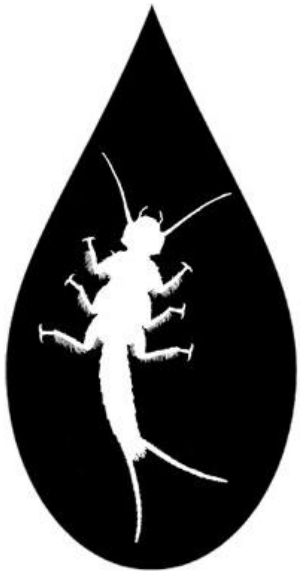
Macroinvertebrate Sample Processing and Reporting Methodologies of the National Aquatic Monitoring Center



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Last Updated: 10 October, 2011

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Table of Contents

Introduction

This document outlines the methodologies used by the National Aquatic Monitoring Center (NAMC) for the processing and reporting of macroinvertebrate samples. The documentation is to be used in conjunction with the standard Microsoft Excel spreadsheet report provided to each customer. The NAMC's output for macroinvertebrate samples aims to support both (multi-) metric and multivariate approaches. Specifically, our standard output consists of four excel worksheets and an optional fifth worksheet:

1. *Metadata*: Summary of information contained herein, including data dictionary for all worksheet columns.

2. *Metrics*: A suite of compositional, richness, tolerance and functional metric, commonly used to determine freshwater biological condition. A summary and description of commonly used metrics is available in Barbour et al. (1999) and Karr and Chu (1999). Note that all richness based metrics are standardized to Operational Taxonomic Units (OTUs; sensu Cuffney et al. 2007) and a fixed count of 300 individuals (unless otherwise requested). In contrast, density based metrics are not standardized to operational taxonomic units (OTUs) or a fixed count (i.e., rarefraction). Density metrics are the estimated number of individuals per square meter for quantitative samples or the estimated number of individuals per sample for qualitative samples.

   The use of standardized metrics is highly recommended when comparing metrics among sites or sampling events, as well as for use in multi-metric indices. Many states have developed regionally calibrated multi-metric indices, which select a suite of metrics that maximizes the discriminatory efficiency between test and reference sites. Alternatively, one can identify the particular metric(s) thought to be most sensitive to a particular pollutant or stressor (e.g., clingers or scrapers as indicators of fine sediment loading). Regardless, assessments are best made by comparing test samples to similarly collected samples at reference sites or from samples collected prior to impacts or management actions at a location. Please note that the standardized data presented herein is not compatible with all multimetric or RIVPAC type models.

3. *Species Matrix (Raw)*: Raw taxonomic and abundance data for sampled sites. Abundance data is the estimated number of individuals per square meter for quantitative samples OR the estimated number of individuals per sample for qualitative samples. Note that the taxonomic data in this worksheet has not been standardized to operational taxonomic units (OTUs), thus considerable redundancy likely exists in the taxonomic hierarchy.

4. *Species Matrix (Standardized)*: Taxonomic and abundance data standardized to Operational Taxonomic Units (OTUs) but not standardized to fixed count. Please note that data has not been standardized to a fixed count as in the 'Standardized Metrics worksheet'. Also, abundance data is the estimated number per square meter

for quantitative samples OR is the estimated number per sample for qualitative samples.

5. *OE Output*: For an additional fee, the NAMC will compute Observed/Expected (O/E) values for available national or state-based O/E models. The ratio O/E is an easily understood and ecologically meaningful measure of the biological condition at a site with the observed taxa being those predicted to naturally occur at a site based on GIS-derived habitat variables. Values can theoretically vary from 1 (equivalent to reference condition) to zero (completely degraded, i.e., all expected taxa are missing) and can be assigned a classification of "Good", "Fair", or "Poor". More detailed documentation can be found at http://www.cnr.usu.edu/wmc/htm/predictive-models/predictive-models-primer and specific model parameters will be available in the future.

Sample Details

Customer provided geographic and methodological information for each sample.

Related fields in Excel Output:

[SampleID]

A unique tracking number, or primary key, assigned by NAMC to manage samples. Typically, samples within a submitted set are assigned sequential sample identification numbers. This number should be provided for all data inquiries and/or corrections to collection methodologies.

[Station_NAMC]

A unique tracking id assigned by NAMC that identifies a distinct geographic site as distinguished by XY coordinates. This unique station name allows multiple samples with disparate customer assigned station names to be assigned to the same location.

[Station_Customer]

The customer provided station name or abbreviation. This often matches the [Station_NAMC] name, but may differ when the customer uses a standardized station name established by their organization or project or when the customer prefers to sequentially number replicate samples at the same station. Both the [Station_NAMC] and [Station_Customer] are provided for clarity and to allow the customer flexibility in customizing their station names while still maintaining the integrity of our database.

[Waterbody]

The customer provided name of the sampled waterbody. We encourage this to include the waterbody name, reference to any nearby tributaries, and other details that would allow someone to easily revisit the site independent of XY coordinates. Example: "ABC Creek, above confluence with XYZ stream, 10 km north of City off Hwy 100."

[County],[State]

The administrative boundaries in which the station is located.

[Latitude], [Longitude]

The XY coordinates in decimal degree units. Coordinates provided in UTM or other formats are converted to decimal degrees. NAMC requires this information for use in additional data analysis and potentially relocating a site, but it is not considered a substitute for other locality information.

[Collection Date]

The calendar date on which the sample was collected.

[Field Notes]

Customer provided sampling or collection notes , as well as specification of "Other" [Habitat Sampled] or "Other" [Collection Method] (see section on [Collection Method].

[Lab Notes]

NAMC generated lab processing notes. This includes any problems with damaged samples and samples without any invertebrates.

[Area Sampled]

The total (cumulative) area sampled in square meters. The NAMC protocol recommends a sample of eight composited Surber samples (1 square foot each) for a total of 0.74 square meters.

[Habitat Sampled]

The micro- or macro-habitat unit targeted for sampling. The values are restricted according to the following criteria:

System type	Habitat unit	Definition
Lentic	Littoral	Near shore zone where light penetrates to the benthos
	Pelagic/Water Column	Open water outside the littoral zone
	Benthos	Benthic substrate outside the littoral zone
	Multiple	Any combination of the above, including qualitative searches of unique habitats
Lotic	Targeted Riffle	Area(s) of fast, turbulent water, often characterized by gravel/cobble substrates; recommended for quantitative stream samples
	Pool	Area(s) of slow, deeper than average water associated with scour/deposition events or the impoundment of water from a flow obstruction
	Reachwide	Any combination of the above, including qualitative searches of unique habitats
Lentic or Lotic	Stomach	Fish or other diet samples
	Other	Any other microhabitat, customer should specify in submitted notes which are then recorded in [Field Notes]

[Collection method]

The sampling device with which the sample was collected. The values allowed during submission are restricted to dominantly used sample devices (in **bold**). Additional samplers, including those listed in regular type, are categorized as "Other" and can be specified further in [Field Notes] during the submission process. The provided list is not intended to be exhaustive, but to document commonly used methods.

Type	Method Name	Description
Aquatic: Benthic	**Surber Net**	Square frame with an area of 1 square foot used for quantitative sampling
	Hess Net	Circular frame of variable area used for quantitative sampling
	Kick Net	Rectangular or D-shaped net used for either qualitative or quantitative sampling
	Ekman or Ponar grab	Hinged closure samplers used for quantitative sampling of softer sediments such as lakes
Aquatic: Water Column	**Drift Net**	Net secured in stream water column for a known duration
	Plankton Net	Net towed through lake or wetland by hand or behind boat
Aquatic: Fish	Stomach Pump	Flushing of fish stomach to eject recent contents
	Dissected Gut	Full dissection of fish gut
Aquatic: Artificial Substrate	Hester Dendy	Stacked plates which invertebrates colonize
Terrestrial	Aerial Net	Circular net used to catch flying insects
	Blacklight	Light used to attract flying insects
	Emergence Trap	Floating or hanging trap to capture newly emerged aquatic insects

Laboratory methods

The NAMC generally follows the procedures recommended by the United States Geological Survey (T. F Cuffney et al. 1993; Moulton et al. 2000) for the processing of aquatic macroinvertebrate samples, which are rationalized in Vinson and Hawkins (1996). Detailed descriptions of NAMC's subsampling, sorting, taxonomic, archiving and QA/QC methodologies can be found in Appendices 1 and 2.

Related fields in Excel Output:

[Field Split]

The percent of the original sample that was preserved and submitted for processing.

[Lab Split]

The percent of the submitted sample that was subsampled and sorted according to procedures described in Laboratory methods (Appendices 1 and 2).

[Split Count]

The number of sub-sampled organisms sorted and identified from the lab split.

[Big_Rare Count]

The number of large and rare organisms non-randomly removed from the sample; the big and rare search is performed after subsampling has occurred(Appendix 1). These taxa are added to the abundance computations to provide a more complete taxa list incorporating rarer taxa that may be missed during subsampling.

Data summarization

Compositional changes in macroinvertebrate assemblages are most frequently used to quantify freshwater ecosystem responses to anthropogenic disturbances (Bonada et al. 2006). Common approaches range from the computation and evaluation of individual metrics characterizing the composition, richness, function or tolerance of invertebrate assemblages to complex multivariate analyses and statistical modeling that aims to predict assemblage composition in the absence of impairment (e.g., RIVPAVS or O/E) (V. H. Resh et al. 1993; Wright et al. 2000; Merritt et al. 2008). Regardless of the analytical approach, determinations of biological condition are generally achieved by comparing the deviation of macroinvertebrate metrics or assemblages composition at test sites (i.e., sampled sites) to that of reference or minimally impacted conditions. The NAMC's output for macroinvertebrate samples aims to support both (multi-) metric and multivariate approaches.

Related fields in Excel Output:

[Fixed Count]

The number of resampled organisms to a fixed count of 300 (unless otherwise requested). If the number of sub-sampled organisms ([Split Count]) was less than the fixed count, the fixed count will be less than the target of 300 and should approximate the [Split Count] but may be slightly lower due to taxa omitted during OTU standardization.

Richness metrics

Richness is a component and estimate of community structure and stream health based on the number of distinct taxa. Taxa richness normally decreases with decreasing water quality. In some situations organic enrichment can cause an increase in the number of pollution tolerant taxa. Taxa richness was calculated for operational taxonomic units (OTUs) and the number of unique genera, and families. The values for operational taxonomic units may be overestimates of the true taxa richness at a site if individuals were the same taxon as those identified to lower taxonomic levels or they may be underestimates of the true taxa richness if multiple taxa were present within a larger taxonomic grouping but were not identified. All individuals within all samples were generally identified similarly according to Standard Taxonomic Effort (see Appendix 1 or NAMC website), so that comparisons in operational taxonomic richness among samples within this dataset are appropriate, but comparisons to other data sets may not. Comparisons to other datasets should be made at the genera or family level.

Related fields in Excel Output:

[Richness]

The number of unique taxa at the lowest possible taxonomic resolution (typically genus or species).

[# of EPT Taxa]

the taxonomic richness for the insect orders Ephemeroptera, Plecoptera, and Trichoptera (EPT). These orders are commonly considered sensitive to pollution

(Karr & Chu 1999). This is reported along with the accompanying density metric, [Abundance of EPT Taxa].

[Shannon's Diversity]

The Shannon-Wiener diversity function is a measure of community structure and heterogeneity defined by the relationship between the number of distinct taxa and their relative abundances. The Shannon's diversity index is noted to weight rare species slightly more heavily than the Simpson's diversity index (Krebs 1999). The calculation is made as follows:

$$-\sum([\text{Relative Abundance}]_{taxa} * \ln([\text{Relative Abundance}]_{taxa}))$$

after Ludwig and Reynolds (1988, equation 8.9, page 92):

$$H' = \sum_{i=1}^{S} (p_i \ln p_i)$$

[Simpson's Diversity]

The Simpson's diversity index is a measure of community structure and heterogeneity defined by the relationship between the number of distinct taxa and their relative abundances. The Simpson's diversity index is noted to weight common species slightly more heavily than the Shannon's diversity index (Krebs 1999). The calculation is provided in the common form as follows:

$$1 - [\text{Simpson's Diversity}] = 1 - \sum([\text{Relative Abundance}]_{taxa})^2$$

after Ludwig and Reynolds (1988, equation 8.6, page 91):

$$\lambda = \sum_{i=1}^{S} ({p_i}^2)$$

Modified to the complement of the Simpson's probability measure as shown in Krebs (1999, equation12.28, page 443):

$$1 - D = 1 - \sum ({p_i}^2)$$

[Evenness]

A measure of the distribution of taxa within a community. Value ranges from 0-1 and approach zero as a single taxa becomes more dominant. The evenness index used in this report was calculated as:

[Shannon's Diversity]/ln([Richness])

following Ludwig and Reynolds (1988, equation 8.11, page 93):

$$E1 = {}^{H'}/_{\ln(S)}$$

Dominance metrics

Metrics used to characterize the absolute or proportional abundance of individual taxa within a sampled assemblage. An assemblage largely dominated (>50%) by a single taxon or several taxa from the same family suggests environmental stress.

Related fields in Excel Output:

[Dominant Family]

The taxonomic family with the highest abundance per sample. The name of this family is given to provide information about the life history and pollution tolerance of the dominant taxa.

[Abundance of Dominant Family]

The density of the most abundant family. This number should be compared to the total abundance for the sample to determine what percent of the total abundance is comprised by the dominant family. An assemblage dominated (e.g., >50%) by a single family suggests environmental stress; although the specific dominant family needs to be considered. For example, dominance by Chironomidae, Hydropsychidae, Baetidae, or Leptohyphidae frequently suggest impaired conditions, while other families within the orders Coleoptera, Ephemeroptera, Plecoptera or Trichoptera may suggest otherwise. Dominance of the macroinvertebrate assemblage by a few taxa can also be evaluated with the Evenness metric.

[Dominant Taxa]

The taxa (usually identified to genus) with the highest abundance in a sample. The name of this taxa is given to provide information about the life history and pollution tolerance of the dominant taxa.

[Abundance of Dominant Taxa]

The density of the numerically dominant taxon. This number should be compared to the total abundance for the sample to determine what percent of the total abundance is comprised by the dominant taxa. An assemblage largely dominated (e.g., >50%) by a single taxon suggests environmental stress. This can also be evaluated in conjunction with the Eveness metric.

Tolerance (Biotic) Indices

Taxa are assigned values based on their tolerance to a single or multiple pollutants (e.g., nutrients, temperature, fine sediment). Pollution tolerance scores are typically weighted by taxa relative abundance and summed among all observed taxa. In the United States the most commonly used biotic index is the Hilsenhoff Biotic Index developed for organic matter enrichment (Hilsenhoff 1987; 1988). The USFS and BLM throughout the western United States have also historically used the USFS Community Tolerance Quotient (Winget & Mangum 1979).

Related fields in Excel Output:

[Hilsenhoff Biotic Index]

The Hilsenhoff Biotic Index (HBI) was originally developed to quantify the tolerance of macroinvertebrate assemblages to organic pollution, but this index has been used to detect nutrient enrichment, fine sediment loading, low dissolved oxygen, and thermal impacts. Families are assigned an index value from 0 (taxa normally found only in unpolluted water) to 10 (taxa found only in severely polluted waters). following Hilsenhoff (1987; 1988) and a family level HBI is calculated using the below equation.Sampling locations with HBI values of 0-2 are considered clean, 2-4 slightly enriched, 4-7 enriched, and 7-10 polluted. The HBI is calculated as:

∑([Abundance]taxa*[Tolerance]taxa)/[Abundance]Total

following the equation presented in Hilsenhoff (1988):

$$FBI = \sum_{i=1}^{s} n_i \times t_i \,/\, \sum_{i=1}^{s} n_i$$

[# of Inolerant Taxa]

Rather than using mean HBI values for a sample, taxon HBI values can also be used to determine the number of pollution intolerant and tolerant taxa occurring at a site. In our report, taxa with HBI values $\leq$ 2 were considered 'intolerant', clean water taxa (Vinson unpublished). The provided value is the richness (count) of taxa with HBI values $\leq$ 2.

[Abundance of Intolerant Taxa]

The abundance of taxa with HBI values $\leq$ 2, which were considered to be 'intolerant', clean water taxa in this report (Vinson unpublished).

[# of Tolerant Taxa]

Rather than using mean HBI values for a sample, taxon HBI values can also be used to determine the number of pollution intolerant and tolerant taxa occurring at a site. In our report, taxa with HBI values $\geq$ 8 were considered pollution 'tolerant' taxa (Vinson unpublished). The provided value is the richness (count) of taxa with HBI values $\geq$ 8.

[Abundance of Tolerant Taxa]

The abundance of taxa with HBI values $\geq$ 8, which were considered to be pollution 'tolerant' taxa in this report (Vinson unpublished).

[USFS Community Tolerance Quotient (d)]

Taxa are assigned a tolerant quotient (TQ) from 2 (taxa found only in high quality, unpolluted waters) to 108 (taxa only found in severely polluted waters). following Winget and Mangum (1979). A dominance weighted community tolerance quotient (CTQd) is calculated according to the equation below where values can range from 20 to 100, with lower values indicating better water quality.

$$\sum([\text{Tolerance Quotient}] * \log([\text{Abundance}]_{taxa}))/\sum \log([\text{Abundance}]_{taxa})$$

Functional Feeding Groups and Traits

Aquatic macroinvertebrates can be categorized by mode of feeding , adaptations to local habitat conditions, time to complete a life cycle, and other life history traits. Such classification schemes attempt to understand how individuals interact with local environmental conditions, with specific emphasis on the functional role of macroinvertebrate assemblages within aquatic ecosystems.

One of the most population classification schemes is functional feeding groups (FFG), which classify individuals based on their morpho-behavioral adaptations for food acquisition (e.g., scraping, piercing, net building); recognizing that all macroinvertebrates exhibit some degree of omnivory. The richness and relative abundance of different FFGs indicate the dependency of observed macroinvertebrate assemblages on different food resources and thus the trophic basis for secondary production. For example, the ratio of scrapers to shredders indicates the degree to which the local macroinvertebrate assemblage depends on instream algal production versus inputs of terrestrial leaf litter.

Functional feeding group designations are derived from Merritt et al (2008). Taxa are not included that are highly variable in their food habits, are parasites, or their primary feeding mode is currently unknown.

Related fields in Excel Output:

Functional feeding group measures

[# of Shredder Taxa] & [Shredder Abundance]

Shredders use both living vascular hydrophytes and decomposing vascular plant tissue - coarse particulate organic matter. Shredders are sensitive to changes in riparian vegetation and can be good indicators of toxicants that adhere to organic matter.

[# of Scraper Taxa] & [Scraper Abundance]

Scrapers feed on periphyton (i.e., attached algae) and associated material. Scraper populations increase with increasing abundance of diatoms and can decrease as filamentous algae, mossesor vascular plants increase, often in response to increases in nitrogen and phosphorus. Scrapers decrease in relative abundance in response to sedimentation and higher levels of organic pollution or nutrient enrichment.

[# of Collector-filterer Taxa] & [Collector-filterer Abundance]

Collector-filterers feed on suspended fine particulate organic matter and often construct fixed retreats or have morpho-behavioral adaptation for filtering particles. Collector-filterers are sensitive highly mobile substrate condition, the quantity of fine particulate organic matter and pollutants that adhere to organic matter.

[# of Collector-gatherer Taxa] & [Collector-gatherer Abundance]

Collector-gatherers feed on deposited fine particulate organic matter. Collector-gatherers are sensitive to deposited toxicants.

[# of Predator Taxa] & [Predator Abundance]

Predators feed on living animal tissue. Predators typically make up about 25% of the assemblage in stream environments and 50% of the assemblage in still-water environments.

Life History Trait measures

[# of Clinger Taxa]

Clingers typically have behavioral (e.g., fixed retreat construction including rock ballasts, silk production) or morphological (e.g., modified gill structures, long curved claws, crochet hooks) adaptations for attachment to the tops of rocks or wood surfaces. Clingers have been found to respond negatively to fine sediment loading or abundant algal growth (Karr & Chu 1999). Clinger taxa were determined using information in Merritt et al. (2008).

[# of Long-lived Taxa]

Taxa that take two or more years to complete their life cycle are considered to be long-lived. Macroinvertebrates with such protracted life cycles are considered good bioindicators since their presence indicates the maintenance of certain water quality or habitat conditions; the number of long-lived taxa typically decreases in response to degraded water quality of physical conditions (Karr & Chu 1999). The classification of long-lived taxa was based on life cycles greater that two years following Merritt et al. (2008).

Taxa Richness and Abundance

For taxa groups that are indicators of water quality or that are commonly used in multimetric indices, richness and abundance within that taxa are given.

[# of ** Taxa]

The richness (count of unique taxa) within each specified group.

[Abundance of ** Taxa]

The abundance, density, or number of aquatic macroinvertebrates of the indicated group per unit area. Invertebrate abundance is presented as the number of individuals per square meter for quantitative samples and the number of individuals collected in each sample for qualitative samples. Abundance is an indicator of habitat availability and fish food abundance. Abundance may be reduced or increased depending on the type of impact or pollutant. Increased organic enrichment typically causes large increases in abundance of pollution tolerant taxa. High flows, increases in fine sediment, or the presence of toxic substances normally cause a decrease in invertebrate abundance.

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Appendix 1:
NAMC Sample Processing:
Subsampling, Sorting and Identification of Macroinvertebrate Samples

Document produced by Scott Miller and Sarah Judson

Last Updated: October, 2011

Table of Contents

Sorting

General procedures for processing invertebrate samples are similar to those recommended by the United States Geological Survey (T. F. Cuffney et al. 1993; Moulton et al. 2000) and are described in greater detail and rationalized in Vinson and Hawkins (1996). A detailed step-by-step, pictorial guide of our laboratory processing is available on our website.

Samples are sub-sampled if the sample appears to contain more than 600 organisms. Sub-samples are obtained by pouring the sample into an appropriate diameter 500 micron sieve, floating this material by placing the sieve within an enamel pan partially filled with water and leveling the material within the sieve. The sieve is then removed from the water pan and the material within the sieve is divided into two equal parts. One half of the sieve is then randomly selected to be processed and the other half set aside. The sieve is then placed back in the enamel pan and the material in the sieve again leveled and split in half. This process is repeated until approximately 600 organisms remain in one-half of the sieve.

The sub-sampled material is placed into a gridded Petri dish and examined systematically under a dissecting microscope with at least 7x magnification until all organisms are removed. As organisms within a sub-sample are removed, they are enumerated and placed into separate vials according to taxonomic Orders. Additional sub-samples are taken until at least 600 total organisms are removed.

When sub-sample sorting is completed, the entire sample is spread throughout a large white enamel pan and searched for 10 minutes to remove any taxa that might not have been picked up during the initial sample sorting process. The objective of this "big/rare" search is to provide a more complete taxa list by finding rarer taxa that may have been excluded during the sub-sampling process. These rarer organisms are placed into a separate vial and the data entered separately from the individuals removed during the sub-sampling process.

Sorted (i.e., clean) and unsorted material are retained in separate jars. The sorted material is subject to random selection for assessing sorting efficiency (Appendix 2). The unsorted material is retained temporarily to resolve any discrepancies in large subsamples (>1000 organisms), unusual lab splits, etc. Once the data had been entered into a computer and checked, the sorted and unsorted portion of the sample are discarded.

Taxonomy

All organisms removed during the sorting process are identified by Society for Freshwater Science (previously the North American Benthological Society) certified taxonomists. We strive to identify organisms to a consistent taxonomic level. Generally, we follow the Southwest Association of Freshwater Invertebrate Taxonomists (SAFIT) Standard Taxonomic Effort Level 1 guidelines in which insects are identified to genus and most non-insects are identified at coarser levels. A complete listing of our Standard Taxonomic Effort can be found on our website. Small (early instar) and poorly preserved specimens may be identified to a higher level than specified. A notable difference between SAFIT recommendations and our identification protocols is that NAMC identifies Chironomidae to subfamily. All specimens are identified to

the lowest taxonomic level feasible without slide-mounting. This specification precludes our ability to identify Chironomidae finer than subfamily, but we are well networked with other labs and regularly subcontract the Chironomidae identifications if requested.

Data Entry

Currently, all sorters and taxonomists enter their processing and identification data directly into an electronic interface which updates a central SQL database. Historically (pre-2011), sample information and invertebrate data were entered into the SQL database via a single data entry employee from taxonomic bench sheets.

Before generating reports, the data is routinely checked for missing information, outliers, and other discrepancies. Once the data is approved, it is inserted into our standard report and is made available online or via specific requests.

Data Ownership Policy

We believe that we should work cooperatively to enhance biomonitoring procedures and to maintain the quality of our nation's waters. We believe this means we should openly share ideas and data. Our policy on data ownership is that we will make all invertebrate, site, and sample data available through online query tools (http://www.cnr.usu.edu/wmc/htm/data) and to external requests. Unless specified otherwise in writing that the data generated from field or laboratory work is proprietary, we will make these data available to requests as soon as we make it available to the original customer following data entry and quality checks. Our policy is the same for the organisms we identify. We archive identified organisms, and unless specified in writing, we will lend these organisms to anyone who requests them, including our internal reference collection and classroom uses.

Sample Archiving

Once the data has been checked, we discard the unsorted portion of the sample unless the customer requests otherwise. The identified portion of the sample is archived in our permanent collection and kept for the foreseeable future, but if we run out of space, a five year storage limit may be imposed. Archived samples are stored in 70% ethanol in glass screw-top vials with polypropylene lids and polypropylene liners. Sample labels are written with fade proof permanent black carbon ink on waterproof paper. Information on each label includes the sampling location, sampling date and a sample ID (unique catalog number). This catalog number is maintained in a computer database. Additional information stored in this computer database includes more specificity on the locality and methods of collection, the agency and personnel who made the collection, the person who identified the specimens, and any additional information associated with a sample. Samples are stored sequentially in a metal cabinet. Retrieval of individual samples or a group of samples with select taxa or by geographic area is made possible by the integration of sample archiving and the computer database. Alternatively, if you would rather keep your samples yourself we will return them in containers as described above.

References Cited

Standard Laboratory Sorting Procedures

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Appendix 2:
National Aquatic Monitoring Center Quality Assurance and Quality Control (QA/QC) Protocols

Document produced by Scott Miller and Sarah Judson

Last Updated: October, 2011

Table of Contents

Introduction

The processing of aquatic invertebrate samples involves a number of distinct operations where data errors may occur. At the National Aquatic Monitoring Center (NAMC) these distinct operations include:

I. Sample sorting – The separation and removal of aquatic invertebrates from organic and inorganic material.

II. Invertebrate identification – The identification of each individual invertebrate to the recommended or lowest practical taxonomic level.

III. Data processing – Data entry into a computer database, the matching of samples to sampling locations and any subsequent data analysis or transformations.

Described below are the ways in which we attempt to reduce these potential errors and quantify the accuracy and precision of our sorting, identification and data processing procedures.

Sorting and Subsampling

The sorting and subsampling of aquatic invertebrate samples involves removing all or a predetermined number (e.g., 600) of randomly selected organisms from the organic and inorganic material within each sample. Error can occur if the sample is not split correctly and/or if all organisms are not removed from a sample split. To reduce error associated with subsampling procedures, we take the following actions:

1. **Use a relatively easy, but effective subsampling method:** see "Subsampling" section in Appendix 1: "NAMC Sample Processing: Subsampling, Sorting and Identification of Macroinvertebrate Samples" or the method pictorial guide our website.
2. **Intensively train all new employees:** includes sorting of a test sample where the type and number of each individual is known and checking all samples (minimum of 15) until 95% sorting efficiency is consistently achieved (see below for explanation).
3. **Record all information associated with each sample:** sorter's name, date processed, time required for sorting, the number of invertebrates removed from the sample and sample split. This information is recorded both on sample vial labels and in our database. Upon set completion, this information is reviewed to identify potential abnormalities (e.g., consistent differences in the percent of the sample sorted among sorters, the time required to sort a sample, the number of invertebrates removed during the sample splitting or the big-rare search) among individual samples within a sample set.
4. **Thoroughly clean all sorting and subsampling equipment:** contamination of samples with individuals from a previous sample is minimized by: 1. thoroughly washing and drying sieves after each sample split; 2. inspecting each sieve prior to splitting a new sample; and 3. noticing if any invertebrates in a sample appear to look "dried out" with respect to other invertebrates in that sample. These desiccated invertebrates may have been leftover from a previous sample (i.e., they were not removed from the sieve during a

previous subsampling event and were inadvertently added to a different sample). and thus are removed from the current sample and discarded as there is no way of determining which sample they were supposed to belong to.

5. **Conduct systematic checks of sorting effectiveness:** 10% of all sorted samples are examined to ensure that at least 95% of the organisms are removed from the examined material. Specifically, a second sorter examines the organic and inorganic matter from which organisms have been removed (i.e., remnant material) for at least 10% of the time that the sample was originally sorted. Remaining organisms are added to sample vials for identification and enumeration. Sorting effectiveness (E_s) is subsequently computed as:

$$E_s = 100 * \frac{S}{R+S}$$

Where R is the total number of organisms obtained during the re-sort of the remnant material and S is the total number of organisms originally obtained from the sample sorting. The goal of this is for $\geq 95\%$ of the organisms be removed during the original sort. Additionally, sorters are evaluated semi-annually in regards to sorting effectiveness and productivity.

6. **Remedial Action**

If sorting efficiency is between 90 and 94.4% one additional sample is checked for that particular sorter, if the second sample does not pass, additional randomly selected samples are checked until three consecutive samples achieve 95% sorting efficiency. If sorting efficiency is below 90% for the initial sample, additional randomly selected samples are checked until three consecutive samples achieve 95% sorting efficiency for a particular sorter.

Verification of Taxonomic Identifications

The NAMC attempts to reduce the number of misidentifications (i.e., accuracy) and improve the consistency (i.e., precision) of taxonomic resolution among taxonomists through a number of conscientious efforts. These efforts include:

1. **Taxonomic certification and workshops:** all NAMC taxonomists are certified in the identification of Western EPT taxa by the Society for Freshwater Science (formally North American Benthological Society). NAMC taxonomists also attend regional taxonomic seminars offered by the Northwest Bioassessment Work Group, Southwest Association of Freshwater Invertebrate Taxonomists, local universities and other entities. Lastly, NAMC taxonomists bring questionable taxa to the taxonomy fairs and workshops to consult taxonomic experts on an approximately annual basis .
2. **Constant and consistent communication among all taxonomists:** questionable specimens are immediately shared with all other taxonomists and compared to voucher specimens from our laboratory and the Utah State University insect collection. If consensus cannot be reached among our taxonomists, the specimens are shown to resident entomologists at Utah

State University. If consensus is still not reached, the taxonomic resolution is left at a coarser level (e.g., genus to family) and the specimen is set aside to be shown to an outside expert.

3. **Review a composite taxa list for each set of samples:** composite taxa lists are reviewed for taxonomic consistency (e.g., all monotypic taxa identified to species) among taxonomists and rare, invasive, or unusual taxa for the habitats or geographical location sampled. Particular attention is given to taxa found at low frequencies within a set or documented by only one taxonomist. These taxa are then re-examined to ensure the accuracy of identifications.
4. **Re-identification and enumeration:** a second taxonomist, who was not responsible for the original identifications, re-identifies and re-enumerates a minimum of 10% of the samples identified per year (and 10% from a given sample set as requested). This allows for the detection of both isolated (single occurrences) and systematic (multiple regular occurrences) taxonomic errors, which dictates the corrective action that should be taken. Similarity between taxonomists is assessed using Sorensen's similarity index (*B*):

$$B = \frac{\sum_{i=1}^{n} | X_{ij} - X_{ik} |}{\sum_{i=1}^{n} (X_{ij} + X_{ik})}$$

   Where X_{ij} and X_{ik} are the number of individuals of species i in the respective samples (j,k). The comparison between both samples is summed for n number of species in the samples. The goal is to have 95% similarity between paired samples. If 95% similarity is not achieved between taxonomists and discrepancies do not result from taxonomic ambiguities, all mis-identified operational taxonomic units identified by the taxonomist in question are independently checked for all samples within a particular sample set.

5. **External taxonomic verification:** 10% of all operational taxonomic units plus 20% of rare OTUs identified within a given calendar year are verified by external experts. The specimens are randomly selected from our database regardless of original taxonomist and excluding taxa identified to a coarser resolution relative to our Standard Effort Levels (see Appendix 1). Prior to sending specimens for verification, at least two taxonomists review the specimen and come to a consensus on the identification. Once specimens are returned, the expert's identification is compared to both the original identification and the consensus identification. If there is a mis-identification bias by a particular taxonomist, the issue is addressed. The verified specimens are retained in the reference collection.

Data processing and sample verification

All sample, site, and invertebrate data is stored in an SQL database. As of 2012, customers can submit sample and site data directly to our lab via an online submission process. Before acceptance into the processing queue, this data is thoroughly checked for common issues, including matching to jar labels, feasible sampling areas, comparison to historical methods, and proximity to existing sites. Customers are contacted when discrepancies arise to clarify current and past methods, station locations, and sample information.

As stated in Appendix 1, all sorters and taxonomists enter their processing and identification data directly into an electronic interface which updates a central SQL database. Before generating reports, the data is checked for missing information, outliers, and other discrepancies. The SQL database has integrity constraints and routine queries for maintaining consistency of data. Customers are contacted for approval before any major changes are made to past data.

Summary

The National Aquatic Monitoring Center staff members have 6-14 years experience tracking, processing and identifying aquatic macroinvertebrate samples. QA/QC is a dynamic process and constructive comments are always welcome.

In 2002, the National Aquatic Monitoring Center recently completed a comprehensive QA/QC evaluation of all of its field and laboratory procedures. Little bias or variation in several measures of accuracy and precision among different field crews, sample sorters, taxonomists and the same taxonomist identifying the same sample several times over a several month period were detected (Table 1).

Table 1. Summary of variability in data attributable to different activities associated with collection of field data, laboratory sample processing and taxonomic identifications. These data were compiled during December of 2002.

Component of Variability Measured	**Range in % Similarity**
Similarity in assemblage composition based on Jaccard Coefficient:	
Among field crews	92-96%
Among sample sorting technicians	Not yet determined
Among taxonomists	98%
Among identifications by the same taxonomist during different weeks	98%
Coefficients of Variation in taxa richness:	Range in Percent
Among field crews	4-7
Among sample sorting technicians	3-6
Among taxonomists	4-6
Among identifications by the same taxonomist during different weeks	2-3