



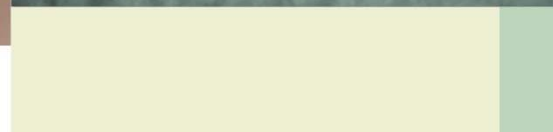
# Standard Operating Procedures Manual

**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

## Sample Processing and Analysis for NEON



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## Summary of Revisions

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Revision Date	Author	Content Revised
12/2014	Michelle Chadwick	Initial Draft authorship
3/2015	Jamie Carmon	Major edits: <ul style="list-style-type: none"><li>• Added Section 3</li><li>• Edited Section 4</li></ul>
7/29/2015	Jamie Carmon	Edit Draft SOP to send to NEON: <ul style="list-style-type: none"><li>• Edit: Section 4</li><li>• Split Section 4 into 4 and 5</li><li>• Edit: Section 7</li></ul>
10/1/2015	Jamie Carmon	Reformat Draft SOP to standardized template <ul style="list-style-type: none"><li>• Edit references</li><li>• Added Appendix B</li></ul>
6/29/2015	Jamie Carmon	Edit SOP to send to NEON and finalize: <ul style="list-style-type: none"><li>• Edit Chironomid/ Oligochaete sections</li><li>• Edit Appendices</li><li>• Edit numbering system</li></ul>

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# 1 Acronyms/Definitions

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**Table 1: Acronyms**

<b>Acronyms</b>	<b>Definition</b>
COC	Chain of Custody Form
GEI	GEI Consultants, Inc.
ITIS	Integrated Taxonomic Information System
NEON	National Ecological Observatory Network
mL	Symbol for mL
SDS	Safety Data Sheets
SOP	Standard Operating Procedure
STE	Standard Taxonomic Effort
μM	Symbol for micrometer

**Table 2: Definitions**

<b>Vocabulary</b>	<b>Definition</b>
Benthic macroinvertebrates	Insects collected from river, lake, or stream samples
Elutriate	Process to remove the organic matrix from the inorganic matrix
Matrix	The organic/inorganic make-up of the sample
Picked portion	The portion of the sample that is analyzed
Unpicked portion	The portion of the sample that is not analyzed
Caton tray	Standardized tray to extract benthic macroinvertebrates
Undenatured ethanol	Pure ethanol
Alcohol Solution	95% undenatured ethanol + 5% glycerol for preservation of samples and vials
Morphotype	Separating taxa by similar type, i.e. family, genus, species etc.
Voucher Collection	Collection of all taxa found in a sample set

## 2 Scope and Applicability

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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, performs all phases of benthic macroinvertebrate analysis, including identification, measurements, and the assembly of voucher collections. The goal of this SOP is to standardize how each task in the laboratory is performed by every technician. This SOP is not a replacement for training. All macroinvertebrate methods are adapted from various sources listed in Appendix E.

### **3 Summary of Method**

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This SOP describes how to log in benthic macroinvertebrate samples collected from NEON, analyze samples using a dissecting microscope, and mount chironomids and oligochaetes. This document also describes benthic macroinvertebrate identification as well as preparing voucher specimens. This SOP only applies to NEON samples.



## 4 Chemical Hazards

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### 4.1 CMC-10 Mounting Media

- 4.1.1 Health Hazard: The primary contacts are through eyes and skin. May cause irritation and/or burning sensation to eyes. Prolonged or repeated contact could cause skin irritation. Material is possibly harmful if swallowed. This chemical, nor any of its components are carcinogenic.
- 4.1.2 First Aid: If inhaled, remove to fresh air. Flush eyes with large amounts of water for 15 minutes. Wash affected skin with soap and water for 15 minutes. If ingested, give 2 glasses of water to drink. Do not induce vomiting. After first aid, seek appropriate medical support. Do not give anything by mouth to an unconscious person.
- 4.1.3 Spill Procedures: Use paper towels or similar absorbent to clean spilled material. Accumulate the absorbed materials and dispose of according to federal, state, and local regulators.

### 4.2 KOPTEC Ethanol 190 Proof

- 4.2.1 Health Hazard: The primary contacts are through eyes and skin. 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.
- 4.2.2 First Aid: Immediately flush eyes with water for 15 minutes. If 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 30 mL syrup of IPECAC. Call doctor.
- 4.2.3 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.

### 4.3 Glycerol

- 4.3.1 Health Hazard: May be irritating to eyes and skin. May cause gastrointestinal discomfort. May cause irritation to respiratory tract.
- 4.3.2 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.
- 4.3.3 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.

### 4.4 Glassware

- 4.4.1 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. Broken glassware with benthic macroinvertebrates should be handled with care to retrieve the macroinvertebrates, then dispose of glass in broken glass trash can.

### 4.5 Safety Data Sheets (SDS)

- 4.5.1 SDS are located in the chemistry preparation room in the laboratory in marked binders.
- 4.5.2 SDS are also located in the L: (Lab) Drive >General Lab>Laboratory Chemicals.

## 5 Materials

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### 5.1 Glassware

5.1.1 Petri dishes (various sizes)

5.1.2 Slides

5.1.3 Cover slips

### 5.2 Microscopes

5.2.1 Dissecting Microscopes (x9)

5.2.2 Compound Microscopes (x3)

5.2.3 Kimwipes

### 5.3 Sieves (multiple sizes, 250 $\mu\text{m}$ , 600 $\mu\text{m}$ , and 1mm)

5.3.1 Brush for washing sieves

5.3.2 Plastic pipettes

5.3.3 Slide boxes for chironomids and oligochaetes

5.3.4 Modified Caton picking tray, with 10 divisions

5.3.5 Designated squirt bottles

5.3.5.1 Labeled with 95% ethanol + 5% glycerol

## 6 Reagents and Chemicals

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### 6.1 Raw water preserved with ethanol solution

#### 6.1.1 Sample collected in field preserved with alcohol 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 sieves and glassware

## 7 Sample Receiving

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### 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 with ethanol preservative (95% ethanol + 5% glycerol)

7.3.2 If samples are not preserved properly, notify NEON

### 7.4 If samples do not arrive with proper preservative, decant the sample into a 250 $\mu$ m sieve

7.4.1 Place the sample into the original labeled container

7.4.2 Preserve with ethanol solution

7.4.3 See Appendix B for ethanol preparation

7.4.4 Log Number Assignment

### 7.5 Assign each sample a unique GEI log number consisting of three parts:

7.5.1 The first two letters of the project name

7.5.2 Domain in parentheses

7.5.2.1 List of domains in Appendix A

7.5.3 Month and year received

7.5.4 Sample number

7.5.4.1 Example: NE[4]1116-2

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

### 7.6 NEON Vial Labels

7.6.1 Create benthic macroinvertebrate labels as per label template

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

7.6.2 Create chironomid and oligochaete labels as per label template

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

7.7 NEON Data Sheet

7.7.1 Create a new folder in the electronic Data folder named:  
GEI ID\_Shipment ID

7.7.2 Locate the electronic NEON Data Sheet Template

7.7.3 Save a new version with the file name: GEI ID\_ Database

7.7.3.1 Save the file in the folder created in 7.7.1

7.7.3.2 All other documentation will be stored in the folder created in 7.7.1

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

7.7.4.1 See field descriptions in NEON 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 GEI Macroinvertebrate Sample Flow Sheet

7.9.2.4 Benthic Macroinvertebrate Extraction Quality Assurance (QA) Bench Sheet

7.9.2.4.1 See Appendix C for examples of documents in 7.9.2

## 7.10 Sample Storage

7.10.1 Benthic macroinvertebrate samples are stored until analysis is complete

7.10.2 After analysis, picked, unpicked, and elutriate portions are placed in separate jars and labeled with pertinent information and re-preserved

7.10.3 Sample jars are stored based on NEON recommendations and GEI procedures for up to six months to allow for post-analytical Quality Assurance Procedure, completed by NEON

7.10.4 After no more than six months, NEON will provide instruction on the disposal of samples

7.10.5 Return voucher collections to the client or retain indefinitely

## 8 Picking Samples

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### 8.1 Picking Trays

8.1.1 Use a U.S. Standard Sieve (No. 60, 250  $\mu\text{m}$ ) and tap water to rinse the sample and remove preservative

8.1.2 Use a modified Caton picking tray for subsampling

### 8.2 Picking trays consist of measured grids used for subsampling

8.2.1 Different size trays are available to the sorter, depending on the sample volume

8.2.2 Prior to picking, any large/rare organisms found in the sample are removed and placed in a separate vial labeled “large/rare” and are not included in the count

8.2.2.1 Sample Analysis

### 8.3 Randomly choose two grid cells and remove all the material from the cells

#### 8.3.1 Elutriation of samples

8.3.1.1 Separate organic and inorganic portions of sample

8.3.1.2 Analyze inorganic portion under the lamp

8.3.1.3 QA the elutriate before placing in separate, labeled jar

8.3.2 Sieve the cells using different size sieves to separate matrix types

8.3.3 Analyze the cells using a dissecting scope

### 8.4 Sort macroinvertebrates by order

8.4.1 Preserve macroinvertebrates in ethanol solution, see Appendix B

8.4.1.1 Do not pick from sample:

8.4.1.1.1 Terrestrials, Vertebrates, Zooplankton, Bryozoa, Porifera, Exuviae, Empty shells, Oligochaete fragments, and Oligochaete anal ends

8.5 Additional cells are randomly chosen until 300 organisms are detected, or the entire sample is processed

8.6 Place the picked, unpicked, and elutriate portions in separate labeled jars



8.7 Complete the Benthic Macroinvertebrate Sample Flow Bench Sheet

8.7.1 See Appendix C for bench sheet example

8.7.2 All samples are QA'ed as described in Section 17

8.8 Vial Labels

8.8.1 Each order is placed in separate vials, and preserved with ethanol solution, using pre-printed labels

8.8.2 All vials for a sample are securely bound together using a rubber band

8.8.3 Vials are placed in a storage container that is labeled with the project name and the GEI ID

## 9 Mounting Chironomidae

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9.1 GEI utilizes two chironomid specialists for identification:

9.1.1 Mr. Uttam Rai a certified GEI taxonomist

9.1.2 Dr. Leonard Ferrington, a third-party consultant certified in taxonomic identification of chironomids

9.2 Isolation of Chironomidae

9.2.1 After sample passes QA, all taxa vials are sorted for any misplaced chironomids

9.2.2 The total number of chironomids is counted and recorded on the vial cap

9.2.3 The data is recorded on the Macroinvertebrate Sample Flow Sheet

9.2.3.1 See Appendix C for flow sheet example

9.3 Subsampling of Chironomidae

9.3.1 When morphotyping, not all chironomids may need to be mounted

9.4 Taxonomist will determine which specimens need to be mounted

9.4.1 If less than 30 chironomids, mount all specimens

9.4.2 If 30-150 chironomids, randomly select 30 specimens to mount

9.5 Obtain Integrid Petri dish

9.5.1 Evenly distribute chironomids in dish

9.5.2 Use random number chart to select chironomids

9.5.3 If more than 150 chironomids, randomly select 20% to mount

## 10 Mounting of Chironomidae: In-House ID Method

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10.1 Chironomids are mounted on 25x75 mm clean glass slides

10.1.1 Place a label corresponding to the sample on the slide (Figure 1)

10.1.2 Sort the specimens by size, and if possible, morphotype

10.1.3 Turn the slide around so the label is upside down (Figure 1)

10.1.3.1 Compound microscopes invert the image so the label will appear right side up for specimen identification

10.2 Place a drop of CMC-10 Mounting Media on the slide

10.2.1 Take specimen with forceps and dip in water, then transfer to CMC-10

10.2.2 Transfer and orient the specimen vertically, with ventral side up

10.3 Mount specimens of similar size together to reduce air bubbles

10.3.1 After the mounting media becomes tacky (approx. 10-20 minutes):

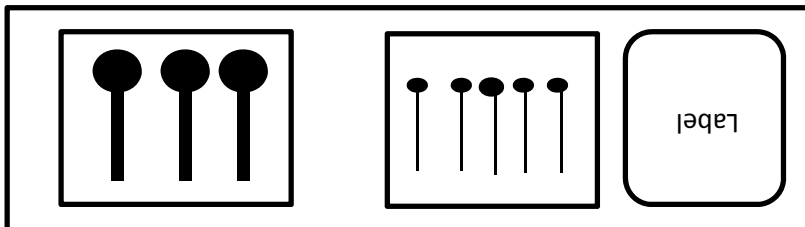
10.3.2 Put CMC on a cover slip and lower it gently on the slide at an angle to prevent air bubbles

10.3.2.1 Let the CMC spread out and fill the cover slip

10.3.2.2 Using the blunt end of the forceps, press on the posterior portion of the chironomids and slide toward the head to open up the mandibles

10.3.3 Slide is ready for analysis after a few hours

**Figure 1: In-House ID Slide Mounting Technique**

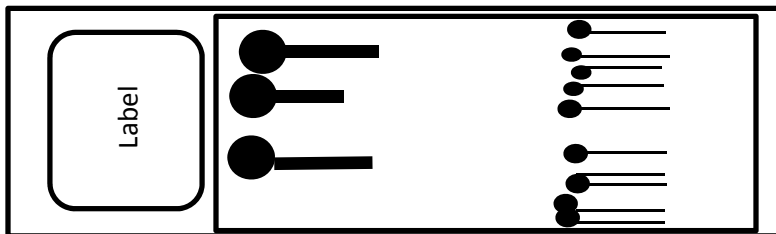


## 11 Mounting of Chironomidae: Dr. Leonard Ferrington ID Method

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- 11.1 Chironomids are mounted on 25x75 mm clean glass slides
  - 11.1.1 Place a label corresponding to the sample on the slide (Figure 2)
  - 11.1.2 Sort the specimens by size, and if possible, morphotype
- 11.2 Place a drop of CMC media and mount the largest specimens on the top of the slide and the smallest specimens on the bottom of the slide (Figure 2)
  - 11.2.1 Mount up to 10 chironomids in a row, maximum 70 per slide
  - 11.2.2 Allow slide to dry for 2 days
- 11.3 After 2 days, place CMC on a cover slip and the slide, and gently lower at an angle to prevent air bubbles
  - 11.3.1 Let the CMC spread out and fill the cover slip
  - 11.3.2 Let dry for 3 days and ship to Dr. Leonard Ferrington

**Figure 2: Dr. Leonard Ferrington Slide Mounting Technique**



## 12 Identification of Chironomidae

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12.1 Place slide under the compound microscope

12.1.1 Enumerate and identify chironomids to lowest possible taxonomic level

12.1.2 Determine length of chironomids to nearest millimeter

12.1.3 Record data in the Chironomid & Oligochaete Bench Sheet

## 13 Mounting Oligochaeta

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### 13.1 Isolation of Oligochaeta

13.1.1 After sample passes QA, all taxa vials are sorted for any misplaced oligochaetes

### 13.2 Oligochaetes that are too big mount are placed in the Miscellaneous vial

13.2.1.1 Oligochaete fragments, or anal ends are removed and not mounted

### 13.3 The total number of oligochaetes is counted and recorded on the vial cap

13.3.1 The data is recorded on the Macroinvertebrate Sample Flow Sheet

### 13.4 Subsampling of Oligochaeta

13.4.1 If less than 30 oligochaetes, mount all specimens

13.4.2 If 30-150 oligochaetes, randomly select 30 specimens to mount

13.4.2.1 Obtain Integrid Petri dish

13.4.2.2 Evenly distribute oligochaetes in dish

13.4.2.3 Use random number chart to select oligochaetes

13.4.3 If more than 150 oligochaetes, randomly select 20% to mount

13.4.3.1 Obtain Integrid Petri dish

13.4.3.2 Evenly distribute oligochaetes in dish

13.4.3.3 Use random number chart to select oligochaetes

13.4.3.3.1 Maximum 500 individuals

13.4.4 Oligochaetes not selected are returned to vial and preserved with ethanol solution

### 13.5 Mount Oligochaeta

13.5.1 Oligochaetes are mounted vertically on 25x75 mm clean glass slides

13.5.1.1 Place a label corresponding to the sample on the slide (Figure 3)

13.5.2 Sort the specimens by size, head facing the top of the slide (Figure 3)

13.5.2.1 Mount up to 10 oligochaetes in a row

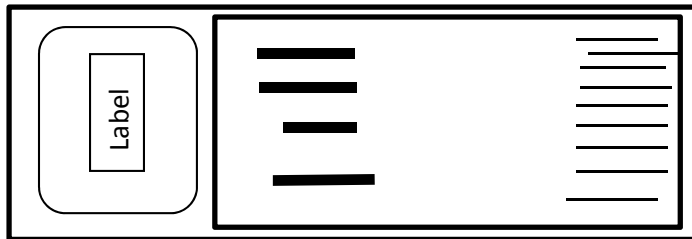
13.6 Allow slide to dry for 2 days

13.6.1 After 2 days, place CMC on a cover slip and the slide, and lower it gently at an angle to prevent air bubbles

13.6.1.1 Let the CMC spread out and fill the cover slip

13.7 Let dry for 3 days before identification

**Figure 3: GEI Oligochaete Slide Mounting Technique**



## 14 Identification of Oligochaeta

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14.1 Place slide under the compound microscope

14.1.1 Enumerate and identify head end of oligochaetes to lowest possible taxonomic level

14.1.2 Record fragments, but do not identify

14.1.3 Determine length of oligochaetes to nearest millimeter

14.1.4 Record data in the Chironomid and Oligochaete Bench Sheets



## 15 Identification of Benthic Macroinvertebrates

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### 15.1 Identify remaining taxa to the lowest Standard Taxonomic Effort (STE)

15.1.1 The STE is the lowest taxonomic level of common taxa

15.2 The GEI-developed STE defines the lowest taxonomic level to which identifications of benthic macroinvertebrate taxa should be attempted and identifies the primary literature to use in making those identifications

15.3 STE is continuously updated as new literature provides newly discovered taxa, range expansions and contractions, new taxonomic rearrangements, and new methods/identification keys for invertebrate identification

15.4 Such identifications are contingent upon the maturity of each specimen, how intact/undamaged each specimen is, and whether or not it is geographically reasonable to use regional identification keys

15.4.1.1 See Appendix D for commonly used identification keys

### 15.5 Benthic Macroinvertebrate Bench Sheet

15.5.1 Taxonomists record the taxon code into the Benthic Macroinvertebrate Bench Sheet

15.5.2 The taxon codes are located on the Integrated Taxonomic Information System (ITIS) database

15.5.3 ITIS provides valid taxonomic names maintained by the U.S. Department of Agriculture

15.6 GEI created taxon codes are indicated by a letter, for internal purposes, in cases when the ITIS has not been updated, etc.

### 15.7 Specimens are returned to the vial and refilled with ethanol solution

15.7.1 Vials are placed in storage container that is labeled with the project name and the GEI ID

## 16 Voucher Collection

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16.1 A voucher collection of specimens allows for verification

16.1.1 Benthic macroinvertebrates

16.1.2 Oligochaetes

16.1.3 Chironomids

16.2 Create a voucher collection for each of the NEON Domains

16.2.1 Update the voucher collection

16.3 Add newly identified taxa as subsequent samples from the same Domain are processed

16.3.1 Use pre-printed labels for each vial for the voucher collection

16.3.1.1 2 labels are printed – one is placed inside the vial, and one is taped to the outside of the vial

16.4 GEI assigns a unique code for each taxon in each Domain

16.5 Vial cap labeled with this code using a silver Sharpie marker

16.5.1 Place up to 5 specimens in the vial and fill with ethanol solution

16.5.1.1 Do not include degraded/damaged specimens

16.6 Verification of voucher collection

16.6.1 After creating a voucher collection, internal verification by a second invertebrate taxonomist may occur

16.6.2 External review may be performed by Dr. Boris Kondratieff (Colorado State University, Fort Collins, Colorado)

16.6.2.1 Record voucher information on NEON Data Sheet

## 17 Quality Assurance/Quality Control Procedures

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### 17.1 Picking QA

17.1.1 QA technicians check all samples for completeness of extraction

17.1.2 Check 25% of the extracted portion under a dissecting microscope

17.1.2.1 Return the sample to the sorter if more than 5% of the total extracted organisms are found.

17.1.2.2 The sorter continues extraction in those portions picked, until more than 95% of the extracted organisms are found.

17.1.3 Randomly select and record 10% of the samples for a reported QA/QC

17.1.4 Record information on the QA bench sheet

17.1.4.1 See Appendix C for example of bench sheet

### 17.2 Identification QA

17.2.1 Perform a random QA on 10% of the sample set.

17.2.2 A trained invertebrate taxonomist confirms the taxa, counts, and sizes to the nearest millimeter

17.2.3 Reject the original identification if the percentage similarity is less than 95% similar.

17.2.4 The QA taxonomist will update the sample identifications, and choose another sample for QA from the identifying taxonomist

17.2.4.1 See Appendix C for example of bench sheet

### 17.3 Data Entry QA

17.3.1 Before data is entered into the perTaxon tab of the NEON Data Sheet, taxonomists verify:

17.3.2 Benthic macroinvertebrate taxon codes, sizes of specimens, and overall formatting

17.3.2.1 Oligochaete taxon codes, sizes of specimens, and overall formatting

17.3.2.2 Chironomid taxon codes, sizes of specimens, and overall formatting

## 18 Finalize Data

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### 18.1 NEON Data Sheet

18.1.1 Save all data from processed samples for individual sample set in the NEON Data Sheet

18.1.2 The laboratory manager, an invertebrate taxonomist, the project manager, and laboratory director, complete the QA for the data

### 18.2 Data Transmittal

18.2.1 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: MITaxID, SITE NAME, DATE DATA SUBMITTED (i.e. MITaxID PRPO PRLA 20150601 consists of benthic macroinvertebrate data, from sites PRLA and PRPO, that were submitted to NEON on June 1, 2015)

18.2.2 Upload the completed data to the NEON Dropbox site.

18.2.3 Should changes to the original data set be required, a new folder on the GEI server is created with the following file name: MITaxID, 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 number

## 19 Sample Storage

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### 19.1 Storage of NEON samples in ethanol solution

19.1.1 Picked, unpicked, and elutriate subsamples will be stored after analysis in ethanol solution

### 19.2 NEON will provide instruction

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

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

19.2.3 Ship completed vials of sorted, identified invertebrates and slide-mounted Chironomidae/Oligochaeta to NEON, based on schedule provided

19.2.3.1 Return voucher collections to NEON

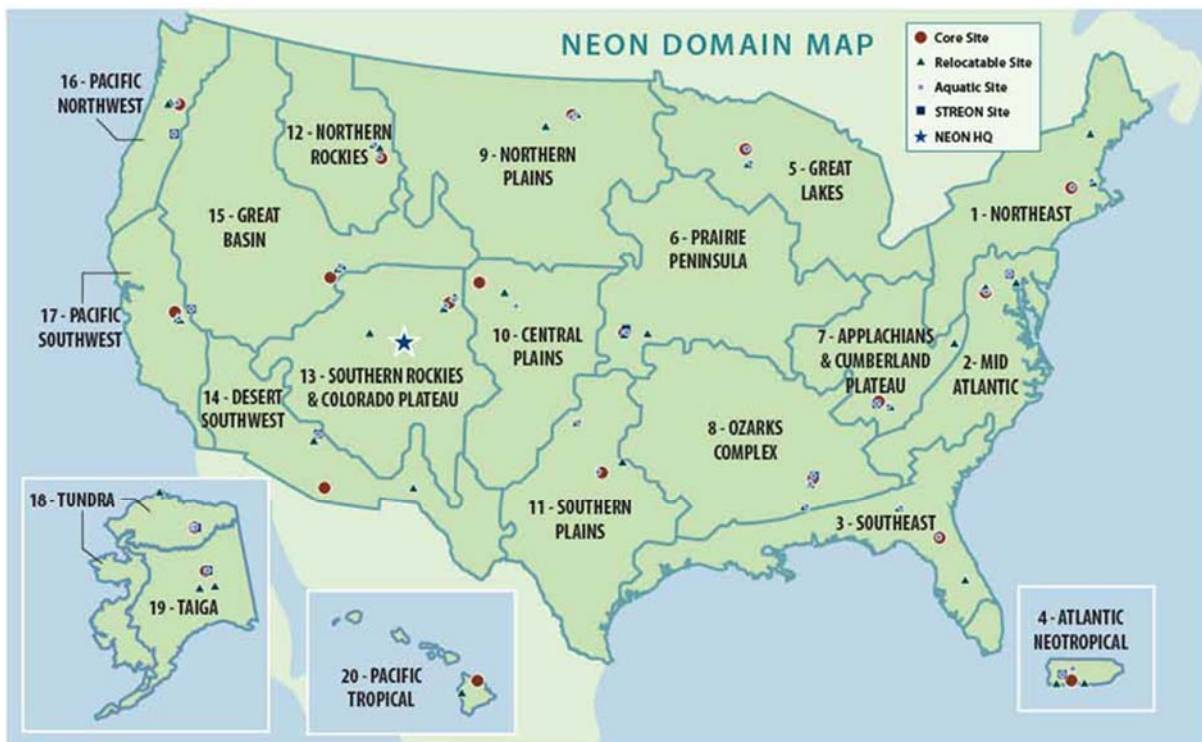
### 19.3 Voucher may be retained indefinitely by GEI

## 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

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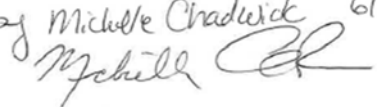
1. To preserve unpicked portion, picked portion, and taxa vials
  - a. 95% pure ethanol + 5% glycerol
2. Example: In a 10 L container, add 400 mL of glycerol to 7600mL of pure ethanol.

## Appendix C Miscellaneous Documents

### 1. Hard Copy of COC

NEON Inc. Domain 04  
 Puerto Rico

Shipment ID	Site ID	Sample Date	Sample ID list	Station ID	Habitat type	Sampler type	Type of preservative used	Volume of preservative used (mL)	Remarks
12097EV90390749021	STCU	20150508	STCU.20150508.riffles.1		riffles	surber	ethanol 95%/glycol 5%		
12097EV90390749021	STCU	20150508	STCU.20150508.riffles.2		riffles	surber	ethanol 95%/glycol 5%		
12097EV90390749021	STCU	20150508	STCU.20150508.riffles.3		riffles	surber	ethanol 95%/glycol 5%		
12097EV90390749021	STCU	20150508	STCU.20150508.riffles.4		riffles	surber	ethanol 95%/glycol 5%		
12097EV90390749021	STCU	20150508	STCU.20150508.riffles.5		riffles	surber	ethanol 95%/glycol 5%		
12097EV90390749021	STCU	20150508	STCU.20150508.runs.1		runs	surber	ethanol 95%/glycol 5%		
12097EV90390749021	STCU	20150508	STCU.20150508.runs.2		runs	surber	ethanol 95%/glycol 5%		
12097EV90390749021	STCU	20150508	STCU.20150508.runs.3		runs	surber	ethanol 95%/glycol 5%		
12097EV90390749021	CUPE	20150508	CUPE.20150508.riffles.1		riffles	surber	ethanol 95%/glycol 5%		
12097EV90390749021	CUPE	20150508	CUPE.20150508.riffles.2		riffles	surber	ethanol 95%/glycol 5%		
12097EV90390749021	CUPE	20150508	CUPE.20150508.riffles.3		riffles	surber	ethanol 95%/glycol 5%		
12097EV90390749021	CUPE	20150508	CUPE.20150508.riffles.4		riffles	surber	ethanol 95%/glycol 5%		
12097EV90390749021	CUPE	20150508	CUPE.20150508.riffles.5		riffles	surber	ethanol 95%/glycol 5%		
12097EV90390749021	CUPE	20150508	CUPE.20150508.runs.1		runs	surber	ethanol 95%/glycol 5%		
12097EV90390749021	CUPE	20150508	CUPE.20150508.runs.2		runs	surber	ethanol 95%/glycol 5%		
12097EV90390749021	CUPE	20150508	CUPE.20150508.runs.3		runs	surber	ethanol 95%/glycol 5%		
12097EV90390749021	GUIL	20150520	GUIL.20150520.surber.1		riffles	surber	ethanol 95%/glycol 5%		
12097EV90390749021	GUIL	20150520	GUIL.20150520.surber.2		riffles	surber	ethanol 95%/glycol 5%		
12097EV90390749021	GUIL	20150520	GUIL.20150520.surber.3		riffles	surber	ethanol 95%/glycol 5%		
12097EV90390749021	GUIL	20150520	GUIL.20150520.surber.4		riffles	surber	ethanol 95%/glycol 5%		
12097EV90390749021	GUIL	20150520	GUIL.20150520.surber.5		riffles	surber	ethanol 95%/glycol 5%		

Received by Michelle Chadwick 6/11/15 @ 1720  




## 2. GEI Project Protocol Sheet

### PROJECT PROTOCOL

Log Prefix: \_\_\_\_\_ Project Number: \_\_\_\_\_  
Date samples received: \_\_\_\_\_ Task: \_\_\_\_\_

Zooplankton samples taken  Y  N Periphyton samples taken  Y  N

Project Name (For Report Header): \_\_\_\_\_

Project Manager: \_\_\_\_\_

Sample Date(s): \_\_\_\_\_

Study Area (State, County):  
\_\_\_\_\_

Type(s) of Samples (check all that apply):

Hess  Surber  Sweep  TimedKick  Ponar  Ekman  Other: \_\_\_\_\_

Number of Sites: \_\_\_\_\_ Sample Preservative: \_\_\_\_\_

Number of Samples: \_\_\_\_\_

#### PROJECT TASKS

Pick Samples:  Y  N

Save Sediment:  Y  N

Mount/ID Midges and Oligochaetes:  Y  N

Special Picking Instructions: Hess/Surber/etc. Sweep  
G Default ----- min 300 pick in 1/10 min 350

Minimum # of organisms \_\_\_\_\_

Biomass:  Y  N

Identify samples:  Y  N

Reference Collection:  Y (new)  U (update)  N

Additional Instructions

### 3. GEI Macroinvertebrate Sample Flow Sheet

MACROINVERTEBRATE SAMPLE FLOW SHEET																					Page: <u>  </u> / <u>  </u>
Project: _____																					
Log Prefix: _____																					
LOG #	SORTING							CHIRON. & OLIGO. COUNT				CHIRON. & OLIGO. MOUNT				IDENTIFICATION			OLIG. ID		
	Date	Time	Picked	L & R	Zoop	Size	Init	Date	#	Time	Init	Date	#	Time	Init	Date	Time	Init	Date	Time	Init

#### 4. QA Sheet Picker

	A	B	C	D	E	F	G	H
1	NEON BENTHIC MACROINVERTEBRATE EXTRACTION QA BENCH SHEET							
2		Project:			Log #:			
3	Log #			Total Bugs before QC (S1)	$\frac{(QC*4)}{\text{Sort 1}}$	Accept (Y/N)	Bugs left in sample (75%) (QC*3)	
4	Date Sort 1:	Sort 1	QC (25%)			%		
5	Date QC1:							
6	Date Sort 2:	Sort 2	QC (25%)	Total Bugs before QC (S1+QC1+S2)	$\frac{(QC*4)}{\text{Total Bugs}}$	Accept (Y/N)	Bugs left in sample (75%) (QC*3)	
7	Date QC2:					%		
8	Date Sort 3:	Sort 3	QC (25%)	Total Bugs before QC (S1+QC1+S2+QC2+S3)	$\frac{(QC*4)}{\text{Total Bugs}}$	Accept (Y/N)	Bugs left in sample (75%) (QC*3)	
9	Date QC3:					%		
10	Date Sort 4:	Sort 4	QC (25%)	Total Bugs before QC (S1+QC1+S2+QC2+S3+QC3+S4)	$\frac{(QC*4)}{\text{Total Bugs}}$	Accept (Y/N)	Bugs left in sample (75%) (QC*3)	
11	Date QC4:					%		
	Extracted By: _____			QC Performed By: _____				

### 5. QA Sheet Taxonomy

	A	B	C	D	E	F	G	H	I	J
1	BENTHIC MACROINVERTEBRATE IDENTIFICATION QA BENCH SHEET									
2										
3	Project:	NEON					ID Date:		Total %	Bench Sheet
4	Log Number:	NE(6)1215-1							Similarity	Corrected
5	Analyzed By:						QA Date:		#DIV/0!	
6	QA by:									
7										
8	TSN	Taxon	Size	Original #	L&R	VC	%	QA #	%	% Similarity
9			0				#DIV/0!		#DIV/0!	#DIV/0!
10			0				#DIV/0!		#DIV/0!	#DIV/0!
11			0				#DIV/0!		#DIV/0!	#DIV/0!
12			0				#DIV/0!		#DIV/0!	#DIV/0!
13			0				#DIV/0!		#DIV/0!	#DIV/0!
14			0				#DIV/0!		#DIV/0!	#DIV/0!
15			0				#DIV/0!		#DIV/0!	#DIV/0!

## Appendix D Commonly Used Identification Keys

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Following is a list of general keys used for identification of benthic macroinvertebrates. Numerous regional and specific keys are also used; a list of literature resources is maintained, updated continuously, and available separately. Updated taxonomic references available by request.

- Brigham, A.R., W.U. Brigham, and A. Gnilka. 1982. *Aquatic Insects and Oligochaetes of North and South Carolina*. Midwest Aquatic Enterprises, Mahomet, IL.
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- Peckarsky, B.L., P.R. Fraissinet, M.A. Penton, and D.J. Conklin, Jr. 1990. *Freshwater Macroinvertebrates of Northeastern North America*. Cornell University Press, Ithaca, NY.
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- Stewart, K.W., and B.P. Stark. 2002. *Nymphs of North American Stonefly Genera (Plecoptera)*, 2<sup>nd</sup> Edition. The Entomological Society of America.
- Thorp, J.H., and A.P. Covich (eds.). 2001. *Ecology and Classification of North American Freshwater Invertebrates*, 2<sup>nd</sup> Edition. Academic Press, Inc., San Diego, CA.
- Usinger, R.L. (ed). 1974. *Aquatic Insects of California*. University of California Press, Berkeley, CA.
- Westfall, M.J., Jr., and M.L. May. 1996. *Damselflies of North America*. Scientific Publishers, Gainesville, FL.
- Wiggins, G.B. 1998. *Larvae of the North American Caddisfly Genera (Trichoptera)*, 2<sup>nd</sup> Edition. University of Toronto Press, Toronto, Canada.
- Wold, J.L. 1974. *Systematics of the Genus Rhyacophila (Trichoptera: Rhyacophilidae) in Western North America with Special Reference to the Immature Stages*. M.S. Thesis. Oregon State University, Corvallis, OR.

## Appendix E Research Literature

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- Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish*, 2<sup>nd</sup> Edition. EPA 841-B-99-002. Office of Water, U.S. Environmental Protection Agency, Washington, DC.
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