and/or macrozooplankton were counted and identified. Taxonomic references are located in Appendix A.

11. Count data sheets: Record all species counts on a laboratory count data sheets (Fig. 5). Fill in all information completely.

Figure 5. Zooplankton monitoring laboratory counting sheets

12. Settle volumes: After an entire month has been processed and QC'd, place zooplankton samples in the graduated Imhoff cones to determine the settled volume (ml/m^3).

13. Archiving samples: Retain all counted zooplankton samples for future reference and archive according to project specifications (five years for the DNR mesozooplankton project).

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

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

where

A = the number of individuals counted in the subsample

B = dilution volume (ml)

C = subsample volume (ml)

D = volume of water filtered ($m^{3)}$

2.2 QA/QC Procedures for Enumeration

1. Standard quality control for laboratory processing of zooplankton samples consists of recounting at least 10% of the samples. Recounts are selected at random and processed by a different technician using the original dilution volume.

2. After recounting, calculate the relative abundance (#s/m^3). Do the same for the original sample. Then calculate the percent error using the following formula:

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

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

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

4. Compare the original list of species with the QC list (Fig. 6) to make certain that the same species were found in the recount and that the counts within species are similar. Add new species found in QC to original data sheet.

5. If gross differences exist, examine the species to determine if the species identifications are correct and notify your supervisor. Your supervisor will determine if a problem with taxonomy exists and will schedule retraining of the original technician if necessary.

6. When a sample fails QC, actions taken depend on the contract requirements. Check with the program manager for the project specifications. Possible corrective actions are listed below:

   - Recount another sample from the batch of ten and if it passes, stop and replace original data of failed QC with recounted data. If it doesn't, recount another randomly selected sample until a sample passes or all samples are recounted.

   - Recount the QC sample a third time. If the two recounts agree, delete the original count data and use the data from the first recount. Recount the station replicate and if it passes, stop. If it doesn't, follow the procedure outlined below.

   - Recount all samples processed the day of the original count (assumes technician had an "off" day).

7. Recounting samples affects the project budget. Be sure that the program manager provides specific QC guidelines with regards to time and is aware of the QC costs during the course of sample processing.

Figure 6. Zooplankton QC sheet

2.3 Data Verification Procedures

1. Field and laboratory data sheets are coded and punched directly from the originals.

2. After punching, printouts are verified and corrected against the originals and rechecked.

3. SAS verification: A SAS job is run on each new data set which is designed to check value ranges, valid species, stations, and dates, and various combinations of conditions unique to each data set (Appendix B).

4. After SAS verification and subsequent corrections, the SAS verification is run again until no errors result.

5. Convert SAS data sets to ASCII files for submissions to the Living Resources program and annotate any changes to the program in the Maryland Chesapeake Bay Program Mesozooplankton Taxonomic Data Dictionary (Appendix C).

APPENDIX A

TAXONOMIC REFERENCES

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APPENDIX B

SAS DATA VERIFICATION PROGRAMS

APPENDIX C

MARYLAND CHESAPEAKE BAY PROGRAM MESOZOOPLANKTON TAXONOMIC DATA DICTIONARY