GEI Consultants

Consulting Engineers and Scientists

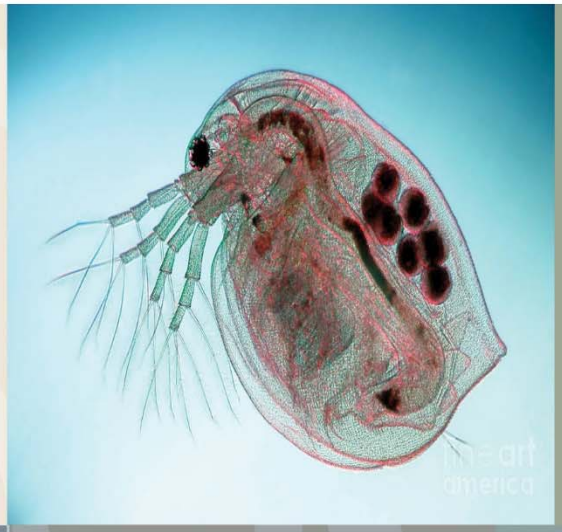
Standard Operating Procedures Manual

Zooplankton Sample Preparation and Analysis for
National Ecological Observatory Network (NEON)

Revision No. 5

Effective: July 2016

Manual Approved by: Natalie Love, Laboratory Director



Standard Operating Procedures Manual

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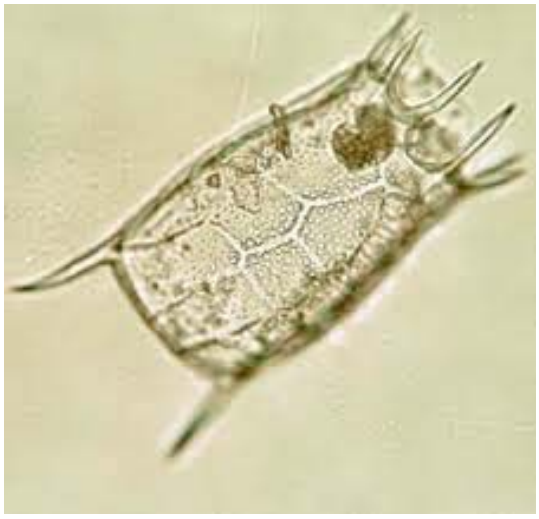

Submitted to:
National Ecological Observatory Network

1685 38th Street, Suite 100
Boulder, CO 80301

Submitted by:
GEI Consultants, Inc.

Ecological Division
4601 DTC Boulevard, Suite 900
Denver, CO 80237

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Summary of Revisions

Revision Date	Author	Content Revised
2008	Michelle Chadwick	Initial authorship
7/2015	Jamie Carmon	Major edits: • Edited Draft SOP for NEON project • Sent to NEON
7/2016	Jamie Carmon	Edit SOP to send to NEON: • Reformat SOP to standardized template

Table of Contents

List of Appendices

1 Acronyms/Definitions

Table 1: Acronyms

Acronyms	Definition
COC	Chain of Custody Form
GEI	GEI Consultants, Inc.
NEON	National Ecological Observatory Network
SDS	Safety Data Sheets
SOP	Standard Operating Procedure
mL	Symbol for milliliter
mm	Symbol for millimeter
µL	Symbol for microliter
µM	Symbol for micrometer

Table 2: Definitions

Vocabulary	Definition
Aliquot	A measured portion removed from a sample for analysis
Ethanol Solution	95% undenatured ethanol + 5% glycerol for preservation of samples and vials
Morphospecies	Morphologically similar taxa
Macrozooplankton	Large zooplankton, Copepods/Cladoceran
Microzooplankton	Small zooplankton, Rotifers/Nauplii
Sedgewick Rafter Slide	Slide with raised edges to hold 1mL aliquots of liquid
Undenatured ethanol	Pure ethanol
Voucher Collection	Collection of all taxa found in a sample set

2 Scope and Applicability

This standard operating procedure (SOP) is used to establish a uniform format for sample handling and analysis for samples from the National Ecological Observatory Network (NEON). This method is applicable to all samples received from NEON. GEI Consultants, Inc. (GEI), Denver CO, in conjunction with Water's Edge Scientific LLC (Water's Edge Scientific), Baraboo, Wisconsin, performs all phases of macro- and microzooplankton identification and measurements. The goal of this SOP is to standardize how zooplankton samples are analyzed by GEI and Water's Edge Scientific. This SOP is not a replacement for training. See Appendix D and E for reference materials.

3 Summary of Method

This SOP describes how to: log in zooplankton samples collected from NEON, ship the samples to Water's Edge Scientific, and upon return perform the QA procedure using a dissecting, and compound microscope. This SOP only applies to NEON samples.

4 Chemical Hazards

4.1 KOPTEC Ethanol 190 Proof

4.1.1 Health Hazard: Acute symptoms may include, shortness of breath, confused behavior, redness of skin, swelling of tissue, watery eyes, and nausea. Can cause moderate eye irritation, a high vapor concentration could be irritating. Can cause mild skin irritation, prolonged exposure could result in drying of the skin, which could lead to skin irritation. May cause irritation to nose, throat, and respiratory tract if inhaled, which could lead to depression of the central nervous system. If ingested, irritation of the digestive tract and signs of nervous system depression could occur.

4.1.2 First Aid: Immediately flush eyes with water for 15 minutes. It contact with skin, flush skin with water while removing clothing. Do not reuse clothing or shoes until cleaned. Move to fresh air and provide oxygen if breathing is difficult after inhalation. If ingested, do not give liquids if victim is unconscious or drowsy. Otherwise, give two glasses of water and induce vomiting by giving 30mL syrup of IPECAC. Call doctor.

4.1.3 Spill Procedures: Warning Flammable. Eliminate all ignition sources. Only qualified personnel should handle the spill. Contain liquid with absorbent material and place in a non-leaking container. Seal tightly for disposal. Refer to federal, state, and local regulators for disposal information.

4.2 Glycerol

4.2.1 Health Hazard: May be irritating to eyes and skin. May cause gastrointestinal discomfort. May cause irritation to respiratory tract.

4.2.2 First Aid: In case of contact with eyes, rinse immediately with water. After skin contact, take off all contaminated clothing and wash with plenty of water. If swallowed, do not induce vomiting. If inhaled, remove victim to fresh air and keep at rest. Call doctor.

4.2.3 Spill Procedures: Ventilate area of spill, and remove all sources of ignition. Absorb material with suitable absorbent for disposal. Refer to federal, state, and local regulators for disposal information.

4.3 Glassware

4.3.1 In case of broken glassware, obtain the dustpan and broom, along with the small trash can for broken glass labeled “Broken Glass”. Do not handle broken glass by hand.

4.4 Safety Data Sheets (SDS)

4.4.1 SDS are located in the chemistry preparation room in the laboratory in marked binders.

4.4.2 SDS are also located in the L: (Lab) Drive >General Lab>Laboratory Chemicals.

5 Materials

5.1 Glassware

5.1.1 Petri dishes (various sizes)

5.2 Microscopes

5.2.1 Dissecting Microscopes

5.2.2 Compound Microscopes

5.2.3 Kimwipes

5.3 Plastic pipettes

5.4 Designated squirt bottles

5.4.1 Labeled with 95% pure ethanol + 5% glycerol (Ethanol solution)

6 Reagents and Chemicals

6.1 Raw water preserved with ethanol solution

6.1.1 Sample collected in field preserved with ethanol solution

6.2 Ethanol Solution (95% ethanol + 5% glycerol)

6.2.1 Preserves sample and taxa. See Appendix B for instructions

6.3 Dish soap

6.3.1 Clean and decontaminate glassware

7 Sample Receiving

7.1 Receive Samples

7.1.1 Unpack samples from box, locate chain of custody (COC) form

7.1.2 Sign, date, and enter time on the COC

7.2 If labels are not legible, or in poor condition, note on COC and notify NEON

7.2.1 If samples are leaking, note on COC and notify NEON

7.3 Sample Preservation

7.3.1 All samples should arrive preserved with ethanol solution (95% ethanol + 5% glycerol)

7.3.2 If samples are not preserved properly, notify NEON

7.3.2.1 If samples do not arrive with proper preservative, filter using 64µm mesh

7.3.2.2 Place the sample into the original labeled container

7.3.2.3 Preserve with ethanol solution

7.3.2.3.1 See Appendix B for ethanol preparation

7.4 Log Number Assignment

7.4.1 Assign each sample a unique GEI log number consisting of four parts

7.4.1.1 The first two letters of the project name

7.4.1.2 Domain in parentheses

7.4.1.2.1 List of domains in 0

7.4.1.3 Month and year received

7.4.1.4 “Z” designates a zooplankton sample

7.4.1.5 Sample number

7.4.1.5.1 Example: NE[4]1116Z-2

7.5 Write the log number on the sample bottles and any forms accompanying samples

7.6 NEON Zooplankton Labels

7.6.1 Create zooplankton labels as per GEI label template

7.6.1.1 Computer>Lab(L)>BUGS>Projects>NEON>Labels for NEON BUGS

7.7 NEON Zooplankton Data Sheet

7.7.1 Create a new folder in the electronic Data folder named:
GEI ID_NEON Shipping ID

7.7.2 Locate the electronic NEON Zooplankton Data Sheet Template

7.7.2.1 Save a new version with the file name: GEI ID_ Database

7.7.2.2 Save the file in the folder created in 7.7

7.7.2.3 All other documentation will be stored in the folder created in 7.7

7.7.3 Data is entered in the data sheet after identifications are completed as per NEON instructions

7.7.3.1 See field descriptions in NEON Zooplankton Data Sheet

7.8 NEON Sample Tracker

7.8.1 Locate the electronic NEON Sample Tracker in the NEON folder and record:

7.8.1.1 GEI ID

7.8.1.2 NEON Shipment ID

7.8.1.3 Domain

7.8.1.4 Date sampled

7.8.1.5 Date received

7.8.1.6 Number of samples

7.8.1.7 Sample type (macroinvertebrates, zooplankton)

7.8.1.8 Shipment packaging

7.9 Project Folder

7.9.1 Label a card stock file folder with the project name and the log prefix

7.9.2 Include the following information:

7.9.2.1 Hard copy of the COC

7.9.2.2 GEI Project Protocol Sheet

7.9.2.3 Zooplankton Quality Assurance (QA) Sheet, See Appendix C

8 Ship Samples to Water's Edge Scientific

8.1 Ship preserved and labeled samples to Water's Edge Scientific

8.1.1 Sign and date the hard copy of the COC that the samples were relinquished to Water's Edge Scientific

8.1.1.1 Use standard GEI shipping practices

8.1.1.2 Send an electronic version of the NEON Zooplankton Data Sheet to Water's Edge Scientific

9 Analysis: Water's Edge Scientific

9.1 Water's Edge Scientific enumerates and identifies zooplankton

9.1.1 Use Folsom splitter to subsample large samples

9.1.1.1 More than 400 individuals per species is present

9.2 Macrozooplankton (Copepods, Cladocerans) identification

9.2.1 Use Ward counting wheel and dissecting scope to enumerate and identify specimens

9.2.2 Dissect 5 organisms from each morphologically similar taxon (morphospecies)

9.2.2.1 Examine with a compound scope for identification to species level

9.2.3 Measure 15-20 organisms from each species (both micro- and macrozooplankton) and determine

9.2.3.1 Maximum length to nearest 0.01mm (millimeter)

9.2.3.2 Minimum length to nearest 0.01mm (millimeter)

9.2.3.3 Mean length to nearest 0.01mm (millimeter)

9.3 Microzooplankton (Rotifers, Nauplii) identification

9.3.1 Analyze using 1 milliliter (mL) aliquot from the original sample bottle

9.3.1.1 Use a Sedgewick Rafter slide and a compound scope to enumerate and identify specimens

9.3.1.2 Identify rotifers to genus or species level based on type and condition of specimen

9.3.1.3 Enumerate nauplii, but do not identify

9.3.2 Measure 15-20 organisms from each species (both micro- and macrozooplankton) and determine

9.3.2.1 Maximum length to nearest 0.01 mm (millimeter)

9.3.2.2 Minimum length to nearest 0.01 mm (millimeter)

9.3.2.3 Mean length to nearest 0.01 mm (millimeter)

9.4 Voucher collection photos will be taken by Water's Edge Scientific

9.4.1 Electronically send photos to GEI Consultants, Inc.

9.5 Water's Edge ships samples back to GEI Consultants, Inc.

9.5.1 Original sample bottle

9.5.2 Analyzed subsample from Ward counting wheel (subsample)

9.5.3 COC

9.5.4 Send email with completed NEON Zooplankton Data Sheet

10 Sample Receiving by GEI Consultants, Inc.

10.1 Samples returned to GEI

10.1.1 Save the NEON Zooplankton Data Sheet from Water's Edge as GEI ID_WE

10.1.1.1.1 Indicates the data is directly from Water's Edge Scientific and the QA has not been completed

10.1.2 Sign and date the COC and place a copy in the Project Folder. See Section 7.9

11 NEON Zooplankton QA: Macrozooplankton

11.1 GEI Consultants, Inc. performs a QA on 10% of the samples

11.1.1 Follow Water's Edge Scientific SOP to duplicate analysis

11.1.2 Determine a percent similarity between Water's Edge Scientific identification, enumerations and measurements

11.2 Enumeration and Identification of Macrozooplankton

11.2.1 The bottle containing the subsample is placed in a Ward plankton counting wheel

11.2.2 All macrozooplankton are enumerated and identified to morphospecies

11.2.3 Dissect 5 individuals of each morphospecies to replicate species level identification

11.2.4 Determine the mean, maximum, and minimum lengths of 15 organisms to verify data from Water's Edge Scientific

11.3 Verify data from NEON Zooplankton Data Sheet

11.3.1 Determine the percent similarity between the original identifications, enumerations, and measurements, from the data collected in the QA

11.3.1.1 See Appendix C for Macrozooplankton QA sheet

11.4 Reject the QA

11.4.1 Percent similarity is less than 75% similar

11.4.2 The QA biologist will update the sample identifications and choose another sample for QA

11.4.2.1 Reanalyze all other samples with the misidentified taxa to ensure the mistake is corrected

11.5 After analysis

11.5.1 Return the subsample from the Ward counting wheel to the sample bottle

12 NEON Zooplankton QA: Microzooplankton

12.1 Enumerate and Identify Microzooplankton

12.1.1 Shake the original sample bottle to distribute contents

12.1.2 Remove 1 mL aliquot and place in Sedgewick Rafter slide

12.1.3 Analyze slide under compound microscope

12.1.4 Identify rotifer to genus or species based on condition of specimen

12.1.4.1 Enumerate nauplii, but do not identify further

12.1.5 Randomly select 15-20 organism from each morphospecies

12.1.5.1 Determine maximum, minimum, and mean lengths to verify data from Water's Edge Scientific

12.2 Verify data from NEON Zooplankton Data Sheet

12.2.1 Determine the percent similarity between the original identifications, enumerations, and measurements, from the data collected in the QA

12.2.1.1 See Appendix C for Microzooplankton QA sheet

12.3 Reject the QA

12.3.1 Percent similarity is less than 75% similar

12.3.2 The QA biologist will update the sample identifications and choose another sample for QA

12.3.2.1 Reanalyze all other samples with the misidentified taxa to ensure the mistake is corrected

12.4 After analysis

12.4.1 Return the subsample, from the Sedgewick Rafter slide, to the sample bottle

13 QA NEON Zooplankton Data Sheet

13.1 Add voucher photos to NEON Zooplankton Data Sheet

13.2 QA biologist reviews all data in the NEON Zooplankton Data Sheet for completeness

13.2.1 Verify sample ID numbers

13.2.2 Review all measurements

13.2.3 Review all identifications

14 Finalize Data

14.1 NEON Zooplankton Data Sheet

14.1.1 Resave completed and QA NEON Zooplankton Data Sheet as GEI ID_QA Data Sheet to indicate the QA has been completed

14.1.2 The laboratory manager, an invertebrate taxonomist, the project manager, and laboratory director also review the NEON Zooplankton Data Sheet for errors

14.2 Data Transmittal

14.2.1 Upon completion of the QA process, the final data set is saved on the server in the folder with the appropriate sample ID with the following file name: ZITaxID, SITE NAME, DATE DATA SUBMITTED (ZITaxID PRPO PRLA 20150601 consists of zooplankton data, from sites PRLA and PRPO, that were submitted to NEON on June 1, 2015)

14.2.2 Should changes to the original data set be required, a new folder on the GEI server is created with the following file name: ZITaxID, SITE NAME, NEW DATE DATA SUBMITTED; the revised data will be placed in the new folder, but the data set will maintain the original file name; the revisions are automatically tracked in the NEON Dropbox with a version

15 Sample Storage

15.1 Storage of NEON zooplankton samples in ethanol solution

15.2 NEON will provide instruction for disposal and transfer

15.2.1 After no more than 6 months, NEON will decide on the disposal or transfer of samples

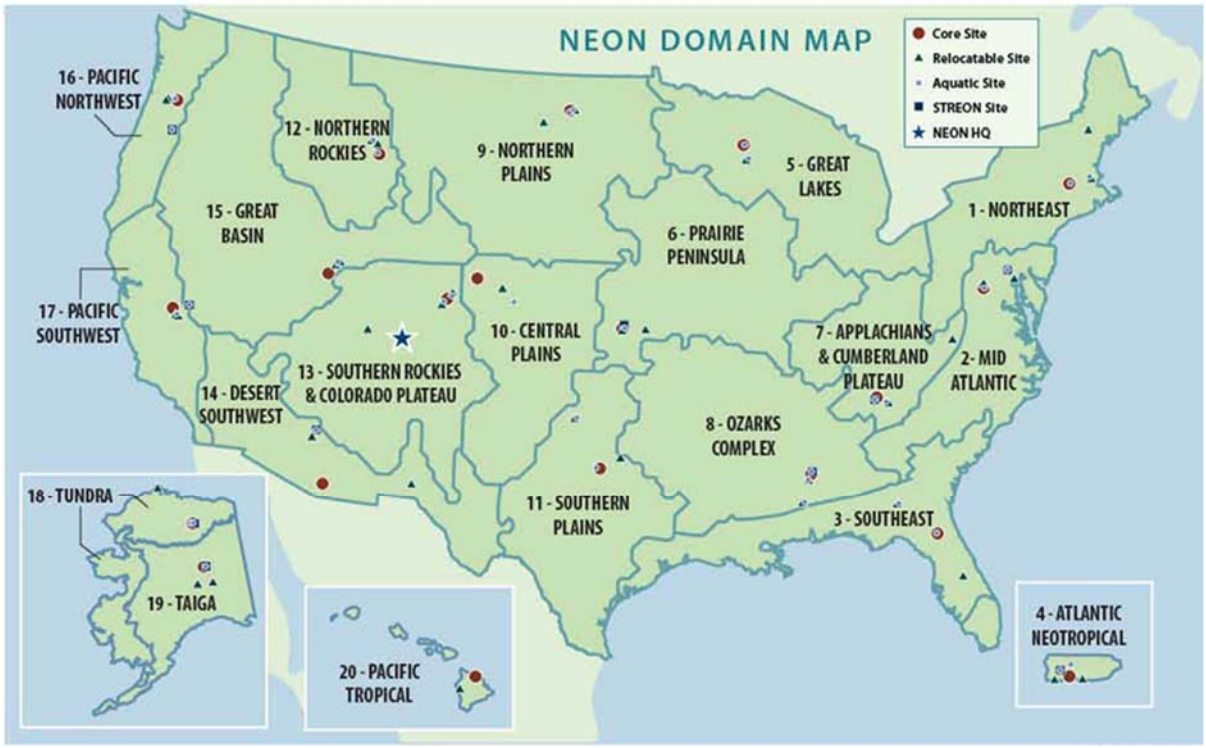
15.2.2 GEI will return coolers and/or reusable shipping containers to NEON Domain Support Facilities within two weeks of designated sample schedule provided by NEON

Appendix A List of Domains

Table A-1: List of Domains GEI may Receive Samples From

Number	Domain Name
2	Mid-Atlantic
4	Atlantic Neotropical
5	Great Lakes
6	Prairie Peninsula
8	Ozarks Complex
9	Northern Plains
10	Central Plains
13	Southern Rockies & Colorado Plateau
14	Desert Southwest
15	Great Basin
17	Pacific Southwest

Photo A-1: Domain Map



Appendix B Ethanol Solution

1. To preserve unpicked portion, picked portion, and taxa vials
    a. 95% pure ethanol + 5% glycerol
        i. Example: In a 10 L container, add 400 mL of glycerol to 7600mL of pure ethanol.

Appendix C Miscellaneous Documents

1. Macrozooplankton QA Sheet

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	MACROZOOPLANKTON IDENTIFICATION QA BENCH SHEET																	
2						Poplulation Similarity						Size Similarity						
3	Project:	NEON		ID Date:		Total %	Bench Sheet					Total %	Bench Sheet					
4	Log Number:	NE(5)615-2				Similarity	Corrected					Similarity	Corrected					
5	Analyzed By:			QA Date:		#DIV/0!	NA					#DIV/0!	NA					
6	QA by:																	
7	Macrozooplankton Population Community Similarity							Macrozooplankton Size Community Similarity										
8	TSN	Taxon	Original #	%	QA #	%	% Similarity	Min orig	Max orig	Mean orig	Width	%	Max QA	Min QA	Mean QA	Width	%	% Similarity
9				#DIV/0!		#DIV/0!	#DIV/0!					#DIV/0!					#DIV/0!	#DIV/0!
10				#DIV/0!		#DIV/0!	#DIV/0!					#DIV/0!					#DIV/0!	#DIV/0!
11				#DIV/0!		#DIV/0!	#DIV/0!					#DIV/0!					#DIV/0!	#DIV/0!
12				#DIV/0!		#DIV/0!	#DIV/0!					#DIV/0!					#DIV/0!	#DIV/0!
13				#DIV/0!		#DIV/0!	#DIV/0!					#DIV/0!					#DIV/0!	#DIV/0!
14				#DIV/0!		#DIV/0!	#DIV/0!					#DIV/0!					#DIV/0!	#DIV/0!

2. Microzooplankton QA Sheet

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	MICROZOOPLANKTON IDENTIFICATION QA BENCH SHEET																	
2						Poplulation Similarity						Size Similarity						
3	Project:	NEON		ID Date:		Total %	Bench Sheet					Total %	Bench Sheet					
4	Log Number:	NE(5)615-2				Similarity	Corrected					Similarity	Corrected					
5	Analyzed By:			QA Date:		#DIV/0!	NA					#DIV/0!	NA					
6	QA by:																	
7	Microzooplankton Population Community Similarity							Microzooplankton Size Community Similarity										
8	TSN	Taxon	Original #	%	QA #	%	% Similarity	Min orig	Max orig	Mean orig	Width orig	%	Max QA	Min QA	Mean QA	Width QA	%	% Similarity
9				#DIV/0!		#DIV/0!	#DIV/0!					#DIV/0!					#DIV/0!	#DIV/0!
10				#DIV/0!		#DIV/0!	#DIV/0!					#DIV/0!					#DIV/0!	#DIV/0!
11				#DIV/0!		#DIV/0!	#DIV/0!					#DIV/0!					#DIV/0!	#DIV/0!
12				#DIV/0!		#DIV/0!	#DIV/0!					#DIV/0!					#DIV/0!	#DIV/0!
13																		
14																		
15																		
16																		

Appendix D Keys Used for Identification

Balcer, M. D., Korda, N. L., & Dodson, S. I. (1984). Zooplankton of the Great Lakes. Madison, WI: University of Wisconsic Press, Ltd.

Pennak, R. W. (1989). Fresh-Water Invertebrates of the United States: Protozoa to Molluska. *3rd Edition*. New York, NY: John Wiley and Sons, Inc. Publishers.

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Thorp, J. H., & Covich, A. P. (2001). *Ecology and Classification of North American Freshwater Invertebrates.* Waltham, Massachusetts: Academic Press.

Appendix E References

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