Rhithron Associates, Inc.
Standard Operating Procedures:
National Environmental Observation Network (NEON)
Benthic Macroinvertebrate Indicator

Version 1.0 March 2019

Corporate Approval

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Introduction

Rhithron processes and identifies macroinvertebrate, zooplankton, diatom and non-diatom algal (periphyton, phytoplankton) samples from clients throughout North America. The data generated from these samples need to be consistently and reliably generated to support the uses to which the data are put, typically, to assess water quality and habitat integrity in surface water systems. The methods and protocols applied to samples vary and depend on client-specifications and project goals. Thus, samples must be handled with the utmost attention and care and the client-specified protocol, including the required taxonomic resolution, must be faithfully followed. This document outlines the procedures used in the analyses of macroinvertebrate samples collected as part of the National Environmental Observation Network (NEON) program, and are adapted from Rhithron's Macroinvertebrate SOP ver.17.2.b. Rhithron's internal data quality objectives (see QAP Ver 18.1.a) are maintained by following these procedures.

All staff working on the NEON project samples, in any capacity , must review the version controlled Standard Operating Procedure in its entirety prior to the staff member working on the project. Additionally, if greater than two months lapse without a review of the NEON SOP then each staff member shall review the SOP in its entirety.

Laboratory organization

The organizational chart in Figure 1 shows the Rhithron personnel responsible for the various tasks associated with macroinvertebrate sample intake and analysis, and illustrates the pathways of communication that are used to assure the quality of Rhithron's work.

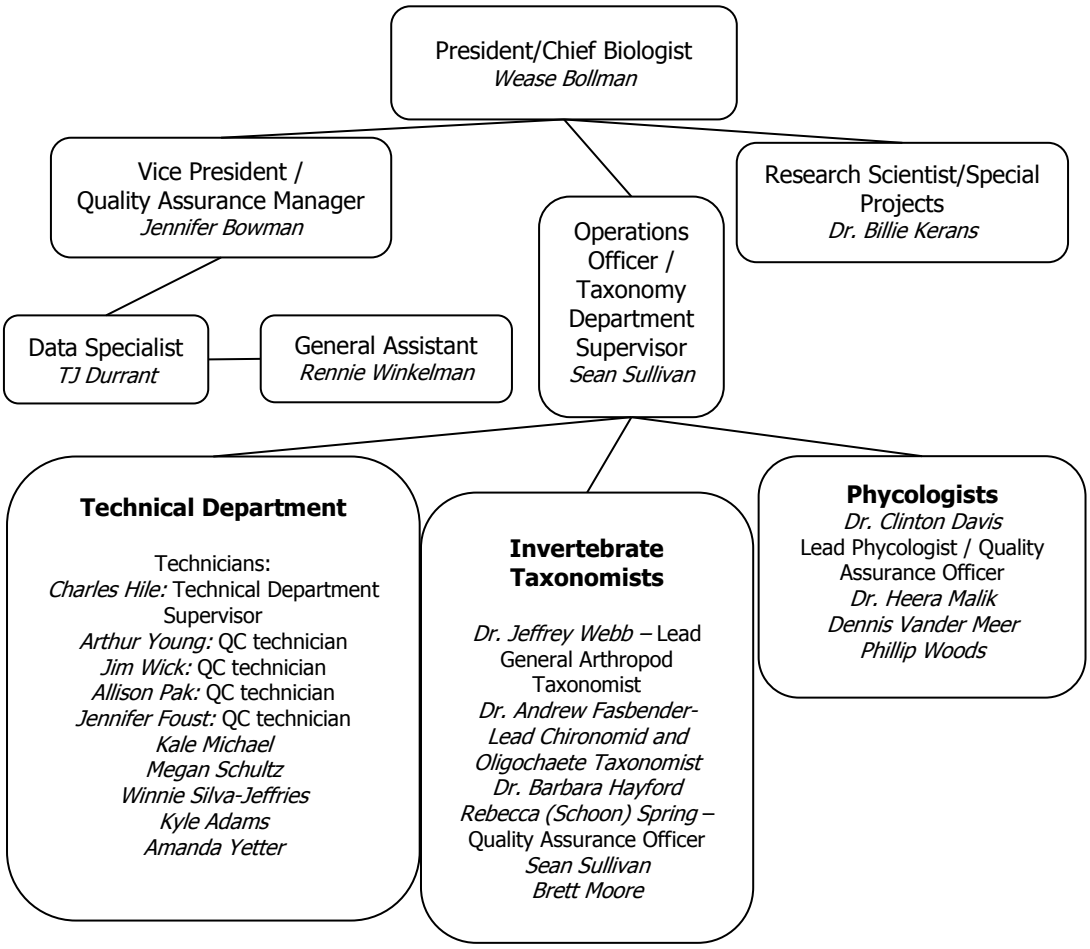


Figure 1. Rhithron Associates, Inc. organizational chart: November 2018

Macroinvertebrate samples

Health and safety warnings

In addition to the laboratory's usual requirements, the following health and safety procedures must be followed. All proper personal protection clothing and equipment (e.g., lab coat, protective eyewear/goggles must be worn or applied. When working with potential hazardous chemicals (e.g., 95% ethanol) or biological agents (benthic organisms or sediment) avoid inhalation, skin contact, eye contact, or ingestion. If skin contact occurs remove clothing immediately and wash/rinse thoroughly. Wash the affected skin areas thoroughly with large amounts of soap and water.

Project set-up

Goals

The goals of invertebrate project set-up procedures are to prepare samples for processing by the Technical Department and the Taxonomists while maintaining the integrity of the samples and to generate the required paperwork and computer files. The General Assistant (Figure 1) receives the samples when they arrive and is responsible for making sure that required procedures are followed.

Sample intake, inventory and chain of custody

Samples that Rhithron receives for processing are collected by clients, and delivered by commercial or postal carriers. Samples generally arrive at the laboratory's front door and the General Assistant signs delivery documents.

Scope

Using the following procedures, the General Assistant assesses the condition of the samples and preservative needs, makes sure that all samples correspond with a chain of custody or inventory (COC) provided by the client, and that all expected parts of the delivery have arrived safely. The procedures in this section pertain to macroinvertebrate sample deliveries, with alcohol (ethanol solution) preservation.

Personnel

The General Assistant is responsible for the completion of sample intake procedures, including generation of the project inventory report (which serves as the sample sign-out document for the Technical Department) upon completion of the intake and inventory procedures.

Procedures

When packages arrive, all shipping containers should be opened by the General Assistant and the COC should be located. The COC should be referred to during all following steps. Each sample jar should be removed and the level and integrity of preservative must be checked, recharging ethanol solution when required. The label of each sample container must be checked against the COC, and marked off as they are identified. The NEON provided barcode label is scanned. For samples with multiple jars, all jars need to be organized together, and scanned appropriately. Any leakage or damage, and any discrepancies between sample labeling and the COC document must be noted. This information must be reported to the Quality Assurance Manager (Figure 1) immediately who reports to NEON by telephone or email immediately. Any discrepancies must be rectified before custody documents are signed, copied, and returned to the client.

Once all samples have been checked off against the COC, and all discrepancies have been rectified by the client, the client-provided COC must be signed and copied. The original COC is returned to the client via fax, data upload to NEON data repository, email or USPS by the Quality Assurance Manager.

The General Assistant creates the Rhithron Associates Incorporated (RAI) Inventory file (an Excel file saved to the client folder). Entries in this file include the site name, client sample identifiers, replicate numbers, sample collection dates, the number of jars in each sample (e.g.,

1 of 2, 2 of 2, etc.), and any other distinguishing data. The RAI Inventory file contains a number of worksheets: 1) "*Client*" Client COC information, or information gathered by the General Assistant from jar labels, 2) "*Pre-check-in"* Client information that is copied into Rhithron-Laboratory Information Management System (LIMS) format for subsequent upload and to create sample jar labels and RAI numbers (internal identifiers) that are assigned to each sample, 3) "*Check-in*" Client information with an additional column with correct client info and consistent with the jar labels, it is a record of the jar/COC checking activity and also for recording discrepancies (communicates to Quality Assurance Manager the discreps he/she has to rectify with NEON) and also the Data Technician (Figure 1) adds discrepancies to the macroinvertebrate project LIMS (RAILIS) for documentation and 4) "*Sample Upload*" This sheet is used by the Data Technician. The General Assistant also places the physical copy of the COC into the General Assistant's filing cabinet

The General Assistant uploads the internal inventory into the Rhithron Associates, Inc. Laboratory Information System (RAILIS)and makes the sample jar labels (RAILIS output) which consist of the unique laboratory sample identification RAI numbers and labels for the all the sample jars. The General Assistant attaches these labels to the sample jars during check-in.

The General Assistant takes samples to the storage site. He/she records the location of the samples in the storage site, the type of preservative, whether the samples were decanted by the client in the project table in RAILIS. The General Assistant then notifies the Data Technician (and copies the Lead Technician (Figure 1)) that the sample data are ready for upload.

The Data Technician then gets custody of the samples and data. He/she uploads the internal inventory created by the General Manager into RAILIS and sample metadata into Rhithron's proprietary Electronic Periphyton and Invertebrate Cloud software (EPIC). RAILIS also generates the subsampling benchsheets, Project Inventory Report and vial labels for use during sorting that are printed by Lead Technician when the samples are about to be processed.

Sample storage and transfer to the Technical Department

Samples and projects awaiting processing are stored in the storage building behind the Rhithron laboratory. The storage building is locked and alarmed for unauthorized entry, and is equipped with a fire suppression system and fire alarms.

Procedures

The General Assistant or designee transfers samples to the secure storage area. Each project is assigned to an individual storage shelf or area designated only for that project and the location is noted in RAILIS. The samples and project remain in the custody of the Technical Department until samples have been processed, at which time custody of the samples and project is transferred to the Taxonomy Department.

Upon completion of taxonomy procedures, custody transfers back to the Technical Department, which transfers all completed sample materials to the secure storage area. Stored samples and sample fractions are checked monthly for sample integrity, and preservative is added as needed. Sample and project custody are tracked and recorded using the custom LIMS project mapping function.

Sorting for a subsample

Goal

The goal of invertebrate sample processing procedures is to produce a random sub-sampling of a raw benthic sample as delivered to Rhithron by NEON. Sub-samples must be produced in a standardized, repeatable manner, and sorting is quality-assured by the application of QC procedures to at least 10% of sorted samples. Sample sorting procedures must be applied so as to achieve the following outcomes:

- The target count of organisms is achieved within the specified tolerance limits (300 minimum).
- Sorting efficiency is maintained at an average level no lower than 90% for each project, thus assuring sorting accuracy and precision.
- The appropriate paperwork is associated with the correct sample.
- All data pertinent to the sub-sampling procedure, including fraction of sample used to obtain the target number of organisms, condition of the sample, any problems associated with sorting, and quality assurance procedure outcomes and statistics, etc. are recorded on sample benchsheets. See Figure X. for an example of the sub-sampling portion of a sample benchsheet.
- Cross-contamination between samples does not occur.

Considerations

The Protocol and Procedures

Rhithron's standard operating procedure for sorting/subsampling aquatic invertebrate samples is described in the following sections. These procedures are applicable to ethanol-preserved samples which are to be sorted to a 300-organism sub-sample. A complete project procedures page accompanies each project and every technician assigned to a project must review the project-specific protocols before beginning to process any samples. If there are any questions or uncertainty about any procedure or protocol detail, the Lead Technician should be consulted for clarification before proceeding.

The sample inventory and sign-out sheet

Each project has an associated internal sample inventory, which provides spaces for sample sign-out, located in the unsorted sample staging area. See an example of a project inventory/sign-out sheet in Figure Y. The General Assistant prints the inventory sheet during the process of sample intake, and delivers it to the technical laboratory with the benchsheets and labels for the project. The inventory sheet serves as an internal chain-of-custody document for the Technical Department and includes the protocol for that project. When a project is ready to be sorted, the inventory sheet is posted. A Technician signs out a sample when processing commences, a second Technician is required to double check all components of the sample metadata and jars to ensure sample completeness and integrity. When all sorting for a project has been completed, the inventory is placed into the project folder, and transferred to the Taxonomy Department.

Technical Department sample metadata

Technicians record sorting information by entering the information into an electronic data entry interface (EPIC). Each sample is identified uniquely within the interface, in order to prevent data association with the wrong sample. Metadata for each sample is recorded by the Data Technician immediately after all steps of sample check-in. All interface fields pertaining to sample preparation and sorting, and QC procedures must be filled out completely.

Scope and personnel qualifications

These procedures may be used by any person who has successfully completed the technical department training program. A laboratory staff member qualified to perform quality control (QC) checks (see below) must be present when samples are processed by an inexperienced staff member, or when QC checks are needed for an experienced sorter's samples.

QC procedures are performed by QC Technicians (Figure 1), who have received additional training and have at least 1 year of experience in the Technical Department and have achieved a mean sorting efficiency of at least 90% over the previous 6 months. QC systems in the Technical Department include examining the sorted substrate from at least 10% of samples in order to determine sorting efficiency for those samples. The fewer pickable organisms missed by the sorting technician, the better the sorting efficiency. QC technicians check all sorted substrate for technicians in training, and check a minimum of 20% of sorted substrate for experienced technicians. The QC technician calculates the Percent Sorting Efficiency (PSE) for each QC sample, and records it on the benchsheet. The QC technician also participates in QC procedures for sample check-out, by double-checking sample identifiers, number of jars, and other parameters for each sample that is checked out prior to sorting and sub-sampling.

The Lead Technician serves as the department quality assurance officer. The Lead Technician provides oversight of daily operations related to sample processing, monitors QC activities to ensure conformance, periodically conducts performance and system audits, verifies the entry of data on benchsheets for completeness and appropriateness, determines sorting efficiency for each technician, performs evaluations to ensure that QC is maintained throughout the sorting and sub-sampling procedures and that the appropriate protocols are applied to all aspects of sample processing.

Materials and equipment

Caton tray (Caton 1991)
Plastic holding tray(s) for Caton screen(s)
1000 ml Nalgene jars
Ethanol solution (95% ethanol + 5% glycerol), in wash bottle
scissors
scoops, spoons or spatula
Caton squares (6cm x 6cm)
nitrile examination gloves
stereoscopic microscope (Leica S6)x 13
Fiberoptic or LED illuminator
3x lighted magnifier
Std. US #60 250 micron soil sieve
specimen cup
specimen handling tools (forceps, needles, pipette)
petri dishes
LIMS-generated sample labels (see Figure Z)
benchsheet (pre-printed and specific to a particular sample)

Methods

Selecting a sample and sample sign-out

Before a Technician (Figure 1) begins sample selection, he/she ensures that his/her workstation has been cleared of any and all materials related to another sample, including jars, vials, benchsheets, and labels. The Technician must determine the next sample to be sorted and check the inventory/sign-out sheet for protocols. The Technician initials the inventory/sign-out

sheet indicating that he/she has reviewed the protocols specific to the project. The Technician then removes the correct sample from the sample staging area project shelf.

The Technician must check the inventory/sign-out sheet to make sure that the correct sample and all jars associated with that sample have been obtained and must compare the outside and inside sample labels with the inventory/sign-out sheet information for that sample. If the Technician discovers any discrepancies, he/she must notify the Lead Technician immediately. Samples that have discrepancies cannot be processed further until the problems are rectified. The Technician then selects the bench sheet associated with the sample, matching RAI numbers and other sample identifiers.

Finally, the Technician must obtain a second check of his/her work to this point from a QC Technician. The QC Technician checks to see that all jars are collected, that all jar identifiers match one another and the benchsheet. The QC Technician checks all information against the inventory and checks off each item on the inventory/sign-out sheet. The QC Technician checks that the proper bench sheet and labels for the sample have been selected. When all of the information has been checked, the QC Technician initials next to the RAI number on the top left hand corner of front page of benchsheet.

Sample preparation

Before beginning the preparation procedure, the Technician should be sure that all sorting equipment is thoroughly cleaned and free of organisms. He/she should carefully examine sieves, Caton tray components, and all other sorting equipment using a 3x lighted magnifier before proceeding.

The Technician should wear nitrile gloves while preparing samples. The sample should be gently mixed in its jar(s). The Technician decants the alcohol preservative while pouring the sample out of each jar, using the 250-micron soil sieve (US #60) and the plastic Caton holding tray in the rinsing sink. If the alcohol is not excessively stained or diluted, retain it for reuse as preservative for the unsorted portion of sample, otherwise, discard the alcohol down the rinsing sink drain. Pour the sample out into the 250-micron sieve. Retrieve all internal sample labels and rinse them of all debris and organisms into the sieve. Check once again to make sure that the internal labels correspond with the bench sheet and the inventory. Save all labels and staple them to the back lower left of bench sheet once they are dried. Gently rinse the sample jar, retaining all contents on the sieve. All material from the jar(s) is now contained on the 250-micron sieve.

Using the 250-micron sieve, gently wash the sample, running cold tap water over it to remove any fine material. Transfer the sieve contents onto the Caton screen. Rinse the sieve onto the Caton screen to collect any organisms or debris that may have been retained in the sieve. Inspect the sieve with the 3x lighted magnifier. Be sure the sieve is clean to prevent cross contamination between samples. Place all organisms retrieved from the sieve onto the Caton screen.

Place the Caton screen into the plastic holding tray. Add enough water to spread the sample evenly over the Caton screen. Move the sample into the corners of the pan using gloved hands, forceps or other equipment. Agitate the tray and screen to help spread the sample. If the sample is composed of different types of material, be sure that there is thorough mixing of all types. Lift the Caton screen out of the plastic tray to drain. Pour off the water from the plastic tray and set the screen back into the tray. Add just enough water to the tray so that it barely covers the screen while it is in the tray. Be careful not to add so much water that the sample material floats around.

Precautions

Never allow a sample to dry out during any stage of preparation or sorting. To ensure that there is no cross-contamination between samples, before beginning sample preparation, and after completion of preparation, be sure to examine sieves, Caton screens, spatulas, spoons, scoops, and all other materials to make sure that no organisms or sample residues are adhering to surfaces. Sample preparation and sorting is often complicated by the materials present in the samples. In every case, your goal is to mix materials as thoroughly as possible and randomly distribute mixed materials over the Caton screen. Do not separate different kinds of materials in the Caton tray.

Obtaining the sub-sample by sorting

Use a random number generator, such as a pair of dice, to select a grid for sorting. Use the Caton cookie-cutter device to delineate the selected grids, moving the sample material very slightly to push the material in the selected grid together, in order to make it easier to get it out of the tray. Using a scoop, scraper, spoon, or other appropriate equipment, lift the contents of the selected grids into petri dishes, one grid in each dish, and add water from a wash bottle to the sample material to avoid desiccation and to disperse the material in the petri dishes. Depending on the consistency of the sample material, it may be necessary to use scissors during these steps.

Examine the Caton screen for any organisms remaining with in each sampled grid. Use the following rules when dealing with organisms that lie on the line between two grids. First, an organism belongs to the grid where its head is. Second, if you can't determine where the head is, the organism belongs to the grid containing most of its body. Third, if part of an organism's head is on either side of the line, pick the organism if the line is on the "top" of the grid or the right side of the grid. Be sure not to let any sample fractions dry out or get spilled. Cover the Caton tray while sorting the selected grids, and do not allow the material in the Caton tray to move around.

Examine the contents of each of the selected grids under the microscope, using at least 10x total magnification. All organisms from the selected fractions, or grids, must be sorted to minimize bias. Technicians are trained to pick and count all: obligate benthic macroinvertbrates, Collembola, sub-aquatic adults (e.g. Gerromorpha), Hydra and Oligochaeta. Technicians will not remove or count: terrestrial invertebrates, vertebrates, Zooplankton, empty mollusk shells, empty Trichoptera cases, exuviae, and headless organisms. Technicians will note the presence of Byrozoa and Porifera in the sample comments on the benchsheets and EPIC.

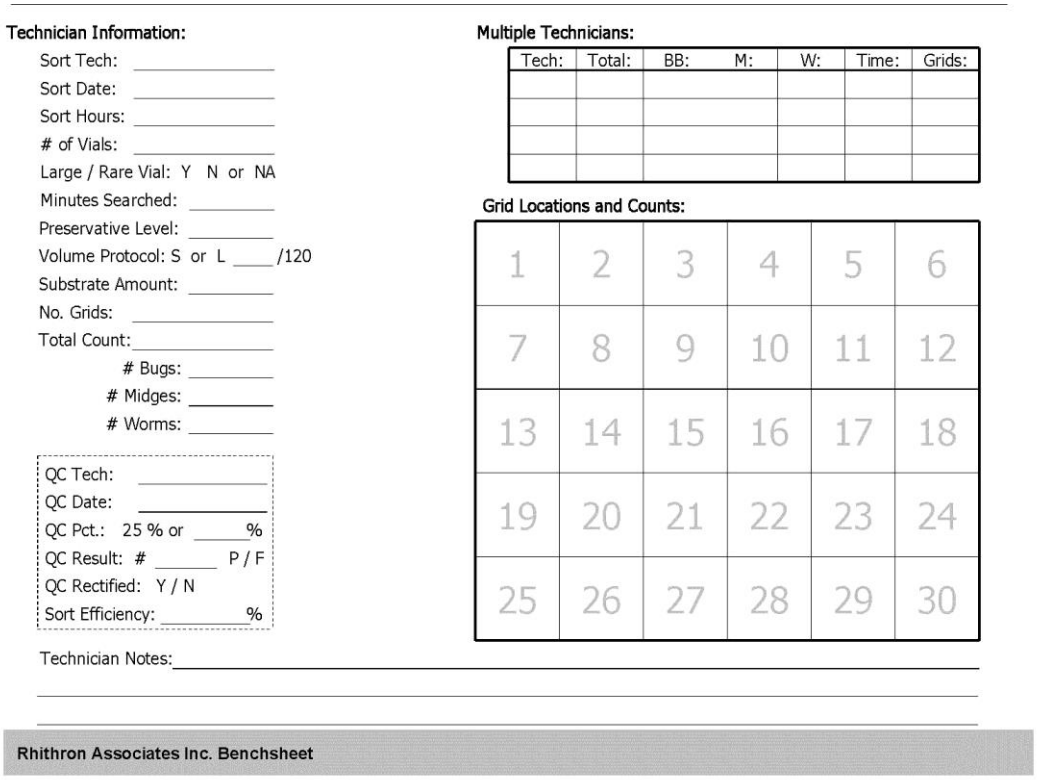
If the target is not reached when the first grid is completely picked and fully processed (including organisms recovered during QC checks), subsequent grids would be randomly selected and each picked to completion until a minimum of organisms is reached.

All samples are sorted using 6.3-60x magnification (Leica S6 stereomicroscopes).
Remove the invertebrates from the sample material in each grid, using forceps. Place organisms for identification in appropriately labeled 1 dram snap cap vial(s). Sort through the substrate material thoroughly.

Using mechanical counters, keep a running count of the total number of organisms picked as well as a separate count of the number of chironomids and the number of Oligochaeta.

If a sorter is unsure as to whether a specimen should be counted or not, he or she should place the organism in the sort vial without counting it (the final count is made by the taxonomist).

QC Technicians will perform sorting efficiency checks on a minimum of 20% of sample substrate of every sample (100% of samples are subject to sorting QC). See procedures for QC in Rhithron's Laboratory Quality Assurance Plan document. All material that is not sorted must be returned to the original sample jar(s) and preserved with fresh ethanol solution. Place the original sample jar(s) on the shelves reserved for processed sample fractions. Unsorted and sorted sample fractions are archived for QC and potential return to the client. Place subsampled organisms into the labeled specimen cup, and place the specimen cup in the sorted sample storage area.



Technician Information:

Sort Tech: _____
Sort Date: _____
Sort Hours: _____
# of Vials: _____
Large / Rare Vial: Y N or NA
Minutes Searched: _____
Preservative Level: _____
Volume Protocol: S or L ____/120
Substrate Amount: _____
No. Grids: _____
Total Count: _____
Bugs: _____
Midges: _____
Worms: _____

QC Tech: _____
QC Date: _____
QC Pct.: 25 % or _____%
QC Result: # _____ P / F
QC Rectified: Y / N
Sort Efficiency: _____%

Multiple Technicians:

Tech:	Total:	BB:	M:	W:	Time:	Grids:

Grid Locations and Counts:

1	2	3	4	5	6
7	8	9	10	11	12
13	14	15	16	17	18
19	20	21	22	23	24
25	26	27	28	29	30

Technician Notes: _____

Rhithron Associates Inc. Benchsheet

Figure X. Sample benchsheet detail: Technician information and sorting/sub-sampling data fields.

Project Inventory Report

Project ID: USGS14NES
Project Name: Northeast U.S. Streams 2014

Customer Information
USGS
Jim Coles
603.226.7845
jcoles@usgs.gov

Project Management Information
Date Arrived: 9/16/2014
Date Due: 1/16/2015
No. Samples: 66
No. Jars: 76

Project Arrival Information
Preservative: Formalin
Arrival Notes:
Storage Location: U2 Bottom

Project Location Information
USA
Multiple States

Project Archive Information

Sample Portion:	Initials	Date	Archive/Ship Location	Disposal: Initials	Disposal: Date
Unsorted remainder					
Sorted remainder					
Identified organisms					
Reference/voucher					

Sample Information

Tech	RAI Number	Station Name	Client ID	Date Coll.	No. Jars	Inventory Discrepancies
	USGS14NES001	Diamond River near Wentworth Location, NH	1052500	8/27/2014	1	
	USGS14NES002	Wild River at Gilead, Maine	1054200	8/28/2014	1	
	USGS14NES003	Swift River near Roxbury, Maine	1055000	8/27/2014	1	

Figure Y. Project inventory/sign-out sheet

Figure Z. LIMS-generated labels, including RAI number label for specimen cup lid, sample label for inside the specimen cup, and RAI number labels for sample fraction vials.

Identification and enumeration

Goal

The goal of the taxonomic portion of sample processing is to identify and enumerate organisms accurately and precisely, to the NEON specific taxonomic effort. Bias is minimized, data are reported completely, and the quality and integrity of data are consistent. Materials related to the project, including labeled microscope slides, labeled vials with identified organisms, and laboratory benchsheets are handled carefully and are archived on the completion of identification and enumeration, and after all QA/QC procedures and data reviews have been completed. Deliverables such as reference collections and photo collections are assembled accurately and completely. Higher levels of taxonomy applied to organisms that cannot be identified to taxonomic targets are explained and qualified in all cases. Life stages are accurately recorded in the data. A citation list of primary and secondary taxonomic literature sources is maintained for each project, to maximize comparability of data.

Considerations

The Protocol and Procedures document

Before a Taxonomist (Figure 1) begins any sample, he/she should review the NEON specific taxonomic requirements: requirements for each project are located on the P&P (Protocol and Procedures) document. P&P documents are found in the "Protocol and Procedures" binder, which is kept in the sorted sample storage area. For reference purposes, Taxonomists can also access P&P documents on the Rhithron network server at \\RHITHRON1\Data\Taxonomy\Taxonomic resolution templates. Look for the Rhithron project name to retrieve the appropriate document. Every Taxonomist assigned to a project must sign off on the appropriate P&P document before beginning any sample.

The sample sign-out sheet

Each project has an associated sample sign-out sheet, which is located in the sorted sample staging area. When a Taxonomist obtains a sample to begin identification and enumeration, he/she should consult this sheet for an available sample. Locate the sample, and put his/her initials and the date in the appropriate column next to his/her sample's RAI number. This form serves as an internal chain-of-custody document for the Taxonomy Department. Make sure that you check the RAI number on the specimen cup against the RAI number on the sign-out sheet. When identification of the sample is completed, initial the sample sign-out sheet, indicating that the sample has been returned to the sorted sample storage area.

The Taxonomist must ensure that his/her workstation is clear of any material related to any other sample before beginning. Projects requiring reference collections will have related procedures described in a later section. Taxonomists will record their data in the EPIC data entry software (EPIC v. 1.7). Procedures for the use of the EPIC data entry software (EPIC v. 1.7) are included later in this document. The EPIC data entry software application uploads data automatically to the Rhithron LIMS. When available ITIS (http://www.itis.usda.gov) taxonomic serial numbers (TSN) are associated with each taxon in the Rhithron LIMS. Taxon entries in the Rhithron LIMS are periodically matched to the ITIS TSNs. Each new taxon entry into the Rhithron LIMS includes the ITIS TSN when it is available. Some taxonomic entries in the Rhithron LIMS do not have associated ITIS TSNs. Such entries include valid names which occur in the literature but are not included in ITIS "Slashed" taxonomies, such as *Pericoma/Telmatoscopus, Chelifera/Metachela*, etc., taxonomic groups, such as the *Rhyacophila vemna* Group, *Cricotopus tremulus* Group, etc. and taxa that are apparently undescribed, such as *Nanocladius* sp. D (Epler). For these, no ITIS TSN is reported with the taxon entry.

Technical Department benchsheets

Technicians record sorting information on paper benchsheets (Figure x), which are delivered to the sorted sample storage area along with the sorted samples. Records from these benchsheets that are pertinent to the Taxonomists are: the number of vials used for the sorted sample, the count of general arthropods, the count of chironomids, and the count of oligochaetes. Technical Department benchsheets are located in file folders in the sorted sample storage area.

Transfer of sorted samples to the Taxonomy Department

Sorted samples are delivered from the Technical Department to the Taxonomy Department by the Project Manager or his/her designee. Sorted samples are stored in green-lidded medical specimen cups. The RAI sample identification number (RAI number) is located on a label on the specimen cup lid. The specimen cup contains vials with sorted specimens; each vial contains a small label with the RAI number. Typically, there is a vial with chironomids, a vial with oligochaetes, one or more vials with general arthropods, and potentially other vials as well. There is also a large sample label inside the specimen cup (Figure Z). All RAI numbers on and in the specimen cup should be identical. Immediately report any discrepancies to the Taxonomy Department Supervisor (Figure 1), who will resolve these problems. Samples with label discrepancies cannot be processed further until problems are resolved.

General arthropod identifications

Scope, related procedures and personnel qualifications

Rhithron's standard operating procedure for identification and enumeration of the general arthropod portions of aquatic invertebrate samples is described in the following sections. The review includes the taxa lists from previous projects from the same area or client when available, the protocols specified for the project, and the quality control results from previous projects from the same area or client when available. The Taxonomy Department Supervisor oversees this review; in addition, taxonomic protocols for each project are documented in a project log, which is available in the taxonomy laboratory throughout the progress of the project. Project-specific protocols are also available on the Rhithron network server.

These procedures are applicable to pre-sorted non-chironomid, non-oligochaete portions of benthic collected as part of the NEON project.

These procedures apply to the Taxonomy staff who report to the Taxonomy Department Supervisor. Taxonomy staff members who identify general arthropod portions of invertebrate samples hold SFS Level II-certifications in Eastern and Western EPT and General Arthropod taxa groups. Under the guidance of the Taxonomy Department Supervisor, Taxonomists are responsible for working as a team to ensure staff are current with changes in taxonomic nomenclature, geographic distributions, and other issues relevant to performing these procedures. Taxonomists interact with other professionals in the field via listservs, meetings and workshops offered by professional societies (e.g. SFS, NBAW, SAFIT, etc.), and informal communications.

Quality control (QC) systems include "blind" re-identification of at least 10% of samples (see procedures for QC in the Rhithron Laboratory Quality Assurance Plan document). The Taxonomy Department Supervisor is responsible for the oversight for these procedures.

Materials and equipment

20mL scintillation vial with cap
Specimen handling tools (forceps, pipette, needles, etc.)
Ethanol solution wash bottle with ethanol
Watch glasses
Sorting palette
Vial or tube rack
Label supplies
Stereoscopic microscope (Leica S8A) x6
Compound Microscope (Leica DM1000)x6
Fiberoptic illuminator (Dolan-Jenner MI 150 or MI 151)x6
Networked computer, located at the microscope, for access to EPIC data entry software

Methods

To prevent any sample cross-contamination, a Taxonomist should ensure that hi/her workstation is clear of all material related to any other sample before beginning. The Taxonomist should obtain a sample from the sorted sample storage area. The Taxonomist puts his/her initials and the date on the sample sign-out sheet, to indicate that he/she has accepted custody of, and responsibility for, the sample. Obtain the Technical Department benchsheet for that sample.

At the workstation, carefully open the specimen container and remove the large sample label and the vials of sorted specimens. Make sure that all RAI numbers on labels, in vials, and on benchsheets correspond with the sample that was signed out. Make sure that the number of vials entered by the Technician on the Technician benchsheet corresponds with the number of vials found in the specimen cup. Immediately notify the Taxonomy Department Supervisor if any discrepancies are encountered. Samples with discrepancies cannot be processed further until problems are resolved.

The Taxonomist initials the front of the large label and places it in the 20mL scintillation vial so that it can be read from the outside of the vial. Fill the vial about halfway with fresh ethanol. He/she should locate the vials with general arthropods. These are usually labeled "BB" with marker pen on the cap of the vial. Carefully spill the contents into a watch glass, checking for organisms that may stick to the cap or to the vial. Add ethanol as needed to keep the sample organisms covered.

Using 10x – 80x magnification, the Taxonomist sorts the organisms, using the sorting palette as needed. He/she should examine each organism and identify each to the required taxonomic resolution, referring as needed to the P&P document on the server or in the Protocol and Procedures binder. Identifications are made with reference to resources in Rhithron's taxonomic resource library, which is a collection of books and documents in hard copy (in the Taxonomy Department Library) and/or in electronic form on Rhithron's network server at: \\SERVER1\Data\Taxonomy\Taxonomic Resources. Do not allow any sample portion to dry out at any time.

The Taxonomist counts each taxon. Record taxa names, counts, life stage, uniqueness, qualifiers, and reference collection information in the EPIC data entry program, using the procedures found in a later section of this document. Terms such as "uniqueness" and "qualifiers" are defined there. Place identified organisms into the 20mL scintillation vial. Add ethanol to the vial as needed.

Taxonomists measure each individual's total body length (excluding cerci) to the nearest 1mm and record the measurements in EPIC. Measurements are made through the microscope at 10x magnification using an ocular micrometer, or a stage micrometer for larger specimens. Specimens that are damaged to an extent such that it precludes total body measurements are estimated based on other specimens of the same taxon from within the same sample, or best professional judgement.

When the Taxonomist has completed the general arthropod identification for the sample, cap the 20mL scintillation vial and place it into the green-lidded specimen cup. Make sure that all other vials (chironomids, oligochaetes, etc.) are replaced in the specimen cup. Return the cup and the technical department benchsheet to the sorted sample storage area.

The Taxonomist should clean-up after the general arthropods of a sample have been identified includes thorough washing and drying of watch glasses, sorting palettes, forceps and needles, and any other equipment that was used in the identification process.

Chironomid identifications

Scope, related procedures and personnel qualifications

Rhithron's standard operating procedure for identification and enumeration of the chironomid portions of aquatic invertebrate samples is described in the following sections. The review includes the taxa lists from previous projects from the same area or client when available, the protocols specified for the project, and the quality control results from previous projects from the same area or client when available. The Taxonomy Department Supervisor oversees this review; in addition, taxonomic protocols for each project are documented in a project log, which is available in the taxonomy laboratory throughout the progress of the project. Project-specific protocols are also available on the Rhithron network server.

These procedures are applicable to pre-sorted chironomid-only portions of benthic samples collected as part of the NEON project. These procedures are applicable when the client-specified taxonomic resolution for chironomids is genus resolution or finer. If chironomids are to be identified to family or sub-family/tribe, follow the procedures for "General arthropod identifications" above. Information about taxonomic resolution specifications for a project may be found in the Protocol and Procedure manual or on the Rhithron network server.

These procedures apply to the Taxonomy staff, which reports to the Taxonomy Department Supervisor. Taxonomy staff members who analyze chironomid sample portions hold SFS Level II-certifications in Chironomidae. Under the guidance of the Taxonomy Department Supervisor, Taxonomists are responsible for working as a team to ensure staff are current with changes in taxonomic nomenclature, geographic distributions, and other issues relevant to performing these procedures. Taxonomists interact with other professionals in the field via listservs, meetings and workshops offered by professional societies (e.g. SFS, NBAW, SAFIT, etc.), and informal communications.

Quality control (QC) systems include "blind" reidentification of at least 10% of samples (SEE PROCEDURES FOR QC IN RHITHRON'S LABORATORY QUALITY ASSURANCE PLAN DOCUMENT). Internal tracking and chain-of-custody documentation is required in the Taxonomy Department The taxonomy department supervisor is responsible for the oversight for these procedures.

Materials and equipment

1 dram snap cap vials
Specimen handling tools (forceps, pipette, needles, etc.)
Ethanol solution wash bottle with ethanol
Watch glass
Sorting palette
Vial or tube rack
Stereoscopic microscope (Leica S8A) x6
Fiberoptic illuminator (Dolan-Jenner MI150 or MI151)
Standard microscope slides
Standard microscope slide cover slips
CMC-10 mounting medium (Masters Chemical Company)
Mounting medium applicator
Glass marking pen
Label supplies
Slide map benchsheet
Compound microscopes (Leica DM1000 x 6, Olympus BX51) with 40x, 60x and 100x magnification, and other objectives as needed
Networked computer, located at the microscope, for access to EPIC data entry software

Methods

The Taxonomist should ensure that his/her workstation is clear of all material related to any other sample. This step is intended to prevent any potential sample cross-contamination. The Taxonomy staff member should obtain a sample from the sorted sample storage area. The Taxonomist puts his/hers initials and the date on the sample sign-out sheet and obtains the Technical Department benchsheet for that sample.

At his/her workstation, carefully open the specimen container and remove the vial of sorted chironomid specimens. This vial is usually labeled "M" with marker pen on the vial cap. The Taxonomist should make sure that all RAI numbers on labels, in vials, and on benchsheets correspond with the sample that was signed out and that the number of vials entered by the technician on the Technician benchsheet corresponds with the number of vials found in the specimen cup. The Taxonomist should immediately notify the Taxonomy Department Supervisor if any discrepancies are encountered. Samples with discrepancies cannot be processed further until problems are resolved.

The Taxonomist should carefully spill the contents into a watch glass, checking for organisms that may stick to the cap or to the vial. If the "morphotyping" protocol is used, replace the small vial label and fill the vial about halfway with fresh ethanol. If the "complete slide mounting" protocol is used, the label may be discarded.

Using 10x – 80x magnification, the Taxonomist should sort the chironomids, using the sorting palette as needed. He/she should make slide mounts as needed (minimum 10% of Chironomidae), using CMC-10 mounting medium, applying a coverslip. A label, indicating the sample identifier (RAI number) and the number of the slides in the sequence for that sample, should be placed on each slide. Allowing CMC mounted material to cure overnight enables the medium to digest soft tissues that may interfere with identification. If permanent slide mounts are required, clear enamel is used to ring the coverslip.

The Taxonomist should examine slide-mounted organisms under the compound microscope. Locate all essential diagnostic characteristics, using the appropriate key or taxonomic resource literature. Identify each to the required taxonomic resolution, referring as needed to the P&P document on the server or in the Protocol and Procedures binder. Identifications are made with reference to resources in Rhithron's taxonomic resource library, which is a collection of books and documents in hard copy (in the Taxonomy Department Library) and/or in electronic form on Rhithron's network server at: \\SERVER1\Data\Taxonomy\Taxonomic Resources.

The Taxonomist should use the slide map benchsheet to record identifications for slide-mounted chironomids. The slide map serves as a record of the number of midges on slides, and their locations, which facilitates QC procedures. Recording the location of each slide-mounted midge also allows taxonomists to easily locate problematic organisms for discussions and rectifications.

When the Taxonomist is identifying chironomids he/she should use the following conventions, unless the client-specified protocol calls for something different. Damaged organisms are identified and counted only if a chironomid fragment includes the head.

The Taxonomist counts each taxon and records taxa names, counts, life stage, uniqueness, qualifiers, and reference collection information in the EPIC data entry program, using the procedures found in a later section of this document. Terms such as "uniqueness" and "qualifiers" are defined there. Midges not mounted on slides are placed in a 1 dram snap cap vial with the sample identifier label. The Taxonomist should initial this label on the back.

Prior to any temporary or permanent slide mounting, Taxonomists measure each individual's total body length (excluding cerci) to the nearest 1mm and record the measurements in EPIC. Measurements are made through the microscope at 10x magnification using an ocular micrometer, or a stage micrometer for larger specimens. Specimens that are damaged to an extent such that it precludes total body measurements are estimated based on other specimens of the same taxon from within the same sample, or best professional judgement.

Reference collection specimens are slide-mounted organisms that are to be included in the collection. After identification and when the CMC medium is no longer fluid, the Taxonomist should organize slides by sample number in a slide box exclusive for the project.

When the Taxonomist has completed the chironomid identification for the sample, he/she caps the 1 dram snap cap vial and places it into the green-lidded specimen cup. He/she should make sure that all other vials (general arthropods, oligochaetes, etc.) are replaced in the specimen cup. The specimen cup and the technical department benchsheet are returned to the sorted sample storage area.

Clean-up after a sample's chironomids are identified includes thorough washing and drying of watch glasses, sorting palettes, forceps and needles, and any other equipment that was used in the identification process.

Oligochaete identifications

Scope, related procedures and personnel qualifications

Rhithron's standard operating procedure for identification and enumeration of oligochaete portions of aquatic invertebrate samples is described in the following paragraphs. A review of protocols includes the taxa lists from previous projects from the same area or client when available, the protocols specified for the project, and the quality control results from previous projects from the

same area or client when available. The Taxonomy Department Supervisor oversees this review; in addition, taxonomic protocols for each project are documented in a project log, which is available in the taxonomy laboratory throughout the progress of the project. Project-specific protocols are also available on the Rhithron network server.

These procedures are applicable to pre-sorted oligochaete-only portions of benthic samples collected as part of the NEON project. These procedures are applicable when the client-specified taxonomic resolution for oligochaetes is genus resolution or finer. If oligochaetes are to be identified to sub-class (i.e. "Oligochaeta"), follow the procedures for "General arthropod identifications" in Procedure 1. above. Information about taxonomic resolution specifications for a project may be found in the Protocol and Procedure manual or on the Rhithron network server. See part b.1 below to locate this information.

Two procedures for oligochaete identification are described here: one protocol uses morphotyping as a time-and-effort saving procedure; the other protocol calls for slide mounting of each and every specimen. Client specifications may require complete slide mounting, otherwise, Rhithron's standard procedure is to carefully morphotype specimens, slide mounting representatives of each taxon or identifying unmounted specimens when possible.

These procedures apply to the Taxonomy staff, which reports to the Taxonomy Department Supervisor. Taxonomy staff members who analyze oligochaete sample portions hold SFS Level II-certifications in Oligochaeta. Under the guidance of the Taxonomy Department Supervisor, Taxonomists are responsible for working as a team to ensure currency with changes in taxonomic nomenclature, geographic distributions, and other issues relevant to performing these procedures. Taxonomists interact with other professionals in the field via listservs, meetings and workshops offered by professional societies (e.g. SFS, NBAW, SAFIT, etc.), and informal communications.

Quality control (QC) systems include "blind" reidentification of at least 10% of samples. See procedures for QC in Rhithron's Laboratory Quality Assurance Plan document. Internal tracking and chain-of-custody documentation is required in the Taxonomy Department. The Taxonomy Department Supervisor is responsible for the oversight for these procedures. These procedures were revised in February 2013, and replace all preceding protocol documents.

Materials and equipment

1 dram snap cap vials
Specimen handling tools (forceps, pipette, needles, etc.)
Ethanol wash bottle with ethanol
Watch glass
Sorting palette
Vial or tube rack
Stereoscopic microscope (Leica S8A x6)
Fiberoptic illuminator (Dolan-Jenner 150 or 151)
Standard microscope slides (part number)
Standard microscope slide cover slips (part number)
CMC-10 mounting medium (Masters Chemical Company)
Wintergreen oil (effective Oligochaeta clearing medium)
Mounting medium applicator
Glass marking pen
Label supplies
Slide map benchsheet
Compound microscope (Leica DM1000 x 6, Olympus BX51) with 40x, 60x and 100x magnification, and other objectives as needed
Networked computer, located at the microscope, for access to EPIC data entry software

Methods

The Taxonomist should ensure that his/her workstation is clear of all material related to any other sample. This step is intended to prevent any potential sample cross-contamination. The Taxonomy staff member should obtain a sample from the sorted sample storage area. The Taxonomist puts his/hers initials and the date on the sample sign-out sheet and obtains the Technical Department benchsheet for that sample.

At his/her workstation, carefully open the specimen container and remove the vial of sorted oligochaete specimens. This vial is usually labeled "W" with marker pen on the vial cap. The Taxonomist should make sure that all RAI numbers on labels, in vials, and on benchsheets correspond with the sample that was signed out and that the number of vials entered by the technician on the Technician benchsheet corresponds with the number of vials found in the specimen cup. The Taxonomist should immediately notify the Taxonomy Department Supervisor if any discrepancies are encountered. Samples with discrepancies cannot be processed further until problems are resolved.

The Taxonomist should carefully spill the contents into a watch glass, checking for organisms that may stick to the cap or to the vial. If the "morphotyping" protocol is used, replace the small vial label and fill the vial about halfway with fresh ethanol. If the "complete slide mounting" protocol is used, the label may be discarded.

Using 10x – 80x magnification, the Taxonomist should sort the oligochaetes, using the sorting palette as needed. Slide mounts should be made by the Taxonomist as needed, using CMC-10 mounting medium and a coverslip. A label should be placed on each slide, indicating the sample identifier (RAI number) and the number of the slide in the sequence for that sample. Allowing CMC mounted material to cure overnight enables the medium to digest soft tissues that may interfere with identification. If permanent slide mounts are required, clear enamel is used to ring the coverslip.

The Taxonomist should examine slide-mounted organisms under the compound microscope. And locate all essential diagnostic characteristics, using the appropriate key or taxonomic resource literature. The Taxonomist should identify each to the required taxonomic resolution, referring, as needed, to the P&P document on the server or in the Protocol and Procedures binder. Identifications are made with reference to resources in Rhithron's taxonomic resource library, which is a collection of books and documents in hard copy (in the Taxonomy Department Library) and/or in electronic form on Rhithron's network server at: \\SERVER1\Data\Taxonomy\Taxonomic Resources.

The Taxonomist should use the slide map benchsheet to record identifications for slide-mounted oligochaetes. The slide map serves as a record of the number of worms on slides, and their locations, which facilitates QC procedures. Recording the location of each slide-mounted worm also allows taxonomists to easily locate problematic organisms for discussions and rectifications.

The Taxonomist should use the following conventions, unless the client-specified protocol calls for something different. First, damaged organisms are only identified and counted if the head and enough additional segments for identification are present .

The Taxonomist should count each taxon and record taxa names, counts, life stage, uniqueness, qualifiers, reference collection information, and non-slide-mounted oligochaetes in the EPIC data

entry program, using the procedures found in a later section of this document. Terms such as "uniqueness" and "qualifiers" are defined there. Prior to any temporary or permanent slide mounting, Taxonomists measure each individual's total body length (excluding cerci) to the nearest 1mm and record the measurements in EPIC. Measurements are made through the microscope at 10x magnification using an ocular micrometer, or a stage micrometer for larger specimens. Specimens that are damaged to an extent such that it precludes total body measurements are estimated based on other specimens of the same taxon from within the same sample, or best professional judgement.

Worms not mounted on slides are placed in a 1 dram snap cap vial with the sample identifier label. The Taxonomist should initial this label on the back.

Reference collection specimens are slide-mounted organisms that are to be included in the collection. After identification and when the CMC medium is no longer fluid, the Taxonomist should organize slides by sample number in a slide box exclusive for the project.

When the Taxonomist has completed the oligochaete identification for the sample, he/she should cap the 1 dram snap cap vial and place it into the green-lidded specimen cup. In addition, he/she should make sure that all other vials (general arthropods, chironomids, etc.) are replaced in the specimen cup. The cup and the Technical Department benchsheet should be returned to the sorted sample storage area.

Clean-up after a sample's oligochaetes are identified includes thorough washing and drying of watch glasses, sorting palettes, forceps and needles, and any other equipment that was used in the identification process.

Quality Assurance/Quality Control

Quality Assurance

Quality assurance is described in the Rhithron QAP (ver 18.1.a), and is maintained through the adherence to the processes and procedures described in this SOP to ensure sample integrity throughout sample processing

Quality Control

Quality control measures are described the Rhithron QAP (ver 18.1.a), and is maintained through routine checks of sorting efficiency taxonomic precision, accuracy, and data accuracy.

Performance objectives: Technical laboratory (sample sorting)

The goal of sample processing is to sort invertebrates from substrate in such a manner that results in an unbiased, representative subsample containing the appropriate number of organisms. The number of organisms is typically determined by the project specifications.

Objectives

There are several aspects of sample processing by Rhithron's technical staff that are important to subsequent data quality and thus, provide the objectives for each project. First, the target count of organisms is achieved within the specified tolerance limits. Second, the client-specified protocol is faithfully followed. Third, sorting efficiency is maintained at an average level no lower than 90% for each project, thus assuring sorting accuracy and precision. Fourth, the appropriate paperwork is associated with the correct sample. Finally, all data pertinent to the sub-sampling procedure, including fraction of sample used to obtain the target number of organisms, condition of the sample, any problems associated with sorting, and quality assurance procedure outcomes and statistics, etc. are recorded on sample benchsheets.

QA/QC plan

Accomplishment of the performance objectives is evaluated by a QA/QC plan that examines the adherence to Rhithron-specific and client-specific protocols.

Target count: Under-processed samples are detected at the time of taxonomic identifications by the taxonomists or at the time of data entry by the Lead Technician. If the sample was not fully picked in the processing stage, under-processed samples are revisited by the sorting technician, who distributes the unpicked sample portion into the appropriate number of Caton tray grids, and sorts the sample until the target count is reached.

Adherence to specified procedure: Daily oversight by the Lead Technician assures that client-specific protocols are followed in the technical department. Documentation for each project in progress is reviewed periodically.

Sorting efficiency: Quality control procedures for initial sample processing and subsampling involves checking sorting efficiency. These checks are conducted on at least 10% of the samples by independent observers who microscopically re-examine

100% of sorted substrate from each QC sample. All organisms that were missed are counted. Sorting efficiency is evaluated by applying the following calculation:

$$SE = \frac{n_1}{n_2} \times 100$$

where: SE is the sorting efficiency, expressed as a percentage, n_1 is the total number of specimens in the first sort, and n_2 is the total number of specimens in the second sort plus the first sort. Sorting efficiency is recorded on the benchsheet, and these data are entered into the Rhithron database.

Correspondence of sample and paperwork: Two technicians check the correspondence of sample and paperwork before each sample is processed. Technicians check the RAI number, the client's sample identifiers, and the number of jars associated with that sample. Both technicians sign the benchsheet, which is generated by Rhithron's database for each specific sample, when this step is completed. Using a "buddy" system insures that there are no mismatches between labels, spreadsheets, other data materials and the corresponding sample. Correct labeling of the sample fractions resulting from the processing procedures is assured by the provision of database-generated labels, which are attached to the benchsheet for each sample.

Complete recording of appropriate sub-sampling data: Benchsheets for samples that have been processed are collected daily by the Lead Technician, who checks for completeness of sub-sampling data, checks for missing data, and enters these data into the Rhithron database. Since these checks are performed daily, obtaining the data for each sample is assured.

Corrective actions: If 90% sorting efficiency is not achieved for a given sample, a failure is recorded on the benchsheet and in the database. Failure of any sample to achieve data quality objectives, triggers assessment of an additional 100% of samples. For large projects, additional QC samples may be stratified by the technician whose sample failed the QA/QC check. Sorting efficiency statistics for each technician and for the entire laboratory are reviewed monthly. Sorting efficiency for each project is reported to the client in the technical summary document. Technicians who do not maintain the target sorting efficiency are given remedial training, and larger portions of the samples they process are examined for the sorting efficiency test until they are able to maintain the target sorting efficiency.

Performance objectives: Taxonomy Department (macroinvertebrate identification and enumeration)

The goal of the taxonomic portion of sample processing is to accurately and precisely identify and enumerate organisms to the taxonomic resolution required by the project. Bias is minimized and data are reported completely. Materials related to the project, including labeled microscope slides, labeled vials with identified organisms, and laboratory benchsheets are handled carefully and are archived on the completion of identification and enumeration, and after all QA/QC procedures and data reviews have been completed. Deliverables such as voucher collections are assembled accurately and completely. Higher levels of taxonomy applied to organisms that cannot be identified to

taxonomic targets are explained and qualified in all cases. Life stages are accurately recorded in the data.

Objectives

There are several aspects of invertebrate identification and enumeration by Rhithron's taxonomy staff that are important to subsequent data quality. First, the accuracy and precision of identifications and enumerations are maintained such that the Percent Difference in Enumeration (PDE) is 5% or less, and the Percent Taxonomic Disagreement (PTD) is 15% or less. Percent Taxonomic Completeness (PTC) is assessed to minimize between taxonomist bias. Third, the client-specified protocol, including specified target number of organisms and the required taxonomic resolution, is faithfully followed. Fourth, all client-requested deliverables are provided, including reference collections. Finally, summaries of QA/QC procedures and results, and sample processing procedures are documented and delivered along with client-requested deliverables.

QA/QC plan

Accomplishment of the performance objectives is evaluated by the following QA/QC plan that examines the adherence to Rhithron-specific and client-specific protocols.

Accuracy of taxonomy is evaluated by adherence to target taxonomic resolution requirements, and by the use of appropriate technical taxonomic literature or other references (e.g., identification keys, voucher specimens). Bias is minimized by the use of taxonomic literature and resources that are accepted by the industry and reflects the most current accepted nomenclature. A bibliography of Rhithron's taxonomic library is maintained in a literature database. Consultation with experts and systematists occurs frequently. High quality optical equipment is used and regularly maintained. Geographic distributions of identified animals are checked and experts consulted when uncertainties arise, to assure credible identifications. Taxonomic discrepancies are examined and discussed by the original taxonomist and the QC taxonomist. Discussions may include the Taxonomy Department Supervisor, Project Manager, Quality Assurance Officer as well as other staff taxonomists. Discrepancies and disagreements that cannot be resolved internally are submitted via vouchered specimens or digital photographs to experts or systematists for resolution. Taxa lists may be changed when disagreements are resolved.

Taxonomic precision is assessed by the re-identification of a randomly-selected 10% of samples in a blind procedure. The results of the QC process are evaluated by the calculation of the PDE,PTC and the PTD. The percent taxonomic disagreement (PTD) is calculated by the following equation:

$$PTD = (1 - \left(\frac{comp_{pos}}{N}\right)) \times 100$$

where $comp_{pos}$ is the number of agreements and N is the total number of organisms in the larger of the 2 counts. The lower the PTD, the more similar are taxonomic results and the overall taxonomic precision is better. Rhithron's quality objective for

PTD is 15% or less. The percent difference in enumeration (PDE) is calculated by the following equation:

$$PDE = \frac{|n1 - n2|}{n1 + n2} \times 100$$

where *n1* is the number organisms counted by the original taxonomist, and *n2* is the number of organisms counted by the QC taxonomist. The lower the PDE, the more precise the enumeration. Rhithron's quality objective for PDE is 5% or less. The Percent Taxonomic Completeness is calculated by the following equation:

$$PTC = \frac{x}{N} * 100$$

Where x is the number of organisms within a sample that were identified to taxonomic targets and N is the total number of organisms within the sample. No MDQO has been established for this metric but literature suggests that samples with >10% PTC should be re-examined to identify any issues within the sample (Stribling *et al.* 2008).
When QC parameters exceed Rhithron's quality objectives, additional samples are randomly selected and re-identified.

Data completeness is addressed by indicating reasons why taxonomic targets are occasionally not met. These are essential data components that are required by the EPIC (v.1.7) data entry program. Reasons include: damage to specimens, poor preservation, early instar or immaturity, and life stage. When metric calculation is required by a project scope, these specimens are included in the calculation of compositional metrics or tolerance indices, but are not included in calculations of richness metrics unless their uniqueness from other specimens is confidently ascertained.

Instrument and Equipment Testing, Inspection and Maintenance

All microscopes and equipment needed to process benthic macroinvertebrate samples are inspected regularly according to manufacture specifications. Rhithron contracts with Pacific Microsystems to perform annual maintenance on all microscopes.

Instrument Calibration Frequency

All microscopes and laboratory equipment, including digital imaging, balances, and combustion and desiccation ovens are calibrated regularly according to manufacturer recommendations. Ocular micrometer calibration is routinely checked against stage micrometers throughout projects to insure zero drift in equipment accuracy.

Electronic data

Data entry

Taxonomic data are entered by taxonomists, using a proprietary data-entry software application (EPIC v.1.7). The EPIC software uses drop-down taxa lists and incorporates several required fields for each taxonomic data entry. Required fields include correctly-spelled taxonomic name, count, uniqueness code, life stage, qualifier, and comments. Direct data entry by taxonomists minimizes errors due to misspellings, data loss or corruption at transfer, and maximizes completeness and thoroughness of the data.

The procedures for data entry are as follows:

1. Access log-in screen using Firefox at www.rhithron.managementsolutionsofva.com.
2. Enter your user name and password.
3. Select the appropriate taxonomist type from the drop down menu.
4. Select the "Sample Entry" Tab from the upper right hand corner buttons.
5. Select the appropriate Project ID from the drop down menu.
6. Select the appropriate Sample ID from the drop down menu.
7. Move your cursor to the highlighted row and Taxon column.
8. Begin typing your taxon's name, a list of approved taxa will appear, select your taxon from the dropdown menu, then press Tab.
9. Enter the count of organisms belonging to this taxon, then press Tab.
10. Select the "Uniqueness" from the drop down menu of the taxon, then press Tab.
11. Select the life stage from the drop down menu of the taxon, then press Tab.
12. Select the "Qualifier" from the drop down menu, if one is needed, then press Tab.
13. Select the "Portion" from the drop down menu of the sample the organisms came from, then press Tab.
14. Type any comments regarding the identification, then press Tab.
15. Press Enter and/or Click the "Save" button and the line of data is saved.
16. Repeat this process until all data have been entered for the sample.
17. To end the sample Click the "Sample Comments" tab and fill in the required data and comments about the sample as a whole. Click "Save Changes". Check the box for sample portion completion under your name and taxonomist type. The sample is now complete and you can move to the next assigned sample.

Reference collection specimens

Data for an individual taxon must be entered prior to entering reference collection data.

1. After data for the taxon has been entered select the ✚ icon in the left most column of the appropriate taxon's row.
2. A pop-up window will appear.
3. Select the number of organisms you are depositing into the reference collection from the drop down menu.
4. Enter the Vial and or Slide number for the appropriate taxon.
5. Optional*** include any narrative comments regarding the specimen(s).
6. Press "Save".

Editing Lines of Data

Data are saved line by line and you can only edit data that you have created.

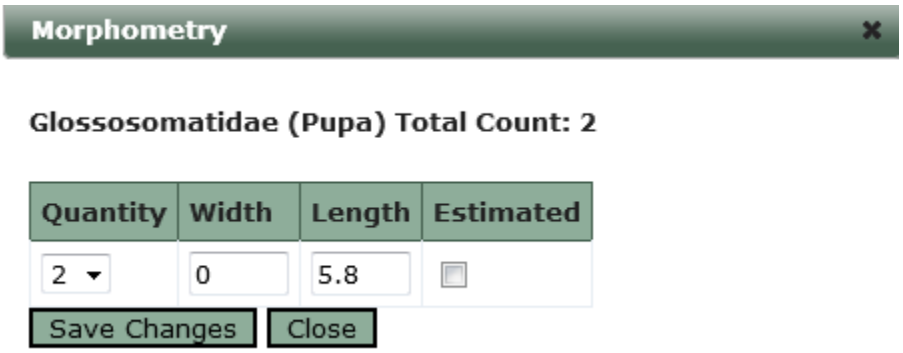
After having saved a line of data, to reactivate the line of data select the  icon in the furthest right column of the line you wish to edit. This will reactivate the line of data and allow you to edit any of the column data. Select Save to save the changes.

Deleting data

To delete a line of data, select the  icon; a pop-up box will appear asking you if you wish to delete the line of data. DOUBLE CHECK THE DATA, and press "OK".

Morphometric data

To add measurement data to a given taxon, select the  icon; a pop-up box (below) will appear and you will be required to enter the quantity, width (if necessary to nearest mm) and length (nearest mm). Multiple lines of data can be entered for binned organisms to the nearest mm. A check box can be used to indicate that the specimens were damaged and an estimated length was recorded.



References

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