Standard Operating Procedure for Zooplankton Analysis

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December 14, 1994

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1.0 Scope and Application

This method is utilized to identify and enumerate zooplankton populations from the Great Lakes. Zooplankton taxa are identified to the lowest taxonomic rank possible. A listing of all organisms identified and their respective densities and morphometric measurements for biovolume calculations are generated and reported.

2.0 Summary of Method

The method, as developed from Gannon (1971), Stemberger (1979) and Evans et al. (1982), is used to examine a preserved zooplankton sample from a conical net towed vertically through a water column. Microcrustacea are examined in four stratified aliquots under a stereoscopic microscope at 20x magnification. The Rotifera are examined in two equal volume subsamples under a compound microscope at 100x magnification,

3.0 Sample Collection and Preservation

See United States Environmental Protection Agency Central Regional Laboratory Standard Operation Procedure for Zooplankton Sample Collection And Preservation.

4.0 Apparatus

4.1 Stereozoom stereoscopic microscope with 10x to 70x magnification (Bausch and Lomb or another equal quality stereoscopic microscope)

4.2 Compound microscope with 100x to 400x magnification (Bausch and Lomb or another equal quality compound microscope)

4.3 Calibrated Hensen-Stempel pipettes or large bore calibrated automatic pipettes: 1, 2, and 5 mL sizes

4.4 Graduated cylinders: 100, 250 and 500 mL

4.5 Folsom plankton splitter

4.6 Ward counting wheel

4.7 Sedgwick-Rafter counting cell

4.8 Cover glass for Sedgwick-Rafter counting cell

4.9 Microscope slide, 1 x 3 inch

4.10 Cover slip, thickness: #1, 22 mm diameter
4.11 Condenser tubes with 64 pm mesh - over end

4.12 Rubber bulb for condenser tubes

4.13 Microprobe

4.14 Micro-transfer loop

4.15 Micro-forcep

4.16 400 mL glass jars with split fractions written on labels

4.17 2 L waste container

Note: Condenser tube is constructed of a 30 cm long glass tube with an inside diameter of 1.1 cm. A small piece of "Nytex" mesh (5x5 cm_2 and 64 µm pore size) is used to cover one end of the tube and mesh is secured by an 0-ring or a rubber band. A 150 mL heavy duty rubber bulb is attached at the other end of the glass tube to apply suction.

5.0 Reagents

5.1 Formalin (= 37-40% formaldehyde solution)

5.2 5% Sodium hypochlorite solution (Chlorox bleach)

6.0 Analytical Procedure

A complete zooplankton analysis consists of two parts. In the first part, four subsamples (A, B, C, and D Counts) are examined for microcrustaceans at 10-70x magnification and in the second, two subsamples are examined for rotifers at 100x magnification.

6.1 Microcrustacean Sample Analysis

The microcrustacean stratified counting method is described in 6.1.1 and the splitting procedure is summarized in Figure 1.

Note: All containers from which zooplankton are transferred are to be rinsed thoroughly with RO/Dl/distilled water to remove any residual organisms adhering to the container. This includes the Folsom splitter, glass jars, and Ward counting wheels.

6.1.1 Microcrustacean Stratified Counts

6.1.1.1 Sample is divided into two "equal" portions using a Folsom plankton splitter. One subsample from the split is saved in a labeled jar indicating the fraction of total original volume it contains (½).

6.1.1.2 The second subsample from the split is placed in the Folsom plankton splitter and divided again. One subsample is saved in a labeled jar indicating the fraction of the total original volume it contains (¼).

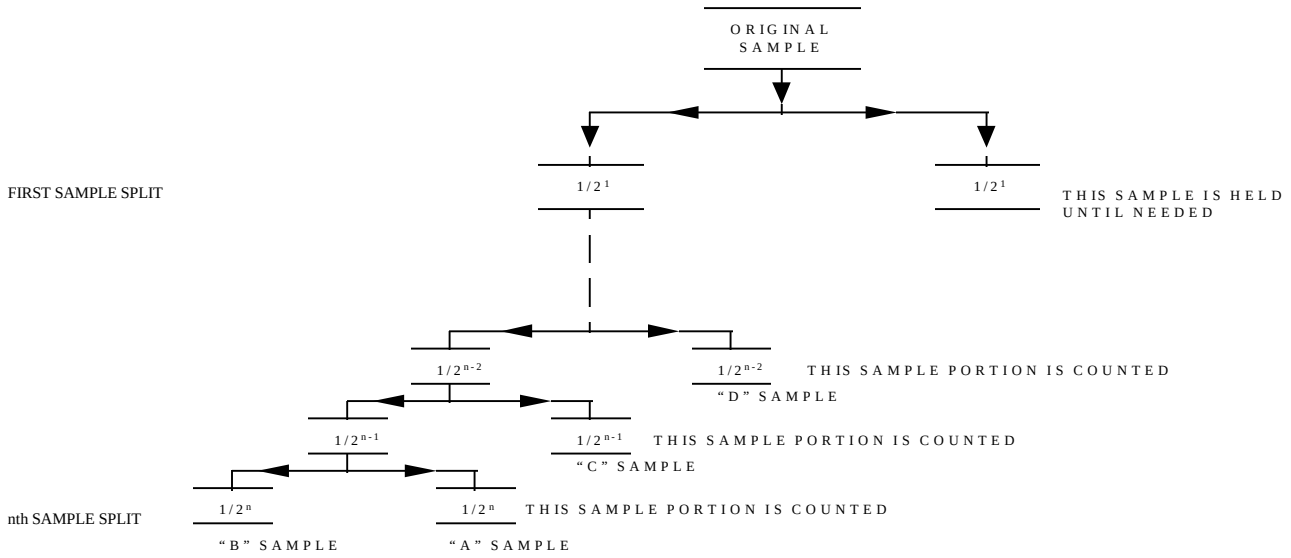
6.1.1.3 Repeat Steps 6.1.1.1 and 6.1.1.2 as many times as necessary until the last two subsamples contain at least 200 and no more than 400 Microcrustacea each (not including nauplii). These two subsamples of equal fraction are saved in appropriately labeled jars.

6.1.2 Four subsamples are to be examined and enumerated. Remove the aqueous portion of the sample with the condensing tube and transfer the remaining organisms in the counting wheel. All Microcrustacea are identified and enumerated under a stereozoom microscope. The four subsamples are listed below in 6.1.2.1, 6.1.2.2 and 6.1.2.3.

6.1.2.1 The final two subsamples which contain 200-400 organisms (see 6.1.1.3) are to be counted first. These are referred to as the A and B Counts. All microcrustaceans are examined and enumerated.

6.1.2.2 A third sample equal in fraction to the sum of the first two (A & B) samples is examined for subdominant taxa (taxa numbered less than 40 in both A and B counts combined). This is the C Count.

6.1.2.3 A fourth subsample equal in fraction to the sum of the first three (A, B and C) counts is examined for large and rare taxa. This is the D count.



LEGEND

"A" AND "B" SAMPLE : THESE SAMPLE PORTIONS ARE THE TWO FINAL SAMPLE VOLUMES.
"C" : THE FIRST PROCEEDING SAMPLE DIVISION.
"D" : THE SECOND PROCEEDUING SAMPLE DIVISION.

*** NOTE: THE ACTUAL FINAL SAMPLE DIVISION ("A" AND "B" COUNTS) WILL BE DETERMINED BY THE DENSITY OF ORGANISMS IN THE ORIGINAL SAMPLE. THE FINAL SAMPLE DIVISION VOLUME MUST HAVE AT LEAST 200 ORGANISMS BUT NOT MORE THAN 400 ORGANISMS IN IT.

6.1.3 Those organisms requiring higher magnification (100-1000x) for identification are mounted on slide and examined under a compound microscope.

6.1.4 When adding the Microcrustacea to the counting wheel make sure that all organisms are settled to the bottom. It is possible to sink the floating Microcrustacea by gently pressing them down using the microprobe.

6.1.5 It is necessary to identify the sex of all mature Copopods encountered.

6.1.6 When duplicate samples are collected in the field, both original and duplicate sample should be analyzed by the same analyst.

6.1.7 If a sample cannot be archived immediately, a few drops of formalin should be added to the sample in order to prevent organisms from clumping.

6.1.8 In order to check for consistency of identification and enumeration, analysts should compare their microcrustacean and rotifer results with historical data. In some occasions, analysts may choose to re-examine archived sample(s) in order to confirm identifications or to clarify some taxonomic problem(s).

6.1.9 After the taxonomic status of a new or unknown organism is decided, the organism should be isolated and placed in a relabeled vial and preserved with 4-6% Formalin. This will serve as the voucher specimen. The label on the vial should include the name of the taxon, date preserved and initials by analysis.

6.1.10 It is important that the voucher specimens are checked periodically so that the lost or damaged ones can be replaced. At least one 'representative' specimen should be available in a vial at all times for examination.

Note: Adult Calanoid and Cyclopoid copepods are identified according to Wilson (1959) and Yeatman (1959) respectively. Adult Harpacticoids are identified to species where possible with the use of Wilson and Yeatman (1959). Immature Copepods are identified at least to suborder (Calanoid, Cyclopoid, or Harpacticoid) and to genus where possible. Nauplii are combined as a group and counted with the rotifers (see Section 6.2). Cladocerans are identified to species except *Diaphanosoma*. Brooks (1957) and Evans (1985) are used for *Daphnia* and Balcer et al. (1984) for *Eubosmina*. The Chydoridae and the remaining Cladocera are identified according to Pennak (1953), Brooks (1959) and Balcer et al. (1984).

6.2 Rotifer Sample Analysis

6.2.1 A jar is selected based on its rotifer density estimated from 6.1.1. The sample is throughly mixed, and a 1 mL subsample is withdrawn with a Hensen-Stempel pipette (or other precalibrated large-bore pipette). The 1 mL subsample described above should contain at least 200 but no more than 400 rotifers and crustacean nauplii. If the subsample contains fewer than 200 organisms, another subsample is taken from a jar with a larger fraction of

the original sample volume. If the subsample contains more than 400 organisms, another subsample from a jar with a smaller fraction is used. If a 1 mL aliquot of the original sample (unsplit) has fewer than 200 organisms, a second 1 mL aliquot reexamined and the results are combined. The volume of the split sample of the jar is then measured in the graduated cylinder.

6.2.2 The subsample is placed in a Sedgwick-Rafter cell and covered with a glass cover slip. All rotifers, microcrustacean nauplii and *Dreissena* veliger and post-veligers are identified and enumerated under a compound microscope at 100x magnification.

6.2.3 After the first rotifer count is completed, a second "duplicate" count from the same jar (6.2.1) equal in volume to the first, is enumerated.

Note: Rotifers are identified to genus and to species where possible according to Pennak (1953), Edmonson (1959), Rutner-Kolisko (1974) and Stemberger (1979). Some rotifers may be indistinguishable by their gross morphology because of their contracted state; therefore, identification of these organisms is determined by examination of their chitinous mouthparts after using sodium hypochlorite bleach as a clearing agent (Stemberger 1979).

6.3 Archiving Microcrustacean And Rotifer Samples

All zooplankton samples are archived after they have been analyzed. These archived samples are an integral part of the quality assurance program of the contract. These samples can be reanalyzed by the contractor (internal) or send to an outside laboratory (external) for quality assurance check(s) or confirmation of identification(s).

6.3.1 All crustacean and rotifer subsamples are combined into a single jar. Depending on the amount of algal materials suspended in the water column, the organisms are allowed to settle (usually from 15 minutes to 1 hour) and the surface water is siphoned off using a condenser tube. The remaining combined sample is transferred to a 125 mL glass "Qorpak" bottle. Fill the sample bottle close to the top with distilled water and add approximately 5 mL of formalin solution to the sample. Label the bottle and the storage box with lake, cruise, station, sampling depth and sample number. All archiving information is computerized using a Word Perfect (Version 5.1) word processing program.

6.3.2 When archiving the sample, the excess water from the splitting jars are placed into a 2 L waste container.

Note: Glass jars and the Folsom plankton splitter should be kept clean to avoid residue buildup.

7.0 Measurements of Microcrustaceans and Rotifers

For the integrated samples (20 m) and B-1 (Lake Erie) samples, it is necessary to take length measurements of microcrustaceans and rotifers:

7.1 Microcrustaceans

The first 20 encounters per species per sample are measured:

Cladocera: Length from the top of the head to the base of the caudal spine.

Copepoda: Length from tip of the head to the insertion of spines into the caudal ramus.

Mysis: Carapace length, or the length from the tip of the head to the cleft in the telson.

Bythotrephes: Body length, excluding the spine.

7.2 Rotifers

The first 20 encounters per species per survey per lake are measured:

Rotifers:

1) Loricate forms: body length from corona to the opposite end at the base of spine (if present).

2) Non-loricate forms: body length from corona to the opposite end, excluding spines, paddles, "toes" or other extensions.

8.0 Calculations

Zooplankton data are reported as number of organisms per cubic meter which are calculated as follows:

8.1 Volume of water filtered

$V = á N_R A$ where;

V = Volume of water filtered (m^3)

á = Flow meter calibration factor (read from the manufacturers calibration graph -varies with flow meter and their respective calibration constants. Some examples of previously used factors:

Meter No.	
4738	0.1408
4473	0.1475
3272	0.1520
3266	0.1465

3099 0.1535

N_R= number of revolutions (read from the flow meter dial)
A = area of the mouth of the net (M2)
= 0. 1 963 m^2 for 0.5 m diameter net

8.2 Microcrustacean densities

$$D = \frac{N \times S}{v}$$

Where D = density of organisms in numbers per cubic meter
N = number of organisms
S = split factor
V = volume of water filtered (from 8.1)

8.3 Rotifer (and nauplii) densities

$$D = \frac{N \times V_s \times S}{N_A \times V}$$

Where D = density of organisms in number per cubic meter
N = number of organisms
N_A= number of 1 mL aliquot examined
V_s = volume of subsamples from which aliquot were removed
S = split factor
V = volume of water filtered (from 8.1)

8.4 Data entry

All microcrustacean and rotifer calculations are made using a Lotus 123 ver. 2.01 worksheet program. Raw data are entered using template file ZOOPLNK.WK1. The following data taken from bench sheets must be entered as "numerical values" (not "labels") onto the worksheet: Number of revolutions; Flow meter calibration factor; Working volume/Subsample volume and Split/Split factor. The following items are to be submitted for data review:

8.4.1 A hard copy of all data entered as well as the calculated results and,

8.4.2 A floppy disk containing all information described in 8.4.1.

Note: Backup/duplicate disks must be made of all data disks submitted to EPA. These disks are to be kept in CRL biology laboratory.

9.0 Quality Control and Methods Precision

9.1 In general, 10% of all samples analyzed are analyzed in duplicate by a second analyst. If a data set has less than 10 samples, at least one sample from that data set is also analyzed in duplicate. Duplicate analyzes include identification and tabulation of data. Data are calculated for the following groups: total immature Copepoda, total mature Copepoda, total Cladocera, total Rotifer and total zooplankton.

The relative percent difference (RPD) between duplicate determinations are compared to guidelines listed below:

Total immature Copepoda	RPD <21%
Total mature Copepoda	RPD <34%
Total Cladocera	RPD <39%
Total Rotifer	RPD <30%
Total Zooplankton	RPD <19%

RPD is calculated as follows:

$$RPD = \frac{(Larger\ Value) - -(Smaller\ Value)}{Average\ Value} \times 100\%$$

9.2 Determinations which exceed control guidelines may require re-analysis unless:

9.2.1 The RPD values are the result of low population for which fewer than 100 individuals have been counted.

9.2.2 The RPD values are the result of a chance occurrence of colonial forms which are enumerated as individuals and which skew the results.

10.0 Safety and Waste Disposal

Proper PPE should be worn in the laboratory while handling and preparing samples for analyses. Follow all laboratory waste disposal guidelines regarding the disposal of Formalin (37% formaldehyde) solutions. Everyday waste should be emptied into a pre-labeled designated waste - drum for Formalin waste. Do not discard samples containing Formalin solutions into the sink.

11.0 References

11.1 Balcer, M.D., N.L. Korda and S.I. Dodson. 1984. Zooplankton of the Great Lakes. A guide to the identification and ecology of the common crustacean species, 174p. Univ. Wisc. Press. Madison.

11.2 Brooks, J.L. 1957. The systematics of North American Daphnia. Mem. Connecticut Acad. Arts and Sci., 13: 1-180.Can. J. Zool. 50: 1373-1403.

11.3 Brooks, J.L. 1959. Cladocera, p. 587-656. *In*: W.T. Edmondson (ed.) Fresh-water Biology, 2nd Ed., Wiley, New York, pp. 1248.

11.4 Edmondson, W.T. 1959. Rotifers, p. 420-494. *In*: W.T. Edmondson (ed.) Fresh-water Biology, 2nd Ed., Wiley, New York, pp. 1248.

11.5 Evans, M. 1985. The morphology of *Daphnia pulicaria*, a species newly dominating the offshore southeastern Lake Michigan summer *Daphnia* community. Trans Amer. Micro. Soc, 104: 223-231.

11.6 Evans, M.S., D.W. Sell and D.I. Page. 1982. Zooplankton studies in 1977 and 1978 at the Donald C. Cook Nuclear Power Plant: Comparisons of preoperational (1971-1974) and operational (1975-1970) population characteristics. Univ. Michigan. Great Lakes Res. Div. Spec. Rep. 89.

11.7 Gannon, J.E. 1971. Two counting cells for the enumeration of Zooplankton micro-Crustacea. Trans Amer. Micros. Soc. 90: 486-490.

11.8 Pennak, R.W. 1953. Freshwater invertebrates of the United States. The Ronald Press Co., N.Y., pp. 769.

11.9 Rutner-Kolisko, A. 1974. Planktonic rotifers: Biology and taxonomy. Die Binnengewasser 26(l) Supplement: 146 pp.

11.10 Stemberger, R.S. 1979. A guide to rotifers of the Laurentian Great Lakes, U.S. Environmental Protection Agency, Rept. No. EPA 600/4-79-021, 185 pp.

11.11 U.S.E.P.A. 1994. United States Environmental Protection Agency Central Regional Laboratory Standard Operation Procedure for Zooplankton Sample Collection and Preservation.

11.12 Wilson, M.S. 1959. Calanoida, P. 738-794. *In*: W.T, Edmondson (ed.) Fresh-water Biology, 2nd Ed., Wiley, New York, pp, 1248.

11.13 Wilson, M.S. and H.C. Yeatman. 1959. Harpacticoida, p. 815-861. *In*: W.T. Edmondson (ed.) Fresh-water Biology, 2nd Ed., Wiley, New York, pp. 1248.

11.14 Yeatman, H.C. 1959. Cyclopoida, p. 795-814. *In*: W.T. Edmondson (ed,) Fresh-water Biology, 2nd Ed., Wiley, New York, pp. 1248.

Appendix 1.

Date Received	Data Set	Sample Type	Lab Number	River or Lake	Station	Depth	CRL Number	Notes

Appendix 2.

ZOOPLANKTON ANALYSIS: ROTIFERS

Lake: _____ Sample No.: _____ Lab No.: _____
Date Collected: _____ Station: _____ St. Captain: _____
Depth of tsw (m): _____ Revs: _____ FM #: _____
Analyzed by: _____ Analyzed: _____

Working Volume (mL): _____ Split: _____
Mililiters in subsample: _____ _____

ORGANISM	A	B
Ascomorena ovalis		
Aspianenna priodonta		
Bdailoid Rotifera		
Bracniamus		
Collethaca		
Conocailcicas		
Conocailas unicornis		
Filinia longiseta		
Gastrosus stylifar		
Kelliserssia longispina		
Keratalla cocalearsis		
Keratalla crassa		
Keratalla eartinae		
Keratalla nimnalis		
Keratalla quadrats		
Nothsica folicae		
Nothsica laurantiia		
Nothsica squmula		
Plossoma truncarum		
Polyarenra colicrsstari		
Polyarena major		
Polyarena remata		
Polyarena vulgaris		
Syncnista spp.		
Trisassarci similis		
Trisassarci cylinarica		
Trisassarci multisariais		
Copepod nauplii:	_____	_____
Braissana polymorena:		
Veligar	_____	_____
Post-Veligar		

Appendix 3.

MICROCRUSTACERAS

Sample no.: _____ Lab. no.: _____

Split factor:

	FA	MA	FB	MB	FC	MC	FD	MD
Cyclops bicuspidamus tn.								
Cyclops vernalis								
Eucyclops agalis								
Eurycamera affinis								
Mesocyclops edax								
Tropocyclops orasims m.								
Diaptomas ashlandi								
Diaptomas minnons								
Diaptomas oragonansis								
Diaptomas sicillis								
Diaptomas siciloidas								
Epischura lacusteris								
Limnocaalanus macruras								
Senecaila calandidas								
TOTAL MATURE COPEPODA:								
Sosmina longirostria								
Chycorus spasarious								
Dapania longiramis								
Dapania pulicaria								
Dapania retrucurva								
Diapnanosoma birgil								
Eubosmina corageni								
Holopadium gibberum								
Lapeseora kingstil								
Polypnamus pediculus								
Eythessrapnes cadarstream								
Capnnia scnoadlar								
TOTAL CLADOCIRA:								
Cyclops copepoditas								
Mesocyclops copepoditas								
Tropocyclops copepoditas								
Diatemus copepoditas								
Episcnura copepoditas								
Limnocaianus copepoditas								
Senecalla copepoditas								
Eurytamora copepoditas								
TOTAL IMMATURE COPEPODA (including naupilli):								
Other organisms								

Appendix 4.

Sample #: _____ Analyst: _____
Lake: _____ Station: _____
Cruise: _____ Run: _____

Microcrustacean Measurements

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	°Cal
Cyclops biscuspidieus (homas) ♀																					
♂																					
Cyclops vernalis ♀																					
♂																					
Eucyclops agilis ♀																					
♂																					
Mesocyclops edas ♀																					
♂																					
Tropocyclops prasimus mesicams ♀																					
♂																					
Diaplomus aslandi ♀																					
♂																					
Diaplomas minmims ♀																					
♂																					
Diapolmas oregonensis ♀																					
♂																					
Diaplomus sicilis ♀																					
♂																					
Diaplomus siciloides ♀																					
♂																					
Epishura lacustras ♀																					
♂																					
Eurytemora affinis ♀																					
♂																					
Limnocaianus macrurus ♀																					
♂																					
Senecella calanoides ♀																					
♂																					
Bythotrephes cedarstroemi																					
Besmina logiresiris																					
Ceriodaphnia																					
Chydorus spheecricus																					
Daphnia galcaia mendelee ♀																					
♂																					
Daphnia longiremis ♀																					
♂																					

Appendix 5.

Sample #: _____

Microcrustacean Measurements

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	°Cal
Daphnia pulicaria ♀																					
♂																					
Daphnia retrocurpe ♀																					
♂																					
Eubesmina coregena																					
Ileopedium gibberum																					
Laptodera																					
Polyphemus pediculus																					
Cyclops copepoditas																					
Mesocyclops copepoditas																					
Tropocyclops copepodites																					
Diacyclops copepodites																					
Epishara copepoditas																					
Limnocalanus copepodites																					
Senacotta copepodites																					