

Standard Operating Procedure for Zooplankton Analysis

LG403

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Standard Operating Procedure for Zooplankton Analysis

1.0 SCOPE AND APPLICATION

- 1.1 This method, as developed from Gannon (1971), Stemberger (1979) and Evans *et al.* (1982), is used to identify and enumerate the zooplankton populations from the Great Lakes.

2.0 SUMMARY OF METHOD

- 2.1 The method involves microscopic examination of preserved zooplankton samples collected with a conical net towed vertically through a water column. Microcrustacea are examined in four stratified aliquots under a stereoscopic microscope. Rotifera are examined in two equal volume sub-samples under a compound microscope.

3.0 SAMPLE COLLECTION AND PRESERVATION

- 3.1 See U.S. EPA GLNPO Standard Operation Procedure (SOP) for Zooplankton Sample Collection and Preservation (LG 402).

4.0 APPARATUS

- 4.1 Most supplies can be acquired from biological supply companies (such as Wildlife Supply Company). The supplies needed are as follows:

Dissecting microscope with 10x to 50x magnification
Compound microscope with 100x to 1000x magnification 1-mL
Calibrated Hensen-Stempel pipette
100-, 250- and 500-mL graduated cylinders
Folsom plankton splitter
Ward counting wheel or other suitable counting chamber
Sedgwick-Rafter counting cell
Cover glass for Sedgwick-Rafter counting cell
Microscope slides, 1 x 3 inch
Cover slips
Tubes for concentrating plankton samples (see below)
Small sieves with 63- and 500- μ m mesh
63- μ m Nitex mesh
Heavy duty rubber bulb
Microprobe
Micro-forceps
100- to 500-mL glass jars with split fractions written on labels (2-2048)

- 4.2 The plankton concentrating tube is constructed by covering one end of a wide glass tube (such as a chromatography tube) with 64- μ m mesh. The mesh is secured with O-rings and a heavy-duty bulb is attached to the other end to provide suction.

5.0 REAGENTS

5.1 Reagents can be ordered through chemical supply companies. CMC has been acquired through Master's Company, Inc.

5.2 The reagents needed are as follows:

Formalin (37% formaldehyde solution)

Ethanol

5% Sodium hypochlorite solution (Clorox bleach)

CMC-9, CMC-10, Hoyer's or other suitable mounting medium for mounting and clearing slide-archived specimens

Rose Bengal stain dissolved in ethanol

Dilute solution of laboratory detergent

6.0 ANALYTICAL PROCEDURE: MICROCRUSTACEAN SAMPLE ANALYSIS

6.1 Microcrustacean Stratified Splitting

6.1.1 When zooplankton samples are returned to the lab, approximately 1 to 3 mL of Rose Bengal stain solution may be added to each sample to aid in finding the smaller organisms. Samples should be processed one at a time. Under the hood, rinse the sample from the sample bottle through a 63- μm (or smaller) mesh sieve with DI water to remove the formalin. Set the formalin aside under the hood in a sealed labeled bottle to use at the completion of analysis (see 7.4)

6.1.2 Be sure to rinse the sample bottle thoroughly with DI water into the 63- μm (or smaller) mesh sieve to remove any residual organisms adhering to walls of the bottle. All containers from which zooplankton are transferred are to be rinsed thoroughly, including the Folsom splitter, glass jars, and counting chambers. Wash the sample into a glass jar. Adding a small amount of dilute laboratory soap to each sample at this time aids in preventing organisms from sticking to the sides of the containers and from floating at the surface of the sample.

6.1.3 If the sample contains large clumps of *Cercopagis*, then:

6.1.3.1 Carefully remove the clumps with forceps and place them in a glass tray.

6.1.3.2 Gently rinse the clumps with a stream of DI water in the tray, while pulling at the clumps with forceps to free trapped organisms.

6.1.3.3 Return "freed" organisms other than *Cercopagis* and *Bythotrephes* and rinse water to the sample and split as usual.

6.1.3.4 Reserve the clumps of *Cercopagis* in a separate jar and subsample if abundant (> 250 individuals, see 6.2.1.5). Also note all *Bythotrephes* identified during this counting process.

- 6.1.4 Stir the sample gently to break up algal clumps and then pour the entire sample into the Folsom plankton splitter. Stir the sample again in a figure-8 pattern to distribute animals uniformly and split the sample by immediately rotating the splitter before the organisms can settle. Rinse the inside of the splitter well to remove organisms that may stick to the sides. Rinse one sub-sample from the splitter receiving trays and save it in a labeled jar indicating the fraction of total original volume it contains.
- 6.1.5 The second sub-sample from the split is placed in the Folsom plankton splitter and divided again. One sub-sample is saved in a labeled jar indicating the fraction of the total original volume it contains.
- 6.1.6 Repeat Steps 6.1.4 and 6.1.5 as many times as necessary until the last 2 sub-samples contain at least 200 and no more than 400 microcrustaceans each (not including nauplii). These 2 sub-samples represent equal fractions of the original sample. One sub-sample is saved in a jar with the appropriately labeled split, and the other sub-sample is saved in a jar labeled “B”.

6.2 Microcrustacean Enumeration

- 6.2.1 Four sub-samples are to be examined and enumerated. The sub-sample is concentrated by using the small sieve or the condensing tube and placed in a circular (or other suitable) counting chamber. All microcrustaceans are identified and enumerated under a dissecting microscope. The four sub-samples are counted using the criteria listed below in 6.2.1.1, 6.2.1.2, and 6.2.1.3. Refer to Figure 1 for a diagram of the splitting process.
- 6.2.1.1 The final two sub-samples which contain 200 - 400 organisms (see 6.1.6) are to be counted first. These are referred to as the A and B Counts. All microcrustaceans (except nauplii) are examined and enumerated. The first twenty intact individuals for each SPECCODE are measured for length (Section 8.1). If the sub-samples contain a large amount of algae, it may be necessary to pick out the organisms and transfer them to a clean counting chamber prior to identification.
- 6.2.1.2 A third sample equal in fraction to the sum of the first two (A + B) samples is examined for subdominant taxa (taxa encountered less than 40 times in A and B counts combined). This is the C Count.
- 6.2.1.3 A fourth sub-sample equal in fraction to the sum of the first three (A, B, and C) counts is examined for large taxa. This is the D count. Large taxa include *Limnocalanus macrurus*, *Senecella calanoides*, *Epischura lacustris*, *Holopedium gibberum*, *Diaphanosoma birgei*, *Leptodora kindti* and *Polyphemus pediculus*. If a taxon defined as “large” has a sum of more than 40 individuals in counts A, B, and C, it is not necessary to enumerate them in D count.
- 6.2.1.4 When a taxa is detected during this sequence of subsampling, all subsamples that this taxa was searched for but not found are referred to as true zero counts.

Subsamples for which these taxa were not searched for should be identified with a “-1” in the submission file. In the data submission, number of a taxa for the entire sample (NUMJAR) is calculated by dividing the sum of all subsample counts for a taxa by the proportion of the sample searched.

- 6.2.1.5 The entire sample must be examined for *Mysis*, *Bythotrephes* and *Cercopagis*. All individuals are counted, but only the first twenty individuals encountered for these three taxa are measured. As previously described, they can be removed from the sample prior to splitting if they are numerous. Otherwise they should be enumerated from A, B, C and D splits as they are examined. After these splits have been counted, pour the uncounted portion through a 500- μ m mesh sieve. Examine the organisms trapped in the sieve for the three taxa. All counts for these three taxa refer to the entire sample (WHOLE, split factor 1). If *Cercopagis* is numerous (> 250), subsampling of the clump is appropriate. Weighing the clump and the subsample removed provide an estimate of proportion counted, enabling the expansion to an estimate of the whole sample count. The balance used should have precision of at least 0.001g. Remove as much water as possible from the wet clump before weighing and subsampling. The number reported in the data submission is the estimate for the whole sample, but sample notes should report that this subsampling occurred and include the weights of the clump and subsample and the count of the subsample.
- 6.2.1.6 If a backlog in sample analysis occurs, the GLNPO project officer can give permission for a deviation in the protocol for the analysis of D20 counts that omits the “B” subsample. NUMJAR is calculated from only the subsamples searched as described in 6.2.1.4. In this case, if 20 or more individuals of a taxa are counted in the “A” subsample, the analyst does not need to search for the taxa in the “C” subsample. This deviation in sample analysis was granted for: Lake Michigan 2012 Summer, Lake Erie 2012 Spring and Summer, all lakes excepting Lake Ontario 2013 Spring and Summer, Lake Superior 2014 Spring.

6.2.2 General Analysis Guidelines

- 6.2.2.1 Those organisms requiring higher magnification for identification are examined at 100 - 1000x magnification under a compound microscope.
- 6.2.2.2 While counting Microcrustacea, make sure that all organisms are settled to the bottom. It is possible to sink floating Microcrustacea by gently pressing them down using the microprobe or by adding a drop of dilute laboratory detergent.
- 6.2.2.3 It is necessary to identify and record the sex of all mature Copepods encountered.
- 6.2.2.4 If replicate samples are collected in the field, all samples from that station should be analyzed by the same analyst. Shallow and deep tows from each station should also be analyzed by the same analyst.
- 6.2.2.5 If a sample cannot be completely counted and archived within 2 days, the

sample should be kept in the refrigerator and a few drops of formalin can be added to the jars to prevent organisms from clumping. Sample analysis should not extend beyond four days.

- 6.2.2.6 In order to check for consistency of identification and enumeration, analysts can compare their microcrustacean and rotifer results with historical data. On some occasions, analysts may choose to re-examine archived samples in order to confirm identifications or to clarify taxonomic problems.
- 6.2.2.7 Occasionally, organisms are encountered which do not already appear on the species list. After the taxonomic status of such an organism is determined, the organism should be placed in a labeled vial and preserved with ethanol. The label in the vial should include genus/species name, date preserved, analyst initials, station number, and sample number. This will serve as a voucher specimen. The voucher specimen should be sent out for external confirmation, then a report made to the Work Assignment Manager (WAM) including the distinguishing characteristics used to identify the new organism, and suggestions as to why it has not been encountered in the past (e.g., it is primarily benthic or littoral). Only AFTER written notification of acceptance of the new organism by the WAM should that species be added to the species list.
- 6.2.2.8 In order to help assure consistency of identification, a voucher specimen collection, based on the species list, should be maintained. Voucher specimens are preserved and stored as stated in 6.2.2.7.
- 6.2.2.9 It is important that the voucher specimens are checked periodically so lost or damaged animals can be replaced. At least one male and one female (preferably 3 - 5) representative specimen should be available at all times for examination.

6.3 Taxonomic References

- 6.3.1 Adult calanoids are identified according to Balcer *et al.* (1984). Adult cyclopoids and Harpacticoids are identified according to Hudson *et al.* (1998). Immature calanoids and cyclopoids are identified to the lowest taxonomic level possible, usually suborder or genus (Czajka 1982). Nauplii are counted with rotifers. Hudson and Lesko (2003) provides supplementary information on Great Lake copepod identification. Malacostracans (i.e., *Mysis relicta*) are identified according to Mauchline (1980). Malacostracans are not collected efficiently with our zooplankton tows but they are enumerated and measured for historical purposes. The following cladocerans are identified according to Balcer *et al.* (1984): *Leptodora kindtii*, *Polyphemus pediculus*, *Holopedium gibberum*, and *Diaphanosoma birgei*. Brooks (1959) and Evans (1985) are used for all Daphnidae. The remaining cladocerans (Chydoridae, Bosminidae, and Macrothricidae) are classified according to Brooks (1959). Members of Cercopagidae (i.e., *Bythotrephes longimanus*, and *Cercopagis pengoi*) are identified according to Rivier (1998).

7.0 ANALYTICAL PROCEDURE: ROTIFER SAMPLE ANALYSIS

7.1 Rotifer, Nauplii and Veliger Sub-sampling

- 7.1.1 Rotifers, nauplii, and veligers are only counted from the tow taken with the 63- μm mesh net. Tows taken with the larger mesh (153- μm) will not capture sufficient numbers of these small taxa.
- 7.1.2 Selection of the split level from which aliquots for rotifer enumeration are taken is based on estimates from previous samples within the data set, or from estimates made during microcrustacean enumeration (rotifers are visible in the dissecting microscope).
- 7.1.3 Separate 1-mL aliquots are taken from the appropriate split, and rotifers, nauplii and veligers are counted from these aliquots. The goal is to count 200 to 400 animals (rotifers and nauplii, not including veligers). The sample should be mixed thoroughly, and the 1-mL aliquot withdrawn with a Hensen-Stempel pipette (or other precalibrated large-bore pipette). In cases when less than 200 animals are encountered in the 1- mL aliquot, additional 1-mL aliquots can be used for each count (see 7.1.5).
- 7.1.4 Repeat this process for a duplicate count. These are referred to as A and B counts.
- 7.1.5 In cases of extremely low rotifer densities, the sample may be concentrated prior to taking aliquots with the pipette. The maximum number of 1-mL aliquots counted from an unsplit sample is 3 per count (i.e., a total of 6 mL), even if the sum does not reach 200 organisms.

7.2 Sedgwick-Rafter Cell Preparation and Rotifer Enumeration

- 7.2.1 The 1-mL aliquot is placed in a Sedgwick-Rafter cell and covered with a glass cover slip.
- 7.2.2 All rotifers, microcrustacean nauplii, and *Dreissena* veligers are identified and enumerated under a compound microscope at 100x magnification. Length measurements are done on the first 20 intact individuals encountered. Some rotifer species require width measurements (Section 8.2).
- 7.2.3 Veligers are enumerated for historical record with recognition of the variability in the reproductive cycle of *Dreissena*. If veligers are extremely abundant (> 100) in the A count, they do not need to be counted in the B count but that deviation must be noted. In those cases, a “-1” should be entered in the ROTB field and ROTAB=ROTA.
- 7.2.4 After the counts are completed, volume of the split used, including the volume of the aliquots, is measured, and this information and the split factor is recorded in the count spreadsheet.

7.3 Taxonomic References

7.3.1 Rotifers are identified to genus and to species where possible according to Edmondson (1959) and Stemberger (1976). 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). This is a time-consuming process that destroys the rotifer and does not often produce clear results. Therefore, in an effort to use lab time efficiently, the bleaching process is most commonly used only as a training technique or in the instance of fairly common organisms with questionable identification.

7.4 Archiving Microcrustacean and Rotifer Samples

7.4.1 After analysis all subsamples are concentrated and returned to the original labeled sample bottle with the original formalin solution from that sample (see 6.1.1).

7.4.2 Sample archiving for a sample set is done after the data submission has been reviewed and accepted. When time to archive, the surface water is siphoned off using a condenser tube or the sample is concentrated using the 63- μm mesh (or smaller) sieve. The concentrated sample is transferred to a 125- mL glass Qorpak bottle.

7.4.3 Fill the sample bottle close to the top with DI and add approximately 5 mL of 37% formalin solution to the sample.

7.4.4 Label the bottle and the storage box with lake, station, lab number, and sample number. All archiving information should be computerized using a word processing program.

8.0 CALCULATION OF MICROCRUSTACEAN AND ROTIFER BIOMASS

8.1 Microcrustacean Biomass

8.1.1 Biomass (dry weight in μg) of microcrustaceans is calculated from formulas relating some linear measurement (usually body length in mm) to body weight. A compilation of the formula references and constants can be found in Appendix 1. Formulas are derived from a number of sources, but are of the general form:

$$\ln W = \ln a + b \ln L$$

Where:

$\ln w$	=	natural logarithm of the dry weight estimate (μg)
$\ln a$ and b	=	species specific constants (listed in appendix)
$\ln L$	=	the length of a measured individual (mm).

Overall biomass for a taxa is calculated by averaging the dry mass of up to the first 20 measured individuals and multiplying by the density.

- 8.1.2 Zooplankton may be measured by use of a calibrated eyepiece micrometer or camera lucida/tablet system during the identification and enumeration process, or they may be removed from the sample, photographed with a digital camera, and measurements calculated from the images. The first twenty intact individuals are measured for each taxa.

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

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 caudal process.

Cercopagis: Body length, from the top of the eye to the end of the caudal claws.

NOTE: *If the organisms are curved or bent, several straight line measurements should be made and summed to obtain total length.*

- 8.1.3 Since the length/weight relationship for *Holopedium gibberum* was developed based on the length of the foot, the equation from the original reference (Persson and Ekbohm 1980) has been modified for input of the total length, that is assumed to be 4 times the foot length.
- 8.1.4 *Copepoda nauplii* follow a length-weight equation appropriate for all life stages of copepods developed by Bottrell et al. 1976. The equation coefficients are in the crustacean factor list (Appendix 1).
- 8.1.5 Dreissenid veligers follow a length-weight equation developed by Kelly Bowen DFO Canada (personal communication).

$$\text{weight } (\mu\text{g}) = 0.025e^{17.5(L \text{ in mm})}$$

This equation is appropriate for individuals smaller than 300 μm .

8.2 Rotifer Biomass

- 8.2.1 Rotifer biomass (μg) is calculated according to A. Ruttner-Kolisko (appendix in Bottrell *et al.* 1976). For most rotifers, calculations use a formula (EQ #1 in Appendix 2) with the general form:

$$\text{Rotifer biomass } (\mu\text{g}) = [(\text{length}^3 \times \text{FF}) + (\% \text{BV} \times \text{length}^3 \times \text{FF})] \times 10^{-6} \times \text{WW} : \text{DW}$$

Where:

$$\mu\text{g} = \text{biomass of individual}$$

Length	=	total length in μm
FF	=	species specific formula for factor (see Appendix 2)
% BV	=	volume of appendages as a percent of body biovolume (see Appendix 2)
10 ⁻⁶	=	conversion to wet weight; assuming a density of 1
WW:DW	=	wet weight to dry weight conversion (see 8.2.2)

Note that the units of length and width measurements are in μm in the rotifer formulas and in mm in the crustacean formulas.

8.2.2 A wet weight/dry weight conversion factor of 0.1 (Doohan, 1973) is used for all genera except *Asplanchna*, for which a factor of 0.039 (Dumont *et al.*, 1975) is used.

8.2.3 For the genus *Collotheca*, width is measured, and the following formula (EQ #2 in Appendix 2) used:

$$\text{Collotheca biomass } (\mu\text{g}) = (\text{width}^3 \times \text{FF}) \times 10^{-6} \times \text{WW} : \text{DW}$$

8.2.4 For the genera *Conochiloides*, *Conochilus*, *Filinia*, and *Trichocerca*, both length and width are measured, and the following formula (EQ #3 in Appendix 2) used:

$$\text{Biomass } (\mu\text{g}) = [(\text{length} \times \text{width}^2 \times \text{FF}) + (\% \text{BV} \times \text{length} \times \text{width}^2 \times \text{FF})] \times 10^{-6} \times \text{WW} : \text{DW}$$

8.2.5 The genera *Conochiloides* and *Conochilus*, have no appendages (%BV=0) that removes one term from the equation above:

8.2.6 Measurements are made for all intact individuals as follows:

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

8.2.6.2 Non-loricated forms: body length from corona to the opposite end, excluding spines, paddles, “toes” or other extensions.

9.0 CALCULATIONS AND REPORTING

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

9.1.1 Volume of water filtered:

$$V = \alpha N R A$$

Where:

- V = Volume of water filtered (m³)
- A = Flow meter calibration factor (see 9.2)
- N_R = Number of revolutions (read from the flow meter dial)
- A = Area of the mouth of the net (m²) = 0.19634954 m² for 0.5-m diameter net

9.1.2 Microcrustacean Densities

$$D = N/(P_T * V)$$

Where:

- D = Density of organisms in numbers per cubic meter
- N = Number of organisms counted in all splits searched
- P_T = The proportion of the total sample used to enumerate the taxon (sum of all inverse split factors).
- V = Volume of water filtered (from 9.1.1)

The overall count for a specific taxa in an entire sample is calculated by dividing the total number of organisms counted in all searched splits by the proportion of the sample in which each taxa was enumerated.

9.2 Flowmeters are calibrated during each cruise (see Zooplankton Sample Collection SOP, LG402). The calibration factor is calculated by dividing tow depth in meters by the average number of flowmeter counts recorded during the tows. This information should be recorded in the field notebook for each cruise, and also entered into the field sampling spreadsheet and database.

9.2.1 The formula for flowmeter calibration is as follows:

$$\alpha = \frac{d}{N_{RR(average)}}$$

Where:

- α = Flowmeter calibration
- d = Sample depth in meters
- N_{R(ave)} = Number of flowmeter counts, averaged for 20 calibration tows

9.3 Rotifer (and Nauplii) Densities

9.3.1 Calculate the densities of rotifers and nauplii using the following formula:

$$D = (N * V_S) / (N_A * V * P_S)$$

Where:

- D = Density of organisms in number per cubic meter
- N = Number of organisms
- N_A = Number of 1-mL aliquots examined
- V_S = Volume of split from which aliquots were removed in ml (including aliquots)
- P_S = The proportion of the total sample represented by the volume from which aliquots were removed (inverse of split factor).
- V = Volume of water filtered in m³ (from 9.1.1)

Density is calculated from the sum of individual counts (ROTAB) taking in consideration the total subsample volume searched (sum of SUBMLA and SUBMLB). For veligers where no B count is done, ROTA is multiplied by 2.

9.4 Data Entry

9.4.1 All microcrustacean and rotifer calculations are made using a spreadsheet program such as Excel or a database program such as Microsoft Access.

9.5 Data Submission to GLNPO

9.5.1 A multi-worksheet Excel data file can be initially submitted electronically for initial review. After final approval the following items are to be submitted on a CD including a cover letter. The date written on the CD should be the date of last changes to the approved data file on the disc.

9.5.2 The data as an Excel file in the GLNPO zooplankton template format.

9.5.3 Electronic copies of all field sheets (pdf format), laboratory count sheets (Excel) and Access database.

9.5.4 Backup/duplicate CD's or DVD's must be made of all data disks submitted to EPA.

10.0 QUALITY CONTROL AUDITS AND METHODS PRECISION

10.1 In general, ten percent 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 should also be analyzed in duplicate.

10.2 For QA counts, the second analyst counts the same split for crustaceans and the same Sedgewick Raftercell aliquots for rotifers, so that only interanalyst variation is quantified,

and not variation associated with sub-sampling. The length measurements should be done on the same individuals, again to ensure that only interanalyst variation is quantified.

- 10.3** Results from the second analyst are reported under the same sample number as the original sample, with the exception that the seventh character is replaced by a “Q.”
- 10.4** Percent similarity for density and biomass will be calculated for the samples analyzed in duplicate by two analysts, according to the following formula:

$$PSC = 1 - 0.5 \sum_{i=1}^K |a - b|$$

Where:

a and *b* are, for a given species, the relative proportions of the total samples *A* and *B*, respectively, which that species represents.

- 10.5** It is expected that the two counts should have a similarity of 90%. If not, the reasons for the discrepancies between analysts should be discussed. If a major difference is found in how the two analysts have been identifying or measuring organisms, the last batch of samples that have been counted by the analyst under review may have to be recounted. Even if a density-based PSC passes, the biomass-based PSC could fail if interanalyst variation in length measurements is large.
- 10.6** For D20 samples, the PSC should be calculated from the entire sample, including both the crustacean and rotifer count.

11.0 SAFETY AND WASTE DISPOSAL

- 11.1** 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. **Do not discard formalin solutions into the sink unless previously diluted as directed by your laboratory health and safety officer.**

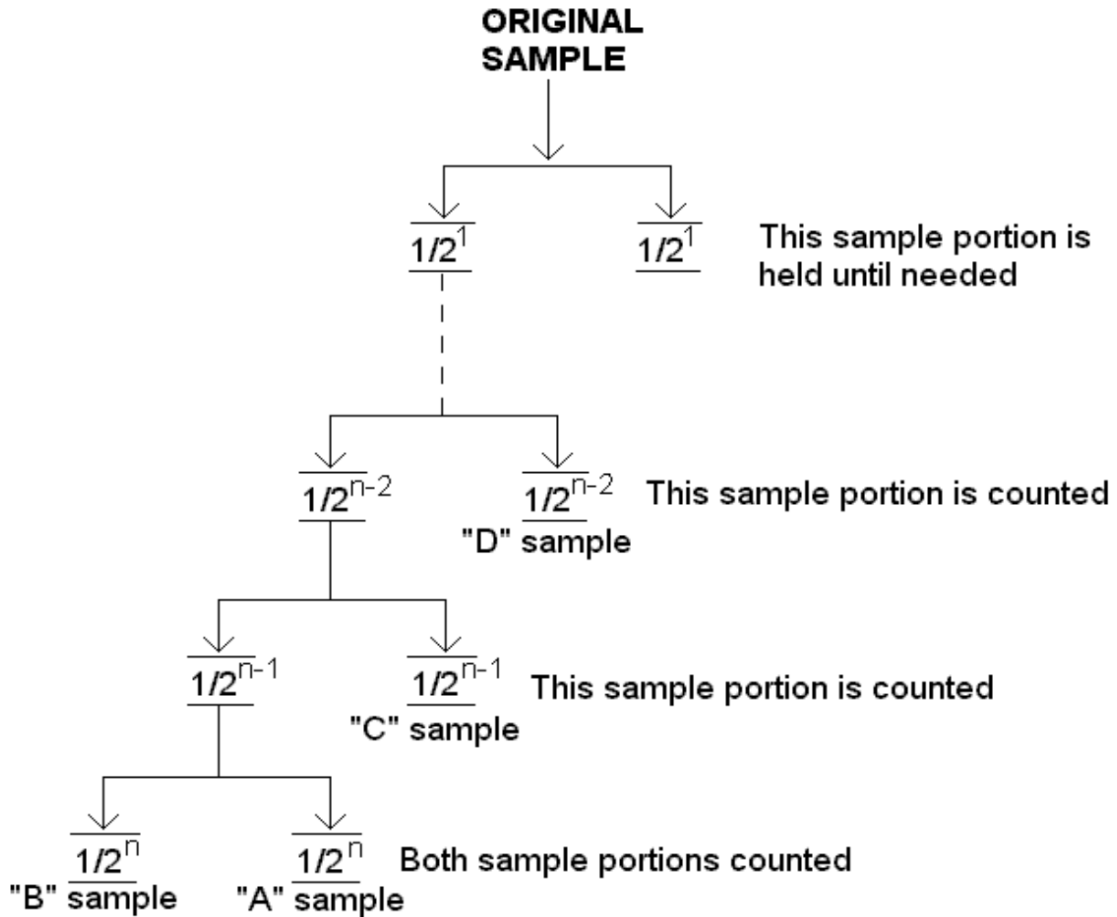
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FIGURE 1: ZOOPLANKTON SAMPLE SPLITTING DIAGRAM



LEGEND:

- "n": the final split level
- "A" and "B" sample: these sample portions are the two final sample volumes
- "C": the first preceding sample division
- "D": the second preceding sample division

NOTE: The actual final sample division will be determined by the density of the organisms in the original sample. The first sample volume must have at least 200 organisms but not more than 400 organisms.

APPENDIX 1: CRUSTACEAN FORMULA FACTORS

SPECCODE	Species	LNA	B	Species Used	Reference
ACAVERN	<i>Acanthocyclops vernalis</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981
ACRHARP	<i>Acroperus harpae</i>	1.167	0.85	<i>Acroperus harpae</i>	Dumont et al., 1975
ALNGLOB	<i>Alonella globulosa</i>	2.1	2.26	<i>Alonella exigua</i> w eggs	Dumont et al., 1975
ALOAFFI	<i>Alona affinis</i>	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
ALOGUTT	<i>Alona guttata</i>	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
ALOQUAD	<i>Alona quadrangularis</i>	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
ALORECT	<i>Alona rectangula</i>	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
ALOSP	<i>Alona spp.</i>	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
BOSLONG	<i>Bosmina longirostris</i>	2.712	2.53	<i>Bosmina longirostris</i>	Bottrell et al., 1976
BOSSP	<i>Bosmina spp.</i>	2.712	2.53	<i>Bosmina longirostris</i>	Bottrell et al., 1976
BYTLONG	<i>Bythotrephes longimanus</i>	2.83	2.09	<i>Bythotrephes cederstroemi</i>	Makarewicz & Jones (1990)
CALCOPE	Calanoid copepodites	1.59	2.59	generic calanoid	Burgess et al. 2015
CAMRECT	<i>Camptocercus rectirostris</i>	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
CAMSP	<i>Camptocercus spp.</i>	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
CANCOPE	<i>Canthocamptus</i> copepodites	1.59	2.59	generic calanoid	Burgess et al. 2015
CANOREG	<i>Canthocamptus oregonensis</i>	1.59	2.59	generic calanoid	Burgess et al. 2015
CANROBE	<i>Canthocamptus robertcokeri</i>	1.59	2.59	generic calanoid	Burgess et al. 2015
CANSP	<i>Canthocamptus spp.</i>	1.59	2.59	generic calanoid	Burgess et al. 2015
CECPENG	<i>Cercopagis pengoi</i>	0.488	2.98	<i>Cercopagis pengoi</i>	Ojaveer et al. 2001
CERLACU	<i>Ceriodaphnia lacustris</i>	2.83	3.15	<i>Ceriodaphnia reticulata</i>	Pace & Orcutt 1981
CERRECT	<i>Ceriodaphnia reticulata</i>	2.83	3.15	<i>Ceriodaphnia reticulata</i>	Pace & Orcutt 1981
CERSP	<i>Ceriodaphnia spp.</i>	2.83	3.15	<i>Ceriodaphnia reticulata</i>	Pace & Orcutt 1981
CHYDORI	Chydoridae	4.543	3.64	<i>Chydorus sphaericus</i>	Rosen 1981
CHYSPHA	<i>Chydorus sphaericus</i>	4.543	3.64	<i>Chydorus sphaericus</i>	Rosen 1981
COPNAUP	Copepod nauplii	1.953	2.4	pooled copepod	Bottrell et al., 1976
CYCCOPE	<i>Cyclops copepodites</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981

SPECCODE	Species	LNA	B	Species Used	Reference
CYCSTRE	<i>Cyclops strenuus</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981
DAPAMBI	<i>Daphnia ambigua</i>	1.603	2.84	Daphnia 'pooled'	Dumont et al., 1975
DAPCATA	<i>Daphnia catawba</i>	1.603	2.84	Daphnia 'pooled'	Dumont et al., 1975
DAPDUBI	<i>Daphnia dubia</i>	1.603	2.84	Daphnia 'pooled'	Dumont et al., 1975
DAPGALEM	<i>Daphnia galeata mendotae</i>	1.603	2.84	Daphnia 'pooled'	Dumont et al., 1975
DAPLAEV	<i>Daphnia laevis</i>	1.603	2.84	Daphnia 'pooled'	Dumont et al., 1975
DAPLONG	<i>Daphnia longiremis</i>	1.603	2.84	Daphnia 'pooled'	Dumont et al., 1975
DAPLUMH	<i>Daphnia lumholtzi</i>	0.056	2.84	Daphnia 'pooled' with correction for spine	Dumont et al., 1975, with a correction removing proportion of length representing spine (0.58*length) (Cornell)
DAPMIDD	<i>Daphnia middendorffiana</i>	1.603	2.84	Daphnia 'pooled'	Dumont et al., 1975
DAPPARV	<i>Daphnia parvula</i>	1.603	2.84	Daphnia 'pooled'	Dumont et al., 1975
DAPPULE	<i>Daphnia pulex</i>	1.603	2.84	Daphnia 'pooled'	Dumont et al., 1975
DAPPULI	<i>Daphnia pulicaria</i>	1.603	2.84	Daphnia 'pooled'	Dumont et al., 1975
DAPRETR	<i>Daphnia retrocurva</i>	1.603	2.84	Daphnia 'pooled'	Dumont et al., 1975
DAPROSE	<i>Daphnia rosea</i>	1.603	2.84	Daphnia 'pooled'	Dumont et al., 1975
DAPSCHO	<i>Daphnia schoedleri</i>	1.603	2.84	Daphnia 'pooled'	Dumont et al., 1975
DAPSP	<i>Daphnia spp.</i>	1.603	2.84	Daphnia 'pooled'	Dumont et al., 1975
DIACOPE	Diaptomid copepodites	1.59	2.59	generic calanoid	Burgess et al. 2015
DIAPT	Diaptomidae	1.59	2.59	generic calanoid	Burgess et al. 2015
DIPBIRG	<i>Diaphanosoma birgei</i>	1.289	3.04	<i>Diaphanosoma brachyrum</i>	Rosen 1981
DIPSP	<i>Diaphanosoma spp.</i>	1.289	3.04	<i>Diaphanosoma brachyrum</i>	Rosen 1981
DIYNANU	<i>Diacyclops nanus</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981
DIYTHOM	<i>Diacyclops thomasi</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981
EPICOPE	<i>Epischura copepodites</i>	1.59	2.59	generic calanoid	Burgess et al. 2015
EPILACU	<i>Epischura lacustris</i>	1.59	2.59	generic calanoid	Burgess et al. 2015
EUBCORE	<i>Eubosmina coregoni</i>	2.712	2.53	<i>Bosmina longirostris</i>	Bottrell et al., 1976
EUELAME	<i>Eurycercus lamellatus</i>	2.1	2.26	<i>Alonella exigua</i> w eggs	Dumont et al., 1975
EURAFFI	<i>Eurytemora carollea</i>	1.59	2.59	generic calanoid	Burgess et al. 2015
EURCOPE	<i>Eurytemora copepodites</i>	1.59	2.59	generic calanoid	Burgess et al. 2015

SPECCODE	Species	LNA	B	Species Used	Reference
EUYAGIL	<i>Eucyclops agilis</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981
EUYCOPE	<i>Eucyclops</i> copepodites	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981
EUYPRIO	<i>Eucyclops</i> <i>prionophorus</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981
EUYELEG	<i>Eucyclops</i> <i>elegans</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981
GRASP	<i>Graptoleberis</i> sp.	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
HALSP	<i>Halicyclops</i> spp.	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981
HARCOPE	Harpacticoid copepodites	1.59	2.59	generic calanoid	Burgess et al. 2015
HARSP	Harpacticoida spp.	1.59	2.59	generic calanoid	Burgess et al. 2015
HOLGIBB	<i>Holopedium</i> <i>gibberum</i>	2.073	3.19	<i>Holopedium</i> <i>gibberum</i>	Persson & Ekbohm 1980 original coefficients modified for total length (foot * 4)
ILYACUT	<i>Ilyocryptus</i> <i>acutifrons</i>	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
ILYSP	<i>Ilyocryptus</i> spp.	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
ILYSPIN	<i>Ilyocryptus</i> <i>spinifer</i>	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
LATSETI	<i>Latona setifera</i>	1.289	3.04	<i>Diaphanosoma</i> <i>brachyrum</i>	Rosen 1981
LEOASHL	<i>Leptodiaptomus</i> <i>ashlandi</i>	1.59	2.59	generic calanoid	Burgess et al. 2015
LEOMINU	<i>Leptodiaptomus</i> <i>minutus</i>	1.59	2.59	generic calanoid	Burgess et al. 2015
LEOSICI	<i>Leptodiaptomus</i> <i>sicilis</i>	1.59	2.59	generic calanoid	Burgess et al. 2015
LEOSICO	<i>Leptodiaptomus</i> <i>siciloides</i>	1.59	2.59	generic calanoid	Burgess et al. 2015
LETKIND	<i>Leptodora kindti</i>	-0.822	2.67	<i>Leptodora kindti</i>	Rosen 1981
LEYQUAD	<i>Leydigia</i> <i>quadrangularis</i>	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
LEYSP	<i>Leydigia</i> spp.	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
LIMCOPE	<i>Limnocalanus</i> copepodites	1.59	2.59	generic calanoid	Burgess et al. 2015
LIMMACR	<i>Limnocalanus</i> <i>macrurus</i>	1.59	2.59	generic calanoid	Burgess et al. 2015
MACLAT	<i>Macrothrix</i> <i>laticornis</i>	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
MACSP	<i>Macrothrix</i> spp.	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
MARALBI	<i>Macrocyclus</i> <i>albidus</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981

SPECCODE	Species	LNA	B	Species Used	Reference
MESCOPE	<i>Mesocyclops copepodites</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981
MESEDAX	<i>Mesocyclops edax</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981
MICRUBE	<i>Microcyclops rubellus</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981
MOIMICR	<i>Moina micrura</i>	1.889	2.57	<i>Moina micrura</i>	Dumont et al., 1975
MYSRELI	<i>Mysis diluviana</i>	1.266	2.72	<i>Mysis relicta</i>	Rudstam et al. 2008
ORTMODE	<i>Orthocyclops modestus</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981
PARCHIT	<i>Paracyclops chittoni</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981
PLEDENT	<i>Pleuroxus denticulatus</i>	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
PLEPROC	<i>Pleuroxus procurvus</i>	2.768	3.84	<i>Alona affinis</i>	Dumont et al., 1975
POYPEDI	<i>Polyphemus pediculus</i>	2.779	2.15	<i>Polyphemus pediculus</i>	Rosen 1981
SCAPSP	<i>Scapholeberis spp.</i>	2.416	2.7	<i>Scapholeberis mucronata</i>	Dumont et al., 1975
SCAPAURI	<i>Scapholeberis aurita</i>	2.416	2.7	<i>Scapholeberis mucronata</i>	Dumont et al., 1975
SENCALA	<i>Senecella calanoides</i>	1.59	2.59	generic calanoid	Burgess et al. 2015
SENCOPE	<i>Senecella copepodites</i>	1.59	2.59	generic calanoid	Burgess et al. 2015
SIDCRYS	<i>Sida crystallina</i>	2.054	2.19	<i>Sida crystallina</i>	Rosen 1981
SKIOREG	<i>Skistodiaptomus oregonensis</i>	1.59	2.59	generic calanoid	Burgess et al. 2015
SKIPALL	<i>Skistodiaptomus pallidus</i>	1.59	2.59	generic calanoid	Burgess et al. 2015
SKIREIG	<i>Skistodiaptomus reighardi</i>	1.59	2.59	generic calanoid	Burgess et al. 2015
THECRAS	<i>Thermocyclops crassus</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981
TROCOPE	<i>Tropocyclops copepodites</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981
TROPASM	<i>Tropocyclops prasinus mexicanus</i>	1.66	3.97	<i>Mesocyclops edax</i>	Rosen 1981

APPENDIX 2: ROTIFER BIOMASS FORMULA FACTORS

SPECCODE	Species	EQ#	FF	%BV	WW:DW
ASCECAU	<i>Ascomorpha ecaudis</i>	1	0.12	0	0.1
ASCOVAL	<i>Ascomorpha ovalis</i>	1	0.12	0	0.1
ASCSALT	<i>Ascomorpha saltans</i>	1	0.12	0	0.1
ASCSP	<i>Ascomorpha spp.</i>	1	0.12	0	0.1
ASPBRIG	<i>Asplanchna brightwelli</i>	1	0.23	0	0.039
ASPHERR	<i>Asplanchna herricki</i>	1	0.23	0	0.039
ASPPRIO	<i>Asplanchna priodonta</i>	1	0.23	0	0.039
BRAANGU	<i>Brachionus angularis</i>	1	0.12	0.1	0.1
BRABIDE	<i>Brachionus bidentata</i>	1	0.12	0.1	0.1
BRABUDA	<i>Brachionus budapestinensis</i>	1	0.12	0.1	0.1
BRACALC	<i>Brachionus calyciflorus</i>	1	0.12	0.1	0.1
BRACAUD	<i>Brachionus caudatus</i>	1	0.12	0.1	0.1
BRADIVE	<i>Brachionus diversicornis</i>	1	0.12	0.1	0.1
BRAHAVA	<i>Brachionus havanaensis</i>	1	0.12	0.1	0.1
BRAQUAD	<i>Brachionus quadridentatus</i>	1	0.12	0.1	0.1
BRARUBE	<i>Brachionus rubens</i>	1	0.12	0.1	0.1
BRASP	<i>Brachionus spp.</i>	1	0.12	0.1	0.1
BRAURCE	<i>Brachionus urceolaris</i>	1	0.12	0.1	0.1
BRAVARI	<i>Brachionus variabilis</i>	1	0.12	0.1	0.1
CEPINTU	<i>Cephalodella intuta</i>	1	0.1	0.05	0.1
CEPSP	<i>Cephalodella spp.</i>	1	0.1	0.05	0.1
COLMUTA	<i>Collotheca mutabilis</i>	2	1.8	0	0.1
COLPELA	<i>Collotheca pelagica</i>	2	1.8	0	0.1
COLSP	<i>Collotheca spp.</i>	2	1.8	0	0.1

SPECCODE	Species	EQ#	FF	%BV	WW:DW
CONNATA	<i>Conochiloides natans</i>	3	0.26	0	0.1
CONSP	<i>Conochiloides spp.</i>	3	0.26	0	0.1
COOUNIC	<i>Conochilus unicornis</i>	3	0.26	0	0.1
COUSP	<i>Colurella spp.</i>	1	0.1	0.05	0.1
DICSP	<i>Dicranophorus spp.</i>	1	0.035	0	0.1
ENCSP	<i>Encentrum spp.</i>	1	0.1	0.05	0.1
EUCALAT	<i>Euchlanis alata</i>	1	0.1	0.05	0.1
EUCCALP	<i>Euchlanis calpidia</i>	1	0.1	0.05	0.1
EUCDILA	<i>Euchlanis dilatata</i>	1	0.1	0.05	0.1
EUCMENE	<i>Euchlanis meneta</i>	1	0.1	0.05	0.1
EUCSP	<i>Euchlanis spp.</i>	1	0.1	0.05	0.1
FILLONG	<i>Filinia longiseta</i>	3	0.52	0.01	0.1
FILTERM	<i>Filinia terminalis</i>	3	0.52	0.01	0.1
GASSP	<i>Gastropus spp.</i>	1	0.2	0	0.1
GASSTYL	<i>Gastropus stylifer</i>	1	0.2	0	0.1
HEXMIRA	<i>Hexarthra mira</i>	1	0.13	0.33	0.1
KELBOST	<i>Kellicottia bostoniensis</i>	1	0.03	0.015	0.1
KELLONG	<i>Kellicottia longispina</i>	1	0.03	0.015	0.1
KERCOCH	<i>Keratella cochlearis</i>	1	0.02	0	0.1
KERCOCHH	<i>Keratella cochlearis hispida</i>	1	0.02	0	0.1
KERCOCHT	<i>Keratella cochlearis f. tecta</i>	1	0.02	0	0.1
KERCRAS	<i>Keratella crassa</i>	1	0.02	0	0.1
KEREARL	<i>Keratella earlinae</i>	1	0.02	0	0.1
KERHIEM	<i>Keratella hiemalis</i>	1	0.22	0.05	0.1
KERQUAD	<i>Keratella quadrata</i>	1	0.22	0.05	0.1
KERSP	<i>Keratella spp.</i>	1	0.02	0	0.1
KERTAUR	<i>Keratella taurocephala</i>	1	0.22	0.05	0.1
KERTEST	<i>Keratella testudo</i>	1	0.22	0.05	0.1
KERVALGT	<i>Keratella valga f. tropica</i>	1	0.22	0.05	0.1
LECFLEX	<i>Lecane flexilis</i>	1	0.1	0.05	0.1

SPECCODE	Species	EQ#	FF	%BV	WW:DW
LECSP	<i>Lecane spp.</i>	1	0.1	0.05	0.1
LECTENU	<i>Lecane tenuiseta</i>	1	0.1	0.05	0.1
LECTUDI	<i>Lecane tudicola</i>	1	0.1	0.05	0.1
LEPSP	<i>Lepadella spp.</i>	1	0.1	0.05	0.1
LEPTRIP	<i>Lepadella triptera</i>	1	0.1	0.05	0.1
MONCOPE	<i>Monostyla copeis</i>	1	0.12	0.1	0.1
MONLUNA	<i>Monostyla lunaris</i>	1	0.12	0.1	0.1
MONSP	<i>Monostyla spp.</i>	1	0.12	0.1	0.1
MYTSP	<i>Mytilina spp.</i>	1	0.1	0.05	0.1
NOOSP	<i>Notommata spp.</i>	1	0.035	0	0.1
NOTACUM	<i>Notholca acuminata</i>	1	0.035	0	0.1
NOTCAUD	<i>Notholca caudata</i>	1	0.035	0	0.1
NOTFOLI	<i>Notholca foliacea</i>	1	0.035	0	0.1
NOTLABI	<i>Notholca labis</i>	1	0.035	0	0.1
NOTLAUR	<i>Notholca laurentiae</i>	1	0.035	0	0.1
NOTMICH	<i>Notholca michiganensis</i>	1	0.035	0	0.1
NOTSP	<i>Notholca spp.</i>	1	0.035	0	0.1
NOTSQUA	<i>Notholca squamula</i>	1	0.035	0	0.1
NOTSTRI	<i>Notholca striata</i>	1	0.035	0	0.1
PLAPATU	<i>Platytias patulus</i>	1	0.22	0.05	0.1
PLOHUDS	<i>Ploesoma hudsoni</i>	1	0.1	0	0.1
PLOLENT	<i>Ploesoma lenticulare</i>	1	0.1	0	0.1
PLOSP	<i>Ploesoma spp.</i>	1	0.1	0	0.1
PLOTRUN	<i>Ploesoma truncatum</i>	1	0.1	0	0.1
POLDOLI	<i>Polyarthra dolichoptera</i>	1	0.28	0.1	0.1
POLEURY	<i>Polyarthra euryptera</i>	1	0.28	0.1	0.1
POLMAJO	<i>Polyarthra major</i>	1	0.28	0.1	0.1
POLREMA	<i>Polyarthra remata</i>	1	0.28	0.1	0.1
POLSP	<i>Polyarthra spp.</i>	1	0.28	0.1	0.1
POLVULG	<i>Polyarthra vulgaris</i>	1	0.28	0.1	0.1
POMSULC	<i>Pompholyx sulcata</i>	1	0.15	0	0.1
PROSP	<i>Proalea spp.</i>	1	.035	0	0.1

SPECCODE	Species	EQ#	FF	%BV	WW:DW
SYNSP	<i>Synchaeta spp.</i>	1	0.1	0	0.1
TESPARV	<i>Testudinella parva</i>	1	0.08	0.1	0.1
TRCPOCI	<i>Trichotria pocillum</i>	1	0.1	0.05	0.1
TRCPOCI	<i>Trichotria pocillum</i>	1	0.1	0.05	0.1
TRCSP	<i>Trichotria spp.</i>	1	0.1	0.05	0.1
TRCTETR	<i>Trichotria tetractis</i>	1	0.1	0.05	0.1
TRICAPU	<i>Trichocerca capucina</i>	3	0.52	0.006	0.1
TRICYLI	<i>Trichocerca cylindrica</i>	3	0.52	0.006	0.1
TRIELON	<i>Trichocerca elongata</i>	3	0.52	0.006	0.1
TRIIERN	<i>Trichocerca iernis</i>	3	0.52	0.006	0.1
TRIINSO	<i>Trichocerca insolens</i>	3	0.52	0.006	0.1
TRILATA	<i>Trichocerca lata</i>	3	0.52	0.006	0.1
TRIMULT	<i>Trichocerca multicroinis</i>	3	0.52	0.006	0.1
TRIPORC	<i>Trichocerca porcellus</i>	3	0.52	0.006	0.1
TRIPUSI	<i>Trichocerca pusilla</i>	3	0.52	0.006	0.1
TRIRATT	<i>Trichocerca rattus</i>	3	0.52	0.006	0.1
TRIROUS	<i>Trichocerca rousseleti</i>	3	0.52	0.006	0.1
TRISIMI	<i>Trichocerca similis</i>	3	0.52	0.006	0.1
TRISP	<i>Trichocerca spp.</i>	3	0.52	0.006	0.1
TYLMONO	<i>Tylotrocha monopus</i>	1	0.035	0	0.1