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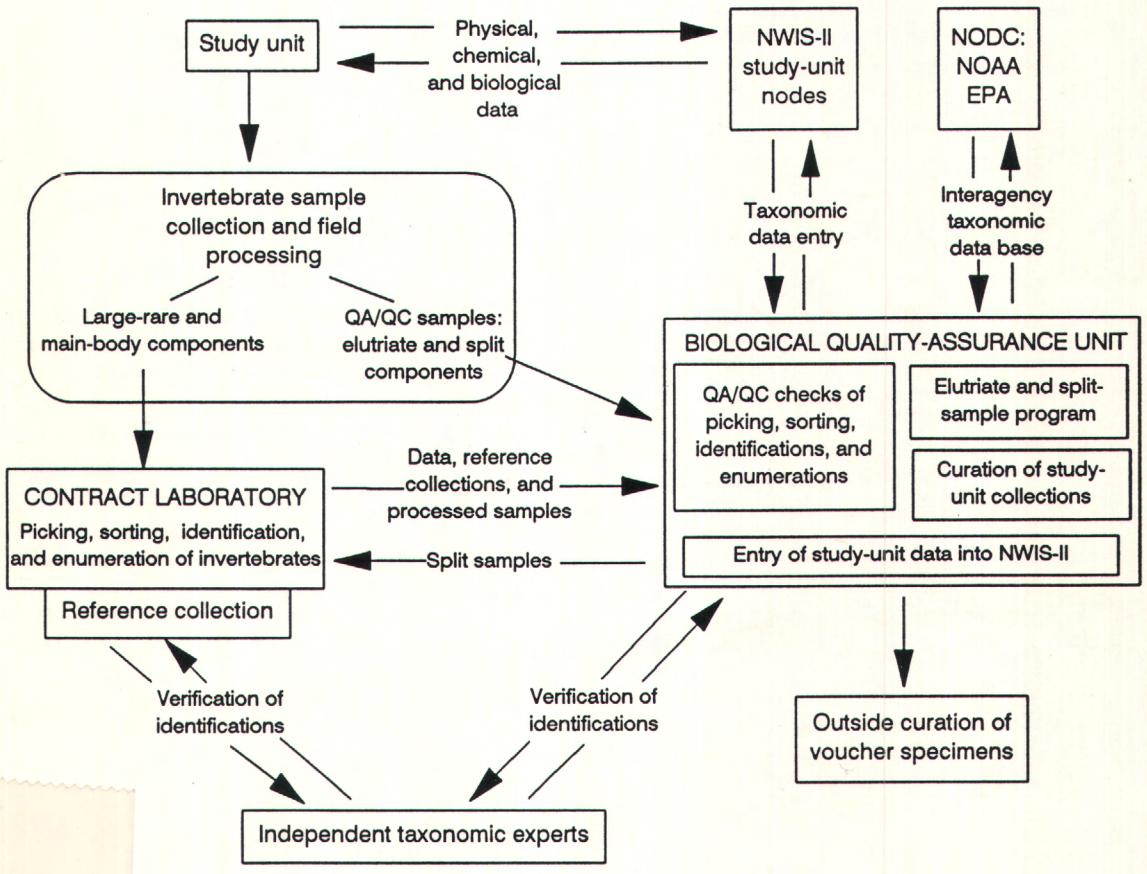
GUIDELINES FOR THE PROCESSING AND QUALITY ASSURANCE OF BENTHIC INVERTEBRATE SAMPLES COLLECTED AS PART OF THE NATIONAL WATER-QUALITY ASSESSMENT PROGRAM

U.S. Geological Survey

Open-File Report 93-407



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COVER PHOTOGRAPH: Flow chart showing the benthic invertebrate sample processing strategy for the National Water-Quality Assessment Program (modified from figure 1, page 4).

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CONVERSION FACTORS

Multiply	By	To obtain
	Length	
micron (µm)	0.00003937	inch
millimeter (mm)	0.03937	inch
centimeter (cm)	0.3937	inch
meter (m)	3.281	foot
	Area	
square centimeter (cm^2)	0.001076	square foot
	Volume	
liter (L)	0.264	gallon
milliliter (mL)	0.000264	gallon
	Mass	
gram (g)	0.03527	ounce, avoirdupois

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GUIDELINES FOR THE PROCESSING AND QUALITY ASSURANCE OF BENTHIC INVERTEBRATE SAMPLES COLLECTED AS PART OF THE NATIONAL WATER-QUALITY ASSESSMENT PROGRAM

By Thomas F. Cuffney, Martin E. Gurtz, and Michael R. Meador

ABSTRACT

Benthic invertebrate samples are collected as part of the U.S. Geological Survey's National Water-Quality Assessment Program. This is a perennial, multidisciplinary program that integrates biological, physical, and chemical indicators of water quality to evaluate status and trends and to develop an understanding of the factors controlling observed water quality. The Program examines water quality in 60 study units (coupled ground- and surface-water systems) that encompass most of the conterminous United States and parts of Alaska and Hawaii.

Study-unit teams collect and process qualitative and semi-quantitative invertebrate samples according to standardized procedures. These samples are processed (elutriated and subsampled) in the field to produce as many as four sample components: large-rare, main-body, elutriate, and split. Each sample component is preserved in 10-percent formalin, and two components, large-rare and main-body, are sent to contract laboratories for further processing. The large-rare component is composed of large invertebrates that are removed from the sample matrix during field processing and placed in one or more containers. The main-body sample component consists of the remaining sample materials (sediment, detritus, and invertebrates) and is subsampled in the field to achieve a volume of 750 milliliters or less. The remaining two sample components, elutriate and split, are used for quality-assurance and quality-control purposes.

Contract laboratories are used to identify and quantify invertebrates from the large-rare and main-body sample components according to the procedures and guidelines specified within this document. These guidelines allow the use of subsampling techniques to reduce the volume of sample material processed and to facilitate identifications. These processing procedures and techniques may be modified if the modifications provide equal or greater levels of accuracy and precision. The intent of sample processing is to determine the quantity of each taxon present in the semi-quantitative samples or to list the taxa present in qualitative samples. The processing guidelines provide standardized laboratory forms, sample labels, detailed sample processing flow charts, standardized format for electronic data, quality-assurance procedures and checks, sample tracking standards, and target levels for taxonomic determinations. The contract laboratory (1) is responsible for identifications and quantifications, (2) constructs reference collections, (3) provides data in hard copy and electronic forms, (4) follows specified quality-assurance and quality-control procedures, and (5) returns all processed and unprocessed portions of the samples.

The U.S. Geological Survey's Quality Management Group maintains a Biological Quality-Assurance Unit, located at the National Water-Quality Laboratory, Arvada, Colorado, to oversee the use of contract laboratories and ensure the quality of data obtained from these laboratories according to the guidelines established in this document. This unit establishes contract specifications, reviews contractor performance (timeliness, accuracy, and consistency), enters data into the National Water Information System-II data base, maintains in-house reference collections, deposits voucher specimens in outside museums, and interacts with taxonomic experts within and outside the U.S. Geological Survey. This unit also modifies the existing sample processing and quality-assurance guidelines, establishes criteria and testing procedures for qualifying potential contract laboratories, identifies qualified taxonomic experts, and establishes voucher collections.

INTRODUCTION

The U.S. Geological Survey's (USGS) National Water-Quality Assessment (NAWQA) Program is a perennial program designed to produce a comprehensive, multifaceted assessment of the quality of the Nation's flowing water resources (Hirsch and others, 1988; Leahy and others, 1990). NAWQA Program activities center on 60 study units (coupled ground- and surface-water systems) located in the conterminous United States, Alaska, and Hawaii. Investigations within each study unit use consistent national guidelines for selecting sampling sites and collecting physical, chemical, and biological data. This national consistency allows an integrated assessment of the status and trends in the Nation's water quality and the development of an understanding of the major factors that affect observed water-quality conditions and trends.

Background

Ecological surveys are a major component of the biological part of the NAWQA Program (Gurtz, 1993) and consist of stream habitat assessment (Meador, Hupp, and others, 1993), and community characterizations of fish (Meador, Cuffney, and Gurtz, 1993), algae (Porter and others, 1993), and benthic invertebrates (Cuffney and others, 1993). Ecological surveys are a part of all NAWQA Program activities, including occurrence and distribution assessments; assessments of long-term trends and changes; and studies of sources, transport, fate, and effects. Each of these sampling activities addresses a different set of objectives and varies in the number of sites sampled, the biological constituents measured, and the frequency and intensity of sampling done at each site.

Components of the ecological surveys (habitat and community assessments) are conducted at fixed and synoptic-survey sites within each study unit. Generally, ecological surveys are conducted only once at a site during a NAWQA Program cycle. However, to meet some Program objectives, particularly for trends analysis, some sites are sampled annually for 3 or more years. Consequently, the design of the NAWQA Program ensures that a large number of ecological survey samples will be collected from a broad geographic region over a long period of time.

Nationally consistent guidelines for sampling biological communities have been developed (Cuffney and others, 1993; Meador, Hupp, and others, 1993; Porter and others, 1993) for the NAWQA Program to ensure that the study units collect comparable data. These guidelines call for the processing of samples by contract laboratories that are responsible for all phases of sample processing, such as identifying and quantifying benthic invertebrates, or that cooperate with USGS personnel to process samples (for example, fish identification

and quantification). National consistency in the processing of ecological survey samples is as essential to regional and national synthesis as are nationally consistent collection methods. Consequently, guidance and standardization are needed for the processing of samples, particularly for those constituents, such as benthic invertebrate samples, that are totally processed by contract laboratories. These guidelines need to address suitable processing methods and quality-assurance (QA) and quality-control (QC) methods to ensure the accuracy of taxonomic identifications and quantifications. Furthermore, coordination and oversight of taxonomy are needed at a national level to ensure a consistency in identifications across all study units and to track changes in taxonomy through time.

Purpose and Scope

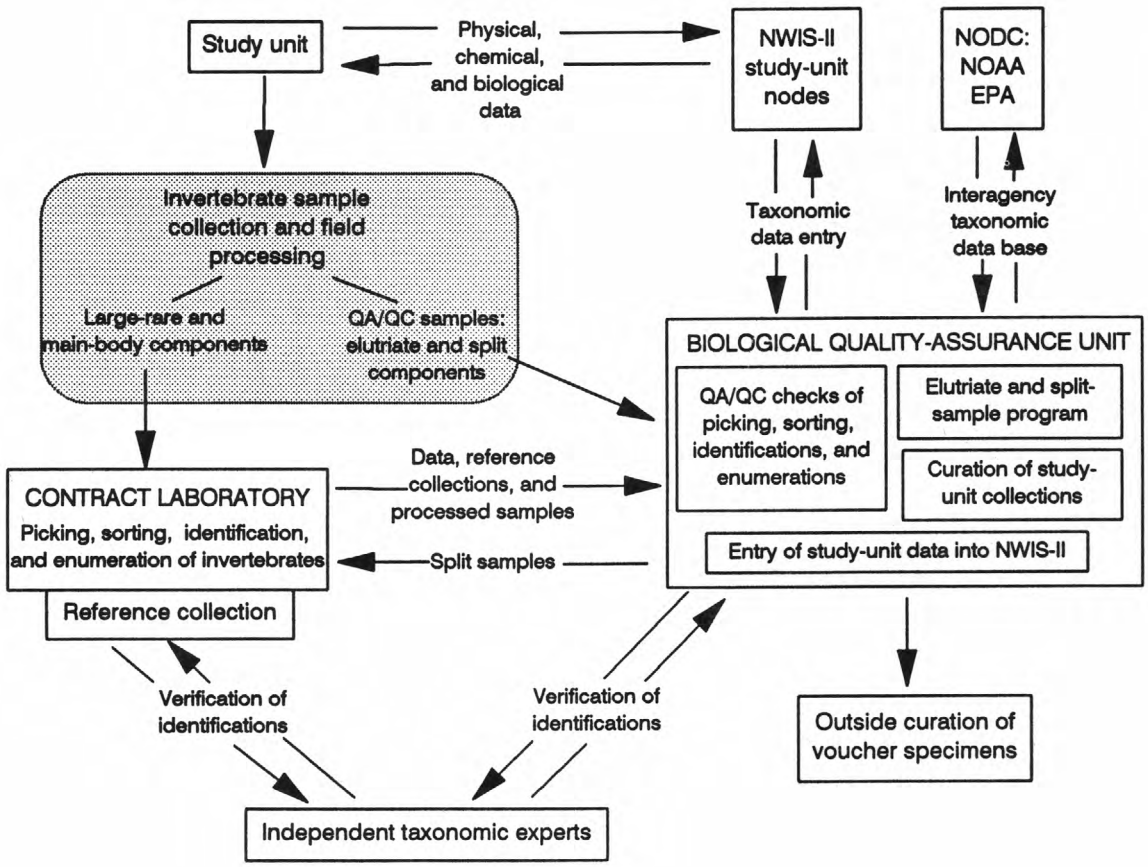
The purpose of this report is to provide nationally consistent guidelines and criteria for the processing of benthic invertebrate samples collected as part of the USGS's NAWQA Program. The guidelines presented here also are used to support other Water Resources Division (WRD) projects that involve the identification and quantification of benthic invertebrates.

These guidelines include the use of standard methods and equipment (for example, sieve sizes) for removing benthic invertebrates from the sample matrix, procedures for the labeling and tracking of samples, subsampling procedures, target levels of identification for major groups of invertebrates, quantification procedures, QA/QC procedures, and standard formats for reporting data on paper and in computer files. In addition, standards and procedures for the initial qualification and continual review of contract laboratories are presented, and the role of the USGS's Biological Quality-Assurance Unit (BQAU) is described.

SAMPLE PROCESSING STRATEGY

The processing of NAWQA Program benthic invertebrate samples depends upon three groups--the study unit, the BQAU, and contract laboratories (fig. 1). The study unit has overall responsibility for the collection, field processing, field QA, and analysis of data from invertebrate samples. Study-unit field activities (shaded area in fig. 1) involve collecting and processing samples and can produce as many as four components for each sample. Two components, the large-rare and main-body sample components, are sent by the study unit to the contract laboratory for analysis. The elutriate and split-sample components are sent to the BQAU for use in evaluating field-related QA/QC issues.

The BQAU has responsibility for establishing and monitoring contracts with laboratories, enacting QA/QC programs to monitor contract work and field sampling activities, coordinating taxonomy among study units, curating study-unit reference collections, and entering data into the National Water Information System-II (NWIS-II) data base. The BQAU receives field QA/QC samples (elutriate and split-sample components) from the study units. At least 10 percent of the elutriate samples are examined to determine the efficacy of the field elutriation procedures. Similarly, the split-sample components are used to evaluate how effective splitting procedures are in producing sample components that are similar. Split-sample components are either processed by the BQAU or sent to contractors for processing.



EXPLANATION

QA/QC	QUALITY ASSURANCE AND QUALITY CONTROL
NWIS-II	NATIONAL WATER INFORMATION SYSTEM II
NODC	NATIONAL OCEANOGRAPHIC DATA CENTER
NOAA	NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
EPA	ENVIRONMENTAL PROTECTION AGENCY
	FIELD PROCESSING
	LABORATORY PROCESSING

Figure 1.--Overview of benthic invertebrate sample processing strategy.

The BQAU reviews sample materials and data returned from the contract laboratories. These reviews include conducting QA/QC checks on sample picking, identifications, and quantifications using the same QA/QC criteria as specified for the contract laboratories. Data forms, QA/QC reports, electronic data files, and study-unit reference collections also are reviewed for accuracy, consistency, and completeness. Reviews of taxonomic identifications are conducted using taxonomic resources within the BQAU and independent taxonomic experts to verify identifications and resolve taxonomic issues among study units. The BQAU places voucher specimens (verified representatives of each taxon) in outside repositories, typically museum or university collections, for review and use by the general scientific community. These reviews are conducted to ensure data quality and to improve sample processing efficiency and effectiveness by working with the contract laboratories to develop better sample processing techniques and QA/QC criteria.

The BQAU enters biological data into the USGS's national data base (NWIS-II) for use by the study unit and other elements of the USGS. Entry of these data includes checking the names of organisms against an interagency taxonomic data base derived from the National Oceanic and Atmospheric Administration's (NOAA) National Oceanographic Data Center's (NODC) taxonomic data base. This data base is currently (1993) under development by the USGS, U.S. Environmental Protection Agency (EPA), and NOAA. Taxonomic names are checked for errors in spelling, authority names, and taxonomic classification. In addition, the data base provides a mechanism to track changes in nomenclature and maintains a hierarchy of taxonomic classification. The BQAU updates the USGS part of the taxonomic data base and coordinates updates to the national-interagency data base. The BQAU places contractor data into NWIS-II for immediate use by the study unit. General release of the data for other NWIS-II users is withheld until the BQAU resolves any issues regarding the quality of sample processing, including picking effectiveness and accuracy of identifications and quantifications, and the study-unit chief authorizes release of the data. Once loaded into the study-unit computer system (study-unit node), the biological data are combined with the physical and chemical data for analyzing water-quality conditions within the study unit.

Contract laboratories are responsible for identifying and quantifying specimens from samples according to technical guidelines established by the BQAU in consultation with study units and other appropriate groups within the NAWQA Program. The contract laboratory processes the samples using specified QA/QC procedures, produces data in standardized paper and electronic formats, and prepares a study-unit reference collection that validates taxonomic determinations. Independent taxonomic experts are used by the contractor to verify identifications of study-unit reference-collection specimens. The study-unit reference collections, QA/QC records, sample remnants, data, and reports are returned directly to the BQAU by the contract laboratory.

Study-Unit Sample Collection and Processing Activities

NAWQA Program study-unit teams collect benthic invertebrates using a standard sampling protocol (Cuffney and others, 1993) that provides guidelines for collecting, processing, and labeling field samples. Typically, one qualitative and two semi-quantitative samples are collected within each sampling reach associated with an ecological survey site. The qualitative samples, together with the semi-quantitative samples, provide data on taxa that are found at a site (presence or absence), whereas the semi-quantitative samples provide data on the structure (percentage of total invertebrates represented by each taxon) of the invertebrate community in two contrasting habitats. Samples are collected using either

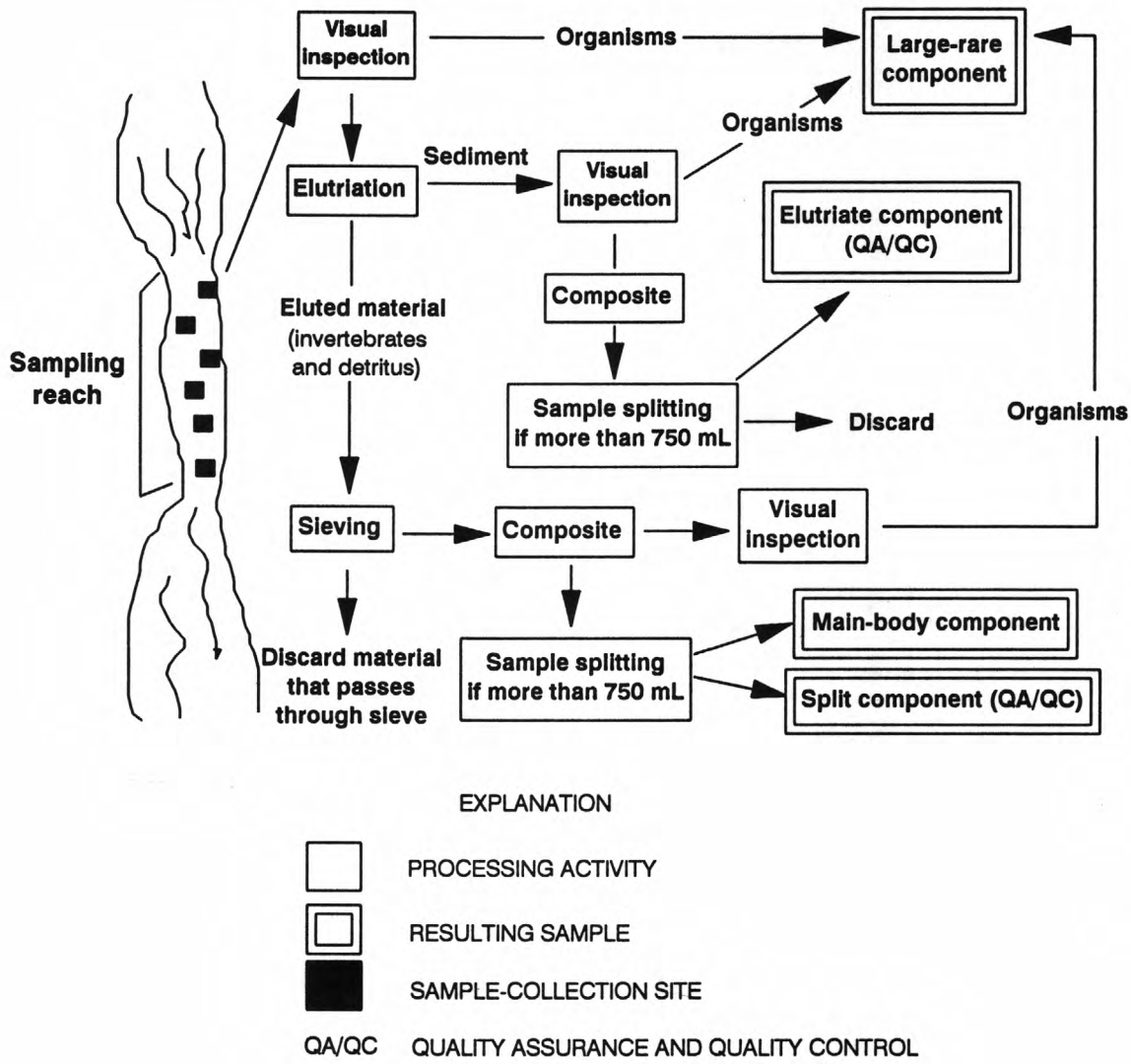


Figure 2.--Overview of field sample processing activities.

210-µm mesh (qualitative samples) or 425-µm mesh (semi-quantitative samples) nets. Typically, five individual samples are collected and composited to form each "richest-targeted habitat" (RTH) and "depositional-targeted habitat" (DTH) semi-quantitative sample. Each qualitative multihabitat sample (QMH) is a composite sample formed from multiple collections taken from as many instream habitats as are present and as can be practicably sampled within the sampling reach.

Study-unit teams prepare qualitative and semi-quantitative samples for shipment to the contract laboratory according to standardized field processing procedures (Cuffney and others, 1993). Field processing (fig. 2) consists of three activities: visual inspection, elutriation, and sample splitting. Visual inspections are conducted at several points during sample processing to remove and preserve separately large specimens, such as megalopterans, case-bearing Trichoptera, unionid mollusks, and crayfish that might physically interfere with subsequent sample splitting or that are sufficiently rare that they might be underrepresented in the split sample. Organisms that are removed during visual inspections are incorporated in the large-rare sample component, which can occupy multiple sample containers. Elutriation (the repetitive washing and decanting of the sample) is used to separate the denser sand and gravel from the less dense invertebrates and detritus. Sample splitting is used to reduce each sample fraction that results from elutriation to a manageable volume (750 mL or less). Collectively, these three steps standardize the volume of sample processed by the contract laboratory, minimize physical damage to specimens during shipment, and provide a representative subsample of the original composite sample.

Field processing can result in four components for each composite sample (table 1) depending upon the volume of the composite sample. Two components (large-rare and main-body) are sent by the study unit directly to the contract laboratory for identification and quantification. The other two components (elutriate and split) are sent by the study unit to the BQAU for use as QA/QC samples. All samples are fixed in 10-percent buffered formalin, labeled, and sealed.

Table 1.--*Descriptions of sample components generated during field processing of benthic invertebrate samples*

[mL, milliliter; QA/QC, quality assurance/quality control]

Component	Description
Large-rare	Obvious, large organisms that might be lost during subsampling. These organisms are removed from the matrix of sediment and detritus and preserved. A single sample can produce multiple containers of this component depending upon the number and size of large-rare organisms.
Main-body	Sample component that remains after removing the large-rare organisms. This component is shipped to the contract laboratory, which removes organisms from the sediment/detritus matrix, identifies, and counts them. This sample component is elutriated and subsampled in the field to limit volume to 750 mL or less.
Elutriate	Sediment remaining after the field sample is elutriated. This sample should be searched for organisms and then split to a volume of 750 mL or less. At least 1 in every 10 of these samples is processed for QA/QC purposes.
Split	Produced when the main-body sample component is split to limit volume to 750 mL or less. This sample is assumed to be similar to the main-body sample and is retained as a QA/QC sample. At least 1 in every 10 of these samples is processed for QA/QC purposes.

Standard sample labels (internal and external) are used to uniquely identify each sample component. The internal label (fig. 3) contains collection information, such as site, collection date, type of sample, reach identifier, and names of the sampling team, and processing information, such as subsample amount, sample subtypes, and sample identification code. A 16-character sample identification code (fig. 4) is used as an external label and contains information on the study-unit name (table 2), date of collection, sample type and subtypes, sample number, and bottle sequence. Sample components that are sent directly to the contract laboratory for analysis (large-rare and main-body) are coded first; components destined for the BQAU for QA/QC purposes (elutriate and split components) are coded last and receive higher bottle-sequence characters (fig. 5). Assigning bottle-sequence characters in this fashion prevents the contract laboratory from determining whether a sample has supporting QA/QC samples by looking for gaps in the bottle sequence codes.

NAWQA INVERTEBRATE SAMPLE

Site Name: S.F. Ahtanum Cr, Tampico, WA

Site ID No.: 12500900

Date: 10 / 28 /90 Subsample 1/2

Collected by: C.F. Jones, M.E. Smith, M.R. Wesson, T.F. Blakely

Type of Sample:

QMH (RTH) DTH

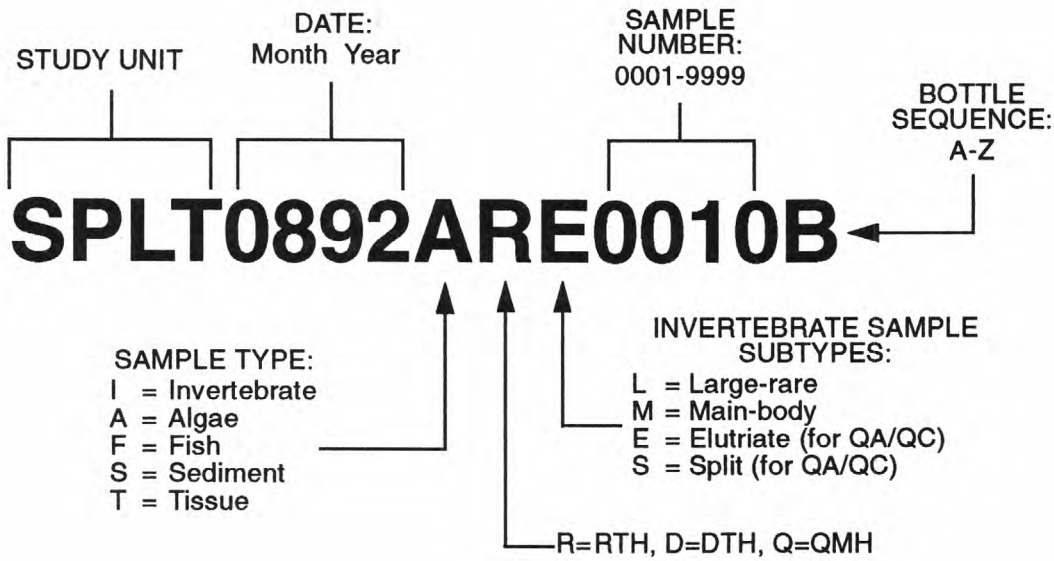
Large-rare (Main) Elutriate Split

Mesh: (425 µm) 210 µm Reach: A

ID Code: YAKI1090IRM0070C

Figure 3.--Example of an internal label for a main-body component of a benthic invertebrate sample.

An invertebrate sample field log (fig. 6) is completed to aid in tracking samples throughout sample processing. A complete copy of the field sample log, including information on QA/QC samples, is sent to the BQAU. Copies of an abbreviated list that excludes references to QA/QC samples are sent to the contract laboratory and packed with the samples as a packing list. The BQAU uses the study unit's field sample log to (1) track samples and monitor the progress of the samples through the contract laboratory, and (2) select elutriate and split-sample components for use in monitoring the quality of field elutriation and splitting techniques. The study units are responsible for the proper packaging and shipment of preserved specimens as established by the U.S. Department of Transportation and the selected shipping company.



	EXPLANATION
QA/QC	QUALITY ASSURANCE AND QUALITY CONTROL
RTH	RICHEST-TARGETED HABITAT
DTH	DEPOSITIONAL-TARGETED HABITAT
QMH	QUALITATIVE MULTIHABITAT

Figure 4.--Example of an external label for the main-body component of a benthic invertebrate sample showing the structure of the NAWQA Program 16-character sample identification code.

Overview of Contract Laboratory Processing Activities

Benthic invertebrate sample processing requires a great deal of labor (sample "picking") and technical expertise (identifications) to produce consistent, high-quality data in a timely manner. Consequently, the NAWQA Program relies on contract laboratories to provide the capability and expertise needed to process the large number of benthic invertebrate samples produced by the Program. Field elutriation and sample splitting provide a sample that is relatively consistent in volume and condition. This consistency allows the contract laboratory to make precise estimates of processing costs per sample (combined large-rare and main-body sample components) and makes it easier to use standardized processing procedures more effectively. Processing procedures center around the following three activities: (1) removal of invertebrates from the sediment and organic matrix of the sample, (2) identification of the organisms, and (3) quantification of the organisms.

Table 2.--*Abbreviations of study-unit names used in the 16-character sample identification codes*

Study Unit	Abbreviation	Study Unit	Abbreviation
Albemarle-Pamlico Drainage	ALBE	Northern Rockies Intermontaine Basins	NROK
Allegheny and Monongahela Basins	ALGH		
Apalachicola-Chattahoochee-Flint River Basin	ACFB	Oahu	OAHU
		Ozark Plateaus	OZRK
Central Columbia Plateau	CCPT	Potomac River Basin	POTO
Central High Plains	CHPL	Puget Sound Drainages	PUGT
Central Nebraska Basins	CNBR	Red River of the North	REDN
Central Oklahoma	COKL	Rio Grande Valley	RIOG
Cheyenne and Belle	CHEY	Sacramento Basin	SACR
Chicot-Evangeline	CHEV	San Joaquin-Tulare Basins	SANJ
Connecticut, Housatonic, and Thames River Basins	CONN	Santa Ana Basin	SANA
Cook Inlet	COOK	Santee Basin and Coastal Drainage	SANT
Delaware River Basin	DELR	South Central Texas	SCTX
Delmarva Peninsula	DLMV	South Platte River Basin	SPLT
Eastern Iowa Basins	EIWA	Southeastern New England	SENE
Georgia-Florida Coastal Plain	GAFL	Southern Arizona	SOAZ
Great and Little Miami River Basin	MIAM	Southern Florida	SOFL
Great Salt Lake Basin	GRSL	Southern High Plains	SHPL
Hudson River Basin	HDSN	Southern Illinois	SILL
Kanawha-New River Basin	KANA	Trinity River Basin	TRIN
Kansas River Basin	KANS	Upper Arkansas River	UARK
Kentucky River Basin	KNTY	Upper Colorado River Basin	UCOL
Lake Erie-Saint Clair Drainage	LERI	Upper Illinois River Basin	UIRB
Long Island-New Jersey Coastal Plain	LINJ	Upper Mississippi River Basin	UMIS
		Upper Snake River Basin	USNK
Lower Susquehanna River Basin	LSUS	Upper Tennessee River Basin	UTEN
Lower Tennessee River Basin	LTEN		
Mississippi Embayment	MISE	Western Lake Michigan Drainages	WMIC
Mobile River	MOBL	White River Basin	WHIT
Nevada Basin and Range	NVBR	Willamette Basin	WILL
New Hampshire and Southern Maine Basins	NHME	Yakima River Basin	YAKI
		Yellowstone River Basin	YELL
North Platte Basin	NPLT		

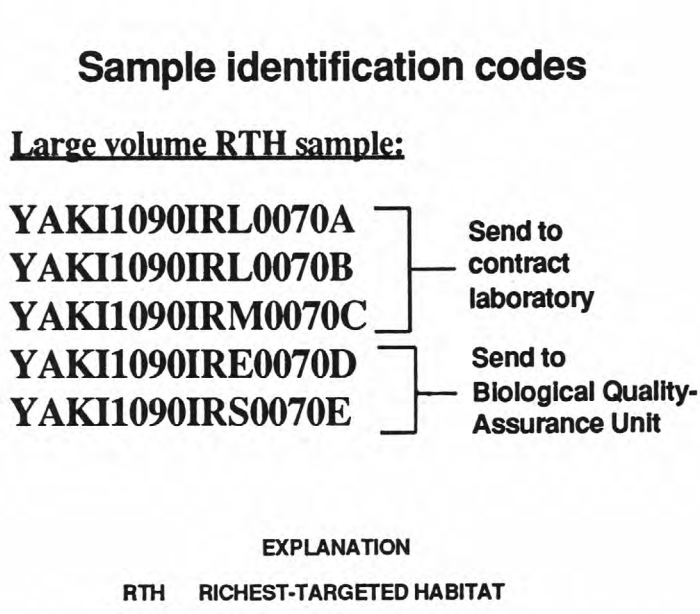


Figure 5.--Examples of sample identification codes generated from the field processing of one semi-quantitative invertebrate sample.

Sample Picking, Sorting, and Subsampling

Picking invertebrates, which may range in length from more than 10 cm (crayfish) to less than 0.5 mm (midge larvae), typically involves removing large specimens followed by the use of a microscope or other magnifying device to spot and remove smaller organisms. Samples that contain relatively large volumes of fine detritus or large numbers of small organisms may need to be subsampled to expedite sample processing. Organisms that are "picked" from the sample are sorted into taxonomic categories (for example, order or family), identified, and quantified.

Various sample picking, sorting, and subsampling methods exist, each of which may or may not produce comparable results on the same sample. In addition, shortcuts in sample processing (for example, heavy subsampling) can be used by contractors to decrease sample processing times and costs. The diversity of processing methods available, the incentive for contractors to minimize processing time, and the likelihood that multiple contractors will be used to process samples make it very important that standards for sample processing techniques be established and followed. Only in this way can consistent and comparable results be ensured.

Organism Identification and Quantification

Sample identification requires individuals who have adequate training and expertise in the identification of benthic invertebrates. Identification typically occurs through the use of dichotomous keys, which offer a formalized step-by-step method for arriving at a name for an organism based on its morphological characteristics. Progression through the dichotomous key results in classification of the specimen according to a nomenclatural hierarchy (for example, Order→Family→Genus→Species) of increasing morphological

NAWQA PROGRAM INVERTEBRATE SAMPLE FIELD LOG

Study Unit: Lower Susquehanna River Basin **Sampling Dates:** **Start:** 09/25/92

Finish: 09/26/92

Sample identification code	Site code	Collection date (MM/DD/YY)	Sample description		Disposition
			Type	Component	
LSUS0992IQL0001A	12510500	09/25/92	QMH	Large-rare	XYZ Labs, 10/1/92
LSUS0992IQM0001B	12510500	09/25/92	QMH	Main-body	XYZ Labs, 10/1/92
LSUS0992IQE0001C	12510500	09/25/92	QMH	Elutriate	BQAU, 10/1/92
LSUS0992IQS0001D	12510500	09/25/92	QMH	Split	BQAU, 10/1/92
LSUS0992IRL0002A	12510500	09/25/92	RTH	Large-rare	XYZ Labs, 10/1/92
LSUS0992IRM0002B	12510500	09/25/92	RTH	Main-body	XYZ Labs, 10/1/92
LSUS0992IRE0002C	12510500	09/25/92	RTH	Elutriate	BQAU, 10/1/92
LSUS0992IRS0002D	12510500	09/25/92	RTH	Split	BQAU, 10/1/92
LSUS0992IDL0003A	12510500	09/25/92	DTH	Large-rare	XYZ Labs, 10/1/92
LSUS0992IDM0003B	12510500	09/25/92	DTH	Main-body	XYZ Labs, 10/1/92
LSUS0992IDE0003C	12510500	09/25/92	DTH	Elutriate	BQAU, 10/1/92
LSUS0992IDS0003D	12510500	09/25/92	DTH	Split	BQAU, 10/1/92
LSUS0992IQL0004A	12510600	09/26/92	QMH	Large-rare	XYZ Labs, 10/1/92
LSUS0992IQM0004B	12510600	09/26/92	QMH	Main-body	XYZ Labs, 10/1/92
LSUS0992IQE0004C	12510600	09/26/92	QMH	Elutriate	BQAU, 10/1/92
LSUS0992IQS0004D	12510600	09/26/92	QMH	Split	BQAU, 10/1/92
LSUS0992IRL0005A	12510600	09/26/92	RTH	Large-rare	XYZ Labs, 10/1/92
LSUS0992IRL0005B	12510600	09/26/92	RTH	Large-rare	XYZ Labs, 10/1/92
LSUS0992IRL0005C	12510600	09/26/92	RTH	Large-rare	XYZ Labs, 10/1/92
LSUS0992IRM0005D	12510600	09/26/92	RTH	Main-body	XYZ Labs, 10/1/92
LSUS0992IRE0005E	12510600	09/26/92	RTH	Elutriate	BQAU, 10/1/92
LSUS0992IRS0005F	12510600	09/26/92	RTH	Split	BQAU, 10/1/92
LSUS0992IDL0006A	12510600	09/26/92	DTH	Large-rare	XYZ Labs, 10/1/92
LSUS0992IDM0006B	12510600	09/26/92	DTH	Main-body	XYZ Labs, 10/1/92
LSUS0992IRL0007A	12510700	09/26/92	RTH	Large-rare	XYZ Labs, 10/1/92
LSUS0992IRM0007B	12510700	09/26/92	RTH	Main-body	XYZ Labs, 10/1/92

Figure 6.--Example of an invertebrate sample field log showing the sample identification code assigned to each sample container and information on collection site, date, sample description, and disposition.

similarity. It is desirable to achieve the lowest level of taxonomic classification possible because ecological characteristics and reactions to water-quality changes are more specific at lower taxonomic levels (species) than at higher levels (genus or family) (Resh and Unzicker, 1975). Consequently, desired levels of taxonomic resolution are specified in the sample processing guidelines.

The process of identification can require viewing the whole organism under low magnification, or it can require clearing and mounting of specimens for viewing at high magnification (for example, midge larvae). Different specimen-mounting techniques are required depending upon the size, type, and use of the specimen. Quantification of semi-quantitative samples consists of counting the number of organisms of each type in each vial and then calculating sample totals by compensating for any subsampling done in the field and laboratory. Qualitative samples are quantified by simply noting the presence of each taxon on the appropriate data sheet.

Initially, identification can appear to be a simple process requiring knowledge of the taxonomic keys and invertebrate morphology. However, keys are written for mature specimens with all of their morphological features intact and fully developed. In reality, specimens obtained from field samples can be very young or damaged during collection or shipment. Consequently, the morphological features required to identify the organism can be missing or obscured, and the identification of the specimen terminates at a higher taxonomic level than desired. In spite of this, a person who is sufficiently familiar with the local fauna and who has access to a suitable reference collection could make an identification at a lower taxonomic level. Thus, local (State or regional) expertise is a desirable attribute for persons involved in invertebrate identification.

Further complicating taxonomic identification is the dynamic nature of the science of taxonomy. New species are constantly being described and old ones revised, resulting in the construction of new keys and changes in the names of organisms. Therefore, it is necessary to use up-to-date keys, specify the taxonomic keys used in identification, include authority names, and incorporate and track changes in taxonomy to ensure national consistency in the identification of biological specimens. A centralized taxonomic data base, study-unit reference collections, and voucher specimens all help to update identifications through time and compensate for changes in nomenclature.

Quality Assurance and Quality Control

Field and laboratory QA/QC procedures are important aspects of sample processing that are crucial to establishing the credibility of the resulting data. Laboratory QA/QC includes sample labeling, documentation of procedures and methods, establishment of empirically derived processing criteria, and test procedures to establish that processing criteria have been met. For example, samples must be labeled in such a manner as to unambiguously identify sample fractions at each stage in the processing sequence. Sample tracking and custody records are kept to document when samples are received, when they enter each processing step, and who has custody of the sample. Specific QA/QC checks are performed to ensure that sample picking and subsampling meet established criteria. Identifications and counts also are checked by having another taxonomist within the contract laboratory confirm the identifications and counts on a subset of samples. In addition, study-unit reference collections are verified by independent experts who are familiar with the appropriate taxonomic group or regional fauna. Specimens confirmed in this manner become part of the USGS's permanent collections against which the identification of other specimens are compared.

QA/QC checks are structured to support a block of 10 or fewer samples (fig. 7) that are processed as a unit. At least 1 sample in every block of 10 samples is checked to ensure that identifications, quantifications, and picking effectiveness meet the criteria specified in figure 7. The sample that is used for the QA/QC checks is selected randomly after the entire block of samples has been processed. If the selected QA/QC sample fails to meet the QA/QC criteria, then the entire block of samples related to that QA/QC sample (invertebrates or sample remnants) must be re-processed and re-checked until the criteria are met. Under certain circumstances, such as when a sample contains very few taxa or organisms, it may not be possible to meet the QA/QC criteria, and a written waiver must be obtained from the BQAU. The QA/QC criteria presented in figure 7 are initial criteria that must be evaluated on the basis of empirical results. The expectation is that continual refinement of sample processing methods will allow contractors to meet or exceed these criteria.

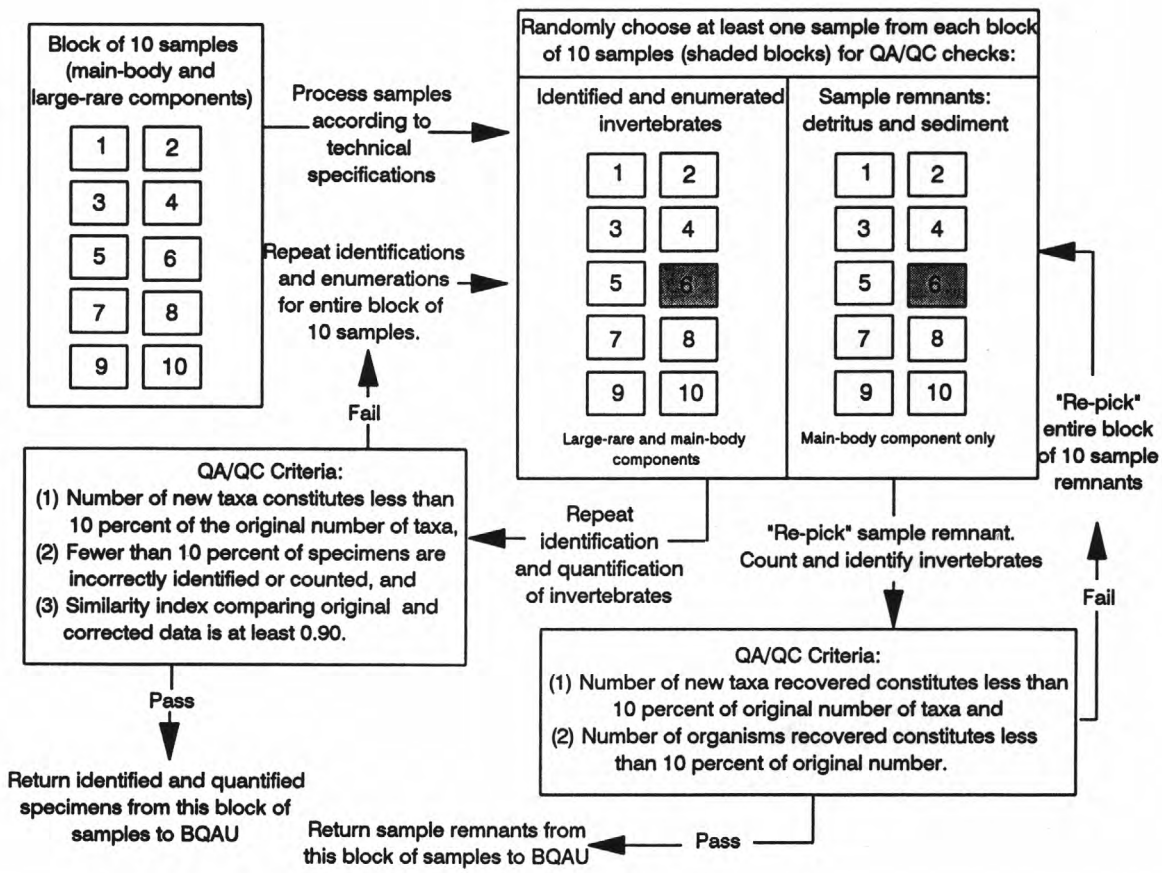


Figure 7.--Quality-assurance (QA) and quality-control (QC) criteria for the laboratory processing of benthic invertebrate samples.

Threatened and Endangered Species

The geographic extent of biological collections within the NAWQA Program raises the possibility of inadvertently collecting State and Federal threatened and endangered species. Study-unit biologists work closely with State and Federal authorities, particularly the U.S. Fish and Wildlife Service, to identify sampling sites that may harbor threatened and endangered invertebrates and to minimize the incidental collection of these taxa. When there is a possibility that threatened and endangered species may be collected from a site, the study-unit biologist notifies the BQAU and the contract laboratory and provides a list of the sites that are affected, the taxa involved, and the identification codes for the samples collected from those sites. The contract laboratory processes these samples ahead of other submissions to accommodate report deadlines requested by the U.S. Fish and Wildlife Service.

All members of a taxonomic group (family or genus) that may contain a threatened or endangered species are sent out to be confirmed by an expert on that species. If the organism in question can be readily identified to species, all members of the appropriate genus are sent for confirmation. Otherwise, all specimens of the appropriate family are sent for confirmation. An appropriate taxonomic expert is chosen by the BQAU in consultation with the U.S. Fish and Wildlife Service, the study-unit biologist, appropriate State officials, and the contract laboratory. If a threatened or endangered species is identified, the BQAU is immediately informed. The BQAU coordinates the notification of the appropriate authorities and oversees the proper disposition of these specimens. The BQAU, study-unit biologist, regional biologist, and the U.S. Fish and Wildlife Service work together with the contract laboratory to obtain any permits necessary for handling threatened and endangered species.

SPECIFICATIONS FOR SAMPLE PROCESSING BY CONTRACT LABORATORIES

The following specifications and procedures for processing qualitative and semi-quantitative benthic invertebrate samples form the basis for establishing standardized procedures that allow multiple contractors to produce data with sufficient accuracy, consistency, and comparability to meet local, regional, and national data needs. Alternative methods may be developed and substituted for the procedures presented here if they provide comparable data that meet or exceed the levels of accuracy and precision specified in the following guidelines. Such substitutions are to be made only with the written approval of the BQAU and are to include adequate documentation of the alternative method and its efficacy. Alternative methods that are approved by the BQAU and that are effective will be recommended to all laboratories processing NAWQA Program samples in an effort to continually improve the data generated by contractors.

Contractor's Responsibilities

The contractor provides all facilities, equipment, materials (with the exception of the original sample containers), and personnel needed to process the invertebrate samples and returns the data, reference collections, invertebrates, data sheets, work sheets, check lists, computer files, and all sample remnants to the BQAU. The contractor is also responsible for following applicable State, Federal, and local regulations related to the storage of preserved samples, safe and proper disposal of chemicals and materials generated as a consequence of processing samples, proper packaging and shipping of specimens, and the safety of all personnel involved in processing samples.

The contractor provides the BQAU with data as each block of samples (fig. 7) is completed and passes all QA/QC checks. Copies of relevant computer files and paper documents (table 3) are shipped to the BQAU along with specimens, sample remnants, and taxonomic data reports (hard copy and Lotus spreadsheet or ASCII files in MS-DOS format) at the contractor's expense. Taxonomic data reports contain the following information: (1) for semi-quantitative samples (RTH and DTH samples)--a taxonomic list, in phylogenetic order, giving the number of individuals in each taxon recovered from the sample, and (2) for qualitative multihabitat samples (QMH)--a taxonomic list, in phylogenetic order, listing the taxa found in each sample. In addition, copies of all QA/QC documents are submitted to the BQAU, including those relating to sample tracking and custody, picking, subsampling, identification, quantification, and reference specimen verifications.

Table 3.--*Names and descriptions of documents used to record information during sample processing*

[NAWQA, National Water-Quality Assessment Program; QA/QC, quality assurance/quality control]

Name of document	Description of document
NAWQA Program study-unit reference-collection specimen source list (fig. 9)	Used to record the identification of the sample component and fraction from which reference-collection specimens originate.
NAWQA Program study-unit reference-collection data sheet (fig. 10)	Used to list the specimens constituting the study-unit reference collection. Lists the taxonomic keys used, the authority name, and person who verified the identification for each taxon.
Summary of taxonomic references used to identify invertebrates (fig. 11)	Bibliographic list of taxonomic references used to identify specimens in the study-unit reference collections.
List of independent taxonomists and affiliations (fig. 12)	List of the names and addresses of the people used to verify specimens.
NAWQA Program sample tracking and custody work sheet (fig. 13)	Used to record when samples arrive at and leave the contract laboratory and who has custody of the samples during processing.
NAWQA Program benthic invertebrate sample processing QA/QC sample block data sheet (fig. 14)	Used to identify QA/QC blocks, list the identification codes of samples in each block, and identify samples randomly chosen for QA/QC checks.
NAWQA Program invertebrate identification and quantification data sheet (fig. 16)	Used to record data (taxa and counts) obtained from the large-rare and main-body sample components.
NAWQA Program invertebrate identification subsampling data sheet (fig. 23)	Used to record data (taxa and counts) and calculate sample totals when subsamples of small, abundant invertebrates are removed from the main-body sample component to speed identifications.
NAWQA Program benthic invertebrate sample tabulation work sheet (fig. 25)	Used to calculate the number of organisms in the field sample by summing sample components and compensating for subsampling in the laboratory and field.

Table 3.--*Names and descriptions of documents used to record information during sample processing*--Continued

[NAWQA, National Water-Quality Assessment Program; QA/QC, quality assurance/quality control]

Name of document	Description of document
NAWQA Program benthic invertebrate sample processing subsampling QA check sheet (fig. 26)	Used to record the data and calculations used to determine the number of subsamples to process from the main-body sample component. Lists QA/QC criteria and records results of QA/QC checks.
Community similarity index work sheet for subsampling (table 8)	Computerized work sheet that calculates the similarity index used to determine how many C-fractions to process when subsampling a main-body sample component (fig. 26).
NAWQA Program invertebrate sample processing picking effectiveness work sheet (fig. 27)	Used to evaluate the effectiveness of procedures to remove invertebrates from the sample matrix. Lists QA/QC criteria and records results of QA/QC checks.
NAWQA Program invertebrate sample processing identification and quantification QA/QC data sheet (fig. 28)	Used to record changes to invertebrate identifications or counts that arise from reviewing the invertebrate elements of the large-rare and main-body sample components.
Community similarity index work sheet for invertebrate identifications and quantifications (table 9)	Computerized work sheet that calculates the similarity index used to determine if invertebrate identifications and quantifications meet criteria levels (fig. 30).
NAWQA Program invertebrate sample processing identification and quantification QA/QC summary check list (fig. 30)	Used to calculate QA/QC check values and record the outcome of checks for invertebrate identifications and quantifications.
NAWQA Program benthic invertebrate quality-assurance check sheet. Sample volume QA/QC checks (fig. 31)	Used to compare initial main-body sample component volumes with the combined volume of sample components that remain at the completion of sample processing.
NAWQA Program laboratory processing QA summary check list (fig. 32)	Used to summarize outcome of picking, identification and enumeration, and sample volume QA/QC checks. Coded entries describe the reason for a QA/QC failure and the actions taken to correct the problem. Also used to record any other problems encountered during sample processing.
NAWQA Program summary data computer file (fig. 33)	Computer file used to summarize taxonomic data for each sample.

Study-unit reference collections and supporting documents (lists of taxonomists and keys) are returned to the BQAU upon completion of all study-unit samples collected during a calendar year. If the contractor desires to retain specimens for an in-house reference collection, written approval must be obtained from the BQAU that documents what specimens are retained, how many of each taxon, and the sample and sample fraction from which they originated.

Invertebrate Identifications

Invertebrates are identified to the lowest practical taxon using the specifications set forth in table 4. When more than one target level is shown in table 4, the lowest level is considered the desired level for all sufficiently mature specimens. Specimens that are too small or damaged to identify below the level of genus, or for which the taxonomy is incomplete, are reported at the lowest level known, such as 'Ephemeroptera G. spp.' or 'Baetidae G. spp.' Consistent deviations from the target taxonomic levels presented in table 4 require written approval by the BQAU and must be accompanied by a written justification in the data report. Professional judgment may be used to assign names to small or damaged specimens based on (1) knowledge of the morphology and life history of the taxon, and (2) consideration of the sample location, date of collection, and other taxa known to occur in the same sample or other samples from the same location. Such "professional judgments" are done in consultation with the BQAU and are documented.

Except for oligochaetes, organisms that were obviously dead at the time of collection, exuviae, and body parts constituting less than 50 percent of the organism are discarded unless the taxon is otherwise not present in a qualitative sample. In the latter case, the specimen or fragment (for example, a mussel shell) is identified to the extent possible, noted on the qualitative sample data sheet, and preserved in a separate labeled container. Counts of oligochaetes are based on heads only and fragments without heads are not counted.

Specimens that require mounting for identification are prepared for permanent mounting in euparal mounting medium. Some specimens (large taxa or those with dark heads) are cleared in 2- to 10-percent solution of potassium hydroxide prior to mounting. Mounted specimens are labeled with the sample and identification information on the left side of the slide. The right side of the slide is reserved for the placement of the study-unit reference-collection number for those specimens placed in the reference collection. Wet specimens are preserved in 70-percent alcohol (ethanol or isopropyl) and appropriately labeled. Labels are printed on plastic paper or 100-percent rag, acid-neutralized paper using water- and alcohol-resistant ink or pencil. Labels include relevant taxonomic information (family, order, genus, or species), sample identification codes, and appropriate sample processing information (see following section on sample fractions, labels, and data sheets).

Identifications are to be conducted using up-to-date keys and supporting literature. All specimens are to be returned to the BQAU unless the laboratory has made prior arrangements with the BQAU to retain specimens for their use. Such arrangements must be made in writing and must document what specimens are retained by the laboratory, what sample, component, and fraction they originated from, and how many of each taxon were retained.

Study-Unit Reference Collections

The contract laboratory produces a reference collection for each study unit that supports all the invertebrate identifications associated with each year's submission of samples (based on collection date). Reference-collection specimens, with the exception of organisms mounted on slides, are preserved in 70-percent alcohol (ethanol or isopropyl) and typically placed in 4-dram (7.08-gram) patent lip glass vials (21 x 70 mm) sealed with light-colored neoprene size 0 stoppers or 4-dram (7.08-gram) screw cap vials with polyseal caps. Large specimens, such as crayfish, may require larger vials or museum jars. Labels are printed using black, alcohol-resistant ink on 100-percent rag, acid-neutralized paper or

Table 4.--*Phylogenetic order and recommended levels for reporting taxonomic data* (modified from Hickman, 1973; Ohio Environmental Protection Agency, 1989; Thorp and Covich, 1991)

Phylogenetic level	Reporting level
Porifera	Species
Coelenterata	Genus
Platyhelminthes	
Turbellaria	Class
Rhynchocoela (Nemertea)	Order/Genus
Gastrotricha	Order
Rotifera	Order/Genus
Nematoda	Order/Genus
Nematomorpha	Genus
Entoprocta (Bryozoa)	Species
Priapula	Family/Genus
Echiura	Family/Genus
Sipuncula	Family/Genus
Mollusca	
Gastropoda	Family/Genus/Species
Pelecypoda	Family/Genus/Species
Cephalopda	Genus
Scaphopoda	Order/Genus
Polyplacophora	Family/Genus
Annelida	
Polychaeta	Family/Genus
Oligochaeta	Genus/Species
Brachiobdellida	Genus
Archiannelida	Family
Hirudinea	Species
Arthropoda	
Pycnogonida	Order/Family
Arachnida	
Acarina	Family/Genus
Crustacea	
Branchiopoda	
Anostraca	Family/Genus
Notostraca	Family/Genus
Conchostraca	Family/Genus
Cladocera	Family/Genus
Ostracoda	Order/Family
Copepoda	Order/Family
Branchiura	Family
Cirripedia	Order/Family
Malacostraca	
Mysidacea	Family/Genus

Table 4.--*Phylogenetic order and recommended levels for reporting taxonomic data* (modified from Hickman, 1973; Ohio Environmental Protection Agency, 1989; Thorp and Covich, 1991)--Continued

Phylogenetic level	Reporting level
Cumacea	Family/Genus
Tanaidacea	Family/Genus
Euphausiacea	Family
Isopoda	Genus
Amphipoda	Genus/Species
Decapoda	Genus/Species
Insecta	
Ephemeroptera	
Siphlonuridae	Genus
Baetidae	Genus (Baetis: species/group)
Oligoneuriidae	Genus
Heptageniidae	Genus/Species
Leptophlebiidae	Genus
Ephemerellidae	Species
Tricorythidae	Genus
Caenidae	Genus
Baetiscidae	Species
Potamanthidae	Genus
Ephemeridae	Genus
Polymitarcyidae	Species
Odonata	
Zygoptera	
Calopterygidae	Genus
Lestidae	Species
Coenagrionidae	Family/Genus
Anisoptera	
Aeshnidae	Species
Gomphidae	Species
Cordulegastridae	Species
Macromiidae	Species
Corduliidae	Species
Libellulidae	Species
Plecoptera	
Pteronarcyidae	Genus/Species
Peltoperlidae	Genus
Taeniopterygidae	Genus
Nemouridae	Genus/Species
Leuctridae	Genus
Capniidae	Genus
Perlidae	Species
Perlodidae	Species

Table 4.--*Phylogenetic order and recommended levels for reporting taxonomic data* (modified from Hickman, 1973; Ohio Environmental Protection Agency, 1989; Thorp and Covich, 1991)--Continued

Phylogenetic level	Reporting level
Chloroperlidae	Genus
Hemiptera	
Belostomatidae	Genus
Nepidae	Genus
Pleidae	Genus
Naucoridae	Genus
Corixidae	Genus
Notonectidae	Genus
Megaloptera	
Sialidae	Genus
Corydalidae	Species
Neuroptera	Genus
Trichoptera	
Philopotamidae	Genus/Species
Psychomyiidae	Species
Polycentropodidae	Genus
Hydropsychidae	Genus/Species
Rhyacophilidae	Species
Glossosomatidae	Genus
Hydroptilidae	Genus/Species
Phryganeidae	Genus
Brachycentridae	Genus
Limnephilidae	Genus
Lepidostomatidae	Genus
Beraeidae	Genus
Sericostomatidae	Genus
Odontoceridae	Genus
Molannidae	Genus
Helicopsychidae	Species
Calamoceratidae	Genus
Leptoceridae	Genus/Species
Lepidoptera	Genus
Coleoptera	
Gyrinidae	Genus
Haliplidae	Genus
Dytiscidae	Genus
Noteridae	Genus
Hydrophilidae	Genus
Hydraenidae	Genus
Psephenidae	Species
Dryopidae	Genus

Table 4.--*Phylogenetic order and recommended levels for reporting taxonomic data* (modified from Hickman, 1973; Ohio Environmental Protection Agency, 1989; Thorp and Covich, 1991)--Continued

Phylogenetic level	Reporting level
Scirtidae	Family
Elmidae	Genus/Species
Limnichidae	Genus
Heteroceridae	Family
Ptilodactylidae	Family
Chrysomelidae	Family
Curculionidae	Family
Lampyridae	Family
Diptera	
Tipulidae	Genus/Species "group"
Blephariceridae	Genus
Psychodidae	Genus
Ptychopteridae	Genus
Dixidae	Genus
Chaoboridae	Genus
Culicidae	Genus
Thaumaleidae	Genus
Simuliidae	Genus/Species
Ceratopogonidae	Family/Genus/Species
Chironomidae	
Tanypodinae	Genus/Species/Group
Diamesinae	Genus/Species/Group
Prodiamesinae	Genus/Species/Group
Orthocladiinae	Genus/Species/Group
Chironominae	
Chironomini	Genus/Species
Pseudochironomini	Genus/Species
Tanytarsini	Genus/Species
Tabanidae	Genus/Species
Athericidae	Species
Stratiomyidae	Genus
Empididae	Family/Genus
Dolichopodidae	Family
Syrphidae	Family/Genus
Sciomyzidae	Family/Genus
Ephydridae	Family/Genus
Muscidae	Species

plastic paper. Internal labels for study-unit reference-collection specimens (fig. 8) include the name of the organism (with authority name), the 16-character NAWQA Program sample identification code, the sample fraction from which the organism originated, and the reference-collection specimen identification number. The T-15 in figure 8 indicates that this is the 15th trichopteran contained in the Yakima River Basin (YAKI) study-unit reference collection for samples collected during 1990. The reference-collection specimen number is also placed on the neoprene stopper, vial screw cap, or museum jar cap.

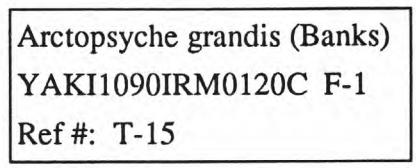


Figure 8.--Example of an internal label for a specimen container in the study-unit reference collection.

All specimens on microscope slides are mounted in euparal or balsam (Hoyer's and other water-based media are not acceptable) and labeled with all pertinent information, including mounting medium, on the left end of the slide. Sufficient space must be maintained for the placement of a reference-collection specimen number on the right side of the slide. Slides must be standard sized (75 x 25 mm or 3 x 1 inch) and dry enough to be stored upright when received by the BQAU.

Multiple specimens are used to represent each taxon, with one taxon represented in each sample container. Specimens representing a single taxon and originating from the same sample and sample fraction may be placed in a single sample container. Specimens representing a single taxon but originating from multiple samples or multiple sample fractions are placed in separate sample containers. Additions to the reference collection are made so that each specimen can be traced back to its original sample container and the sample fraction from which it was removed. This is accomplished by keeping a record of the source for each reference-collection specimen (fig. 9). The reference collections are returned to the BQAU for review at the conclusion of sample processing. These collections are accompanied by paper and electronic forms of the reference-collection data sheet (fig. 10), the source list for reference specimens (fig. 9), the list of reference materials used for identifications (fig. 11), and a listing of the independent taxonomists who confirmed reference-collection identifications for the contract laboratory (fig. 12). The reference-collection data sheet for each taxon includes the authority name, taxonomic reference, and the identity of the person who verified the identification.

Types of Samples and Purpose of Each Type

The contractor can receive three types of invertebrate samples: qualitative, semi-quantitative, and field QA/QC. Qualitative samples are composites of samples taken from the variety of habitats present at a sampling site and are collected on the basis of 210-µm mesh. The purpose of a qualitative sample is to provide, in conjunction with the semi-quantitative samples, a list of taxa present at each site. A semi-quantitative sample is a

NAWQA Program Study-unit Reference-Collection Specimen Source List

Project: GS-NAWQA **Year:** 1990

Study Unit: Yakima River Basin **Lab. Prefix:** YAKI90-

Collection number	Taxon	Lab. no.	NAWQA sample code	Fraction
MO-1	*Corbicula fluminea*	1	YAKI1090IRL0001A	B
AN-1	*Dina anoculata*	44	YAKI1090IQM0016C	F-1
AN-2	*Mooreobdella fervida*	44	YAKI1090IRM0016C	F-2
CR-1	*Hyalella azteca*	16	YAKI1090IDL0006A	B
CR-2	*Pacifasticus leniusculus*	1	YAKI1090IDL0001B	B
EP-1	*Ameletus sp.*	51	YAKI1090IQM0019B	F
EP-2	*Baetis bicaudatus*	26	YAKI1090IQM0009C	F
EP-3	*B. insignificans*	26	YAKI1090IQM0009C	F
EP-4	*B. quilleri*	26	YAKI1090IQM0009C	F
EP-5	*B. tricaudatus*	26	YAKI1090IQM0009C	F
EP-6	*Centroptilum sp.*	26	YAKI1090IQM0009C	F
EP-7	*Cinygmula sp.*	89	YAKI1090IRL0061A	B
EP-8	*Epeorus grandis*	89	YAKI1090IRL0061A	B
OD-1	*Calopteryx sp.*	130	YAKI1090IDL0090B	B
OD-2	*Argia sp.*	130	YAKI1090IDL0090B	B
PL-1	*Yoroperla sp.*	200	YAKI1090IRL0110A	B
PL-2	*Taenionema sp.*	200	YAKI1090IRL0110A	B
PL-3	*Taeniopteryx nivalis*	200	YAKI1090IRL0110A	B
PL-4	*Zapada cinctipes*	200	YAKI1090IRL0110A	B

Figure 9.--Example of a study-unit reference-collection specimen source list used to track the sources of specimens included in study-unit reference collections.

NAWQA Program Study-Unit Reference-Collection Data Sheet

Project: GS-NAWQA **Year:** 1993

Study Unit: Yakima River Basin

Reference number	Taxonomic identification			Authority	Taxonomic Reference	Confirmed by
	Level 1	Level 2	Level 3			
MO-1	Pelecypoda	Corbiculidae	*Corbicula fluminea*	Müller	18	J.D. Smith
AN-1	Annelida	Hirudinea	*Dina anoculata*	Moore	14	A.H. Harrold
AN-2			*Mooreobdella fervida*	Verrill	14	A.H. Harrold
CR-1	Crustacea	Amphipoda	*Hyalella azteca*	Saussure	11	C.D. Paulsen
CR-2		Decapoda	*Pacifasticus leniusculus*	Dana	10	E.F. Williams
EP-1	Ephemeroptera	Siphlonuridae	*Ameletus sp.*	Eaton	12	M.A. Gotz
EP-2		Baetidae	*Baetis bicaudatus*	Dodds	7,12,15,17	M.A. Gotz
EP-3			*B. insignificans*	McDunnough	7,12,15,17	M.A. Gotz
EP-4			*B. quilleri*	Dodds	7,12,15,17	M.A. Gotz
EP-5			*B. tricaudatus*	Dodds	7,12,15,17	M.A. Gotz
EP-6			*Centroptilum sp.*	Eaton	7,12,15	M.A. Gotz
EP-7		Heptageniidae	*Cinygmula sp.*	McDunnough	9,7,12	M.A. Gotz
EP-8			*Epeorus grandis*	McDunnough	7,12	M.A. Gotz
OD-1	Odonata	Calopterygidae	*Calopteryx sp.*	(Selys)	16	C.M. Highlin
OD-2		Coenagrionidae	*Argia sp.*	(Hagen)	16	C.M. Highlin
PL-1	Plecoptera	Peltoperlidae	*Yoroperla sp.*	Ricker	23, 25	G.M. Stoneman
PL-2		Taeniopterygidae	*Taenionema sp.*	Banks	23, 25	G.M. Stoneman
PL-3			*Taeniopteryx nivalis*	Pictet	23, 25	G.M. Stoneman
PL-4		Nemouridae	*Zapada cinctipes*	Banks	23, 25	G.M. Stoneman

Figure 10.--Example of a study-unit reference-collection data sheet used to list specimens in the study-unit reference collection. (Numbers listed in the taxonomic reference column refer to the references listed in figure 11.)

Summary of Taxonomic References Used to Identify Invertebrates

Reference number	Bibliographic reference
7	Edmunds, G.F., Jr., Jenson, S.L., and Berner, L., 1976, The mayflies of North and Central America: Minneapolis, Minn., University of Minnesota Press, 330 p.
10	Hobbs, H.H., Jr., 1972, Crayfishes (Astacidae) of North and Middle America: Washington, D.C., U.S. Environmental Protection Agency, Biota of freshwater ecosystems identification manual no. 9, 173 p.
11	Holsinger, J.R., 1972, The freshwater amphipod crustaceans (Gammaridae) of North America: Washington, D.C., U.S. Environmental Protection Agency, Biota of freshwater ecosystems identification manual no. 5, 89 p.
12	Jensen, S.L., 1966, The mayflies of Idaho (Ephemeroptera) v. 1-5: Salt Lake City, Utah, University of Utah, M.S. Thesis, 365 p.
14	Klemm, D.J. (ed.), 1985, A guide to freshwater Annelida (Polychaeta, Naidid, and Tubificid Oligochaeta, and Hirudinea) of North America: Dubuque, Iowa, Kendall/Hunt, 198 p.
15	McCafferty, W.P., and Waltz, R.D., 1990, Revisionary synopsis of the Baetidae (Ephemeroptera) of North and Middle America: Transactions of the American Entomological Society, v. 116, no. 4, p. 769-799.
16	Merritt, R.W., and Cummins, K.W. (eds.), 1984, An introduction to the aquatic insects of North America (2d ed.): Dubuque, Iowa, Kendall/Hunt, 722 p.
17	Morihara, D.K., and McCafferty, W.P., 1979, The *Baetis* larvae of North America (Ephemeroptera: Baetidae): Transactions of the American Entomological Society. v. 105, p. 139-221.
18	Pennak, R.W. (ed.), 1989, Fresh-water invertebrates of the United States (3d ed.): New York, John Wiley & Sons, Inc., 628 p.
23	Stark, B.P., Szczytko, S.W., and Baumann, R.W., 1986, North American stoneflies (Plecoptera): Miscellaneous Publications of the Entomological Society of America, v. 10, no. 1, p. 1-80.
25	Stewart, K.W., and Stark, B.P., 1988, Nymphs of North American stonefly genera (Plecoptera): Thomas Say Foundation, Entomological Society of America, v. 12, p. 1-436.

Figure 11.--Summary of taxonomic references used to identify invertebrates.

List of Independent Taxonomists and Affiliations

Name	Affiliation
R.B. Buzzy	Institute for the Advancement of Mayfly Research 1245 Aspen Way London, CO 35086 303-555-5758
M.A. Gotz	Center for Taxonomic Excellence University of Wisdom 3 Apian Way Wisdom, IA 69345 987-555-3320
A.H. Harrold	University of Glenn Ellen Department of Entomology 34 Academic Road New Britain, NJ 14454 203-555-1324
C.M. Highlin	McLoughlin Museum of Natural History 989 Museum Drive McLoughlin, TX 94567 517-555-9856
C.D. Paulsen	Institute for Amphipod Research 347 Crustacean Blvd. Crayfish, LA 55578 714-555-0990
J.D. Smith	University of Cairo Department of Biology Cairo, MO 44567 313-555-3985
G.M. Stoneman	University of Academic Studies Department of Entomology 666 Spirit Road Hampton, NY 14055 716-555-4437
E.F. Williams	American Crayfish Breeders Association 134 Carapace Lane Proleg, LA 55568 714-555-8877

Figure 12.--Hypothetical list of independent taxonomists provided by contract laboratories and used to confirm invertebrate identifications.

composite of multiple samples from a single type of habitat and is collected on the basis of 425-µm mesh. The purpose of semi-quantitative samples is to provide a list of taxa present within a specific targeted habitat type (either richest or depositional) and their relative abundances (proportion of total contributed by each taxon). Field QA/QC samples are composed of elutriate or split-sample components obtained from semi-quantitative and qualitative samples. The purpose of providing field QA/QC samples is to monitor the efficacy of field elutriate and splitting techniques. Mesh size differences and the differences in data objectives for each sample type must be considered in selecting techniques and methods for processing qualitative, semi-quantitative, and field QA/QC samples.

Qualitative and semi-quantitative samples are distinguished by the 10th character in the 16-digit code displayed on the external label attached to the outside of each sample container (fig. 4). Semi-quantitative samples are distinguished by an "R" or "D" in this position, which identifies the sample as either a richest-targeted habitat ("R" = RTH) or depositional-targeted habitat ("D" = DTH) sample. Qualitative-multihabitat samples (QMH) are identified by a "Q" in this position. The internal sample label (fig. 3) contains additional information to identify the sample, including the sample identification code and the sample type. Additional codes can be added to identify different types of qualitative and semi-quantitative samples collected as part of study-unit synoptic or case studies. These qualitative and semi-quantitative samples will be processed in accordance with the procedures outlined in this guidance document.

In addition to qualitative and semi-quantitative samples, the BQAU works with the study units to send periodic field QA/QC samples to the contract laboratories as a check on the quality of field sample processing. The split-sample component of qualitative or semi-quantitative field samples is typically used for this purpose, though other types of samples can be used to address specific QA/QC issues. These QA/QC samples are labeled and packaged so as to make them indistinguishable from other samples of the same type: RTH, DTH, or QMH. Field QA/QC samples are mixed with other study-unit samples and sent to the contractor from the study unit.

Sample Receipt, Log-In, and Tracking

The BQAU coordinates the shipment of samples between the study unit and the contractor by matching the study unit with the appropriate contract laboratory. When possible, the study unit will ship samples directly to the contract laboratory rather than to the BQAU. A packing list, derived from the study-unit invertebrate sample field log (fig. 6) and describing the contents of each shipping container, is packed inside every shipping container. A separate master sample list, also derived from the study-unit invertebrate sample field log, is sent in a separate mailing to the contract laboratory. This list is a compilation of all samples shipped to the laboratory and indicates the number of boxes of samples shipped. A copy of the field sample log is also sent to the BQAU. If samples are to be processed in a specific sequence (for example, samples from sites that could have threatened or endangered species will need to be processed before other sites), the desired order of processing is specified on the field sample log sent to the contractor, and a copy is provided to the BQAU.

Samples are logged in upon receipt. During the log-in process, the contractor checks the condition of the shipment and compares the contents of each box against the packing list supplied with the box and the master sample list derived from the invertebrate sample field

log. If samples are missing, damaged, or are not listed on the master sample list, the BQAU is notified immediately. The date of receipt and the initials of the person responsible for the sample are entered on the sample tracking and custody work sheet (fig. 13).

Samples may be given a unique laboratory identification code and project code during the log-in process if this is normal operating procedure for the laboratory. However, documentation must exist that clearly associates the laboratory identification codes with the 16-character NAWQA Program sample identification codes. In the example presented in figure 13, the project code is "GS-NAWQA," and the laboratory sample identification code consists of a laboratory prefix (study-unit abbreviation and year of collection: YAKI90) and a sequential laboratory number (1-15). Consequently, the laboratory identification code for sample YAKI1090IRM0001C is YAKI90-3.

Sample log-in also involves transferring samples from the original fixative (10-percent buffered formalin) to 70-percent alcohol (ethanol or isopropyl). The transfer date is recorded on the sample tracking and custody work sheet and must be accomplished within 10 working days from receipt of the sample. The internal and external labels are compared for accuracy and consistency during the transfer to alcohol, and the volume of the main-body sample component is determined and recorded on the sample tracking and custody work sheet. Volumes may be determined by placing the sample in a graduated cylinder, allowing the material to settle, and then recording the volume of material. Alternatively, the height of settled sample material can be used to measure volume in a standardized sample container if the geometry of the container allows the height of sample material to be measured and converted to volume accurately.

If a discrepancy exists between the internal and external labels, the internal label always takes precedence. When this situation occurs, the contract laboratory contacts the BQAU to document and resolve the labeling problem. A copy of the sample tracking and custody work sheet is sent to the BQAU after each set of samples is received and transferred to alcohol. Receipt of the sample tracking and custody work sheet verifies that the contractor has received the appropriate samples. The sample tracking and custody work sheet is also used to record the date that the sample enters the various processing steps, the initials of the person with custody of the sample during that processing step, and the date that the sample was returned to the BQAU.

Preparing Samples for Processing

A single sample typically consists of one or more containers of large-rare sample-component material (invertebrates) and a single container that holds 750 mL or less of main-body sample-component material (invertebrates, detritus, and sediment). In preparation for processing, samples are assembled into blocks of 10 or fewer samples (fig. 14). Each QA/QC sample block is assigned a unique identification code that identifies the study unit (YAKI for Yakima River Basin), year (90), and block number (B1). The sample identification codes (laboratory prefix, sequential laboratory number, and 16-character NAWQA Program sample identification code) are entered on the QA/QC sample block data sheet for each member of the block. The sample chosen for picking, identification, and quantification QA/QC checks is indicated on this data sheet by placing the appropriate number ("1" for the first check, "2," "3," or "4" if the first, second, or third check is failed) in the QA/QC number column next to the appropriate sample (block sequence number). However, the QA/QC sample is not chosen until all samples in the block have been processed (see section on QA and QC measures).

NAWQA Program Sample Tracking and Custody Work Sheet

Project: GS-NAWQA **Year:** 1990
Lab. Prefix: YAKI90- **Study Unit:** Yakima River Basin

Lab. number	NAWQA identification code	Sample log-in				Sample picking		Identifications and enumerations		Sample remnant volume checks		Sample return	
		Date received	Date to alcohol	Volume (mL)	Initials	Date	Initials	Date	Initials	Date	Initials	Date	Initials
1	YAKI1090IRL0001A	11/1/90	11/5/90		C.D.F			11/24/90	G.L.W	11/30/90	C.D.F	12/10/90	C.D.F
2	YAKI1090IRL0001B	11/1/90	11/5/90		C.D.F			11/24/90	G.L.W	11/30/90	C.D.F	12/10/90	C.D.F
3	YAKI1090IRM0001C	11/1/90	11/5/90	740	C.D.F	11/19/90	T.B.M	11/24/90	G.L.W	11/30/90	C.D.F	12/10/90	C.D.F
4	YAKI1090IDL0002A	11/1/90	11/5/90		C.D.F			11/24/90	G.L.W	11/30/90	C.D.F	12/10/90	C.D.F
5	YAKI1090IDL0002B	11/1/90	11/5/90		C.D.F			11/24/90	G.L.W	11/30/90	C.D.F	12/10/90	C.D.F
6	YAKI1090IDM0002C	11/1/90	11/5/90	450	C.D.F	11/19/90	T.B.M	11/24/90	G.L.W	11/30/90	C.D.F	12/10/90	C.D.F
7	YAKI1090IQL0003A	11/1/90	11/5/90		C.D.F			11/24/90	C.G.M	11/30/90	C.D.F	12/10/90	C.D.F
8	YAKI1090IQL0003B	11/1/90	11/5/90		C.D.F			11/24/90	C.G.M	11/30/90	C.D.F	12/10/90	C.D.F
9	YAKI1090IQM0003C	11/1/90	11/5/90	250	C.D.F	11/20/90	T.B.M	11/24/90	C.G.M	11/30/90	C.D.F	12/10/90	C.D.F
10	YAKI1090IRL0004A	11/1/90	11/5/90		C.D.F			11/25/90	C.G.M	11/30/90	C.D.F	12/10/90	C.D.F
11	YAKI1090IRM0004B	11/1/90	11/5/90	670	C.D.F	11/20/90	T.B.M	11/25/90	C.G.M	11/30/90	C.D.F	12/10/90	C.D.F
12	YAKI1090IDL0005A	11/1/90	11/6/90		C.D.F			11/25/90	C.G.M	11/30/90	C.D.F	12/10/90	C.D.F
13	YAKI1090IDL0005B	11/1/90	11/6/90		C.D.F			11/25/90	C.G.M	11/30/90	C.D.F	12/10/90	C.D.F
14	YAKI1090IDL0005C	11/1/90	11/6/90		C.D.F			11/25/90	C.G.M	11/30/90	C.D.F	12/10/90	C.D.F
15	YAKI1090IDM0005D	11/1/90	11/6/90	735	C.D.F	11/21/90	T.B.M	11/25/90	C.G.M	11/30/90	C.D.F	12/10/90	C.D.F

Figure 13.--Example of a NAWQA Program sample tracking and custody work sheet.

NAWQA Program Benthic Invertebrate Sample Processing
QA/QC Sample-Block Data Sheet

Instructions: Enter the identification code for the sample block and the sample identification codes (laboratory prefix, laboratory sample number, and 16-character NAWQA Program sample identification code) that constitute the samples of the block. Within each block, indicate the sample that was randomly chosen for picking effectiveness and identification/enumeration QA/QC checks by entering a "1" in the box labeled QA/QC number.

QA/QC block identification code: YAKI90-B1

Project: GS-NAWQA | **Laboratory prefix:** YAKI90-

Block sequence number	QA/QC number	Large-rare component		Main-body component	
		Lab no.	NAWQA code	Lab no.	NAWQA code
1		1,2	YAKI1090IRL0001A,B	3	YAKI1090IRM0001C
2		4,5	YAKI1090IDL0002A,B	6	YAKI1090IDM0002C
3	1	7,8	YAKI1090IQL0003A,B	9	YAKI1090IQM0003C
4		10	YAKI1090IRL0004A	11	YAKI1090IRM0004B
5		12,13,14	YAKI1090IDL0005A,B,C	15	YAKI1090IDM0005D
6		16,17	YAKI1090IQL0006A,B	18	YAKI1090IQM0006C
7		19,20	YAKI1090IRL0007A,B	21	YAKI1090IRM0007C
8		22	YAKI1090IDL0008A	23	YAKI1090IDM0008B
9		24,25	YAKI1090IQL0009A,B	26	YAKI1090IQM0009C
10		27	YAKI1090IRL0010A	28	YAKI1090IRM0010B

QA/QC block identification code: YAKI90-B2

Project: GS-NAWQA | **Laboratory prefix:** YAKI90-

Block sequence number	QA/QC number	Large-rare component		Main-body component	
		Lab no.	NAWQA code	Lab no.	NAWQA code
1		29	YAKI1090IRL0011A	30	YAKI1090IRM0011B
2		31,32	YAKI1090IDL0012A,B	33	YAKI1090IDM0012C
3		34,35	YAKI1090IQL0013A,B	36	YAKI1090IQM0013C
4		37,38	YAKI1090IRL0014A,B	39	YAKI1090IRM0014C
5		40	YAKI1090IDL0015A	41	YAKI1090IDM0015B
6		42,43	YAKI1090IQL0016A,B	44	YAKI1090IQM0016C
7	1	45,46	YAKI1090IRL0017A,B	47	YAKI1090IRM0017C
8		48	YAKI1090IDL0018A	49	YAKI1090IDM0018B
9		50	YAKI1090IQL0019A	51	YAKI1090IQM0019B
10		52	YAKI1090IRL0020A	53	YAKI1090IRM0020B

Figure 14.--Example of a quality-assurance (QA) and quality-control (QC) sample-block data sheet.

The main-body sample component is prepared for processing by placing it onto a sieve with the appropriate mesh size (425 µm for semi-quantitative samples and 212 µm for qualitative samples) and rinsing with tap water to remove the preservative. The large-rare sample component is prepared by combining invertebrates from all containers onto a sieve of the appropriate mesh size and rinsing to remove the formalin. The sample tracking and custody work sheet should be consulted to ensure that all sample containers are present and that all large-rare sample components are combined at this stage.

Processing the Large-Rare Sample Component

The large-rare sample component contains invertebrates that were removed from the sample matrix while in the field. Consequently, processing this sample component (fig. 15) is relatively simple because it does not involve separating invertebrates from any surrounding sediment or detritus--that is, it does not involve "picking" of the sample--nor does it involve any subsampling. The combined and rinsed large-rare component is sorted into major taxonomic groups, such as order or family, to produce multiple vials of sample fraction "A" preserved in 70-percent alcohol. Invertebrates in the sample fraction "A" containers are then identified and quantified to produce multiple containers of fraction "B." The name of each taxon is entered on an invertebrate identification and quantification data sheet (fig. 16) along with the relevant information obtained from the sample label and the quantity of each taxon if the sample is a semi-quantitative one, such as RTH or DTH. If the sample is a qualitative one, then the presence of the taxon is noted by entering a "1" in the total count column. If more than one data sheet is needed, then the sequence of data sheets is listed in the space provided at the top of the data sheet (page 1 of 2).

The specimens identified from the large-rare component are compared to the verified specimens in the study-unit reference collection to confirm identifications. If the taxon is not represented in the study-unit reference collection, it is added to the study-unit reference collection and verified by an independent taxonomic expert. If the taxon is represented in the study-unit reference collection, but the specimen in question is of particularly high quality, such as a mature specimen in good condition and exhibiting the morphological features characterizing the taxon, it may be added to the reference collection as an additional representative of that taxon. The source for each reference-collection specimen is recorded on the reference-collection source data sheet (fig. 9).

The processing of the large-rare sample component results in three products: (1) the finalized data, (2) additions to the study-unit reference collection, and (3) multiple containers of sample fraction "B" sorted by major taxonomic group (typically order or family). The preliminary data are finalized (check appropriate box on the invertebrate identification and quantification data sheet) after the sample block has passed all QA/QC checks. The QA/QC criteria used for the large-rare sample component are outlined in figure 7 and discussed in the section on QA and QC measures.

Data sheets, QA/QC forms, electronic data, and all containers of sample fraction "B" are returned when the QA/QC checks have been completed for the block of samples. The study-unit reference collection is returned after all of the study unit's samples have been processed for that collection year. All products returned to the BQAU are subject to additional reviews of identifications, quantifications, and picking effectiveness.

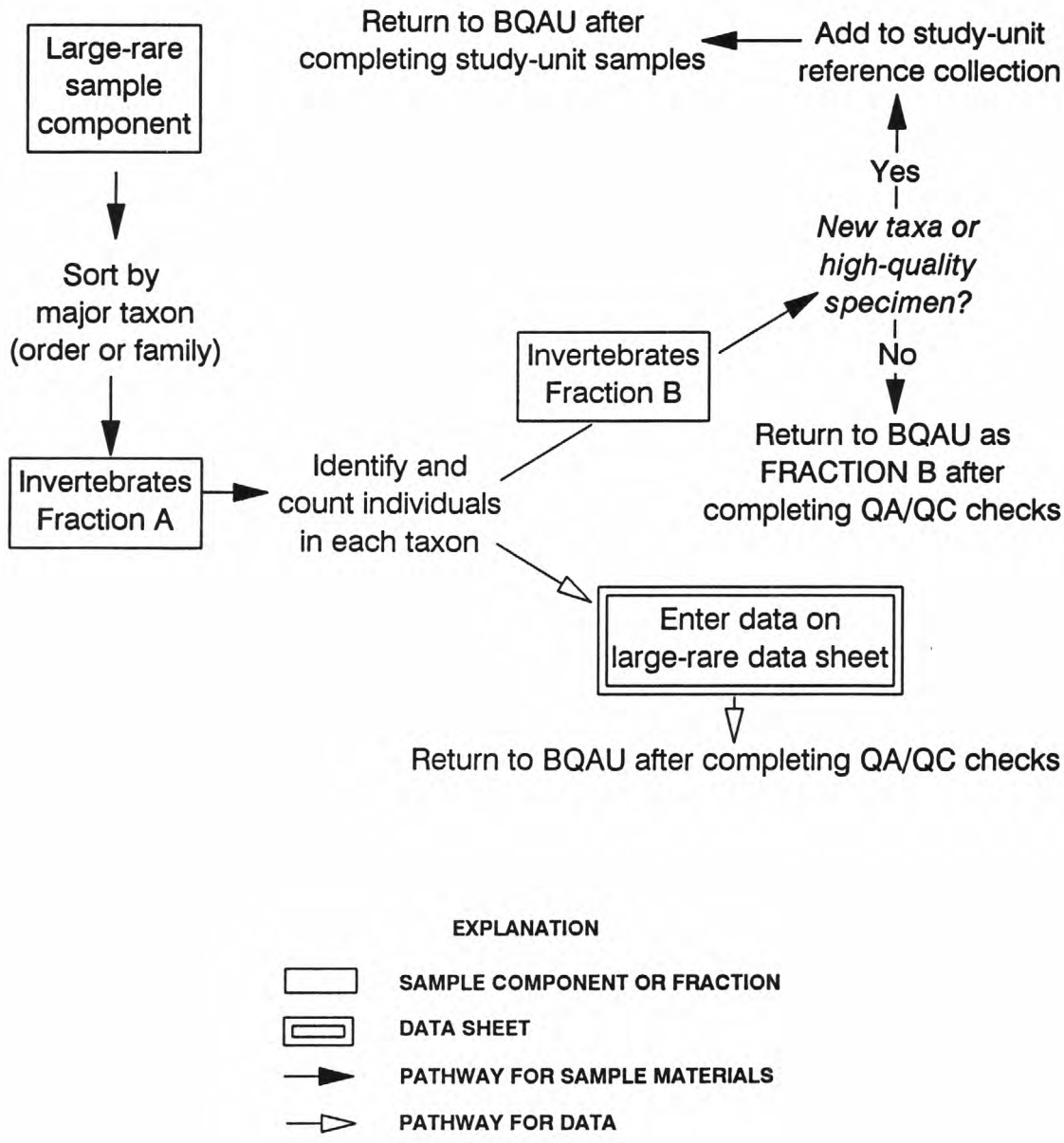


Figure 15.--Processing flow chart for the large-rare sample component of a benthic invertebrate sample.

NAWQA Program Invertebrate Identification and Quantification Data Sheet

Sample information:

Preliminary	✓		
Final	✓		
Page	1	of	1

Study unit: Yakima River Basin
Site name: S.F. Ahtanum Cr. Tampico, WA
Site ID number: 12500900
Date collected: 10/28/90 (month/day/year)
Field subsample: 1/1
Laboratory subsample: 1/1 (0 of 0 subsamples processed)
Reach: A
Sample type: RTH (RTH, DTH, QMH enter one)
Sample identification code: YAKI1090IRL0070A,B
Laboratory code: YAKI90-153, -154
Sample component: Large-rare (large-rare or main-body)
Sample fraction: B (B, F, F-1, F-2, F-3, or F-4)

TAXONOMIC IDENTIFIERS	Total count
Fossaria sp.	2
Lanx sp.	3
Juga sp.	8
Pacifastacus leniusculus	2
Baetis bicaudatus	5
Baetis quilleri	3
Baetis tricaudatus	2
Epeorus longimanus	2
Rhithrogena sp.	4
Stenonema sp.	2
Attenella margarita	3
Leptohyphes sp.	6
Argia sp.	1
Calopteryx sp.	1
Taeniopteryx nivalis	1
Cheumatopsyche sp.	2
Hydropsyche alhedra	3
Hydropsyche amblis	2
Anagapetus sp.	1
Glossoma sp.	6
Leucotrichia pictipes	10
Nectopsyche sp.	2
Dubiraphia givlianii	1
Zaitzevia parvula	6
Antocha saxicola	1
Simulium vittatum	5

Figure 16.--Example of an invertebrate identification and quantification data sheet used to record the presence and quantity of each taxon in the large-rare sample component.

Processing the Main-Body Sample Component

Processing of the main-body sample component requires "picking" to separate the invertebrates from the surrounding matrix of detritus and sediment prior to identification and quantification. Such "picking" is conducted with the aid of an illuminated magnifier or microscope capable of magnifying the sample material 5- to 10-fold. The details of the main-body sample component processing strategy are presented in figure 17. All final and intermediate sample fractions generated during processing are identified with a unique letter identifier (fraction C, D, E, or F) that facilitates sample labeling and tracking.

Processing of the main-body sample component starts by placing the rinsed sample in a white plastic or enamel picking tray. At this point, the contract laboratory has the option of processing the entire sample or dividing the sample into four equal subsamples and processing two or more of these subsamples. The subsampling approach requires more complicated QA/QC procedures than does processing the entire sample; however, it can reduce the volume of sample processed by 25 to 50 percent.

If the laboratory decides to process the entire sample, then processing occurs according to the sequence presented in figure 17. The invertebrates are removed from the sediment and detritus matrix and placed into one or more appropriately sized sample containers that are labeled as the "D" fraction. The detritus and sediment that remain after removing the invertebrates becomes the sample remnant and is labeled as fraction "E." Once the fraction "D" invertebrates are identified, sorted by order or family, and quantified, they are identified as F-fractions. Data generated from the F-fractions are entered on an invertebrate identification and quantification data sheet (fig. 18) as either the number of individuals constituting each taxon (semi-quantitative samples) or as a notation (enter a "1" in the total column) of its presence (qualitative samples). If more than one data sheet is required to record the information, the data sheets are linked by recording the page sequence (page 1 of 3) in the block provided in the top right-hand corner of the data sheet.

As invertebrates are identified, they are checked against the study-unit reference collection. If the specimen is not represented in the reference collection or is a particularly high-quality specimen, it is added to the reference collection. In the latter case, the specimen supplements the specimen that already exists in the reference collection rather than replacing it. The identifiers for the sample providing the reference-collection specimen are entered onto the specimen source data sheet (fig. 9).

If the laboratory decides to subsample the main-body sample component, it must be divided into quarters and processed according to the guidelines set forth in figure 19. Subsampling can be accomplished by using either a standard or modified Waters (1969) sample splitter or an Imhoff cone subsampler (Wrona and others, 1982), or by simply dispersing the sample uniformly over a gridded surface and randomly selecting grids that add up to one-fourth of the surface area (Welch, 1948). Whichever method is selected, the efficacy of the method, as implemented by the contract laboratory, must be demonstrated, and a copy of the method and supporting data must be on file with the BQAU before the subsampling method can be used. Alternative subsampling techniques can be used only with prior written approval from the BQAU and must be accompanied by adequate documentation of the method, its efficacy, and the samples to which the method is applied.

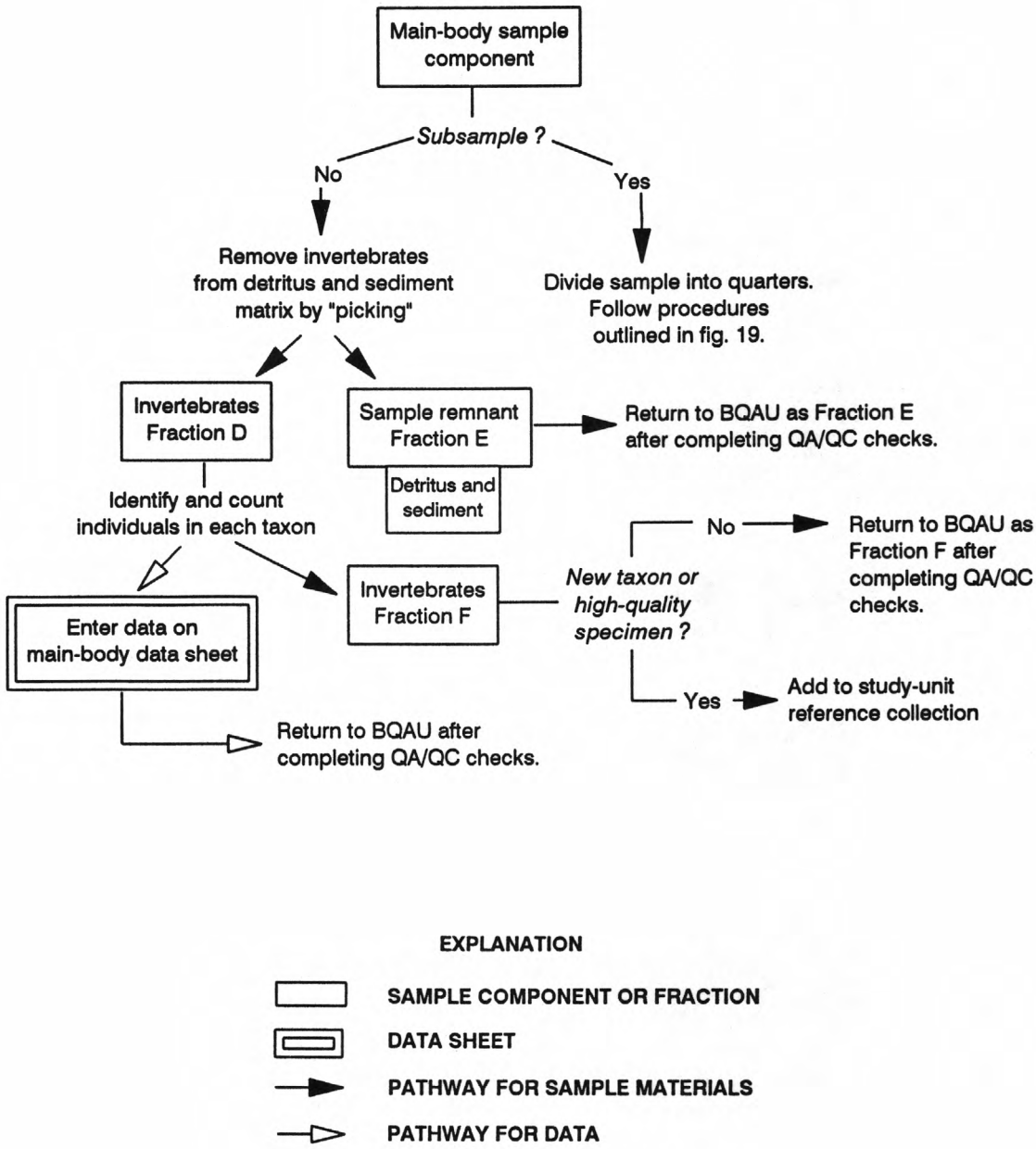


Figure 17.--Processing flow chart for the main-body sample component of a benthic invertebrate sample.

NAWQA Program Invertebrate Identification and Quantification Data Sheet

Sample information:

Study unit: Yakima River Basin
Site name: S.F. Ahtanum Cr. Tampico, WA
Site ID number: 12500900
Date collected: 10/28/90 (month/day/year)
Field subsample: 1/2
Laboratory subsample: 1/1 (0 of 0 subsamples processed)
Reach: A
Sample type: RTH (RTH, DTH, QMH enter one)
Sample identification code: YAKI1090IRM0070C
Laboratory code: YAKI90-155
Sample component: Main-body (large-rare or main-body)
Sample fraction: F (B, F, F-1, F-2, F-3, or F-4

Preliminary	✓		
Final	✓		
Page	1	of	1

TAXONOMIC IDENTIFIERS	Total count
Fossaria sp.	3
Lanx sp.	4
Juga sp.	9
Turbellaria	75
Aulodrilus pluriseta	1
Telmatodrilus vejdovskyi	59
Baetis bicaudatus	359
Baetis quilleri	52
Baetis tricaudatus	135
Attenella margarita	67
Leptohyphes sp.	123
Caenis sp.	13
Argia sp.	15
Calopteryx sp.	11
Hetaerina sp.	1
Taeniopteryx nivalis	75
Cheumatopsyche sp.	1
Hydropsyche alhedra	143
Hydropsyche amblis	205
Hydropsyche sp. B	9
Anagapetus sp.	45
Glossoma sp.	49
Leucotrichia pictipes	160
Dubiraphia givlianii	43
Macronychus sp.	23
Zaitzevia parvula	35
Simulium vittatum	368
Rheotanytarsus sp.	48
Cricotopus sp. grp.	39
Eukiefferiella sp.	37
Corynoneura sp.	4
Thienemanniella sp.	81
Orthocladius sp.	39
Pentaneura sp.	4
Thienemannimyia sp. grp.	40
Hemerodromia sp.	13

Figure 18.--Example of an invertebrate identification and quantification data sheet used to record the presence and quantity of each taxon in the main-body sample component.

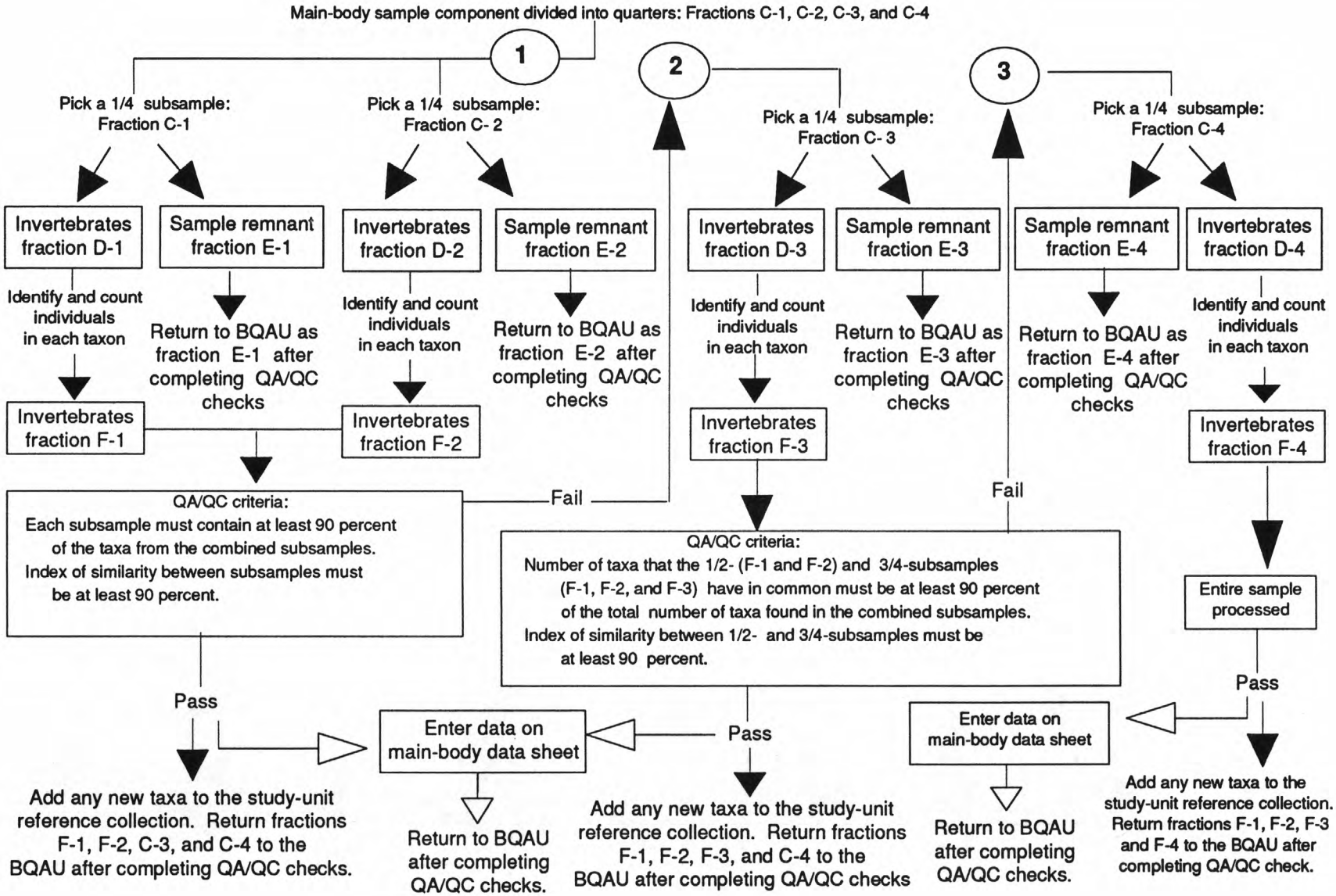


Figure 19.--Processing flow chart for the main-body sample component illustrating the use of subsampling and the accompanying quality-assurance and quality-control measures. Solid arrows indicate sample path; open arrows indicate data path.

Once divided, the 1/4-subsamples are randomly assigned the fraction identifiers C-1, C-2, C-3, and C-4. The invertebrates are removed from two 1/4-subsamples (fractions C-1 and C-2) to produce two invertebrate fractions, D-1 and D-2, and two sample remnants, fractions E-1 and E-2 (see branch 1 in fig. 19). Once the two invertebrate fractions are sorted, identified, and quantified, they are labeled as fractions F-1 and F-2. The data from each "F" fraction are entered on separate invertebrate identification and quantification data sheets (figs. 20 and 21), and new or high-quality specimens are added to the study-unit reference collection and recorded on the specimen source list (fig. 9). If additional data sheets are needed, the appropriate information is entered in the page sequence block at the top of the page.

Subsamples F-1 and F-2 are then compared to determine if the QA/QC criteria are satisfied or if additional subsamples need to be processed. The subsampling QA/QC data and results are recorded on a subsampling quality-assurance data sheet (see section on QA and QC measures). Initial quality criteria require that each subsample contain at least 90 percent of the taxa of the combined F-1 and F-2 subsamples and that the index of similarity (percentage similarity coefficient, Whittaker, 1952) between the two subsamples be at least 90 percent (semi-quantitative sample only). These initial quality criteria will be revised on the basis of results achieved by the contract laboratories and the data needs of the study units.

If the QA/QC criteria are not met, then an additional 1/4-subsample (C-3, branch 2 in fig. 19) is processed to produce an additional identified invertebrate fraction (F-3) and sample remnant (E-3). Data from the F-3 fraction are entered on a third invertebrate identification and quantification data sheet (fig. 22), and any new or high-quality taxa are added to the study-unit reference collection and recorded on the reference-collection specimen source list (fig. 9). If the number of taxa common to the 1/2- (combined F-1 and F-2 fractions) and 3/4-subsamples (combined F-1, F-2, and F-3 fractions) is at least 90 percent of the number of taxa found in the 3/4-subsample and the percentage similarity coefficient (PSC) between the 1/2- and 3/4-subsamples is at least 90 percent (semi-quantitative samples only), then the QA/QC criteria are met and no further processing is needed.

If the QA/QC criteria are not met, then the final 1/4-subsample (C-4, branch 3, fig. 19) is processed, the data from the F-4 fraction are entered on a fourth invertebrate identification and quantification data sheet, and the specimens are added to the study-unit reference collection as appropriate. The number of subsamples processed and the sequence in which they are processed are indicated on the "lab subsample" line of the invertebrate identification and quantification data sheets (for example, 1 of 3, 2 of 3, and 3 of 3). All sample fractions (C, E, and F) and data sheets are returned to the BQAU after all samples in the QA/QC sample block have passed QA/QC checks for picking, identification, and quantification (see section on QA and QC measures).

Occasionally, even a 1/4-subsample will contain large numbers (more than 50 individuals) of small organisms such as chironomids, oligochaetes, or copepods. These small organisms require extra processing steps (clearing and mounting on microscope slides) in order to achieve the desired level of identification. Subsampling may be used to process and identify these small organisms more efficiently. Such subsampling is accomplished by first sorting the specimens into taxonomic categories that can be readily achieved without

NAWQA Program Invertebrate Identification and Quantification Data Sheet

Sample information:

Preliminary	✓		
Final	✓		
Page	1	of	1

Study unit: Yakima River Basin
Site name: S.F. Ahtanum Cr. Tampico, WA
Site ID number: 12500900
Date collected: 10/28/90 (month/day/year)
Field subsample: 1/2
Laboratory subsample: 1/4 (1 of 3 subsamples processed)
Reach: A
Sample type: RTH (RTH, DTH, QMH enter one)
Sample identification code: YAKI1090IRM0070C
Laboratory code: YAKI90-155
Sample component: Main-body (large-rare or main-body)
Sample fraction: F-1 (B, F, F-1, F-2, F-3, or F-4)

TAXONOMIC IDENTIFIERS	Total count
Argia sp.	3
Calopteryx sp.	4
Hetaerina sp.	1
Baetis bicaudatus	107
Baetis quilleri	12
Baetis tricaudatus	30
Attenella margarita	11
Caenis sp.	10
Taeniopteryx nivalis	21
Anagapetus sp.	15
Glossoma sp.	9
Leucotrichia pictipes	53
Hydropsyche alhedra	17
Hydropsyche amblis	52
Dubiraphia givlianii	7
Macronychus sp.	15
Simulium vittatum	89
Rheotanytarsus sp.	1
Cricotopus sp. grp.	9
Eukiefferiella sp.	11
Corynoneura sp.	2
Thienemanniella sp.	19
Pentaneura sp.	1
Thienemannimyia sp. grp.	7
Hemerodromia sp.	3
Fossaria sp.	1
Lanx sp.	1
Juga sp.	2
Turbellaria	35

Figure 20.--Example of an invertebrate identification and quantification data sheet used to record the presence and quantity of each taxon of a subsampled main-body sample component in the F-1 fraction.

NAWQA Program Invertebrate Identification and Quantification Data Sheet

Sample information:

Preliminary	✓		
Final	✓		
Page	1	of	1

Study unit: Yakima River Basin
Site name: S.F. Ahtanum Cr, Tampico, WA
Site ID number: 12500900
Date collected: 10/28/90 (month/day/year)
Field subsample: 1/2
Laboratory subsample: 1/4 (2 of 3 subsamples processed)
Reach: A
Sample type: RTH (RTH, DTH, QMH enter one)
Sample identification code: YAKI1090IRM0070C
Laboratory code: YAKI90-155
Sample component: Main-body (large-rare or main-body)
Sample fraction: F-2 (B, F, F-1, F-2, F-3, or F-4)

TAXONOMIC IDENTIFIERS	Total count
Argia sp.	2
Calopteryx sp.	3
Baetis bicaudatus	83
Baetis quilleri	15
Baetis tricaudatus	25
Leptohyphes sp.	44
Taeniopteryx nivalis	17
Anagapetus sp.	10
Glossoma sp.	10
Leucotrichia pictipes	40
Hydropsyche alhedra	40
Hydropsyche amblis	56
Hydropsyche sp. B	5
Dubiraphia givlianii	13
Zaitzevia parvula	7
Simulium vittatum	95
Cricotopus sp. grp.	7
Eukiefferiella sp.	9
Thienemanniella sp.	15
Orthocladius sp.	15
Pentaneura sp.	1
Thienemannimyia sp. grp.	9
Hemerodromia sp.	1
Lanx sp.	1
Turbellaria	20
Telmatodrilus vejdovskyi	35

Figure 21.--Example of an invertebrate identification and quantification data sheet used to record the presence and quantity of each taxon of a subsampled main-body sample component in the F-2 fraction.

NAWQA Program Invertebrate Identification and Quantification Data Sheet

Sample information:

Preliminary	✓		
Final	✓		
Page	1	of	1

Study unit: Yakima River Basin
Site name: S.F. Ahtanum Cr. Tampico, WA
Site ID number: 12500900
Date collected: 10/28/90 (month/day/year)
Field subsample: 1/2
Laboratory subsample: 1/4 (3 of 3 subsamples processed)
Reach: A
Sample type: RTH (RTH, DTH, QMH enter one)
Sample identification code: YAKI1090IRM0070C
Laboratory code: YAKI90-155
Sample component: Main-body (large-rare or main-body)
Sample fraction: F-3 (B, F, F-1, F-2, F-3, or F-4)

TAXONOMIC IDENTIFIERS	Total count
Argia sp.	6
Calopteryx sp.	1
Baetis bicaudatus	79
Baetis quilleri	12
Baetis tricaudatus	46
Attenella margarita	39
Leptohyphes sp.	48
Taeniopteryx nivalis	18
Anagapetus sp.	9
Glossoma sp.	18
Leucotrichia pictipes	27
Cheumatopsyche sp.	1
Hydropsyche alhedra	50
Hydropsyche amblis	46
Hydropsyche sp. B	2
Dubiraphia givlianii	12
Macronychus sp.	2
Zaitzevia parvula	6
Simulium vittatum	92
Rheotanytarsus sp.	35
Cricotopus sp. grp.	13
Eukiefferiella sp.	8
Corynoneura sp.	1
Thienemanniella sp.	27
Orthocladius sp.	14
Pentaneura sp.	1
Thienemannimyia sp. grp.	14
Hemerodromia sp.	6
Fossaria sp.	1
Lanx sp.	1
Juga sp.	5
Turbellaria	1
Aulodrilus pluriseta	1
Telmatodrilus vejdovskyi	35

Figure 22.--Example of an invertebrate identification and quantification data sheet used to record the presence and quantity of each taxon of a subsampled main-body sample component in the F-3 fraction.

mounting the specimens (table 5) and then selecting a random subset of specimens from each of these taxonomic categories. The random selection technique must not be biased by differences in the size of the organisms. Specimens may be randomly chosen by dispersing the organisms uniformly across a petri dish mounted on gridded graph paper. A grid is chosen at random, and all the individuals within it are removed, mounted, and identified. Additional grids are processed until the total number of organisms identified meets or exceeds the numeric criteria specified in table 5. For example, if more than 50 chironomid larvae are present in a sample, they are divided into subfamilies and each subfamily is subsampled by randomly selecting individuals until either the percentage criterion (at least 10 percent of the organisms in the subfamily) or minimum criterion (25 organisms) is met, whichever criterion is greater. These specimens are then mounted and identified.

Table 5.--*Numeric criteria for subsampling specified taxonomic groups to facilitate identifications*

Taxonomic level	Taxonomic grouping for mounting	Numeric criteria	
		Percent	Minimum
Nematoda	Phylum	10	25
Annelida	Family	10	25
Eubranchiopoda	Order	10	25
Cladocera	Order	10	25
Copepoda	Order	10	25
Ostracoda	Order	10	25
Arachnoidea	Hydracarina	10	25
Chironomidae	Subfamily	10	25
Simuliidae	Family	10	25

Data related to subsampling for identification are entered on the invertebrate identification subsampling data sheet (fig. 23). The proportion of each taxonomic category (subfamily of Chironomidae in fig. 23) that is mounted and identified (A) is recorded in column "a" along with the number of individuals for each taxon (B in column "b"). Total number for each taxon in the laboratory sample or subsample is calculated as Total count = B÷A. If the sample is a qualitative sample, then the total number in the sample component is not calculated, and the presence of the taxon is noted by entering a "1" in column "c" of the data sheet. However, the percentage of the sample processed and the percentage mounted for identification are entered in the appropriate columns ("a" and "b," respectively) even for qualitative samples. Additionally, the list of taxa obtained from qualitative samples may be supplemented by arbitrarily mounting and identifying specimens that appear to be different species. The totals for each taxon (column "c") are transferred to the appropriate invertebrate identification and quantification data sheet (fig. 18). It is recommended that data obtained by subsampling be recorded to at least one decimal point. Appropriate rounding is conducted on the sample tabulation work sheet described in table 3.

NAWQA Program Invertebrate Identification Subsampling Data Sheet

Preliminary	✓		
Final	✓		
Page	1	of	1

Sample information:

Study unit: Yakima River Basin
Site name: S. F. Ahtanum Cr. Tampico, WA
Site ID number: 12500900
Date collected: 11/28/90 (month/day/year)
Field subsample: 1/2
Laboratory subsample: (1 of 3 subsamples processed)
Reach: A
Sample type: DTH (RTH, DTH, QMH enter one)
Sample identification code: YAKI1190IDM0216C
Laboratory code: YAKI90-345
Sample component: main-body (large-rare or main-body)
Sample fraction: F-1 (B, F, F-1, F-2, F-3, or F-4)

	a	b	c
TAXONOMIC IDENTIFIERS	**Proportion mounted (A)**	**Number identified (B)**	**Total count (B÷A)**
Chironominae			
Chironomus sp.	0.115	3	26.1
Cladotanytarsus sp.	0.115	1	8.7
Cryptochironomus sp.	0.115	1	8.7
Endochironomus sp.	0.115	1	8.7
Micropsectra sp.	0.115	1	8.7
Microtendipes sp.	0.115	2	17.4
Paratanytarsus sp.	0.115	12	104.3
Paratendipes sp.	0.115	5	43.5
Polypedilum sp.	0.115	4	34.8
Rheotanytarsus	0.115	5	43.5
Stempellinella sp.	0.115	1	8.7
Stempellina sp.	0.115	1	8.7
Tanytarsus sp.	0.115	4	34.8
Diamesinae			
Diamesa sp.	0.130	1	7.7
Diamesa balcalensis	0.130	2	15.4
Pagastia sp.	0.130	3	23.1
Potthastia longimana	0.130	1	7.7
Orthocladiinae			
Cricotopus sp. grp.	0.122	32	262.3
Cricotopus festivellus	0.122	1	8.2
Cricotopus nostocicola	0.122	22	180.3
Cricotopus tremulus	0.122	1	8.2
Cricotopus trifascia grp.	0.122	57	467.2
Eukiefferiella claripennis	0.122	38	311.5
Heleniella sp.	0.122	1	8.2
Lopesciadius sp.	0.122	1	8.2
Nanocladius sp.	0.122	2	16.4
Orthocladius sp. 1	0.122	29	237.7
	Totals	232	1,918.7

Figure 23.--Example of an invertebrate identification subsampling data sheet.

Sample Fractions and Internal Label

Sample processing can produce as many as 21 sample fractions (table 6) of which 3 to 9 remain at the end of sample processing, depending upon the number of subsamples processed (table 7). For example, processing the large-rare sample component produces only one sample fraction (B), whereas processing the main-body sample component can produce as few as two (F and E) or as many as eight fractions (F-1, F-2, F-3, F-4, E-1, E-2, E-3, and E-4) when subsampling is used. Unprocessed 1/4-subsamples remain only when one-half (two C fractions) or three-quarters (one C-fraction) of the main-body sample component is processed. Invertebrate fractions (B and F) may consist of multiple containers (elements) with one major taxonomic group (for example, order or family) per container. All fractions that remain at the conclusion of sample processing are returned to the BQAU where they are used to determine the quality of sample processing, identification, and quantification.

Table 6.--*Descriptions of sample fractions generated during benthic invertebrate sample processing*

Sample component	Sample fraction	Description of sample fraction
Large-rare	A	Contents of large-rare sample component combined, rinsed, and sorted by major taxonomic group.
	B	Invertebrates from fraction A that have been sorted, identified, enumerated, and checked against the reference collection. Some specimens may have been incorporated into the reference collection.
Main-body	C	Main-body sample component. May be divided into four equal parts designated: C-1 to C-4.
	D	Invertebrates removed from the entire main-body sample component (C) prior to identification.
	E	Material that remains after removal of invertebrates from the entire main-body sample component (C). The sample remnant consists of sediment and detritus.
	F	Invertebrates originating from fraction D after they have been sorted, identified, enumerated, and checked against the reference collection. Some specimens may have been incorporated into the reference collection.
	D-1	Invertebrates removed from the first 1/4-subsample of the main-body sample component (fraction C-1) prior to identification.
	E-1	Material that remains after removal of invertebrates from the first 1/4-subsample of the main-body sample component (fraction C-1). Referred to as the sample remnant. Consists of sediment and detritus.

Table 6.--*Descriptions of sample fractions generated during benthic invertebrate sample processing*--Continued

Sample component	Sample fraction	Description of sample fraction
Main-body	F-1	Invertebrates originating from fraction D-1 after they have been sorted, identified, enumerated, and checked against the reference collection. Some specimens may have been incorporated into the reference collection.
	D-2	Invertebrates removed from the second 1/4-subsample of the main-body sample component (fraction C-2) prior to identification.
	E-2	Material that remains after removal of invertebrates from the second 1/4-subsample of the main-body sample component (fraction C-2). Referred to as the sample remnant. Consists of sediment and detritus.
	F-2	Invertebrates originating from fraction D-2 after they have been sorted, identified, enumerated, and checked against the reference collection. Some specimens may have been incorporated into the reference collection.
	D-3	Invertebrates removed from the third 1/4-subsample of the main-body sample component (fraction C-3) prior to identification.
	E-3	Material that remains after removal of invertebrates from the third 1/4-subsample of the main-body sample component (fraction C-3). Referred to as the sample remnant. Consists of sediment and detritus.
	F-3	Invertebrates originating from fraction D-3 after they have been sorted, identified, enumerated, and checked against the reference collection. Some specimens may have been incorporated into the reference collection.
	D-4	Invertebrates removed from the fourth 1/4-subsample of the main-body sample component (fraction C-4) prior to identification.
	E-4	Material that remains after removal of invertebrates from the fourth 1/4-subsample of the main-body sample component (fraction C-4). Referred to as the sample remnant. Consists of sediment and detritus.
	F-4	Invertebrates originating from fraction D-4 after they have been sorted, identified, enumerated, and checked against the reference collection. Some specimens may have been incorporated into the reference collection.

Table 7.--*Sample fractions remaining after sample processing is completed*

[The number of fractions generated by processing the main-body sample component depends upon whether the main-body sample component is subsampled and the percentage of the sample that is eventually processed to satisfy the quality-assurance/quality-control criteria]

Sample component	Subsampling used?	Percentage processed	Invertebrate fractions	Sample remnant fractions	Unprocessed 1/4-subsample
Large-rare	no	100	B	none	none
Main-body	no	100	F	E	none
	yes	50	F-1, F-2	E-1, E-2	C-3, C-4
	yes	75	F-1, F-2, F-3	E-1, E-2, E-3	C-4
	yes	100	F-1, F-2, F-3, F-4	E-1, E-2, E-3, E-4	none

Each sample fraction is identified by a unique letter (A-F) or letter and number (1-4) code that, in combination with the 16-character NAWQA Program sample identification code, the laboratory-assigned sample code, and any relevant taxonomic information, provides a simple mechanism for labeling sample fractions (fig. 24). Sample fraction labels are placed inside the sample fraction container and simplify sample tracking by identifying both the sample of origin and the processing steps that have been completed. For example, the label shown in figure 24(B) shows that this container houses the chironomid portion of a main-body sample component from a semi-quantitative DTH sample (IDM from sample identification code in fig. 4). The fraction codes indicate that this fraction originated from the first randomly chosen subsample (C-1) to be processed in the subsample processing sequence (branch 1, fig. 19). This produced the unidentified invertebrate fraction (D-1) that was then identified and quantified to become invertebrate fraction F-1. These labels are made from plastic paper or 100-percent rag, acid-neutralized paper. Label information is entered using black alcohol-resistant ink or pencil. Mounted specimens are labeled in a similar manner on the left side of the slide, and the mounting medium is specified. Sufficient space is allowed on the right side of the slide for the placement of a study-unit reference-collection specimen identification number.

Separate invertebrate identification and quantification data sheets are used to record invertebrate data from each B- and F-fraction. If the sample is semi-quantitative, then the number of individuals composing each taxon is entered. Otherwise, a "1" is entered in the "total" column to indicate the presence of the taxon in the qualitative sample. The individual invertebrate identification and quantification data sheets are combined, along with information on field and laboratory subsampling, to tabulate the total number of organisms in the sample.

Sample Tabulations

Totals for the entire sample are calculated on the sample tabulation work sheet (fig. 25) by first listing the taxa comprising the large-rare and main-body sample components on the work sheet in phylogenetic order (see order listed in table 4). Totals from the large-rare data

sheet are transferred to column "a," and totals from the main-body sample component data sheets are entered in column "b." If the main-body sample component was subsampled, then the column "b" entries are the total for all F-fractions divided by the proportion of the main-body sample component that was processed. For example, 107 *Baetis bicaudatus* were counted in fraction F-1 (fig. 20), 83 in fraction F-2 (fig. 21), and 79 in fraction F-3 (fig. 22) accounting for 269 *B. bicaudatus* in the three-fourths of the sample component processed. Consequently, the calculated total for *B. bicaudatus* in the main-body sample is 358.7 (269÷0.75=358.7) as indicated on the sample tabulation work sheet (column "b", fig. 25). If the main-body component were not subsampled, then the total count from the data sheet (359 *B. bicaudatus*, fig. 18) would be transferred to column "b."

A

Lab Code:			Taxon:				
ID Code:							
Large-rare:		A	B				
Main-body	1/4-subsample		C	1	2	3	4
	Invert.	D	F	1	2	3	4
	Remnant		E	1	2	3	4

B

Lab Code: YAKI90-6			Taxon: Chironomidae				
ID Code: YAKI1090IDM0002C							
Large-rare:		A	B				
Main-body	1/4-subsample		~~C~~	~~1~~	2	3	4
	Invert.	~~D~~	(F)	(1)	2	3	4
	Remnant		E	1	2	3	4

Figure 24.--A blank (A) and completed (B) internal label used to identify sample components and fractions during laboratory processing.

NAWQA Program Benthic Invertebrate Sample Tabulation Work Sheet

Sample information:

Preliminary	✓		
Final	✓		
Page	1	of	2

Study unit: Yakima River Basin
Site name: S.F. Ahtanum Cr. Tampico, WA
Site ID number: 12500900
Date collected: 10/28/90 (month/day/year)
Field subsample: 1/2
Laboratory subsample: 1/4 (3 main-body subsamples processed)
Reach: A
Sample type: RTH (RTH, DTH, QMH enter one)
Sample identification code: YAKI1090IRL0070A,B and YAKI1090IRM0070C
Laboratory code: YAKI90-153, -154, -155
Sample component: Large-rare, Main-body (large-rare or main-body)
Sample fraction: B, F-1, F-2, F-3 (B, F, F-1, F-2, F-3, or F-4)

Instructions: *Totals for the main-body sample component (b) are calculated for each taxon by summing abundances obtained from all "F" fractions (F, F-1, F-2, F-3, F-4) and dividing the sum by the proportion of the main-body sample component that was processed, 1, 0.75, or 0.5 (see laboratory subsample). Sample totals (c) are calculated by dividing totals for the main-body sample component (b) by the proportion of the sample sent to the laboratory (field subsample) and adding the large-rare totals to this total.*

	a	b	c
TAXONOMIC IDENTIFIERS	Large-rare totals	Main-body totals	Sample totals
Gastropods, Lymnaeidae, *Fossaria sp.*	2	2.7	8
Lanx sp.	3	4.0	11
Juga sp.	8	9.3	27
Platyhelminthes, Turbellaria	0	74.7	150
Oligochaeta, Tubificidae, *Aulodrilus pluriseta*	0	1.3	3
Telmatodrilus vejdovskyi	0	93.3	187
Decapoda, Astacidae, *Pacifastacus leniusculus*	2	0.0	2
Ephemeroptera, Baetidae, *Baetis bicaudatus*	5	358.7	723
Baetis quilleri	3	52.0	107
Baetis tricaudatus	2	134.7	272
Heptageniidae, *Epeorus longimanus*	2	0.0	2
Rhithrogena sp.	4	0.0	4
Stenonema sp.	2	0.0	2
Ephemerellidae, *Attenella margarita*	3	66.7	137
Tricorythidae, *Leptohyphes sp.*	6	122.7	252
Caenidae, *Caenis sp.*	0	13.3	27
Odonata, Coenagrionidae, *Argia sp.*	1	14.7	31
Calopterygidae, *Calopteryx sp.*	1	10.7	23
Hetaerina sp.	0	1.3	3
Plecoptera, *Taeniopteryx nivalis*	1	74.7	151
Trichoptera, Hydropsychidae, *Cheumatopsyche sp.*	2	1.3	5
Hydropsyche alhedra	3	142.7	289
Hydropsyche amblis	2	205.3	413
Hydropsyche sp. B	0	9.3	19
Glossosomatidae, *Anagapetus sp.*	1	45.3	92
Glossoma sp.	6	49.3	105
Hydroptilidae, *Leucotrichia pictipes*	10	160.0	330

Figure 25.--Example of a two-page sample tabulation work sheet used to calculate the total number of invertebrates in a sample.

NAWQA Program Benthic Invertebrate Sample Tabulation Work Sheet

Sample information:

Preliminary	✓		
Final	✓		
Page	2	of	2

Study unit: Yakima River Basin
Site name: S.F. Ahtanum Cr. Tampico, WA
Site ID number: 12500900
Date collected: 10/28/90 (month/day/year)
Field subsample: 1/2
Laboratory subsample: 1/4 (3 main-body subsamples processed)
Reach: A
Sample type: RTH (RTH, DTH, QMH enter one)
Sample identification code: YAKI1090IRL0070A,B and YAKI1090IRM0070C
Laboratory code.: YAKI90-153, -154, -155
Sample component: Large-rare, Main-body (large-rare or main-body)
Sample fraction: B, F-1, F-2, F-3 (B, F, F-1, F-2, F-3, or F-4)

Instructions: *Totals for the main-body sample component (b) are calculated for each taxon by summing abundances obtained from all "F" fractions (F, F-1, F-2, F-3, F-4) and dividing the sum by the proportion of the main-body sample component that was processed, 1, 0.75, or 0.5 (see laboratory subsample). Sample totals (c) are calculated by dividing totals for the main-body sample component (b) by the proportion of the sample sent to the laboratory (field subsample) and adding the large-rare totals to this total.*

	a	b	c
TAXONOMIC IDENTIFIERS	Large-rare totals	Main-body totals	Sample totals
Trichoptera, Leptoceridae, *Nectopsyche* sp.	2	0.0	2
Coleoptera, Elmidae, *Dubiraphia givlianii*	1	42.7	87
Macronychus sp.	0	22.7	46
Zaitzevia parvula	6	17.3	41
Diptera, Tipulidae, *Antocha saxicola*	1	0.0	1
Simuliidae, *Simulium vittatum*	5	368.0	741
Chironomidae, *Rheotanytarsus sp.*	0	48.0	96
Cricotopus sp. grp.	0	38.7	78
Eukiefferiella sp.	0	37.3	75
Corynoneura sp.	0	4.0	8
Thienemanniella sp.	0	81.3	163
Orthocladius sp.	0	38.7	78
Pentaneura sp.	0	4.0	8
Thienemannimyia sp. grp.	0	40.0	80
Empididae, *Hemerodromia sp.*	0	13.3	27
Total	**84**	**2,404.0**	**4,906**

Figure 25.--Example of a two-page sample tabulation work sheet used to calculate the total number of invertebrates in a sample--Continued.

The totals for the entire sample are calculated by correcting the main-body component totals for the field subsampling and then adding the totals from the large-rare component. These sample totals are entered in column "c" of the sample tabulation work sheet. The field subsample in the example presented in figure 25 is one-half. Therefore, the *B. bicaudatus* total from the main-body sample component (358.7) is divided by one-half to produce an estimate for the entire main-body sample (717.4). The total from the large-rare sample component (5) is then added to the total from the main-body sample component to produce an estimated sample total for *B. bicaudatus* of 722.4 individuals. This value is then rounded to the next highest whole number (723) to avoid losing rare species as a result of rounding errors.

If the sample being tabulated is a qualitative sample, then a "1" or "0" is entered in column "a" or "b" to indicate the presence (1) or absence (0) of the taxon in the respective sample component. A "1" is entered in the sample total column ("c") to indicate the presence of the taxon in the sample. If more than one data sheet is required, as illustrated in this example, the sequence of pages is indicated in the page sequence block at the top of the page. Sample tabulation work sheets, along with other data sheets, sample fractions, and electronic data files, are returned to the BQAU when all samples within the QA/QC sample block pass all QA/QC checks.

Quality-Assurance and Quality-Control Measures

Quality-assurance and quality-control measures are used to evaluate the effectiveness of sample processing procedures used in the field and at the contract laboratory. Field QA/QC samples are used by the BQAU to determine how well the sample elutriation procedure separates invertebrates from stream sediments and how effectively the field subsampling procedure divides samples. Laboratory QA/QC measures guide the subsampling of main-body sample components and evaluate the overall quality of the data produced by the contractor. Field and laboratory QA/QC measures use the number of taxa and their quantity (semi-quantitative samples) to establish the quality of sample elutriation, splitting, picking, identification, and quantification. These measures of data quality are combined with estimates of temporal and spatial variability (Cuffney and others, 1993) to estimate overall data quality.

Laboratory subsampling QA/QC measures compare identifications and quantifications among subsamples whenever a main-body sample component is subsampled. In contrast, the quality of sample picking, identification, and quantification is evaluated after all samples within a QA/QC sample block have been processed by randomly choosing one sample to represent the entire QA/QC sample block (fig. 14). The sample remnants (fraction E or fractions E-1 to E-4) and invertebrate fractions B and F are re-processed to determine the quality of the original data. If the quality-assurance criteria are not met by the randomly chosen sample, then all similar fractions (sample remnants or invertebrate fractions) within the sample block are re-processed.

Field Quality-Assurance and Quality-Control Checks

Initially, 10 percent of the elutriate and split-sample components are randomly selected and subjected to QA/QC checks. Elutriate sample components are typically processed within the BQAU, which determines the number and identity of invertebrates in the elutriate material. The QA/QC criteria for the sample elutriate are that the number of "new" taxa and the number of organisms found in the elutriate must be 10 percent or less of the estimates for the whole sample (combined large-rare and main-body sample components). The elutriate

and sample estimates are corrected for field and laboratory subsampling prior to this comparison. If a sample fails this QA/QC check, the BQAU works with the appropriate study unit to correct elutriation problems through additional training or modifications of elutriation procedures. If the problem cannot be corrected by training or by modifying elutriation procedures, then the criteria must be revised and applied to the overall estimate of data variability.

Split samples can be processed by the BQAU or sent to a contract laboratory for processing. In the latter case, the split sample is modified to resemble a typical study-unit invertebrate sample by adding a large-rare sample component and re-labeling the split sample as a main-body sample component. The large-rare sample component is obtained from invertebrates in the split sample. The desired QA/QC criteria are that the number of taxa be within 10 percent of the estimate for the main-body sample component and that the index of community similarity (complement of the Bray and Curtis dissimilarity index, 1957) between the two be at least 0.90. However, these criteria may not be achievable by field processing; consequently, the results of the split-sample program will be used to empirically derive expectations for sample splits. Based on results obtained from the examination of split samples, the procedures used to split samples could be changed as well as the current QA/QC criteria for laboratory processing. The empirically derived criterion for sample splits will be used to derive overall estimates of sample variability.

Subsampling Quality-Assurance and Quality-Control Checks

QA/QC checks for subsampling are intended to ensure that the estimates of the number of taxa and proportion of each taxon obtained through subsampling meet minimum QA/QC requirements. To meet this intent, consecutive 1/4-subsamples are processed until either the QA/QC criteria are met or the entire sample is processed (fig. 19). Two subsamples (F-1 and F-2) are processed and compared to determine if they meet the QA/QC criteria. If they do not meet the criteria, an additional subsample (F-3) is processed and the resulting data for the 1/2- (F-1 and F-2) and 3/4-subsamples (F-1, F-2, and F-3) are compared to determine if they meet the QA/QC criteria. If the criteria are still not met, the entire sample is processed.

Two QA/QC criteria are used to evaluate subsampling: (1) the number of taxa that the subsamples have in common must be at least 90 percent of the combined number of taxa, and (2) the similarity between the two communities described by the subsamples must be at least 90 percent, as determined using a percentage similarity coefficient (PSC). The BQAU periodically evaluates subsampling QA/QC criteria based on results achieved by the contract laboratories. This evaluation considers the costs and benefits of modifying subsampling procedures, changing similarity indices, and modifying QA/QC criteria. Before any modifications can be implemented, the effects on overall data quality must be considered. NAWQA Program biologists will need to be involved in this evaluation process whenever it is anticipated that these modifications could affect overall data quality or costs.

Subsampling QA/QC checks are based on a compilation of the subsample data recorded on the invertebrate identification and quantification data sheets. For example, subsample data for the main-body sample component YAKI1090IRM0070C are entered on three invertebrate identification and quantification data sheets (figs. 20, 21, and 22). These data are compiled in an electronic spreadsheet (table 8) that serves to record the data, count the number of taxa, and calculate the PSC. The QA/QC test results and summary calculations are then entered on the subsampling quality-assurance check sheet (fig. 26).

Table 8.--*Example of a community similarity index work sheet used to calculate the percentage similarity coefficient for subsampling*

Taxon	Quantity in subsample			Similarity calculations	
				Branch 1	Branch 2
	F-1	F-2	F-3	$\lvert a_i-b_i \rvert$	$\lvert a_i-b_i \rvert$
Argia sp.	3	2	6	0.20	0.17
Calopteryx sp.	4	3	1	.21	.18
Hetaerina sp.	1	0	0	.18	.03
Baetis bicaudatus	107	83	79	5.17	1.95
Baetis quilleri	12	15	12	.41	.23
Baetis tricaudatus	30	25	46	1.15	.72
Attenella margarita	11	0	39	2.01	1.80
Leptohyphes sp.	0	44	48	7.61	1.19
Caenis sp.	10	0	0	1.82	.33
Taeniopteryx nivalis	21	17	18	.89	.27
Anagapetus sp.	15	10	9	1.01	.33
Glossoma sp.	9	10	18	.09	.36
Leucotrichia pictipes	53	40	27	2.75	1.60
Cheumatopsyche sp.	0	0	1	.00	.06
Hydropsyche alhedra	17	40	50	3.82	.87
Hydropsyche amblis	52	56	46	.20	1.05
Hydropsyche sp. B	0	5	2	.87	.06
Dubiraphia givlianii	7	13	12	.97	.00
Macronychus sp.	15	0	2	2.74	.39
Zaitzevia parvula	0	7	6	1.21	.10
Simulium vittatum	89	95	92	.20	1.03
Rheotanytarsus sp.	1	0	35	.18	1.91
Cricotopus sp. grp.	9	7	13	.43	.19
Eukiefferiella sp.	11	9	8	.45	.22
Corynoneura sp.	2	0	1	.36	.01
Thienemanniella sp.	19	15	27	.87	.36
Orthocladius sp.	0	15	14	2.60	.28
Pentaneura sp.	1	1	1	.01	.01
Thienemannimyia sp. grp.	7	9	14	.28	.24
Hemerodromia sp.	3	1	6	.37	.20
Fossaria sp.	1	0	1	.18	.02
Lanx sp.	1	1	1	.01	.01
Juga sp.	2	0	5	.36	.21
Turbellaria	35	20	1	2.93	1.78
Aulodrilus pluriseta	0	0	1	.00	.06
Telmatodrilus vejdovskyi	0	35	35	6.06	.77
Column total	**548**	**578**	**677**	**48.60**	**18.99**
Number of taxa in subsample	**29**	**26**	**34**		
Percentage similarity coefficient				**75.70**	**90.51**

NAWQA Program Benthic Invertebrate Sample Processing

Subsampling Quality-Assurance Check Sheet

Project: GS-NAWQA **Date:** 11/30/90

Lab. no.: YAKI90-155 **NAWQA ID no.:** YAKI1090IRM0070C

QA/QC check results

Comparison of F-1 and F-2 fractions			
Number of taxa			
Number of taxa in either subsample F-1 or F-2 (a)	34	Percentage similarity coefficient (PSC)	
Number of taxa that subsamples F-1 and F-2 have in common (b)	21	$\sum_{i=1}^{K} \lvert a_i - b_i \rvert$	48.6
Percent of taxa in common [(b÷a)x100]	61.8	$PSC = 100 - 0.5 \sum_{i=1}^{K} \lvert a_i - b_i \rvert$	75.7
Pass or fail? (Pass if ≥ 90 %)	Fail	Pass or fail? (Pass if PSC ≥ 90)	Fail

Comparison of F-3 fraction with F-1 and F-2 fractions			
Number of taxa			
Total number of taxa in subsamples F-1, F-2, and F-3 (a)	36	Percentage similarity coefficient (PSC): comparison of 1/2- and 3/4-subsamples	
Number of taxa that the 1/2- and 3/4-subsamples have in common (b)	34	$\sum_{i=1}^{K} \lvert a_i - b_i \rvert$	18.9
Percent of taxa that the 1/2- and 3/4-subsamples have in common [(b÷a)x100]	94.4	$PSC = 100 - 0.5 \sum_{i=1}^{K} \lvert a_i - b_i \rvert$	90.5
Pass or fail? (Pass if ≥ 90 %)	Pass	Pass or fail? (Pass if PSC ≥ 90)	Pass

Figure 26.--Example of a quality-assurance check sheet for subsampling the main-body sample component.

In this example, the F-1 and F-2 fractions contained 29 and 26 taxa, respectively (table 8). Collectively, these two fractions contained 34 taxa. Neither fraction held 90 percent or more of the collective number of taxa, and both fractions failed this QA/QC criterion. The similarity between the F-1 and F-2 fractions (branch 1, fig. 19) is calculated as

$$PSC = 100 - 0.5 \sum_{i=1}^{K} |a_i - b_i| \quad , \tag{1}$$

where K is the number of taxa in fractions F-1 and F-2, and a_i and b_i are the percentages of taxon *i* in the F-1 and F-2 fractions, respectively. The value of $|a_i - b_i|$ for each taxon and the calculated PSC are given in table 8. In this example, the PSC (75.7) is less than the criterion (90 percent) and fails the QA/QC check (fig. 26). Because one or more criteria were failed, an additional C-fraction was processed (fig. 22) to produce the data listed in table 8 for F-3.

The 3/4-subsample (combined F-1, F-2, and F-3 subsamples) contained 36 taxa of which 34 also occurred in the 1/2-subsample (combined F-1 and F-2 subsamples). Consequently, the percentage of common taxa (94.4 percent) exceeded the 90 percent criterion. Similarly, the PSC index (90.5 percent) comparing the 1/2- and 3/4-subsamples (branch 3, fig. 19) exceeded the criterion of 90 percent, and the similarity criterion was passed. Because both QA/QC criteria were passed, processing met the data-quality criteria with three-fourths of this sample component processed. If this sample had been a qualitative one, only the tests relating to the number of taxa would have been performed.

Picking Effectiveness Quality-Assurance and Quality-Control Checks

Two criteria are used to evaluate the effectiveness of sample picking: (1) the number of taxa missed during the original sample picking (new taxa), and (2) the total number of organisms found in the sample remnant. Semi-quantitative samples are evaluated using both criteria, but qualitative samples are evaluated only on the first criterion. Also, because the picking effectiveness check involves re-processing sample remnants, it is applicable only to main-body sample components and includes all E-fractions generated during sample processing. Consequently, a main-body sample component that was subsampled could require that two, three, or four sample remnants be re-checked.

The QA/QC criteria are evaluated against the data as reported on the main-body invertebrate identification and quantification data sheets (figs. 20, 21, and 22). The first criterion specifies that the number of new taxa found in the sample remnant must be 10 percent or less of the number of taxa listed on the original data sheets. A new taxon is one that does not appear on the main-body sample component data sheets for that sample. The example presented in figure 27 illustrates a situation where a main-body sample component has been subsampled, and 75 percent of the sample component has been processed. In this example, two stoneflies (*Nemoura sp.* and *Yoroperla sp.*) were found in the sample remnants (E-1, E-2, and E-3) that were not listed among the 36 taxa found initially (table 8). These two new taxa represent 5.6 percent of the original number of taxa, and the first criterion is passed.

NAWQA Program Invertebrate Sample Processing

Picking Effectiveness QA/QC Work Sheet

Project: GS-NAWQA **Date:** 12/10/90
Lab. Prefix: YAKI90- **NAWQA Sample ID:** YAKI1090IRM0070C
Lab. No.: 155

QA/QC sample block code: YAKI90-B7

Sample fractions evaluated (check):		E	X	E-1	X	E-2	X	E-3		E-4

QA/QC check summary

Percent new taxa		Number of organisms	
Total taxa in sample (a):	36	Total organisms in sample (a):	1,803
New taxa in remnant (b):	2	Number organisms in remnant (b):	19
Percent [(b÷a)x100]:	5.6	Percent [(b÷a)x100]:	1
Pass or fail? (Pass if ≤10 %)	Pass	Pass or fail? (Pass if ≤ 10 %)	Pass

List of taxa recovered from QA/QC sample remnant:

Taxon	Quantity
Diptera, Chironomidae, *Thienemannimyia* sp. grp.	3
Eukiefferiella sp.	4
Cricotopus sp. grp.	2
Platyhelminthes, Turbellaria	3
Plecoptera, Nemouridae, *Nemoura* sp.	1
Peltoperlidae, *Yoroperla* sp.	6

Figure 27.--Example of an invertebrate sample processing picking effectiveness quality-assurance/quality-control (QA/QC) work sheet.

The second QA/QC criterion involves the total number of organisms recovered from the sample remnants. This total must be less than 10 percent of the total number of organisms removed from the sample component. In the example, 19 organisms were recovered from sample remnants E-1, E-2, and E-3. This constitutes about 1 percent of the 1,803 organisms (table 8) originally removed and passes the second criterion for sample picking effectiveness. Because both QA/QC checks were passed, the sample remnants for the QA/QC sample block require no further processing, and the checks for sample identification and quantification can be evaluated.

Identification and Quantification Quality-Assurance and Quality-Control Checks

Identification and quantification QA/QC checks are intended to establish that organisms in the invertebrate fractions (B- and F-fractions) have been correctly identified and counted. Three criteria are used: (1) at least 90 percent of the specimens from the B- and F-fractions must be correctly identified, (2) the number of new taxa added to the sample's taxa list (fig. 25) must be less than 10 percent of the original number of taxa, and (3) the community similarity index comparing the original and corrected data must be at least 0.90 (Bray and Curtis, 1957). The first two criteria are applicable to qualitative and semi-quantitative samples, whereas the last criterion is only applicable to semi-quantitative samples. All invertebrate fractions that originate from the sample chosen to represent the QA/QC block (fig. 14) are checked. A different taxonomist reviews the identifications, counts the taxa, and records any discrepancies on the invertebrate identification and quantification QA/QC data sheets (figs. 28 and 29).

Discrepancies in identification and quantification can take three forms: (1) simple errors in counts, (2) errors in identification that require adjustments to counts but not to the taxa list, and (3) errors in identification that require adjustments both to counts and to the taxa list. An example of an identification and quantification check for a large-rare sample component is provided in figure 28. Sample identification information and the date that the check is conducted are entered at the top of the data sheet. The sample fractions that are evaluated are listed in the next data block, fraction B for the large-rare sample component. The last data block is where discrepancies in counts or identifications that arise from the QA/QC review are recorded. Examples of all three types of discrepancies are illustrated: (1) simple errors in counts without any adjustments to identifications (*Simulium vittatum*), (2) errors in identification that require adjustments to taxon counts but do not add to the taxa list for the sample component (*Baetis bicaudatus*), and (3) errors in identification that require adjustments to taxon counts and add to the taxa list for the sample component (*Hydropsyche amblis; Zaitzevia parvula; Calopteryx* sp.; and *Leptohyphes* sp.). A simple adjustment to the original taxon count requires a single entry that lists the name of the taxon (original identification), the original quantity, and the quantity after the re-count. Changes in identification require multiple lines. The first line records the original and adjusted counts for the taxon as it was originally identified (*Leptohyphes* sp.), while one or more following lines record the corrected identifications and their abundances (*Tricorythodes* sp. and *Caenis* sp.). Figure 28 shows that a total of five taxa (*Hydropsyche* sp. B, *Macronychus* sp., *Hetaerina* sp., *Tricorythodes* sp., and *Caenis* sp.) were found that had not been identified previously in the large-rare sample component (fig. 16), and eight organisms (1- *H. amblis*, 1-*Simulium vittatum*, 1- *Z. parvula*, 1-*B. bicaudatus*, 1-*Calopteryx* sp., and 3-*Leptohyphes* sp.) that were incorrectly identified or counted.

NAWQA Program Invertebrate Sample Processing

Identification and Quantification QA/QC Data Sheet

Project: GS-NAWQA **Date:** 12/10/90
Lab. Prefix: YAKI90- **NAWQA Sample ID:** YAKI1090IRL0070A,B
Lab. No.: 153, 154

QA/QC sample block code: YAKI90-B7

Sample fractions evaluated (check):	X	B		F		F-1		F-2		F-3		F-4

List of revisions to identifications and enumerations:			
Taxon		Quantity	
Original identification	Corrected identification	Original	Re-count
Hydropsyche amblis		2	1
Hydropsyche amblis	*Hydropsyche sp. B*	0	1
Simulium vittatum		5	6
Zaitzevia parvula		6	5
Zaitzevia parvula	*Macronychus sp.*	0	1
Baetis bicaudatus		5	4
Baetis bicaudatus	*Baetis tricaudatus*	2	3
Calopteryx sp.		1	0
Calopteryx sp.	*Hetaerina sp.*	0	1
Leptohyphes sp.		6	3
Leptohyphes sp.	*Tricorythodes sp.*	0	2
Leptohyphes sp.	*Caenis sp.*	0	1

Figure 28.--Example of an invertebrate sample processing identification and quantification quality-assurance/quality-control (QA/QC) data sheet for a large-rare sample component.

NAWQA Program Invertebrate Sample Processing

Identification and Quantification QA/QC Data Sheet

Project: GS-NAWQA
Lab. Prefix: YAKI90-
Lab. No.: 155

Date: 12/10/90
NAWQA Sample ID: YAKI1090IRM0070C

QA/QC sample block code: YAKI90-B7

Sample fractions evaluated (check):		B		F	X	F-1	X	F-2	X	F-3		F-4

List of revisions to identifications and enumerations:

Taxon		Quantity	
Original identification	Corrected identification	Original	Re-count
Thienemaniella sp.		61	70
Eukiefferiella sp.		28	15
Eukiefferiella sp.	*Parakiefferiella sp.*	0	13
Turbellaria		56	45
Baetis bicaudatus		269	260
Baetis bicaudatus	*Callibaetis sp.*	0	9

Figure 29.--Example of an invertebrate sample processing identification and quantification quality-assurance/quality-control (QA/QC) data sheet for a main-body sample component.

The same QA/QC check procedures are used to evaluate the identification and quantification of invertebrates obtained from the main-body sample component. The example presented in figure 29 is for a main-body sample component (YAKI1090IRM0070C) that was subsampled in the field (1/2 field subsample), divided into quarters during laboratory processing, and had three 1/4-subsamples processed. This example differs from that of the large-rare sample component (fig. 28) in that the QA/QC check data include three F-fractions. All counts and QA/QC checks for the main-body sample component are done by summing the data from these F-fractions (figs. 20, 21, and 22). These sums are not corrected for laboratory or field subsampling. In this example, two new taxa (*Callibaetis* sp. and *Parakiefferiela* sp.) were found, and 42 organisms were incorrectly identified or counted (9-*Thienemaniella* sp., 13-*Eukiefferiella* sp., 11-Turbellaria, and 9-*Baetis bicaudatus*).

The effects of identification, quantification, and picking errors are assessed and summarized for each component and for the whole sample. QA/QC checks for the large-rare and main-body sample components are based on the parts of the sample processed in the laboratory and do not include corrections for field or laboratory subsampling. QA/QC checks for the whole sample are intended to represent the cumulative effects of errors on the estimate for the whole sample so estimates are corrected for field and laboratory subsampling.

Identification and quantification QA/QC checks are more accurate and more easily verified if the data and calculations are maintained on a computer spreadsheet, such as illustrated in table 9. The original data are entered in the first three columns, and the original totals for each taxon are calculated in the fourth column taking into account any laboratory or field subsampling. The next three columns are the corrections that were made to counts (additions and reductions) and identifications during the QA/QC checks (figs. 28 and 29), including corrections arising from sample picking effectiveness checks (fig. 27). These corrections are separated into corrections to the counts of the original taxa and additions of new taxa (see bottom of table 9). Corrected values result from summing the original values and the QA/QC adjustments. Adjustments arising from sample picking are added to the main-body sample component values. The "total" column includes corrections for field and laboratory subsampling. The remaining columns are associated with calculating the Bray-Curtis dissimilarity index (Bray and Curtis, 1957). An index of similarity between the original and corrected values is then calculated using the complement of the Bray-Curtis index:

$$1 - BC_{ij} = 1 - \left(\frac{\sum_{k=1}^{S} |n_{ik} - n_{jk}|}{\sum_{k=1}^{S} (n_{ik} + n_{jk})} \right), \quad (2)$$

where BC is the Bray-Curtis index comparing the original i and corrected j values, S is the number of taxa present in the sample, n_{ik} is the number of individuals in taxon k in the original values i, and n_{jk} is the number of individuals in taxon k in the corrected values j.

Table 9.--*Example of a two-page community similarity index work sheet used to calculate the complement of the Bray-Curtis dissimilarity index*

Taxon	Original values from data sheets			QA/QC adjustments			Corrected values			Bray-Curtis index calculations					
										Large-rare		Main-body		Total	
	Large-rare	Main-body	Total	Large-rare	Main-body	Sample picking	Large-rare	Main-body	Total	$\lvert n_{ik}-n_{jk}\rvert$	$n_{ik}+n_{jk}$	$\lvert n_{ik}-n_{jk}\rvert$	$n_{ik}+n_{jk}$	$\lvert n_{ik}-n_{jk}\rvert$	$n_{ik}+n_{jk}$
Fossaria sp.	2	2	8	0	0	0	2	2	8	0	4	0	4	0	16
Lanx sp.	3	3	11	0	0	0	3	3	11	0	6	0	6	0	22
Juga sp.	8	7	27	0	0	0	8	7	27	0	16	0	14	0	54
Turbellaria	0	56	150	0	-11	3	0	48	128	0	0	8	104	22	278
Aulodrilus pluriseta	0	1	3	0	0	0	0	1	3	0	0	0	2	0	6
Telmatodrilus vejdovskyi	0	70	187	0	0	0	0	70	187	0	0	0	140	0	374
Pacifastacus leniusculus	2	0	2	0	0	0	2	0	2	0	4	0	0	0	4
Baetis bicaudatus	5	269	723	-1	-9	0	4	260	698	1	9	9	529	25	1421
Baetis quilleri	3	39	107	0	0	0	3	39	107	0	6	0	78	0	214
Baetis tricaudatus	2	101	272	1	0	0	3	101	273	1	5	0	202	1	545
Epeorus longimanus	2	0	2	0	0	0	2	0	2	0	4	0	0	0	4
Rhithrogena sp.	4	0	4	0	0	0	4	0	4	0	8	0	0	0	8
Stenonema sp.	2	0	2	0	0	0	2	0	2	0	4	0	0	0	4
Attenella margarita	3	50	137	0	0	0	3	50	137	0	6	0	100	0	274
Leptohyphes sp.	6	92	252	-3	0	0	3	92	249	3	9	0	184	3	501
Caenis sp.	0	10	27	1	0	0	1	10	28	1	1	0	20	1	55
Argia sp.	1	11	31	0	0	0	1	11	31	0	2	0	22	0	62
Calopteryx sp.	1	8	23	-1	0	0	0	8	22	1	1	0	16	1	45
Hetaerina sp.	0	1	3	1	0	0	1	1	4	1	1	0	2	1	7
Taeniopteryx nivalis	1	56	151	0	0	0	1	56	151	0	2	0	112	0	302
Cheumatopsyche sp.	2	1	5	0	0	0	2	1	5	0	4	0	2	0	10
Hydropsyche alhedra	3	107	289	0	0	0	3	107	289	0	6	0	214	0	578
Hydropsyche amblis	2	154	413	-1	0	0	1	154	412	1	3	0	308	1	825
Hydropsyche sp. B	0	7	19	1	0	0	1	7	20	1	1	0	14	1	39
Anagapetus sp.	1	34	92	0	0	0	1	34	92	0	2	0	68	0	184
Glossoma sp.	6	37	105	0	0	0	6	37	105	0	12	0	74	0	210

Table 9.--*Example of a two-page community similarity index work sheet used to calculate the complement of the Bray-Curtis dissimilarity index*--Continued

Taxon	Original values from data sheets			QA/QC adjustments			Corrected values			Bray-Curtis index calculations					
										Large-rare		Main-body		Total	
	Large-rare	Main-body	Total	Large-rare	Main-body	Sample picking	Large-rare	Main-body	Total	$\lvert n_{ik}-n_{jk}\rvert$	$n_{ik}+n_{jk}$	$\lvert n_{ik}-n_{jk}\rvert$	$n_{ik}+n_{jk}$	$\lvert n_{ik}-n_{jk}\rvert$	$n_{ik}+n_{jk}$
Leucotrichia pictipes	10	120	330	0	0	0	10	120	330	0	20	0	240	0	660
Nectopsyche sp.	2	0	2	0	0	0	2	0	2	0	4	0	0	0	4
Dubiraphia givlianii	1	32	87	0	0	0	1	32	87	0	2	0	64	0	174
Macronychus sp.	0	17	46	1	0	0	1	17	47	1	1	0	34	1	93
Zaitzevia parvula	6	13	41	-1	0	0	5	13	40	1	11	0	26	1	81
Antocha saxicola	1	0	1	0	0	0	1	0	1	0	2	0	0	0	2
Simulium vittatum	5	276	741	-1	0	0	4	276	740	1	9	0	552	1	1481
Rheotanytarsus sp.	0	36	96	0	0	0	0	36	96	0	0	0	72	0	192
Cricotopus sp. grp.	0	29	78	0	0	2	0	31	83	0	0	2	60	5	161
Eukiefferiella sp.	0	28	75	0	-13	4	0	19	51	0	0	9	47	24	126
Corynoneura sp.	0	3	8	0	0	0	0	3	8	0	0	0	6	0	16
Thienemanniella sp.	0	61	163	0	9	0	0	70	187	0	0	9	131	24	350
Orthocladius sp.	0	29	78	0	0	0	0	29	78	0	0	0	58	0	156
Pentaneura sp.	0	3	8	0	0	0	0	3	8	0	0	0	6	0	16
Thienemannimyia sp. grp.	0	30	80	0	0	3	0	33	88	0	0	3	63	8	168
Hemerodromia sp.	0	10	27	0	0	0	0	10	27	0	0	0	20	0	54
New taxa															
Tricorythodes sp.				2	0	0	2	0	2.00	2	2	0	0	2	2
Parakiefferiella sp.				0	13	0	0	13	34.67	0	0	13	13	35	35
Callibaetis sp.				0	9	0	0	9	24.00	0	0	9	9	24	24
Nemoura sp.				0	0	1	0	1	2.67	0	0	1	1	3	3
Yoroperla sp.				0	0	6	0	6	16.00	0	0	6	6	16	16
							Column totals			**15**	**167**	**69**	**3,623**	**200**	**9,856**
							Bray-Curtis index			**.09**		**.02**		**.02**	
							1-(Bray-Curtis index)			**.91**		**.98**		**.98**	

The absolute values of the differences between the original and corrected values and the sum of the original and corrected values are calculated on the spreadsheet and summed at the bottom (column totals). The column totals are then used to calculate the Bray-Curtis dissimilarity index (BC_{ij}) and its complement. The value of the similarity index ($1\text{-}BC_{ij}$) that compares the original (i) and corrected values (j) must be at least 0.90 for the sample to pass the QA/QC check.

Results from the identification and quantification QA/QC checks are recorded on the identification and quantification QA/QC summary check list (fig. 30). In this example, the large-rare sample component fails the QA/QC check for new taxa. Five taxa were identified that were not part of the 26 taxa originally listed for this sample component (fig. 16). These new taxa represent 19.2 percent of the original number of taxa and exceed the QA/QC criterion of 10 percent new taxa. Eight of the 84 organisms (9.5 percent) were incorrectly identified or counted (fig. 28), and the sample component meets the criterion of 10 percent (or less) incorrect identifications or counts. Finally, the similarity index ($1\text{-}BC_{ij}$) between the original and corrected values for the large-rare sample component (0.91) meets the QA/QC criterion of at least 0.90. Based on these results, all of the large-rare sample components in the QA/QC sample block YAKI90-B7 have to be re-processed until all three of the QA/QC checks are passed.

The main-body sample component passes two of the three QA/QC checks. Four new taxa were identified during the QA/QC checks; two (*Parakiefferiella* sp. and *Callibaetis* sp.) from reviewing the F-fractions and two (*Nemoura* sp. and *Yoroperla* sp.) from re-picking the sample remnants. These additions represent 11.1 percent of the 36 taxa originally recovered from the main-body sample component and exceed the 10-percent QA/QC criterion. Sixty-one organisms were incorrectly identified or counted in the sample remnants (1-*Nemoura* sp., 6-*Yoroperla* sp., 3-*Thienemannimyia* sp. grp., 4-*Eukiefferiella* sp., 2-*Cricotopus* sp. grp., and 3-Turbelleria) and F-fractions (9-*Thienemaniella* sp., 13-*Parakiefferiella* sp. originally identified as *Eukiefferiella* sp., 11-Turbellaria, and 9-*Callibaetis* sp. originally identified as *Baetis bicaudatus*). This is 2.5 percent of the original number of organisms (2,404) recovered from the main-body sample component and is within the QA/QC criterion of 10 percent. The similarity index ($1\text{-}BC_{ij} = 0.98$) is also within the QA/QC limits (0.90), and the sample passes this part of the check. However, the new taxa criterion was not passed, and all main-body sample components within this QA/QC block need to be reviewed.

Unlike the previous QA/QC checks, the data listed for the total sample represent the totals for the entire sample (table 9) rather than data for the individual components (large-rare or main-body). Five new taxa (*Nemoura* sp., *Yoroperla* sp., *Tricorythodes* sp., *Parakiefferiella* sp., and *Callibaetis* sp.) were added to the 42 taxa originally obtained from the large-rare and main-body sample components (fig. 25). This represents an increase of 11.9 percent, which exceeds the QA/QC criterion (10 percent), and the sample fails this check. A total of 170.7 organisms (3.5 percent) were incorrectly identified or counted out of a total of 4,906 (fig. 25), a percentage which is within the QA/QC limits. The total of incorrectly identified or counted organisms includes 8 from the large-rare sample component and 61 from the main-body sample component that were corrected for field (0.5) and laboratory (0.75) subsampling, thus, $[(61 \div 0.75) \div 0.5] + 8 = 170.7$. The community similarity index (0.98) is also within the limits of the QA/QC criterion (0.90). It is not anticipated that a whole-sample QA/QC check would fail unless either or both of the component QA/QC checks fail. However, if the whole-sample QA/QC check fails and neither of the component QA/QC checks fails, then all elements (B-, E-, and F-fractions) of the QA/QC sample block must be re-checked.

NAWQA Program Invertebrate Sample Processing

Identification and Quantification QA/QC Summary Check List

Project: GS-NAWQA **QA/QC Sample Block ID:** YAKI90-B7
Lab. Prefix: YAKI90- **Date:** 12/10/90

QA/QC check summaries

Large-rare sample component

Lab. No.: 153, 154		**NAWQA Sample ID:** YAKI1090IRL0070A,B			
Percent new taxa		Incorrect identifications or counts		Similarity index (1-BC_{ij})	
Total number of taxa (a):	26	Total number of organisms in sample (a):	84	$\sum_{k=1}^{S} \lvert n_{ik} - n_{jk} \rvert$	15
Number of new taxa (b):	5	Number of organisms incorrectly identified or counted(b):	8	$\sum_{k=1}^{S} (n_{ik} + n_{jk})$	167
Percent ([b÷a] x 100)	19.2	Percent ([b÷a] x 100)	9.5	1-BC_{ij}	0.91
Pass or fail? (Pass if ≤ 10 %)	Fail	Pass or fail? (Pass if ≤ 10 %)	Pass	Pass or fail? (Pass if index ≥ 0.90)	Pass

Main-body sample component

Lab. No.: 155		**NAWQA Sample ID:** YAKI1090IRL0070C			
Percent new taxa		Incorrect identifications or counts		Similarity index (1-BC_{ij})	
Total number of taxa (a):	36	Total number of organisms in sample (a):	1,803	$\sum_{k=1}^{S} \lvert n_{ik} - n_{jk} \rvert$	69
Number of new taxa (b):	4	Number of organisms incorrectly identified (b):	61	$\sum_{k=1}^{S} (n_{ik} + n_{jk})$	3,623
Percent ([b÷a] x 100)	11.1	Percent ([b÷a] x 100)	3.4	1-BC_{ij}	0.98
Pass or fail? (Pass if ≤ 10 %)	Fail	Pass or fail? (Pass if ≤ 10 %)	Pass	Pass or fail? (Pass if index ≥ 0.90)	Pass

Total sample

Percent new taxa		Incorrect identifications or counts		Similarity index (1-BC_{ij})	
Total number of taxa (a):	42	Total number of organisms in sample (a):	4,906	$\sum_{k=1}^{S} \lvert n_{ik} - n_{jk} \rvert$	200
Number of new taxa (b):	5	Number of organisms incorrectly identified (b):	170.7	$\sum_{k=1}^{S} (n_{ik} + n_{jk})$	9,856
Percent ([b÷a] x 100)	11.9	Percent ([b÷a] x 100)	3.5	1-BC_{ij}	0.98
Pass or fail? (Pass if ≤ 10 %)	Fail	Pass or fail? (Pass if ≤ 10 %)	Pass	Pass or fail? (Pass if index ≥ 0.90)	Pass

Figure 30.--Example of an identification and quantification quality-assurance/quality-control (QA/QC) summary check list.

When a QA/QC sample block is re-checked due to a QA/QC check failure, a new sample is chosen at random to represent the quality of the re-processed QA/QC sample block. This sample should be identified on the QA/QC sample block data sheet (fig. 14) with a "2" to indicate that this sample is the second sample to represent the sample block. If samples continually exceed the QA/QC limits, the contract laboratory must contact the BQAU to determine the cause for this problem and, if necessary, to seek re-adjustment of the QA/QC criteria.

Sample Volume Quality-Assurance and Quality-Control Checks

The volumes of main-body sample components and the volumes of C- and E-fractions are recorded as part of the QA/QC checks on processed samples (fig. 31). The criterion is that the C- and E-fraction volumes must sum to within 15 percent of the original main-body sample component volume. If this criterion is not met, the laboratory must account for the difference in sample volumes. This check is intended to ensure that subsamples are not lost during laboratory processing, that any lost material is accounted for, and that all sample remnants are returned to the BQAU for review. If the C- and E-fractions are within 15 percent of the volume measured for the main-body sample component, then no action is required by the contract laboratory. If the volumes are not within 15 percent (laboratory numbers 82, 94, and 106, fig. 31), then the laboratory must provide an explanation for the discrepancy. For example, combined volumes of processed materials could be lower than the original volume if large debris, such as sticks, are removed during processing, or they could exceed the original volume if the processed materials are not allowed sufficient time to settle. The primary intent of checking sample volumes is to document when and where sample material is lost in the processing sequence. This information helps assess the effect of lost material on the resultant data and provides guidance for modifying processing steps to avoid processing errors.

Quality-Assurance Summary Check List

QA/QC checks are summarized on the QA summary check list (fig. 32). This check list provides an overview of the results of the identification and quantification QA/QC checks, the picking effectiveness checks, and the sample volume checks. It is also used to record information about processing errors or problems encountered with other samples. The laboratory sample code, 16-character NAWQA Program sample identification code, and sample fraction designation are entered to identify each sample or sample fraction. Letter codes (table 10) are used to identify the types of errors that occurred and the actions that were taken to correct them. For example, WE in the error code column indicates that some of the sample bypassed the sieve when this sample was rinsed. WC in the action code column indicates that this material was caught and added back into the sample. Consequently, no sample material was lost and the data quality was not impaired. If material had been lost and not recovered, then WI would have been entered in the action code column to indicate that the data quality was compromised.

There are three types of errors that are applicable to the identification and quantification QA/QC checks. These include finding too many new taxa (NT), having too many organisms that are incorrectly identified or quantified (IE), and exceeding the allowances for the similarity index (SI). Two actions are appropriate remedies for these errors: review the B-fractions (IB) or review the F-fractions (IF). Similarly, finding too many new taxa (NT) or too many organisms (CC) are errors associated with evaluating picking effectiveness. The appropriate action for picking errors is to re-pick the sample remnants (RP).

NAWQA PROGRAM BENTHIC INVERTEBRATE QUALITY ASSURANCE CHECK SHEET

SAMPLE VOLUME QA/QC CHECKS

Project: GS-NAWQA **Study Unit:** ALBE
Lab. Prefix: ALBE93-

Lab. no.	Original sample volume (mL)	Sample volumes (mL) for fractions and 1/4-subsamples that remain at the completion of sample processing						Ratio of processed to original volume	QA/QC results (pass/fail)
		C	E or E-1	E-2	E-3	E-4	Total		
76	452		400				400	0.88	Pass
78	740	210	190	187	212		799	1.08	Pass
80	250		215				215	.86	Pass
82	410		250				250	.61	Fail
84	670		157	150	163	166	636	.94	Pass
86	139		150				150	1.08	Pass
90	75		70				70	.93	Pass
94	150		90				90	.60	Fail
96	230		210				210	.91	Pass
100	266		234				234	.88	Pass
102	150		135				135	.90	Pass
104	650	333	160	153			646	.99	Pass
106	735		143	147	150	123	563	.77	Fail
108	563		479				479	.85	Pass
110	478		526				526	1.10	Pass
112	225		200				200	.89	Pass
114	250		220				220	.88	Pass

Figure 31.--Example of a sample volume quality-assurance/quality-control (QA/QC) check sheet.

NAWQA PROGRAM
LABORATORY PROCESSING QUALITY-ASSURANCE SUMMARY CHECK LIST

Project: GS-NAWQA **Study Unit:** Yakima River Basin
Laboratory Prefix: YAKI90-

			Quality Assurance Check Summary											QA Officer (initials)
			Identification and enumeration			Picking effectiveness			Volumes			Other		
Lab. number	NAWQA sample identification code	QA/QC block	Pass/ fail ?	Error code	Action code	Pass/ fail ?	Error code	Action code	Pass/ fail ?	Error code	Action code	Error code	Action code	
1	YAKI1090IRL0001A	YAKI90-B1										WE	WC	R.F.G.
7,8	YAKI1090IQL0003A,B	YAKI90-B1	Pass											R.F.G.
9	YAKI1090IQM0003C	YAKI90-B1	Fail	IE	IF	Pass			Pass					R.F.G.
45,46	YAKI1090IRL0017A,B	YAKI90-B2	Fail	NT	IB									R.F.G.
47	YAKI1090IRM0017C	YAKI90-B2	Pass			Fail	NT	RP	Pass					R.F.G.
70	YAKI1090IDL0029A	YAKI90-B3	Pass											R.F.G.
71	YAKI1090IDM0029B	YAKI90-B3	Pass			Fail	CC	RP	Pass					R.F.G.
85,86	YAKI1090IRL0033A,B	YAKI90-B4	Fail	NT	IB									R.F.G.
87	YAKI1090IRM0033C	YAKI90-B4	Pass			Fail	NT	RP	Pass					R.F.G.
110,111	YAKI1090IQL0047A,B	YAKI90-B5	Fail	IE	IB									R.F.G.
112	YAKI1090IQM0047C	YAKI90-B5	Fail	SI	IF	Pass			Pass					R.F.G.
130,131	YAKI1090IRL0054A	YAKI90-B6	Pass											R.F.G.
132	YAKI1090IRM0054C	YAKI90-B6	Pass			Fail	CC	RP	Fail	VE	VC			R.F.G.
153,154	YAKI1090IRL0070A,B	YAKI90-B7	Fail	NT	IB	Pass								R.F.G.
155	YAKI1090IRL0070C	YAKI90-B7	Fail	NT	IF	Pass			Fail	VE	VC			R.F.G.

Figure 32.--Example of a laboratory processing quality-assurance summary check list. (Error and action codes listed in table 9.)

Table 10.--*Error codes and action codes for use on the quality-assurance summary check list*

Code	Description of error and action codes
	Error codes
NT	Number of new taxa recovered is more than 10 percent of the original number of taxa.
CC	Number of organisms recovered from sample remnant represents more than 10 percent of the number of organisms removed in original picking effort.
IE	Number of organisms incorrectly identified or counted is more than 10 percent of the original number of organisms.
SI	Similarity index between original and corrected values is less than criterion (0.90 for the complement of the Bray-Curtis dissimilarity index or 90 percent for percentage similarity coefficient).
LE	Labeling error--contact the Biological Quality-Assurance Unit if error is on the original sample label.
SE	Subsampling error--error during division of sample into four equal subsamples.
WE	Washing error--some sample bypassed washing sieve.
DE	Data entry error on data sheet.
VE	Volume error--original main-body volume and sum of component volumes differ by more than 15 percent.
CE	Calculation error--mathematical error on data sheets.
	Action codes
RP	All sample remnants (fraction E) in quality-assurance/quality-control sample block have been re-picked, invertebrates have been identified and counted, and data sheets corrected.
IB	Invertebrates from B fractions in quality-assurance/quality-control sample block have been reviewed (identified and counted) and data sheets corrected.
IF	Invertebrates from F fractions in quality-assurance/quality-control sample block have been reviewed (identified and counted) and data sheets corrected.
LC	Labels corrected, re-checked, and data sheets corrected.
SC	Sample re-subsampled, processed, re-checked, and data sheets corrected.
WC	Material bypassing sieve caught in washbasin, sample combined and re-washed.
WI	Material bypassing sieve lost, partial sample processed.
DC	Data entry error corrected (strike out incorrect entry with one line and write in corrected entry, initial).
VC	Volumes from all sample fractions have been re-checked and calculations re-checked. Account for and report probable cause for any discrepancy.

Other error and action codes relate to problems with labeling (LE), subsampling (SE), volumes (VE), data entry (DE), and calculations (CE). These errors have matching action codes that document the corrective actions taken (LC, SC, VC, DC, and CR, respectively). Additional error and action codes may be used on the QA summary check list if they are documented in the interim and final reports.

Quality Assurance and Quality Control for Reference Collections

Study-unit reference collections constitute an important and long-term record of the types of organisms present within each study unit. These collections are archived and provide a permanent account of conditions within the study unit that can be compared with future collections from the study unit and with other study units across the Nation. Consequently, the accuracy of these reference collections is very important. Therefore, identifications of the specimens composing the study-unit reference collection must be confirmed by an independent taxonomic expert, and the keys used to identify specimens must be documented. These actions are documented on the study-unit reference-collection data sheet (fig. 10). This data sheet provides entries for a specimen number, three levels of identification (level 3 is typically genus or genus and species), authority name, references to the taxonomic keys used for identifying the specimen, and the name of the taxonomic expert that confirmed the identification. This data sheet is supported by a bibliographic listing of the keys used for identification (fig. 11) and a listing of the independent taxonomic authority used, including addresses and phone numbers (fig. 12).

Return of Materials and Specimens

All materials, with the exception of the study-unit reference collection and supporting materials (taxonomic bibliography and list of taxonomists), are returned to the BQAU upon successful completion of each QA/QC sample block. The study-unit reference collection is returned after processing all study-unit samples for that calendar year is completed. However, a copy of the list of study-unit reference-collection specimens and supporting materials are provided to the BQAU for review at least monthly. In addition, copies of all data sheets, work sheets, check lists, laboratory logs, methods specifications, reference-collection lists, and sample custody records are returned to the BQAU with the sample materials (table 3). Electronic and paper copies of this information are provided.

Electronic forms should be provided in MS-DOS-compatible format, preferably as Lotus 1-2-3 Version 2.x compatible spreadsheets. An MS-DOS-compatible computer file of the summary data for each sample (column "c" of the sample tabulation work sheet, fig. 25) must be provided either in spreadsheet or column-justified ASCII format (fig. 33). Note that the 16-character USGS sample identification codes for the large-rare and main-body components are entered in the data file.

Taxa are entered in phylogenetic order and to the taxonomic levels specified in table 4. Four columns are provided for entering organism identifications. The right two columns are reserved for recording information on genus and species. The left two columns (level 1 and level 2 taxonomic identifiers) are used to record taxonomic identifiers above the level of genus. Typically, level 1 and level 2 identifiers are order and family, respectively. However, family and subfamily may be more appropriate for Chironomidae, and class and order may be appropriate for other groups, such as Gastrotricha (table 4). The last column is used to enter the life stage for the organism (A-adult, P-pupae, or L-larvae, nymph, or naiad) if the

NAWQA PROGRAM SUMMARY DATA COMPUTER FILE

A. Headings (italics) for data entry into the summary computer data file.

Study Unit:	HDSN
Site name:	Little River @ Kingston, NY
Site ID number:	10210441
Date collected (mdy):	09/23/92
Sample type:	RTH
Reach:	A
Sample ID codes	HDSN0992IRL0057A,B HDSN0992IRM0057C

Taxonomic Identifiers

Level 1	*Level 2*	*Genus*	*Species*	*Quantity*	*Life Stage*
Diptera	Chironomidae	Polypedilum	illinoense	10	P

B. Image of completed data summary file.

HDSN
Little River @ Kingston, NY
10210441
09/23/92
RTH
A
HDSN0992IRL0057A,B HDSN0992IRM0057C

Coelenterata	Hydridae	Hydra	americana	21	
Oligochaeta	Lumbriculidae			1	
Oligochaeta	Tubificidae	Limnodrilus		1	
Oligochaeta	Tubificidae	Limnodrilus	hoffmeisteri	43	
Hirudinea	Erpobdellidae	Erpobdella	punctata	8	
Decapoda	Cambarinae			3	
Amphipoda	Talitridae	Hyalella	azteca	23	
Odonata	Aeshnidae	Anax	junius	3	L
Trichoptera	Hydropsychidae			2	P
Trichoptera	Hydropsychidae	Hydropsyche		21	L
Trichoptera	Hydropsychidae	Hydropsyche	venularis	2	L
Diptera	Chironomidae			4	L
Diptera	Chironomidae	Polypedilum		6	L
Diptera	Chironomidae	Polypedilum	convictum	111	L
Diptera	Chironomidae	Polypedium	convictum	17	P

Figure 33.--Specifications for the summary data computer file.

taxon has more than one life stage that is aquatic. Copies of other electronic data sheets used in calculations or QA/QC checks (tables 8 and 9) are also returned for review.

BIOLOGICAL QUALITY-ASSURANCE UNIT

The Quality Management Group's BQAU is located at the USGS National Water-Quality Laboratory, Arvada, Colorado. It includes taxonomic specialists for invertebrates, fish, and algae who, together with a data-base specialist and unit manager, work to ensure the quality and integrity of biological data obtained by the NAWQA Program. This involves working closely with NAWQA Program biologists, contract laboratories, and taxonomic experts outside the USGS to ensure that invertebrate samples are consistently and accurately collected, processed, identified, and enumerated. Accuracy and consistency in taxonomic data are obtained by providing technical oversight to ensure that competent contractors are used (laboratory qualification), that laboratories adhere to contract guidelines (contract development and monitoring), that accurate information is supplied (data checking and taxonomic verifications), and that procedures are continuously improved to better meet the needs of the NAWQA Program (communications with contractors, study units, and the general scientific community).

The activities of the BQAU are intended to provide the NAWQA Program and other WRD projects with accurate, high-quality data that (1) meet the needs of the projects, (2) are accepted by the general scientific community, and (3) retain their value and relevancy through time. The BQAU works with USGS contracting officials, the Branch of Quality Assurance, and other appropriate WRD personnel to provide the technical aspects of contract specifications. This includes specifying methods, data reporting procedures, and QA/QC monitoring programs and techniques. The BQAU also establishes technical criteria for evaluating contract laboratories and assists the contracting officer in evaluating candidate contract laboratories.

Once contracts have been established, the BQAU monitors laboratory performance by reviewing data, QA/QC information, specimens, and sample remnants returned by the contract laboratory. Specimens are reviewed for accuracy in identifications, quantifications, and sample picking using the same criteria as specified in the contract. The unit also reviews the contractor's QA/QC data to ensure that the contractor is adhering to contract specifications, producing data that meet the specified quality criteria, and providing complete documentation of processing activities. The BQAU evaluates and coordinates modifications to existing processing procedures to ensure data continuity and comparability. Processing procedures that provide better data at reasonable costs are incorporated into existing contracts or into new contracts.

The BQAU also coordinates taxonomy nationally to ensure accuracy and consistency in identifications among study units and contract laboratories. This is done by curating study-unit reference collections and samples, placing voucher specimens in repositories (museums, universities, or research institutions) outside the USGS, and maintaining a reference library focused on taxonomic descriptions as well as methods and techniques associated with invertebrate identifications, curation, and QA/QC procedures. The unit works with local, regional, and national taxonomists to verify identifications of taxonomic specimens and maintains the taxonomic data base within NWIS-II. The BQAU also works with NOAA and the EPA in maintaining an accurate and consistent interagency taxonomic data base and integrating it with the NWIS-II taxonomic data base.

The BQAU develops and maintains computer data bases required to administer NAWQA Program study-unit sample collections maintained within the USGS. It enters invertebrate data into NWIS-II for immediate use by study-unit teams and approves such data, in conjunction with the project chief, for general release. The unit also works with the study-unit teams to evaluate field quality-assurance activities by evaluating the efficacy of field elutriation and sample-splitting procedures. In this capacity, it works closely with the study-unit biologists, regional biologists, and national synthesis teams to meet the data needs of the NAWQA Program. It also collaborates with study-unit personnel and taxonomic experts outside the USGS through report preparation, peer-reviewed publications, and participation in professional meetings. The BQAU trains laboratory personnel, NAWQA Program regional biologists, and study-unit personnel in QA/QC procedures related to handling and processing benthic invertebrate samples.

Development of Technical Aspects of Contracts

The BQAU works with the NAWQA Program teams (protocol development, regional biologists, study-unit biologists, and the National Leadership Team) to develop field quality-assurance techniques, recommend appropriate sample handling and processing procedures, define the scope of laboratory services needed to support the sampling protocols, and develop the technical specifications for sample processing, quality assurance, and data formats. The BQAU's technical specifications are incorporated into the Request for Proposals (RFP) that form the basis for all contract work. In this regard, the BQAU works closely with the appropriate contracting office to ensure that the necessary technical specifications are included in the RFP's. The BQAU is responsible for establishing technical and performance criteria for the evaluation of candidate laboratories and oversees a technical review committee that assists the contract office in reviewing the technical merits of contract laboratories.

Guidelines and Testing Procedures for Qualifying Laboratories

The qualifications of any contractor used to process samples or verify identifications (for example, contract laboratory, museum, university, or research institute) need to be carefully examined to ensure that the contractor can provide the necessary services accurately and in a timely manner. The following guidelines establish methods for evaluating potential contractors and help to ensure that they have the personnel, equipment, facilities, experience, and stability to provide the long-term services required by the NAWQA Program. The contractor selection process is conducted by a three- to five-member technical review committee led by a member of the BQAU. The membership of this technical review committee is composed of BQAU personnel, NAWQA Program biologists, District biologists, and other WRD personnel, such as from the Branch of Quality Assurance, as deemed appropriate. The technical review committee works with the appropriate contracting officer to provide technical assistance in the development of contracts and in the evaluation of potential contractors. The contracting officer is the responsible agent for awarding contracts.

The evaluation process begins with the development of an RFP issued by the appropriate contracting office. The BQAU is responsible for developing the technical aspects of the RFP and works with the appropriate contracting officials to help with the development of the RFP. Once the RFP has been released, the BQAU and the laboratory review committee conduct an initial review of the information provided by the laboratories in response to the RFP, such as personnel, physical facilities, and experience, and use this

information to rank laboratories on their ability to provide the prescribed services. Laboratories that rank highest during the initial review are provided with actual samples by which to evaluate their performance. These laboratories are then evaluated and ranked on their performance in processing these samples. Finally, laboratories that rank high in the initial review and performance test are evaluated on the cost of their services. The BQAU and the laboratory review committee are responsible for developing specific point ratings for review criteria that are used to rank the laboratories during the initial review and performance phases. Criteria that should be considered in the initial review and performance- and cost-evaluation processes are outlined below. The relevant information is requested from the laboratories as part of the RFP.

Criteria for the Initial Review of Laboratory Qualifications

The qualifications of personnel who will be involved in processing the samples and administering the laboratory are reviewed. It is particularly important to determine if the personnel who will be involved in identifying and quantifying the invertebrates have relevant training and experience in invertebrate taxonomy. College transcripts and publications are important mechanisms for establishing the existence of such training and can provide insight into the type and amount of training as well as an individual's proficiency at taxonomy. On-the-job training also should be considered; however, it must be demonstrated that the training occurred under the instruction of a competent taxonomist and that the training increased the individual's proficiency in identifying invertebrates.

The laboratory must have sufficient laboratory space to handle the processing and storage of samples. At a minimum, there should be separate areas where samples can be logged in and stored prior to processing, washed and preserved, picked, identified and counted, and stored after processing is completed. The laboratory should include a "wet lab" area with sufficient sink space, counter space, and ventilation to adequately and safely handle formalin- and alcohol-preserved samples. Space should be dedicated to processing taxonomic samples and should not be used for other activities, such as chemical analysis, chemical storage, or toxicity testing. The laboratory should have in place all necessary safety equipment, such as fire extinguishers, ventilation systems, fume hoods, safety showers, and chemical storage cabinets, for the safe handling of preserved samples. The laboratory should have a designated safety officer who is responsible for safety in the laboratory. Plans that meet applicable Federal, State, or local standards should be available for the handling, storage, and disposal of toxic substances associated with the processing of invertebrate samples.

The candidate laboratory needs to demonstrate that it has the necessary equipment for processing invertebrates. This should include

1. Stereoscopic dissecting microscopes capable of 10X-100X magnifications for picking and identifying invertebrates. There should be separate dissecting microscopes available for sample processing and identification purposes.

2. A compound microscope with phase contrast that is capable of 100X-1,000X magnifications for identification of chironomids, oligochaetes, and other small invertebrates.

3. Appropriate sieves (425-µm and 212-µm mesh), acceptable subsampling device(s), forceps, vials, sample jars, microscope slides, and appropriate mounting media available in sufficient quantities to handle the anticipated sample load.

4. Computer equipment capable of storing data files on 3 1/2- or 5 1/4-inch diskettes that are readable by MS-DOS-based personal computers. Acceptable data formats include column-justified ASCII files and spreadsheet files compatible with LOTUS 1-2-3 version 2.0. Special consideration should be given to laboratories that use computers to minimize errors inherent in the hand-entry of data, such as double-entry of data.

The candidate laboratory should provide an overview of their standard sample processing procedures and suggest how these procedures match or differ from sample processing, QA/QC, and data-entry procedures recommended in the RFP. Suggestions for improvement and streamlining USGS-recommended procedures are evaluated and can contribute to a higher ranking of the laboratory. A laboratory reluctant to address the recommended sampling procedures will receive a lower ranking than a more cooperative laboratory.

The candidate laboratory must demonstrate its experience and establish its reputation by providing a list of past clients, who may be contacted as references, and examples of reports and publications produced by the laboratory as a normal part of its activities. Experience is ranked highly in evaluating the laboratory. Laboratory personnel should provide written details of how they routinely monitor the quality of data they produce. This QA/QC plan should address the issues specified in the RFP, including sample-tracking procedures (sample log-in and log-out), documentation of methods, sample picking effectiveness, procedures for verifying identifications and quantifications, labeling procedures, data-entry and data-checking procedures, employee training, and laboratory safety. A QA/QC officer should be identified who has overall responsibility for data quality.

The laboratory should maintain an on-site library of taxonomic literature appropriate to the regional expertise claimed by the laboratory. This library should include not only general taxonomic references (for example, Merritt and Cummins, 1984; Pennak, 1978, 1989; Peckarsky and others, 1990, Thorp and Covich, 1991) but also published species descriptions, regional taxonomic keys, and literature on the occurrence and distribution of aquatic invertebrates. This library should be accessible to those working on identifications and should be properly organized and maintained. The laboratory should be able to provide, by taxonomic group, the names of people not affiliated with the laboratory who have been used to verify identifications. These individuals should be recognized experts on specific taxonomic groups or regional fauna. Publications on invertebrate taxonomy should serve to verify the taxonomic expertise of the independent experts.

Initial review of potential contract laboratories may require follow-up inquiries to obtain more details on their personnel, operations, equipment, and facilities. These follow-ups may include visiting laboratories to review their operations and facilities. Once the initial review has been completed, the top-ranked laboratories enter the next stage of review--performance on field samples.

Criteria for Testing Laboratory Performance

Actual laboratory performance is evaluated by providing the candidate laboratory with several invertebrate samples representative of actual NAWQA Program samples--that is, composed of a large-rare component and a main-body component. Laboratory performance can be evaluated by "spiking" the large-rare sample component with a few organisms that do not occur in the location specified on the sample label or with specimens

deliberately damaged so that they cannot be identified to the level specified in the RFP. Additionally, the main-body sample component can be "spiked" with known numbers of a few distinctive taxa to determine picking and identification effectiveness. These spiked samples are sent to the top ranked contractors for processing according to procedures and criteria established in the RFP. All components of the processed samples, including invertebrates and sample remnants, are returned to the BQAU for evaluation.

The evaluation of potential contract laboratories on the basis of actual performance is an important part of ensuring that laboratories will be able to provide the needed services in an acceptable and timely fashion. The timeframe in which such evaluations can occur during the contracting process is very limited. Consequently, the samples must be prepared well in advance of anticipated use, and expectations for results must be established prior to submitting the sample to the candidate laboratory. Samples should be obtained from representative study units and pre-processed to establish expectations for contractor results. Split samples should be considered for use in this situation. The final product of this performance evaluation is a ranking of the laboratory's performance. The BQAU should consider the following criteria when evaluating contractor performance:

1. Sample turnaround time--the sample must be processed and the data and specimens returned within the time specified by the contract.

2. Picking effectiveness--the sample remnant from the main-body sample component is re-picked and judged to be acceptable if it passes the QA/QC criteria.

3. Condition of samples--the condition of the "picked" invertebrates, including reference collections, is ranked as being in excellent, good, fair, or poor condition for identification. Such condition is determined by the presence of the structures used for identification purposes, such as gills, legs, claws, and setae. Vials of invertebrates should be free of sediment, detritus, or other non-animal debris. The contractor must return the entire sample or account for any sample material lost during processing. This is evaluated by measuring the volume of the main-body sample component and comparing it to the volume of material returned by the contractor and the QA/QC sample volume check sheet.

All methods used by the contract laboratory must be documented in the report received from the contract laboratory. This report must include all QA/QC checks and all requested paper work and documentation. Data must be returned in paper and electronic forms according to specifications for electronic data formats and must be readable by MS-DOS-compatible personal computers.

All "spiked" specimens must have been found and properly identified and quantified. Damaged specimens that were "spiked" in the sample should not have been identified to levels that depend upon missing characters, or an explanation as to how such identifications were made should be included in the final report. Identifications must be consistent with the location of the sample, with the exception of "spiked" specimens. Common taxonomic groups (for example, Chironomidae) should not be missing or in disproportionate abundance. Absence of specific taxonomic groups can indicate an accident during sample processing that resulted in the loss of part of the "picked" taxa. Submitting split samples to candidate laboratories is particularly useful for this purpose. The proper authority should be listed with each taxon in the reference collection. The contractor should list the taxonomic keys used to identify the organisms. These keys should be appropriate for the taxonomic group and the region.

All raw counts and subsampling should be properly recorded and documented for the main-body sample component. Quantifications must meet the QA/QC criteria established for identifications and quantifications. Counts for sample components and the whole sample must be correctly calculated and corrected for field and laboratory subsampling. If computer programs are used to automatically calculate totals, then copies of the program code (for example, a copy of the spreadsheet) and the name and version number of the software should be supplied.

The contractor must supply a reference collection to support the identifications that were made. The independent outside taxonomic expert used to verify each taxon must be identified as well as the literature used to identify the taxon. Study-unit reference-collection specimens must be prepared properly and be in good condition. The authority name must be associated with each reference specimen.

Evaluating Laboratories on Costs

The final element in evaluating candidate laboratories is the consideration of the cost of services. This step is taken after all laboratories have been ranked on the basis of technical services. The top-ranking laboratories in the initial review step are evaluated for performance, and the laboratories ranked highest in performance are then considered on the basis of the cost of their services and the contracts awarded. This approach provides a defensible method for selecting qualified laboratories that takes into consideration performance and price.

Identifying Qualified Regional and National Taxonomic Experts

The BQAU not only relies on its own taxonomic experts but also uses outside experts to confirm identifications and resolve regional and national taxonomic issues. Consequently, it is necessary to be able to identify and interact with qualified regional and(or) national taxonomic experts. Identifying these individuals is a subjective process that requires taxonomic specialists within the BQAU to have a good general background in their areas of taxonomy and to be familiar with local, regional, and national experts. Contacts with recognized experts are strengthened by encouraging collaborative publishing and regular participation in meetings dealing with taxonomic issues.

When deemed necessary, qualification should be done by providing vials of identified specimens to the local expert for verification. A variety of invertebrates should be included in the sample, in keeping with the taxonomic and(or) regional expertise of the expert, and should include some incorrectly identified specimens. Qualification should be based on the following criteria:

1. The outside expert should express a willingness to work with the USGS in establishing correct identification.
2. Incorrectly identified specimens should be reported and corrections made.
3. Supporting arguments and the key characters used in supporting an identification should be reported to the USGS taxonomic specialist.

When new species are discovered, the USGS retains ownership of the specimen, but the BQAU should encourage collaboration between the identifier and the study-unit biologist in publishing their descriptions. Many taxonomic experts will provide limited confirmation

services free of charge. However, this good will should not be abused, and an acceptable arrangement for compensation, either monetary or through specimen exchange, should be sought.

Voucher Collections

The BQAU specialist selects one or more examples of each taxon from the reference collections to be placed in a permanent collection as a voucher specimen. Voucher specimens serve as permanent physical representations that substantiate the names applied to organisms collected as part of the NAWQA Program. Voucher specimens ensure the credibility of research results by documenting the identity of the organisms and making them available for review by the general scientific community. They also enhance the endurance of research results by enabling future researchers to determine identities of specimens in light of intervening changes in taxonomy. Given the long-term nature of the NAWQA Program, voucher collections are essential to prevent ambiguities in nomenclature among study units and between NAWQA Program cycles.

The nature of voucher specimens dictates that the organization responsible for the collection must have experience in the long-term maintenance of wet (alcohol preserved) and mounted specimens, adequate quantity and quality of space to store specimens, an effective mechanism for locating and retrieving specimens upon request, and personnel experienced in invertebrate taxonomy. In addition, the organization should have a history that indicates it will continue into the foreseeable future.

Screening criteria need to be developed for identifying organizations that can fulfill this need. Universities, museums, or research institutes with established regional or national reference collections are good candidates for voucher specimen repositories. Preliminary qualifications (see previous section on evaluating candidate contract laboratories) play a large role in qualifying voucher repositories. Additional criteria include a review of cataloging procedures in present or proposed use and a tour of the facilities, including a spot visual check of the condition of stored specimens. Subsequent performance can be evaluated by periodically reviewing the condition of stored specimens, reviewing cataloging procedures, monitoring the speed with which requested specimens can be obtained, and assessing the computerized depository records and interconnectivity with USGS systems. Costs for curating voucher collections can vary widely. An estimate of annual costs to build and maintain a voucher collection according to USGS specifications should be obtained.

Monitoring Contractor Performance

The BQAU is responsible for the continued monitoring of contractor performance during the life of the contract. Among performance issues that should be evaluated are the (1) accuracy of identifications and quantifications, (2) completeness of records, and (3) conditions of reference collections. Monitoring compliance to contract specifications is only part of the process of achieving reliable, high-quality data. Communicating with contractors to identify, analyze, and resolve problems and inefficiencies associated with sample processing is extremely important. There needs to be a continuing dialog among the BQAU, contractors, and NAWQA Program teams aimed at assessing and modifying the procedures, criteria, and techniques used to process samples with the common goal of producing more reliable data faster and more economically.

The guidelines presented in this document should be viewed as a starting point for this process. The BQAU continually strives to improve the quality of data produced by the contact laboratories. Periodic workshops that bring together NAWQA Program personnel, BQAU personnel, and contractors to discuss the field and laboratory objectives of the Program and to share insights into more effective techniques for sample processing are productive devices for promoting communication and sharing ideas.

SUMMARY

Qualitative and semi-quantitative benthic invertebrate samples are collected as part of the USGS's NAWQA Program. These samples are part of a multidisciplinary approach that provides multiple lines of evidence for evaluating water-quality status and trends, and for refining our understanding of the factors that control water quality. The NAWQA Program is designed as a perennial program that encompasses most of the conterminous United States as well as parts of Alaska and Hawaii. The long-term and broad geographic scope of this Program makes it imperative that standardized procedures for the collection, processing, identification, and quantification of biological samples be implemented to facilitate the production of consistent and accurate data that meet local, regional, and national needs.

Qualitative and semi-quantitative benthic invertebrate samples are collected by local teams according to standardized procedures for sample collection and field processing. The semi-quantitative samples are intended to provide information on the structure of the benthic invertebrate communities within selected habitats. The qualitative samples are intended, in conjunction with the semi-quantitative samples, to provide a comprehensive taxa list for each sampling site. Field processing involves visual inspection of the sample to remove obvious, large invertebrates, elutriation to remove sand and gravel, and splitting to keep sample volumes at 750 mL or less. Four sample components can result from field processing: large-rare, main-body, elutriate, and split. The large-rare and main-body sample components are shipped to a contract laboratory for processing. The elutriate and split-sample components are shipped to the BQAU. The BQAU develops specifications and procedures for the processing of invertebrate samples, develops contracts with laboratories, monitors contract laboratory performance, enters contractor data into USGS data bases, monitors the quality of field samples, coordinates taxonomy on a national basis, and maintains study-unit reference collections.

The contract laboratory processes benthic invertebrate samples according to nationally consistent procedures and must meet quality standards established by the BQAU. Sample processing consists of removing invertebrates from the sample matrix (sample "picking"), identifying the invertebrates, and counting the number of individuals in each taxon (semi-quantitative samples) or noting the presence of each taxon (qualitative samples). Sample processing guidelines include options for subsampling to reduce the volume of sample processed. Processing of benthic invertebrate samples results in a number of end products, including QA/QC documents, data sheets, invertebrates, sample remnants, and a study-unit reference collection. This material is returned to the BQAU, which checks the quality of sample processing (for example, effectiveness of sample "picking" and the accuracy of identifications and quantifications).

Standardized processing procedures developed by the BQAU include specifications for QA/QC checks that are performed by the contract laboratories and reported to the BQAU. Processing guidelines also provide numerous examples of standardized laboratory forms, sample labels, and detailed sample processing flow charts. The sample processing specifications establish the format for the delivery of sample data in computer-readable form, the confirmation of identifications by outside experts, sample-tracking procedures, and target levels for taxonomic determinations. In addition to establishing guidelines for the processing of benthic invertebrate samples, these guidelines present criteria and testing procedures for qualifying potential contract laboratories, methods of identifying qualified taxonomic experts, and procedures for establishing voucher collections.

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