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Procedures for Collecting and Processing Aquatic Invertebrates and Fish for Analysis of Mercury as Part of the National Water-Quality Assessment Program

Open-File Report 2008–1208

U.S. Department of the Interior
U.S. Geological Survey

Cover: Processing invertebrates at Oak Creek, Wisconsin (upper left); sorting invertebrates at St Marys River, Florida (upper right); largemouth bass from St Marys River, Florida (lower right); electroshocking for fish in St Marys River, Florida (lower left); brown trout otoliths (in water droplet next to tweezers) from Pike River, Wisconsin (center). (All photographs by the authors, except largemouth bass by Mandy Annis, U.S. Geological Survey.)

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Foreword

The U.S. Geological Survey (USGS) is committed to providing the Nation with credible scientific information that helps to enhance and protect the overall quality of life and that facilitates effective management of water, biological, energy, and mineral resources (http://www.usgs.gov/). Information on the Nation's water resources is critical to ensuring long-term availability of water that is safe for drinking and recreation and is suitable for industry, irrigation, and fish and wildlife. Population growth and increasing demands for water make the availability of that water, now measured in terms of quantity and quality, even more essential to the long-term sustainability of our communities and ecosystems.

The USGS implemented the National Water-Quality Assessment (NAWQA) Program in 1991 to support national, regional, State, and local information needs and decisions related to water-quality management and policy (http://water.usgs.gov/nawqa). The NAWQA Program is designed to answer: What is the condition of our Nation's streams and ground water? How are conditions changing over time? How do natural features and human activities affect the quality of streams and ground water, and where are those effects most pronounced? By combining information on water chemistry, physical characteristics, stream habitat, and aquatic life, the NAWQA Program aims to provide science-based insights for current and emerging water issues and priorities. From 1991–2001, the NAWQA Program completed interdisciplinary assessments and established a baseline understanding of water-quality conditions in 51 of the Nation's river basins and aquifers, referred to as Study Units (http://water.usgs.gov/nawqa/studyu.html).

Multiple national and regional assessments are ongoing in the second decade (2001—2012) of the NAWQA Program as 42 of the 51 Study Units are reassessed. These assessments extend the findings in the Study Units by determining status and trends at sites that have been consistently monitored for more than a decade, and filling critical gaps in characterizing the quality of surface water and ground water. For example, increased emphasis has been placed on assessing the quality of source water and finished water associated with many of the Nation's largest community water systems. In addition, national syntheses of information on pesticides, volatile organic compounds (VOCs), nutrients, selected trace elements, and aquatic ecology are continuing.

The USGS aims to disseminate credible, timely, and relevant science information to address practical and effective water-resource management and strategies that protect and restore water quality. We hope this NAWQA publication will provide you with insights and information to meet your needs, and will foster increased citizen awareness and involvement in the protection and restoration of our Nation's waters.

The USGS recognizes that a national assessment by a single program cannot address all water-resource issues of interest. External coordination at all levels is critical for cost-effective management, regulation, and conservation of our Nation's water resources. The NAWQA Program, therefore, depends on advice and information from other agencies–Federal, State, regional, interstate, Tribal, and local–as well as nongovernmental organizations, industry, academia, and other stakeholder groups. Your assistance and suggestions are greatly appreciated.

Matthew C. Larsen
Acting Associate Director for Water

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Conversion Factors and Abbreviations

Conversion Factors

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
	Volume	
liter (L)	1.057	quart (qt)
liter (L)	0.2642	gallon (gal)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25°C).

Concentrations of mercury in biological tissues are given in micrograms of mercury per gram of tissue (µg/g).

Other Abbreviations

Abbreviations	Definition
DI	Deionized water
$\delta^{13}C$	Stable isotope ratio of carbon ($^{13}C/^{12}C$) expressed per mil (‰)
$\delta^{15}N$	Stable isotope ratio of nitrogen ($^{15}N/^{14}N$) expressed per mil (‰)
Hg	Mercury
ITIS	Integrated Taxonomic Information System
MeHg	Methylmercury
NAWQA	National Water-Quality Assessment Program
NWIS	National Water Information System
THg	Total mercury
USGS	United States Geological Survey
USEPA	United States Environmental Protection Agency
WMRL	USGS Wisconsin Mercury Research Laboratory

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Technical Reviewers

Ann T. Chalmers, Hydrologist, U.S. Geological Survey, Montpelier, Vt.
Karen R. Murray, Ecologist, U.S. Geological Survey, Troy, N.Y.

Technical Support

Amanda H. Bell, Hydrologist, U.S. Geological Survey, Middleton, Wis.
Daniel T. Button, Physical Scientist, U.S. Geological Survey, Columbus, Ohio
Michelle A. Lutz, Physical Scientist, U.S. Geological Survey, Middleton, Wis.
Karen R. Murray, Ecologist, U.S. Geological Survey, Troy, N.Y.

Editorial and Graphics

Jennifer L. Bruce, Geographer, U.S. Geological Survey, Middleton, Wis.
Michelle Greenwood, Reports Specialist, U.S. Geological Survey, Middleton, Wis.
Dale Simmons, Hydrologist, U.S. Geological Survey, West Trenton, N.J., Approving Official
Kevin J. Breen, Acting Bureau Approving Official, U.S.Geological Survey, New Cumberland, Pa.

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Abstract

Mercury studies conducted as part of the U.S. Geological Survey's National Water-Quality Assessment (NAWQA) Program have included nationwide reconnaissance samplings of hundreds of stream sites, as well as detailed, process-oriented research at selected sites. These reconnaissance and detailed studies are intended to provide a better understanding of methylmercury bioaccumulation in stream ecosystems over a range of environmental settings. This publication describes trace-element-clean techniques used for collection and processing of aquatic invertebrates and fish to be analyzed for total mercury, methylmercury, and stable isotopes as part of NAWQA studies.

Introduction

Mercury (Hg) is one of the most widespread contaminants affecting our Nation's aquatic ecosystems, and methylmercury (MeHg) is the most biologically available and toxic form of Hg. Fish-eating (piscivorous) wildlife are at risk for Hg bioaccumulation and associated sublethal health effects (Wiener and Spry, 1996; Wiener and others, 2002). The Mercury Study Report to Congress (U.S. Environmental Protection Agency, 1997) indicated that the predominant route of exposure to MeHg for humans and piscivorous wildlife is fish consumption; the report reviewed the link between anthropogenic Hg sources and MeHg contamination in fish. Studies of human sensitivities, particularly fetuses and children, prompted the U.S. Environmental Protection Agency (USEPA) to establish a water-quality criterion for MeHg in fish of 0.3 (μg/g) wet weight (U.S. Environmental Protection Agency, 2001). The USEPA set this criterion for States or Tribes to consider as an advisory level for human consumption of fish from coastal waters, rivers or lakes.

Sampling for Hg as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Program has consisted of two efforts: (1) reconnaissance (one-time) samplings of Hg in water, streambed sediment, and fish across the United States, and (2) detailed studies conducted across a range of environmental settings, to focus on processes associated with Hg cycling and bioaccumulation in aquatic ecosystems. The first effort was patterned after a 1998 USGS National Mercury Pilot Study (Krabbenhoft and others, 1999; Brumbaugh and others, 2001). The focus of both efforts is to gain an understanding of the factors and processes that control total Hg (THg) and MeHg concentrations in key components of stream ecosystems, and to better understand factors controlling bioaccumulation of MeHg in top predator (predominantly piscivorous) fish, because studies have shown that mature piscivorous fish generally reflect the highest potential Hg concentrations in aquatic food webs (Francesconi and Lenanton, 1992; Wiener and Spry, 1996; Boudou and Ribeyre, 1997; Morel and others, 1998; Kim and Burggraaf, 1999).

Purpose and Scope

The objective of this protocol is to document procedures and provide guidance for collecting and processing benthic invertebrates, forage fish, and top predator fish for quantification of Hg in tissues of aquatic biota. The procedures described in this protocol represent only one component of a multimedia approach to understanding the dynamics of Hg bioaccumulation in stream ecosystems. Sample collection and processing techniques for other media (periphyton, streamwater, streambed sediment, sediment pore water), which also are being assessed as part of the NAWQA Program for Hg and related biogeochemical constituents, are summarized elsewhere (Bell and Scudder, 2004; Lewis and Brigham, 2004; Lutz and others, 2008). Concurrent sampling for THg and MeHg in surface water and streambed sediment provides information about sources and relative bioavailability of Hg in each stream, and ultimately contributes to an understanding of the relative potential of watersheds to convert inorganic Hg to MeHg.

Overview of Sampling Approach

Trace-element-clean techniques are critical to obtaining accurate data on Hg concentrations in the environment; clean techniques minimize direct contact between the sample and potential contaminant sources, such as personnel, equipment, or other objects in the sampling environment. Additional considerations for collection of samples for low-level Hg concentrations in water, sediment, and biota are outlined in Olson and DeWild (1999) and U.S. Environmental Protection Agency (2000). The field methods for NAWQA Hg bioaccumulation studies were established through collaborative efforts of the NAWQA and Toxic Substances Hydrology Programs and researchers from other disciplines in the USGS.

Reconnaissance Mercury Studies

NAWQA reconnaissance Hg studies have included environmental and biological samples collected once per site at locations across the United States in a wide range of geographic settings. These studies have primarily targeted top predator fish, such as largemouth bass or a functionally equivalent piscivorous species, whenever possible. The descriptor "piscivorous" is used to represent fish that are top predators in a particular stream; although these fish may be opportunistic and ingest a range of prey including invertebrates, their diet is predominantly fish. Fish of lower trophic levels have been collected where largemouth bass and other predominantly piscivorous fish were rare or unavailable. During 1998 and 2002, THg was measured in composite samples of fish, with composite samples typically including skin-off fillets from five or fewer fish. Although, whereas composite sampling reduces the cost of sample analysis, analysis of fillets from individual fish provides useful information on the variability of Hg concentrations in fish at a particular site. Thus, during 2004–05, fish were processed individually for THg in skin-off fillets. Only THg was measured in these skin-off axial muscle (fillets) samples because it has been demonstrated that approximately 95 percent of Hg in fish muscle tissue is MeHg (Huckabee and others, 1979; Bloom, 1992; Wiener and Spry, 1996) and because the cost is much greater for a MeHg determination in fish than for a THg determination.

Detailed Mercury Studies

Detailed studies are ongoing in the NAWQA Program to increase understanding of processes associated with Hg cycling in streams and bioaccumulation and transfer of Hg through riverine food webs to top predators. For these studies, the relations between Hg bioaccumulation and food-web complexity are being investigated by evaluating Hg concentrations in representative organisms from different trophic levels in a study stream: algae, benthic/epibenthic invertebrates, forage fish, and top predator fish. The descriptor "forage fish" represents primary consumers (herbivores) or secondary consumers (omnivores or carnivores), which generally are smaller species that serve as forage (prey) for top predator fish. Although forage fish may ingest some smaller fish prey (for example, larvae or juveniles), plant and invertebrate material make up the majority of their diet. Top predator fish and 1-year-old forage fish, all analyzed separately, were recommended by Wiener and others (2007) as the preferred indicators for monitoring trends in Hg bioaccumulation in freshwater ecosystems.

For detailed studies, whole-body invertebrates are analyzed for both MeHg and THg, because previous studies have suggested that the relative proportion of MeHg to THg may not be consistent among invertebrate taxa or across sites (Mason and others, 2000; Haines and others, 2003; Wiener and others, 2007); whole-body forage fish and fillets of top predator fish are analyzed for THg only. Both invertebrates and fish are analyzed for stable isotope ratios of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) to provide additional information on trophic level and food sources (see, for example, Kidd and others, 1995; Finlay and others, 2002). In addition, age determination and optional gut-content analysis are conducted on top predator fish.

If resources are available, detailed studies in specific watersheds may include additional objectives not covered in this report.

Pre-Field Activities

A brief summary of pre-field activities and a suggested list of supplies and equipment are presented in this section, but these are by no means exhaustive. Sufficient planning and preparation are critical to the collection of high-quality data, and each research group should evaluate these recommendations with respect to their specific study areas and target species.

Select Sampling Sites and Target Species

New sampling sites must be established in the USGS National Water Information System (NWIS) database (http://waterdata.usgs.gov/nwis) **before** sampling so that the database is able to accept site information and analytical results.

Although site-selection criteria are determined by program-level research goals, an important consideration in site selection is the presence of sufficient numbers of the desired top predator fish in the appropriate size/age range. The use of target taxa allows for comparison among sites across geographical areas. Biota collected should reflect, as much as possible, the existing community structure; primary producers (algae) and primary and secondary consumers (invertebrates and forage fish) should be representative of the simplified food chain associated with the target top predator fish.

Once sites are selected, target and alternate species are identified for each trophic level (primary consumer, secondary consumer, and top predator) for each site where historical community data exist. Where community data do not already exist, community structure and feeding relationships for each site may be established through literature review, review of existing data, and consultation with specialists at universities and environmental agencies (such as State and Federal fish and wildlife agencies).

At least two key invertebrate taxa should be targeted for sampling. These taxa should represent different functional feeding categories (for example, scrapers, shredders, grazers, collectors/gatherers) and should be taxa and sizes that are considered important prey items for target forage and (or) top predator fish. At least one of the invertebrate taxa should be in the scraper feeding category, feeding on periphyton (attached algae). Invertebrates are sorted to the lowest practical taxon (preferably at least genus level) and processed as single-taxon composites. For example, one or more species of net-spinning caddisfly larvae in the family Hydropsychidae could form a composite sample and one or more species of tube-case-maker caddisfly larvae in the family Brachycentridae could form another composite sample. Net-spinning and tube-case-maker caddisflies should not be lumped into a single composite sample, however, because of different Hg bioaccumulation potentials. For all sites, alternate species of invertebrates should be identified to serve as functionally equivalent substitutes in case adequate numbers of primary target species are not available. Invertebrates should be removed from their cases (if any) before being put in composite samples. Some invertebrates are unsuitable because of their small size, as expending the time needed to collect sufficient mass may not be feasible; other invertebrates are unsuitable if their large size makes them an unlikely food item for the target fish collected. Note that it is difficult to obtain clean, non-contaminated tissues from molluscs, particularly gastropods (snails); these organisms should be collected only when no alternative taxa can be obtained in sufficient numbers. If molluscs are collected, the soft tissues should be analyzed without shell material; although wildlife ingest the entire snail, for example, dietary contributions are derived primarily from the soft tissues. Freeze-drying prior to forceps extraction of soft tissues produces a clean tissue sample, but this process is difficult and time-consuming.

Two species of forage fish and one species of top predator fish should be collected at each site; these will be identified in the field to the lowest possible taxonomic category and processed as individuals. Alternate species of acceptable forage fish and top predator fish should also be identified for all sites to serve as functionally equivalent substitutes in the case that adequate numbers of primary target species are not collected. Use size/age relationships to target top predators and size (forage size) to target forage fish. In choosing size ranges for top predators, select sizes that approximate mature fish (3-4 years of age). Study personnel should be aware that fish size can vary greatly by age and by region, and by gender within a given region (Carlander, 1969; 1977). Historic size/age relations can be used to determine the appropriate size ranges of target species for a specific site, and state and local environmental agencies commonly will be able to provide information regarding distribution, relative abundance, and size/age ranges of various species at specific sites.

A species list of preferred top predator fish with associated taxonomic codes from the multi-agency Integrated Taxonomic Information System (ITIS) is provided in table 1. Species in table 1 are listed in decreasing order of preference for sampling. The priority list is based on national distribution of each fish species, the degree to which each species is piscivorous, and other criteria. The list is biased toward widely distributed species; other primarily piscivorous species may be used if necessary. Black basses (Centrarchidae: *Micropterus*) should be targeted whenever possible because they are widely distributed and because they are largely piscivorous and relatively long-lived (maximum age is approximately 6-9 years) (Becker, 1983; Hoyer and Canfield, 1994). Because of their diet, size, and longevity, black basses typically have higher body-burdens of Hg than smaller, younger, and less piscivorous fish (Kidd and others, 1995; Stafford and Haines, 1997).

Where no historical community or species-specific data exist, the target top predator should be selected from the priority list (table 1) based on site availability and, if possible, confirmed by reconnaissance visits. One or two species should be identified that can be collected across all study areas, with preference toward collection of the same species used by adjacent NAWQA study areas for other regional and national Hg studies. Invertivorous fish may be collected if piscivorous fish are not available. If no predominantly piscivorous or invertivorous fish are available, yet the site is highly desirable for other reasons, an omnivorous fish species may be collected, although species that are largely omnivorous (for example, carp (*Cyprinus carpio*) or suckers (Catostomidae)) should be avoided if possible. Before resorting to sampling a non-piscivorous fish, extend the sampling effort upstream or downstream within a stream segment (between intervening major inputs or confluences) to make sure that all habitats have been investigated for alternative piscivorous fish. In some cases, targeted environmental settings may occur only in relatively small streams with poor fish communities — for example, small urban streams. After exhausting attempts to obtain target fish species, collection of alternate species may be necessary. Select target fish that are resident species (no anadromous, highly migratory, or recently stocked fish); avoid sampling areas where fish are likely to move in and out of other major bodies of water (for example, near a confluence with lakes or large rivers).

Table 1. Target and alternate top predator fish species and Integrated Taxonomic Information System (ITIS) codes.

[Species listed in decreasing order of preference for sampling. Priority is based primarily on national distribution of each fish species and degree to which each species is piscivorous]

Family name	Common name	Scientific name	ITIS code
		Target	
Centrarchidae	Largemouth bass	*Micropterus salmoides*	168160
	Smallmouth bass	*Micropterus dolomieu*	550562
	Spotted bass	*Micropterus punctulatus*	168161
Esocidae	Northern pike	*Esox lucius*	162139
Percidae	Walleye	*Sander vitreus*	650173
	Sauger	*Sander canadensis*	650171
	Yellow perch	*Perca flavescens*	168469
Salmonidae	Brown trout	*Salmo trutta*	161997
		Alternate	
Esocidae	Chain pickerel	*Esox niger*	162143
	Muskellunge	*Esox masquinongy*	162144
Cyprinidae	Northern pikeminnow[1]	*Ptychocheilus oregonensis*	163523
Moronidae	White bass	*Morone chrysops*	167682
Ictaluridae	Flathead catfish	*Pylodictus olivaris*	164029
	Channel catfish	*Ictalurus punctatus*	163998
Centrarchidae	Black crappie	*Pomoxis nigromaculatus*	168167
	White crappie	*Pomoxis annularis*	168166
	Bluegill	*Lepomis macrochirus*	168141
	Rock bass	*Ambloplites rupestris*	168097
	Redbreast sunfish	*Lepomis auritis*	168131
	Green sunfish	*Lepomis cyanellus*	168132
Salmonidae	Rainbow trout	*Onchorhynchus mykiss*[2]	161989
	Cutthroat trout	*Oncorhynchus clarkii*	161983

[1] Formerly Northern squawfish

[2] Formerly *Salmo gairdneri*

Prepare Sampling Plan

Biological, water, and streambed-sediment samples should be collected concurrently (optimal) or within a sufficiently short time period (2 weeks for invertebrates and forage fish; 4 weeks for top predator fish) of other chemical sampling to minimize hydrologic or other environmental changes that would confound relevant associations among sampled media. Invertebrate and fish taxonomists should be consulted prior to sample collection, and sampling plans for fish must be included in an approved NAWQA quality-assurance/quality-control plan for each study unit, following the guidance provided in Walsh and Meador (1998).

Logistics for sample-collection and processing procedures for all media must be coordinated prior to conducting fieldwork in order to minimize risk of site disturbance and sample contamination. Protocols for sample collection, processing, labeling, and submission and for data management also should be reviewed by all field personnel prior to sample collection (see appendixes 1a-1d). A summary of invertebrate and fish sample-collection and sample-processing procedures is provided in table 2. Field personnel should carefully review sampling plans and forms prior to conducting field work. All personnel must be familiar with trace-element-clean techniques, including “clean hands/dirty hands” methods, to minimize contamination and avoid

compromising sample quality (U.S. Environmental Protection Agency, 1996; Olson and DeWild, 1999). All field personnel should have predefined roles to ensure efficiency and to prevent cross-contamination. For example, one individual might be assigned to note-taking, preparation of sample containers and labels, and washing equipment; another could be assigned to sorting, cleaning, and processing sampled organisms. The order of sites sampled should proceed from lowest to highest suspected Hg contamination. Prior to beginning sampling at a site, potential invertebrate and sediment collection areas should be noted to minimize their disturbance. At each site, a typical order for sample collection would be water, followed by invertebrates, then sediment, and finally fish.

Obtain Permits and Permissions

Federal and State collection permits and additional licenses (for example, sport fishing licenses and trout stamps) may be required for collections of invertebrates and fish. Walsh and Meador (1998, p. 7) provide a table of agencies responsible for permitting in each State. Additionally, working on Federal or Tribal lands will require special permissions that should be sought months in advance of sampling. A consultation with local U.S. Fish and Wildlife Service personnel with regard to Section 7 of the Endangered Species Act (assessment of federally endangered or threatened species in the stream or watershed) is recommended and may be required in some locations; consultation with State and Federal personnel is required for sampling streams with federally listed threatened and endangered species (http://www.fws.gov/endangered/consultations/). Landowner permissions may be required for site access when sampling on private property or when access requires crossing private property. Note that State policies regarding ownership of the stream bottom (public or private) vary.

Prepare Equipment and Supplies

Suggested equipment and supplies are listed in appendix 2. Supplies and equipment that come in contact with organisms should consist of new or clean plastic (Teflon®, polypropylene, polyethylene, or polyethylene terephthalate whenever possible). Small scintillation vials

Table 2. Summary of invertebrate and fish sample collection and processing methods.

[g, gram; THg, total mercury; MeHg, methylmercury; $\delta^{13}C$ and $\delta^{15}N$, stable isotopes of carbon and nitrogen]

Sampling frequency (per year)	Number of taxa	Number and type of sample	Organism age	Sample wet weight (g)	Body part	Laboratory analyses	Laboratory[1]
			Invertebrates				
2	2	3, composite	larvae	1	whole	THg, MeHg,	USGS–WMRL
						$\delta^{13}C$, $\delta^{15}N$	USGS–FL
			Forage fish				
2	2	12, individual	1+ years	5	whole	THg, MeHg,	USGS–WMRL
						$\delta^{13}C$, $\delta^{15}N$	USGS–FL
			Top predator fish				
1	1	12, individual	3 to 4 years	5	fillet (skin off)	THg	TERL
						$\delta^{13}C$, $\delta^{15}N$	USGS–FL
						age/otolith	TBD
						gut[2]	TBD

[1] USGS–WMRL, U.S. Geological Survey Wisconsin Mercury Research Laboratory, Middleton, Wis. (contact: John DeWild, jfdewild@usgs.gov)
USGS–FL, U.S. Geological Survey Florida Integrated Science Center, Tallahassee, Fla. (contact: Lia Chasar, lchasar@usgs.gov)
TERL, Trace Element Research Laboratory, Texas A&M University, College Station, Tex. (contact: Robert Taylor, rtaylor@ocean.tamu.edu)
TBD, to be determined.

[2] Denotes optional gut analysis.

(polyethylene terephthalate, polypropylene, or polyethylene) are also acceptable for invertebrate samples. When in doubt, consult the USGS Wisconsin Mercury Research Laboratory (WMRL) in Middleton, Wisconsin regarding container recommendations. Fine-tipped plastic forceps work well for hand-picking invertebrates. High-grade stainless steel knives may be used for filleting and dissecting fish. A stainless steel dredge, such as an Ekman or a Ponar, may be used to collect benthic invertebrates from non-wadable sites; however, efforts should be made to collect a subsample from dredge materials that do not come into direct contact with the surface of the dredge.

Prepare assembled equipment and supplies to minimize the potential for sample contamination. New, sealed supplies such as zip-seal plastic bags and plastic vials with plastic caps do not need pre-cleaning for the purposes described in this biological sampling protocol. Prior to field work, clean all other supplies and equipment in a dilute (0.1-percent by volume) solution of non-ionic surfactant detergent (Liquinox®) by soaking equipment for 30 minutes and then using a plastic scrub brush to scrub all surfaces. Rinse equipment with copious amounts of tap water because residual detergent on supplies or equipment could contaminate tissues for stable carbon isotope and other chemical analyses. All non-metal equipment (for example, cutting boards, trays, plastic forceps, plastic sieves) should then be soaked in 5-percent hydrochloric acid (trace metal grade such as Omni Trace®) for 8 to 24 hours prior to initial sampling and triple rinsed in deionized (DI) water (<0.055 µS/cm). Fillet knives and other stainless steel tools should not be cleaned with acid; these tools should be cleaned with dilute detergent solution, rinsed with copious amounts of tap water, triple rinsed with DI water, and air dried completely prior to storage in order to minimize rusting. Small, high-grade stainless steel disposable dissecting knives are an option for processing forage fish (Brumbaugh and others, 2001; Wilde and others, 2004). After they are cleaned, supplies and smaller equipment should be double-bagged in new plastic bags and stored in sealed containers to minimize contamination; supplies should be cleaned and packed separately for each site to minimize the need for field cleaning (Brumbaugh and others, 2001; Wilde and others, 2004). Large, new plastic bags can be used to wrap nets and other gear so that they do not directly contact truck beds or other potentially highly contaminating surfaces. In the field, all equipment should be cleaned between sites by scrubbing with dilute Liquinox® and rinsing with copious amounts of tap water, triple rinsing with DI water, rinsing with 5-percent hydrochloric acid (non-metal items only), and again triple rinsing with DI water.

Prepare and Review Field Forms and Labels

Field personnel should preview field forms, sample labels, and laboratory submission forms. See section on Field Data Forms and Sample Labels later in this report. Examples of forms and labels are provided in appendixes 1a-1d and a list is provided in table 3. Field forms and labels should be preprinted with station name, USGS station number, analyte, medium code, and contact information (name and telephone number). This is not only a valuable time-saving measure for field work but an important tool for to minimizing errors on field forms.

Table 3. Appendixes containing forms and labels for invertebrate and fish sample collection and processing methods.

[THg, total mercury; MeHg, methylmercury; $\delta^{13}C$ and $\delta^{15}N$, stable isotopes of carbon and nitrogen; --, not provided in report]

Field form	Laboratory analysis	Sample label	Laboratory submission form
Invertebrates			
Appendix 1a-1	THg, MeHg	Appendix 1b-1	Appendixes 1c-1, 1d-1, 1d-2
	$\delta^{13}C$, $\delta^{15}N$		Appendix 1c-2
Forage fish			
Appendix 1a-2	THg	Appendix 1b-2	Appendix 1c-3
	$\delta^{13}C$, $\delta^{15}N$	Appendix 1b-2	Appendix 1c-4
Top predator fish			
Appendix 1a-2	THg	Appendix 1b-2	Appendix 1c-3
	$\delta^{13}C$, $\delta^{15}N$	Appendix 1b-2	Appendix 1c-4
	age	Appendix 1b-3	Appendix 1c-5
	gut[1]	--	--

[1] Denotes optional gut analyses

Quality Assurance and Quality Control

Guidance provided in this protocol is intended to assure the collection of accurate, high-quality data. Contamination of samples is a possibility at every stage, including equipment preparation and collection, processing, storage, and shipment of samples to analytical laboratories. Therefore, it is critical to ensure proper planning at all stages and to complete training of personnel in trace-element-clean sample-collection and sample-processing techniques. As mentioned earlier, a quality-assurance/quality-control plan for fish sampling that is specific to your NAWQA study unit must be approved by prior to sample collection. Maintaining adequate supplies of gloves and other expendable items during field work further enables personnel to adhere to clean sampling and processing techniques.

Quality-assurance samples are collected to investigate field and laboratory variability. Triplicate invertebrate composite samples and multiple individual fish samples in this protocol serve as quality-assurance replicates for field variability of biological samples. Quality assurance for laboratory analyses should include duplicate runs, use of certified or standard reference sample materials from the National Institute of Standards and Technology or other provider, and notification when quality-control data are outside acceptable ranges. Voucher specimens for taxonomic confirmation should be preserved in 70-percent ethanol (invertebrates) or in 10-percent buffered (pH = 7) formalin (invertebrates or fish) and retained until the conclusion of the study.

Sample Collection and Processing

Biota may be collected by any means that do not result in contamination of their tissues for chemical analyses. Biologists familiar with the study area, such as county and state agency biologists, can be excellent resources for the best sampling strategies for invertebrates and fish at sites within their jurisdictions.

Samples must be processed in a clean environment. Processing at the site, or a nearby outdoor area, is acceptable if a stable, clean work area is available. Processing in an enclosed facility, such as a field laboratory, is also acceptable. Avoid sources of contamination, such as dusty roads, heavy traffic, older field and laboratory vehicles, or older buildings where dust and (or) particle-shedding from building materials (for example, paneling, or ceiling tiles) might be of concern. Avoid facilities or vehicles where any forms of Hg, including Hg-based preservatives and manometers, have been used. Cover all work surfaces with new clear plastic sheeting or bags. Wear disposable, powder-free plastic gloves during all sample processing. Change gloves frequently, particularly after touching any unclean surface. Clean, thicker reusable plastic gloves, such as dishwashing gloves, may be used for fish handling where thinner, disposable gloves would tear upon contact with spines of a fish. For reusable gloves, wash outsides thoroughly with detergent solution, tap water, and DI water between sampling sites.

Care should be taken during sample processing and storage to minimize desiccation as aquatic invertebrates can contain approximately 70 to 90 percent water (Sugden, 1967; Glazier, 1992; Daniel Cain, U.S. Geological Survey, oral commun., 2004), and fish contain approximately 75 to 80 percent water (Lantry and others, 1999). Low sample weights resulting from desiccation will bias the calculation of “wet-weight” Hg concentrations from reported dry-weight values. Therefore, the smallest appropriate sample container should be used, and all air expressed from zip-seal bags when used. Processed samples should be preserved immediately on dry ice for transport to a freezer or analytical laboratory and analyzed as soon as possible, preferably within 6 months.

Invertebrates

Invertebrate sampling should be conducted a minimum of two times per year to account for seasonal variation in species presence or abundance, and in Hg concentration and other biogeochemical parameters in environmental media. Invertebrates may be collected by netting with a clean net, such as a D-frame with mesh size appropriate to the target organism(s) and the field conditions; by using plastic sieves (Nalgene); or by hand picking with gloved hands and plastic forceps into plastic bags or containers. Collect at least 1 g wet weight for each composite sample of invertebrates to ensure sufficient biomass for analyses (minimum of 0.1 g dry weight) (Hall and others, 1998; John DeWild, U.S. Geological Survey, oral commun., 2005). Depending on the size of invertebrate collected, the number of individuals needed to obtain 1 g wet weight will vary but, in any case, should not be less than 15 individuals for large invertebrates such as crayfish or less than 30 individuals for small invertebrates such as caddisfly larvae. Field personnel should attempt to be consistent with selection of species and selection of size classes within a species. To allow for any natural variability in tissue Hg concentrations, collect each taxon from as broad a range of locations within a reach as possible. Artificial substrates may be considered in certain situations if sufficient biomass cannot be collected from natural substrates.

Invertebrates typically are processed as three replicate composite samples for each species with the same number of organisms, of similar size, in each composite sample. Compositing yields two benefits. First, for small organisms, compositing yields sufficient sample mass for chemical analysis. Second, chemical concentrations may vary considerably among individual organisms at lower trophic levels, and compositing effectively averages this variability.

Minimize holding (depuration) times to less than a few hours per site. At least five voucher specimens of each taxon should be collected for taxonomic confirmation. Aerators can help keep invertebrates alive until processing. It also may help to keep them in zip-sealed bags inside containers that are set on wet ice in a cooler.

Single-taxon invertebrate composites are processed prior to leaving the field site to ensure that adequate sample mass has been obtained. Each composite sample of invertebrates is analyzed for $\delta^{13}C$, $\delta^{15}N$, THg, and MeHg (no analyses for isotopes of mercury). The isotope laboratory will freeze-dry and homogenize each composite sample, then subsample the homogenate for analyses of stable isotopes, THg, and MeHg. If sample mass is insufficient for all three analyses, the priority order of analysis is given to MeHg, followed by stable isotopes and finally, THg.

Processing steps for invertebrates:

1. Set up the work surface for processing: The table or counter should be covered with a new plastic sheet or bag. The plastic should be secured (for example, with duct tape) to provide a clean and stable work surface.
2. Preliminary sorting and cleaning of invertebrates may be accomplished with clean plastic ice cube trays (fig. 1). Invertebrates should be sorted to the target taxon, rinsed with DI (for example, by dipping in DI in a clean ice cube tray cube), and picked free of obvious debris.
3. Invertebrates should then be blotted with clean lint-free paper wipes (such as Kimwipe® or Kaydry®) to remove excess water (for smaller organisms, this can be accomplished by placing on lint-free paper wipes), and sorted into three separate tared (to the nearest 0.01 g) plastic or Teflon® containers for three replicate composite samples of 1 g each.
4. Weigh each composite sample to the nearest 0.01 g using an appropriate field-portable scale. Composite field weights (final weight - tare weight) are for general estimates to ensure that minimum weights have likely been achieved in the field; chemical analyses will be performed on freeze-dried tissue. Optimal sample mass is a minimum of 1 g wet weight. Composite field weights can be useful for comparing laboratory-dried weight to field weight in computation of moisture content.

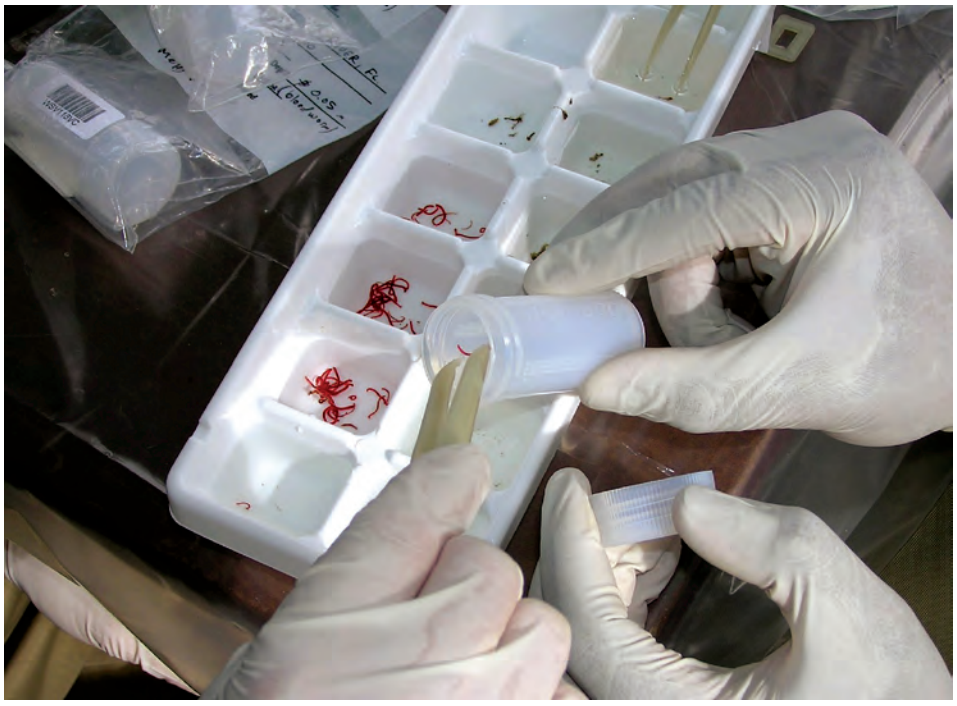


Figure 1. Field-processing methods for invertebrates. Preliminary sorting and cleaning can be done with plastic forceps and clean plastic ice-cube trays filled with deionized water. Photograph by Mark Brigham, U.S. Geological Survey.

Fish

A variety of fish-collection procedures may be appropriate, depending on site conditions and target species. General fish-collection procedures are described elsewhere (Meador and others, 1993; Moulton and others, 2002), and rely primarily on electrofishing (backpack, barge, and boat). Other methods in combination with or in lieu of electrofishing, such as seining or rod-and-reel (with artificial lures), or passive gear such as traps or nets, may be more effective at some sites and are acceptable. After capture, fish should be placed in a live cage/net in the stream (large top predator fish only, as small forage fish may be damaged), a large aerated bucket, or an aerated live well to minimize stress until processing, to keep fish alive, and to allow any unneeded fish to be returned live to the stream. Guidelines for live specimen handling and care are provided in Walsh and Meador (1998). Fish can be anesthetized using carbon dioxide from carbon dioxide-producing tablets, such as Alka-Seltzer® (2-4 tablets per gallon of water in bucket or other container). After anesthetization, fish can be euthanized by additional carbon dioxide (recommended by Walsh and Meador, 1998) or a sharp blow to the base of the skull. If fish are to be processed in a location other than where they were collected, place them in clean zip-seal bags on wet ice and process them within 24 hours to minimize possible loss of sample integrity.

Forage Fish

Twice per year, preferably together with collection of water, invertebrates, and (or) periphyton, collect two species of forage fish that are suspected important prey items of the targeted top predator fish. At each site, the goal is to collect 12 individuals of each species of similar size/length with consideration to sizes of forage fish that the top predator fish could ingest. Avoid very small fry (for example, young of year) fish, primarily because of the greater risk of potential taxonomic misidentification and also the greater possibility of mercury concentrations below analytical detection capabilities. Individual forage fish are analyzed whole for THg and stable isotopes. In general, age determination will not be done on forage fish.

Processing steps for forage fish:

1. Set up the work surface for processing: The table or counter should be covered with a new plastic sheet or bag. The plastic should be secured to provide a clean and stable work surface. All field personnel participating in processing should be gloved. The person measuring and handling the fish should change gloves after touching any surface, other than the fish sample, that is not trace-element clean.
2. Set up a cleaned, plastic cutting surface (sheet or board). Use cleaned knives and dissecting tools for processing the Hg tissue sample.
3. Rinse each fish three times with DI water to remove excess debris and slime; this can be done by placing the whole fish in doubled heavy-weight zip-seal bags filled with DI water and gently shaking the bag while supporting the bottom seam. Smaller fish can be held in a clean gloved hand over a new plastic bag (in case the fish is dropped), or placed directly in the bag, and rinsed with a squirt bottle filled with DI water. The gloved (new gloves for each fish), clean-hands person should remove the fish from the bag. Be careful of dorsal spines during this process. If a fish drops to the ground, use a new fish and dispose of the soiled fish with remains of processed fish.
4. Measure each fish for total length to the nearest 0.1 cm using a clean fish-measuring board or a flat ruler covered with a clean plastic sheet.
5. Weigh each fish to the nearest 0.1 g (or 0.01 g, especially if fish weight is <1.0 g) in a clean tray on the scale; the tray should be covered with a new piece of plastic (such as a heavy-weight zip-seal bag) for each fish. Be sure to tare the scale with the bag in place before weighing the fish.
6. Place whole fish in an appropriately labeled plastic container or heavy-weight zip-seal bag that minimizes trapped air around the tissues. Label the container (vial or zip-seal bag), and place the labeled sample container inside a heavy-weight zip-seal bag; ensure that label and bar code for each sample are adjacent to each other on the inner sample bag. Placing each sample into an outer zip-seal bag minimizes the risk of sample labels becoming detached, as sometimes happens when samples are frozen.
7. In general, age of forage fish will not be determined. If age of forage fish is to be determined, first remove the head of the fish immediately posterior to the outside curve of the operculum (fig. 2) and place head in sample container. Rinse the fish body well with DI water prior to placing it in an appropriately labeled small plastic zip-seal bag. Double bag with second heavy-weight zip-seal bag as described above.
8. Place all separately bagged fish samples of the same species and of similar size from a site together into a larger plastic bag to keep each sample type for each site together in one large plastic bag.
9. After the samples have been processed, immediately place them under dry ice in an insulated portable cooler and keep frozen until shipment to analytical laboratories.

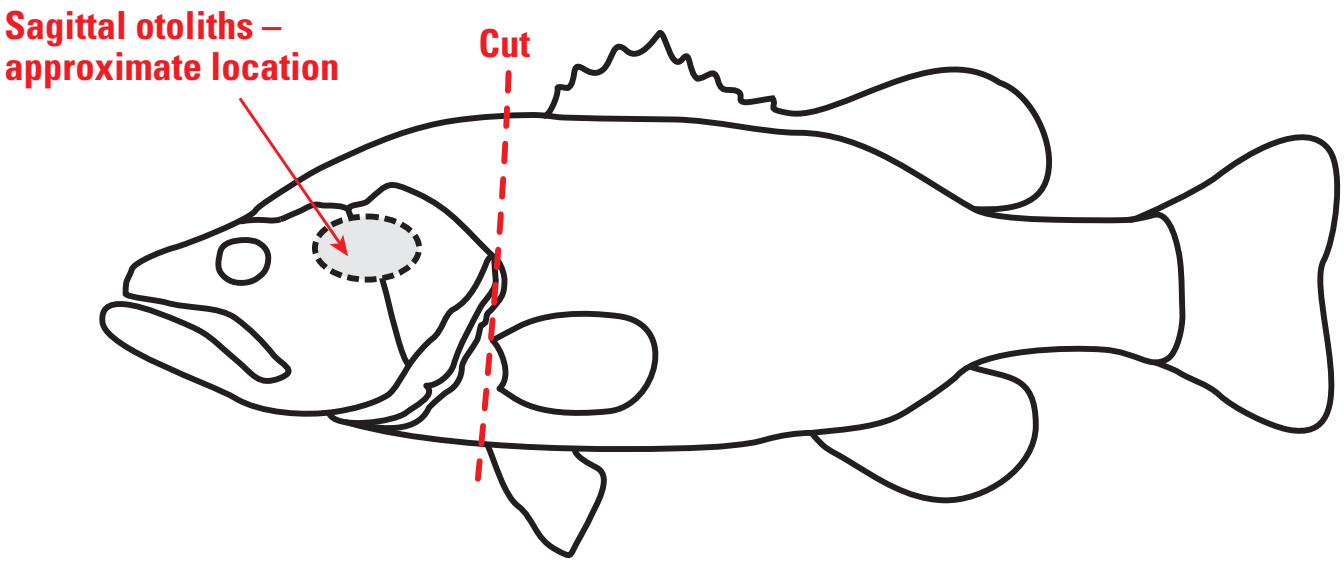


Figure 2. Diagram showing approximate internal location of sagittal otoliths, using largemouth bass as an example. The location of cut shown minimizes potential damage to otoliths when removing the head for subsequent otolith excision or when removing the fillet. Diagram by Lia Chasar, U.S. Geological Survey.

10. Between consecutively processed fish at a site, clean cutting board, knife, and dissecting tools with triple rinses of DI.

11. Place all used disposable sharp items, such as scalpel blades, in a hard plastic container and seal. Place all other waste in doubled plastic trash bags. Transport all waste to appropriate disposal location; do not leave on site.

Top Predator Fish

Once per year, preferably together with the second sampling of invertebrates and forage fish, collect one species of top predator fish for which the feeding preference is known and is well described. Top predator fish generally are longer lived with slower tissue turnover than forage fish and have a temporally integrated diet history (Hesslein and others, 1993), so they do not have to be collected at the same time as the rest of the biota. At each site, collect the target top predator fish with the goal of collecting 12 mature individuals of one species with similar size (approximate age 3-4 years). The Hg content of some fish species changes as the fish matures and grows large enough to change from a diet of invertebrates to a diet of fish (Mathers and Johansen, 1985). If enough of the target fish species in the age range of 3 to 4 years cannot be found, then fill out the remaining number of individuals needed with ones as close to the target age as possible to total 12 individual fish of the target species. A second species of piscivorous fish may be sampled if availability of the primary target species is limited and (or) there is interest in two key species at a site, if time and funding allow. When two or more fish species are sampled, collect at least six individual fish of each species.

Process fish immediately, or keep on wet ice and process within 24 hours. Each fish should be processed and analyzed separately. For top predator fish, otoliths are collected for age determination, and gut contents may be collected for short-term diet information. To help minimize risk of sample contamination, tissues for Hg and isotope analyses are collected first, followed by tissues for age determination and optional gut contents. Extreme care must be taken during dissection, as fluids from punctured internal organs, such as the gall bladder and intestinal tract, can contaminate muscle tissue intended for Hg analyses.

Processing steps for top predator fish:

1. Set up the work surface for processing: The table or counter should be covered with a new plastic sheet or bag. The plastic should be secured to provide a clean and stable work surface. All field personnel participating in processing should be gloved. The person measuring and handling the fish should change gloves after touching any surface, other than the fish sample, that is not trace-element clean.

2. Set up a cleaned, plastic cutting surface (sheet or board). Use cleaned knives and dissecting tools for processing the Hg tissue sample.

3. Rinse each fish three times with DI water to remove excess debris and slime; this can be done by placing the whole fish in doubled heavy-weight zip-seal bags filled with DI water and gently shaking the bag while supporting the bottom seam. The gloved (new gloves for each fish), clean-hands person should remove the fish from the bag. Be careful of dorsal spines during this process. If a fish drops to the ground, use a new fish and dispose of the soiled fish with remains of processed fish.

4. Measure each fish for total length to the nearest 0.1 cm using a clean fish-measuring board or a flat ruler covered with a clean plastic sheet.

5. Weigh each fish to the nearest 0.1 g in a clean tray on the scale; the tray should be covered with a new piece of plastic (such as a heavy-weight zip-seal bag) for each fish. Be sure to tare the scale with the bag in place before weighing the fish.

6. For a right-handed person, place fish on its right side with head pointing to the left (reverse for left-handed person). Before cutting, note location of sagittal otoliths (fig. 2).

7. CUT-1:

    a. Fish with scales: While holding the head with the one hand, position the knife perpendicular to the fish on its dorsal part, with the blade posterior to the gill cover and angled slightly away from the head. Cut through the scales, skin, and flesh of the fish until the knife reaches the backbone (fig. 3). Wipe scales and debris from the knife blade with a lint-free paper towel and rinse the knife with deionized water.

    b. Fish without scales: While holding the head with the left hand, position the knife perpendicular to the fish with the blade behind the left gill cover and pectoral fin and the tip of the knife point forward toward the dorsal part of the head. Cut through only the skin from the backbone down to the bottom of the belly. Use cleaned stainless steel pliers (with serrated teeth on jaws) or “fish-skinning pincers” to grip skin and, while holding head, pull toward tail to remove skin. Continue pulling sections of skin until entire left axial muscle is exposed.

8. CUT-2: Starting at cut-1, slice through the dorsal part of the fish parallel and immediately adjacent to the dorsal fin; cut along the dorsal spines toward the base of the tail fin until just past the posterior edge of the dorsal fin. Cut 2b: Extend Cut 1 ventrally and deepen it to the rib cage (fig. 4, cut 2b).

9. CUT-3: Cut around the rib bones in a slight arc to the ventral part of the fish and near the vent or anus while avoiding cutting near or into the central body cavity where body fluids could contaminate the fillet sample (fig. 5, cut 3a). Continue to extend cut 3a along the ventral part of the fish from the area near the vent toward the tail; also deepen cut 2a along dorsal part of fish and extend the cut to within 1 to 2 cm from the base of the tail fin with the fillet still attached to the tail section (fig. 5, cut 3b). For fish without scales, cut the fillet free from tail and proceed to step 11.

10. CUT-4: Turn the fillet over so the scale side is facing the cutting surface. Starting near the base of the tail fin, slice through the fillet just above the skin layer, letting the knife ride on the inside surface of the skin and leaving the skin and scales attached to the fish (fig. 6). Remove the rib cage and any large bones if still on the fillet.

11. Rinse the fillet copiously with DI water over clean plastic or inside a clean zip-seal plastic bag. Drain water from fillet, tare field scale to a new zip-seal plastic bag that has been double-rinsed with DI water, place fillet in this bag, and weigh to nearest 0.1 g. Push air from bag and seal to minimize trapped air around the tissues. Label bag and place in second heavy-weight zip-seal bag; ensure that label and bar code for each sample are adjacent to each other on the inner sample bag. Placing each sample into an outer zip-seal bag minimizes the risk of sample labels becoming detached, as sometimes happens when samples are frozen. Collect only one fillet from each fish unless sample mass is insufficient.

12. Keep each sample for a site together in one large plastic bag by placing all separately bagged and labeled fish fillets of the same species and of similar size together into a larger plastic bag,

13. Immediately place sample fillets under dry ice in an insulated portable cooler and keep frozen until shipment to analytical laboratories.

14. Incise the abdomen of the filleted fish for gender determination and optional gut content removal (fig. 7).

15. Note the gender of each fish (if mature) or note immature on the field form.

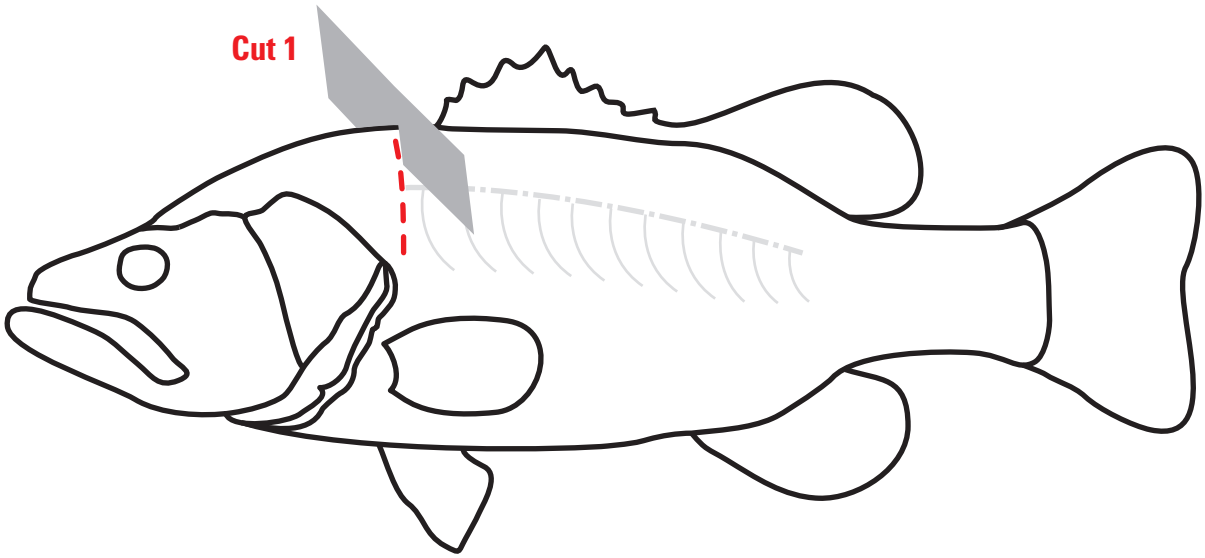


Figure 3. Diagram and photograph illustrating Cut 1 in dissection of top predator fish. While holding the head with the one hand, position the knife perpendicular to the fish on its dorsal part, with the blade posterior to the gill cover and angled slightly away from the head. Cut through the scales, skin, and flesh of the fish until the knife reaches the backbone. Wipe scales and debris from the knife blade with a lint-free paper towel and rinse the knife with deionized water. Diagram by Lia Chasar, U.S. Geological Survey. Photographer unknown.

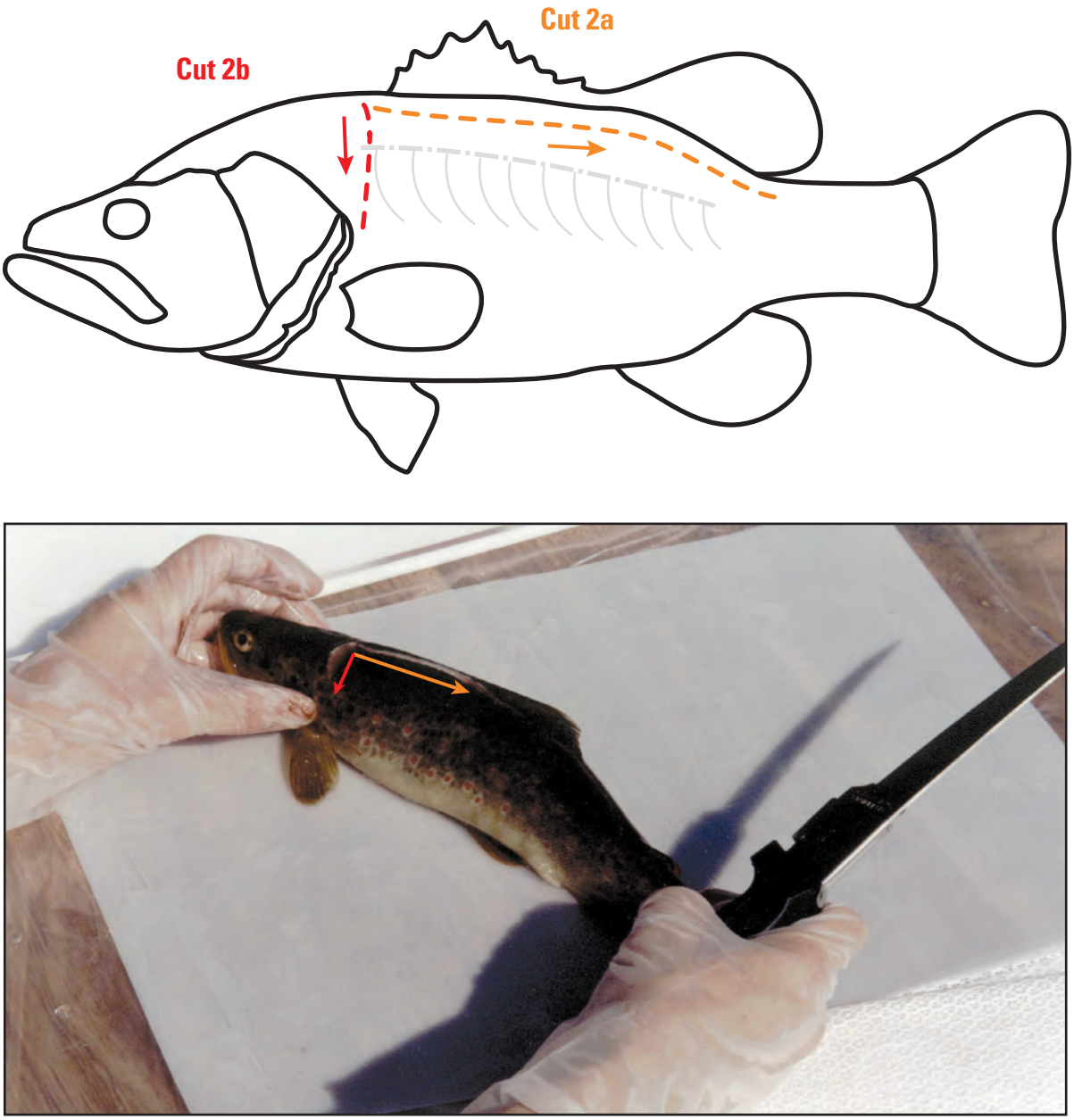
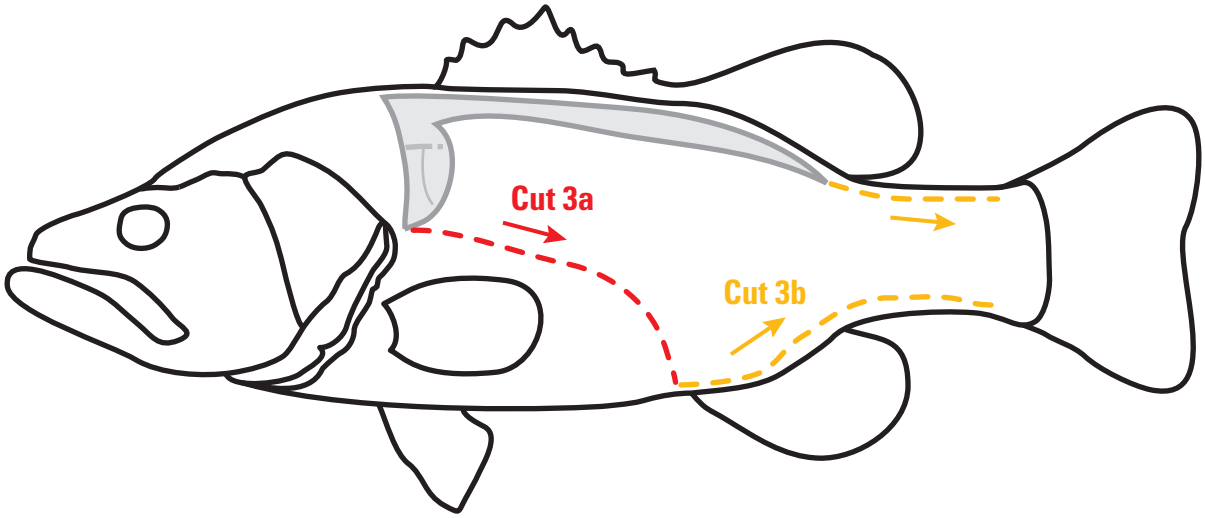


Figure 4. Diagram and photograph illustrating Cut 2a and 2b in dissection of top predator fish. **Cut 2a:** starting at Cut 1, slice through the dorsal part of the fish parallel and immediately adjacent to the base of the dorsal fin; cut along the dorsal spines toward the tail fin until just past the posterior edge of the dorsal fin. **Cut 2b:** extend Cut 1 ventrally and deepen it to the rib cage. Drawing by Lia Chasar, U.S. Geological Survey. Photograph by Mitch Harris, U.S. Geological Survey.



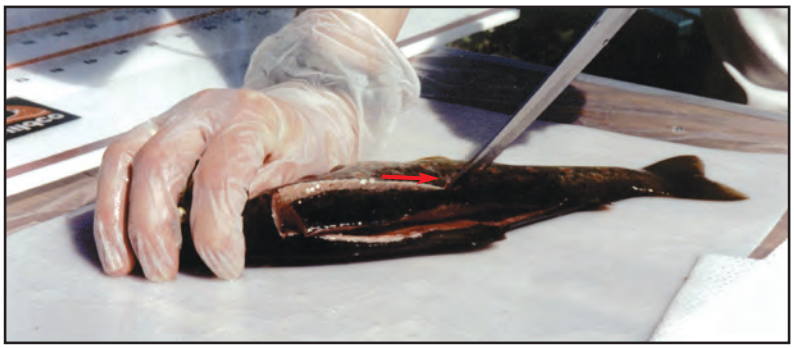


Figure 5. Diagram and photographs illustrating Cut 3 in dissection of top predator fish. **Cut 3a:** cut around the rib bones in a slight arc to the ventral part of the fish and near the vent while avoiding cutting near or into the central body cavity where body fluids could contaminate the fillet sample. **Cut 3b:** continue to extend Cut 3a along the ventral part of the fish from the area near the vent toward the tail; also deepen Cut 2a along dorsal part of fish and extend the cut to within 1 to 2 centimeters from the base of the tail fin with the fillet still attached to the tail section. Drawing by Lia Chasar, U.S. Geological Survey. Photograph by Mitch Harris, U.S. Geological Survey.

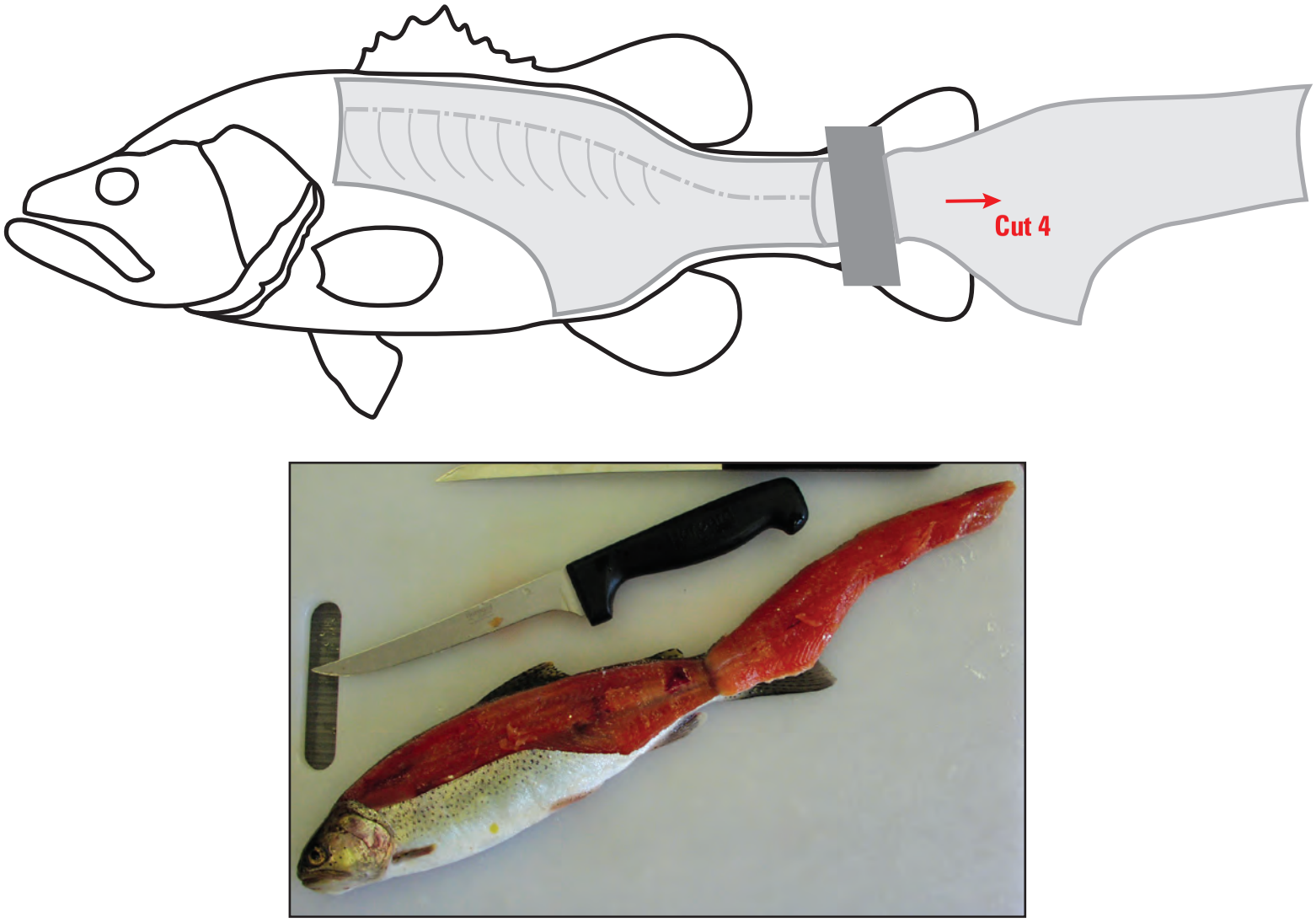


Figure 6. Diagram and photograph illustrating Cut 4 in dissection of top predator fish. Turn the fillet over so the scale side is facing the cutting surface. Starting near the base of the tail fin, slice through the fillet just above the skin layer, letting the knife ride on the inside surface of the skin and leaving the skin and scales attached to the fish. Remove the rib cage and any large bones if still on the fillet. Drawing by Lia Chasar, U.S. Geological Survey. Photograph by Dennis Wentz, U.S. Geological Survey.

Figure 7. Photograph illustrating examination of abdominal cavity of top predator fish for sex identification (female shown, with eggs visible in ovaries). Photograph by Mitch Harris, U.S. Geological Survey.

16. For optional collection of gut contents, cut the esophagus free as close as possible inside the head area while taking care not to cut deeply and risk damage to the otolith sacs. Cut intestinal tract free of body wall above the vent. Immediately preserve stomach contents in a vial or jar with 10-percent buffered (pH = 7) formalin for later analysis. Do not freeze gut contents, as this may preclude identification of some organisms.

17. Finally, for age determination, remove head or otoliths.

18. If otoliths will be excised in the laboratory and not in the field, remove the fish head as follows. Cut at groove at back edge of operculum where fleshy material meets bone (fig. 2). Store head frozen in zip-sealed plastic bag for later excision of otoliths.

19. If otoliths will be excised in the field, do not remove the fish head and instead use a heavy knife or small tin snips to score the skull on the upper inside surface of the mouth cavity just anterior to the estimated location of otolith capsules (fig. 8*A*). Bend upper jaw back to break skull open (fig. 8*A*), and remove otoliths from their capsules (fig. 8*B*). After removal, clean extracted otoliths with DI water and very gentle rubbing (fig. 8*C*); dry well with a clean Kimwipe® or Kaydry® and place in appropriately labeled vial for shipment. Remove all connective tissue from otoliths, and allow otoliths to completely dry before placing them in a vial; failure to do this may result in molding, rapid degradation of otoliths, and loss of ability to age the fish.

20. Between consecutively processed fish at a site, clean cutting board, knife, and dissecting tools with triple rinses of DI.

21. Place all used disposable sharp items, such as scalpel blades, in a hard plastic container and seal. Place all other waste in doubled plastic trash bags. Transport all waste to appropriate disposal location; do not leave on site.

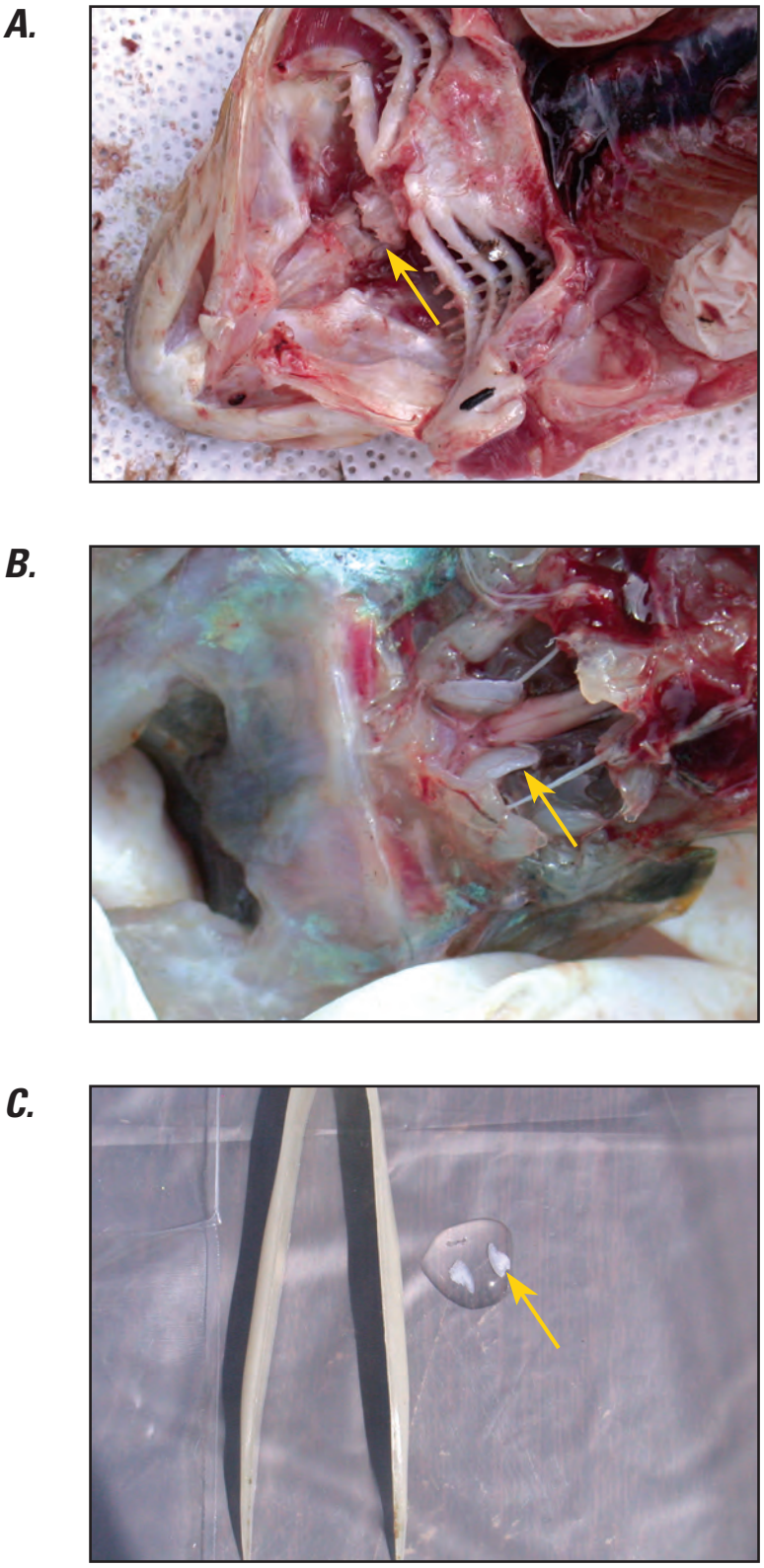


Figure 8. Photographs illustrating otolith extraction (a brown trout is shown as an example): ***A***, ventral view of head cavity with otolith area fractured open; ***B***, closeup view of otoliths in capsules; and ***C***, extracted otoliths. Photographs by Dennis Wentz, U.S. Geological Survey.

Field Data Forms and Sample Labels

Labeling the sample properly and filling out all field forms and other paperwork correctly and completely are critical steps in ensuring proper data management. Blank field forms and labels are presented at the end of this report and are listed in table 3. Separate field forms are used for invertebrates (appendix 1a-1) and fish (appendix 1a-2). Additionally, separate labels are used for different tissues and analyses: whole invertebrates for Hg/isotopes (appendix 1b-1), fish whole-body or fillet tissues for Hg/isotopes (appendix 1b-2), and fish age/otoliths (appendix 1b-3). Example labels and forms for fish gut contents are not provided but may be created by modifying appendixes 1a-2 and 1b-2.

Hg concentrations in biota, ITIS taxonomic code and body part code (NWIS alpha parameter codes "TAXON" and "BDPRT," respectively), as well as number of individuals in a composite sample, and fish weight, length, age, and sex, are stored in the USGS NWIS database (http://waterdata.usgs.gov/nwis). To facilitate this storage, each individual sample must have a unique combination of four key identifiers. For NWIS, these identifiers are site or station identification number, sample date, time, and medium code (NWIS alpha parameter codes "STAID," "DATES," "TIMES," and "MEDIM," respectively). Ensure that these identifiers are filled out on all forms and labels. Sample times (NWIS alpha parameter code "TIMES") for each individual fish are sequential for each species or group of fish and each invertebrate composite sample. For multiple biological samples collected on the same day, offset the TIMES by 1 minute for each sample, including invertebrates, and verify that times for periphyton, invertebrates, and fish do not overlap for a given date at a site to avoid accidental overwriting of results in NWIS. The field crew leader should review all field forms and labels for accuracy and completeness prior to leaving the sampling site.

Analytical laboratories contracted by the USGS require use of a unique field sample identification number for each biological sample. In early NAWQA Hg studies, sample identification numbers were generated by using a simple coding scheme: the study-unit identification code followed by a three-digit numeral (for example, REDN001, REDN002, etc.) For future sampling, an NWIS-compatible sample identifier that is numeric and is associated with a bar-code label likely will be used.

Post-Field Processing Activities

Post-field activities are those associated with data-management tasks that cannot be done prior to field work and sample shipping. Prompt, careful attention to sample documentation is critical.

Data Management

Samples should be logged into NWIS as soon as possible after sample collection. Prior to NWIS sample login, verify station, date, time, and medium code for all samples and ensure that ITIS taxonomic codes for the species of invertebrates and fish you have collected exist in NWIS. If the ITIS code for a collected taxon does not exist in NWIS, recheck spelling of the name, attempt to locate the proper ITIS code (see http://www.itis.gov/), and submit these ITIS codes to your database administrator for addition to the NWIS database. The NWIS parameter codes for biological samples collected using this protocol are shown in appendix 3.

Fill out separate laboratory submission forms for samples of invertebrates (appendix 1c-1 to1c-2.) and fish tissue (appendix 1c-3 to 1c-5) and optional gut content analysis (example laboratory submission form not provided). To minimize data-transcription errors, these forms should be populated with data retrieved from the NWIS database using QWDATA (water-quality database, NWIS subsystem) after sample login is completed. Retrieve STAID, SNAME (site or station name), DATES, TIMES, and MEDIM in tab-delimited format from QWDATA and enter these data into the laboratory submission forms. Field sample identification numbers are required on laboratory submission forms and must be unique for each sample (unique combination of STAID, DATES, TIMES, and MEDIM) in the shipment. When new NWIS-compatible sample numbers are implemented, each number will need to be unique nationally. Verify that key sample data are consistent on all field forms, labels, and laboratory submission forms.

Sample Shipment

Before shipping the samples, verify that they have been recorded on appropriate sample submissions forms and call the analytical laboratory to confirm that the laboratory is prepared to properly receive the samples. Frozen samples must be express-shipped (next-day delivery), and must contain sufficient dry ice so that a 1-day delay in delivery (due to weather, for example) will not compromise sample integrity. Avoid shipping on Thursdays or Fridays or just prior to holidays so that frozen samples will not sit for days while en route to the laboratory. Request a notification of receipt if one is not automatically provided by the laboratories.

Paper copies of completed laboratory submission forms must accompany sample shipments. In addition to paper copies of laboratory submission forms, submit electronic copies to the appropriate laboratory to facilitate data management. Study-unit personnel must retain copies of all forms and record sample shipment date and, if available from the shipping company, the tracking number for each shipment.

Invertebrate samples should be shipped to Dr. Lia Chasar at the USGS, Florida Integrated Science Center (USGS-FISC) in Tallahassee, Fla., where samples will be processed; subsamples will be analyzed for stable isotopes and additional subsamples will be forwarded to the WMRL for THg and MeHg analyses. For invertebrates, include the WMRL Laboratory Request for Analysis (appendix 1d-1) and Cooler Inventory (appendix 1d-2) together with the laboratory submission forms for Hg and isotope analyses. The WMRL forms will be forwarded with the processed subsamples when the isotope laboratory ships them to the WMRL for Hg analyses.

Fish samples, except for age/otolith samples, should be shipped to the appropriate laboratory (as of January 2008, fish-mercury analyses are conducted under contract with the Trace Element Research Laboratory, Texas A&M University in College Station, Tex.) where samples will be processed; subsamples will be analyzed for THg and additional subsamples will be forwarded to Dr. Chasar (USGS-FISC) for stable isotope analyses. Ship age/otolith samples directly to Dr. Chasar.

Ship invertebrate samples, fish age/otolith samples, and laboratory submission forms to:

Dr. Lia Chasar
USGS
Florida Integrated Science Center
2010 Levy Avenue
Tallahassee, FL 32310
Phone: 850/942-9500, extension 3010
Email: lchasar@usgs.gov

Ship fish samples (except age/otolith samples) and laboratory submission forms to:

Dr. Robert Taylor
TERL
TAMUS 4458
VMA Bldg. Room 107
College Station, TX 77843-4458
Phone: 979/845-1568
Email: RTaylor@cvm.tamu.edu

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Appendix 1. Forms and Labels Used for Sample Collection and Processing of Invertebrates and Fish

Appendix 1a. Field data sheets.

1a–1. Field data sheet for invertebrate tissue samples.
This form is available for download at http://pubs.usgs.gov/ofr/2008/1208/

Field data sheet for invertebrate tissue samples

FIELD DATA SHEET (NWIS field name noted in parentheses)					
USGS Mercury studies--Invertebrate tissue samples					**Study Unit (SUID):**
Station Name (SNAME):					
Station ID (STAID):			**Sample date (DATES), YYYYMMDD:**		
Time range, 24h, HHMM - HHMM:				**Medium Code (MEDIM):** C	
Sample Type (STYPE): 9		**Analysis status (ASTAT):** H		**Hydrologic condition (HSTAT):**	
Field Crew:					
Field comments:					
Species Common name:				**Latin name:**	
ITIS Taxonomic code (TAXON):				**Bodypart code (BDPRT):**	
Stream habitat/location where samples collected:				**circle one or more**	
Riffle		**Pool**		**Run**	**Channel margin**
Sample comments:					

Composite #	Sample time (TIMES), *24 h*	# Individuals in composite (P81614)	Sample wet wt, field, g	Sample ID	NWIS Record #
1					
2					
3					

Appendix 1. Forms and labels used for sample collection and processing of invertebrates and fish—Continued.

Appendix 1a. Field data sheets—Continued.

1a–2. Field data sheet for fish tissue samples.

This form is available for download at http://pubs.usgs.gov/ofr/2008/1208/

Field data sheet for fish tissue samples

FIELD DATA SHEET	(NWIS field name noted in parentheses)
USGS Mercury studies--Fish tissue samples	Study Unit (SUID):
Station Name (SNAME):	
Station ID (STAID):	Sample date (DATES), YYYYMMDD:
Time range, 24h, HHMM - HHMM:	Medium Code (MEDIM): C
Sample Type (STYPE): 9	Analysis status (ASTAT): H / Hydrologic condition (HSTAT):
Field Crew:	
Field comments:	
Species Common name:	Latin name:
ITIS Taxonomic code (TAXON):	Body part code (BDPRT):

Fish #	Sample time (TIMES) *(24 h)*	Fish length, total, cm, P91106	Fish wt, g, P91104	Sample wt, g, P91105	Gender, Male, P47463	Gender, Female, P47462	Scale/ Otolith	Sample ID	NWIS Record #
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									

Appendix 1. Forms and labels used for sample collection and processing of invertebrates and fish—Continued.

Appendix 1b. Sample labels.
All labels are 1-1/3" by 4", and are designed to fit Avery weatherproof mailing labels (Avery label number 5522).
All labels are available for download at http://pubs.usgs.gov/ofr/2008/1208/

1b–1. Labels for invertebrate tissue samples to be analyzed for mercury and stable isotopes.

Invertebrates: mercury and stable isotopes sample label

Invertebrates for Hg/Isotopes Station ID: _____
Station Name: _____
USGS Sample ID #: _____
Date: _____ Time (24h): _____Medium: C
Organism Name: _____
Contact: _____Ph: _____

1b–2. Labels for fish tissue samples to be analyzed for mercury and stable isotopes.

Fish: mercury and stable isotopes sample label

Fish for Hg/Isotopes Station ID: _____
Station Name: _____
USGS Sample ID #: _____
Date: _____ Time (24h): _____Medium: C
Organism Name: _____
Contact: _____Ph: _____

1b–3. Labels for fish tissue samples (otoliths) to be analyzed for ageing.

Fish: otolith analysis for ageing label

Fish otolith sample for ageing Station ID: _____
Station Name: _____
USGS Sample ID #: _____
Date: _____ Time (24h): _____Medium: C
Organism Name: _____
Contact: _____Ph: _____

Appendix 1. Forms and labels used for sample collection and processing of invertebrates and fish—Continued.

Appendix 1c. Sample submission and tracking forms.

1c–1. Request for total mercury and methylmercury analysis for invertebrate tissue samples.
This form is available for download at http://pubs.usgs.gov/ofr/2008/1208/

Invertebrates: total mercury and methylmercury analysis submission form

SAMPLE SUBMISSION/TRACKING FORM	**Page ___of ___**
USGS Mercury studies--Invertebrate tissue samples	**Shipper instructions:**
Filename: HgStudy_XXXX_SUID_YYYYMMDD.xls	(1) Save a COPY of this file in the format: HgStudy_XXXX(LAB)_SUID_YYYYMMDD.xls
Study Unit contact:	(2) Enter sample data into this spreadsheet, electronically, exactly as in NWIS.
Fax number:	(3) Sign, date, and ship hard copy of this completed form with samples.
Shipped by:	(4) Email electronic copy to laboratory to facilitate lab log-in.
Shipping date:	**Lab Instructions:**
Received by:	(1) Sign, date, and note comments upon receipt of samples.
Received date:	(2) Save form. Fax or mail copy to Study Unit contact.
Study Unit (SU) notes to lab:	
Analyses requested: Total Mercury and Methylmercury	**Medium Code: C**
Lab notes / condition of samples:	

Sample data

STAID	**DATES**	**TIMES** *(24 h)*	**# Individuals in composite**	**Sample wet wt, field (g)**	**Species Latin name**	**Species Common name**	**Sample ID**	**Comments**

Appendix 1. Forms and labels used for sample collection and processing of invertebrates and fish—Continued.

Appendix 1c. Sample submission and tracking forms—Continued.

1c–2. Request for stable isotope analysis for invertebrate samples.
This form is available for download at http://pubs.usgs.gov/ofr/2008/1208/

Invertebrates: stable isotopes analysis submission form

SAMPLE SUBMISSION/TRACKING FORM	**Page ___of ___**
USGS Mercury studies--Invertebrate tissue samples	**Shipper instructions:**
Filename: HgStudy_XXXX_SUID_YYYYMMDD.xls Study Unit contact: Fax number: Shipped by:	(1) Save a COPY of this file in the format: HgStudy_XXXX(LAB)_SUID_YYYYMMDD.xls (2) Enter sample data into this spreadsheet, electronically, exactly as in NWIS. (3) Sign, date, and ship hard copy of this completed form with samples. (4) Email electronic copy to laboratory to facilitate lab log-in.
Shipping date:	**Lab Instructions:**
Received by: Received date:	(1) Sign, date, and note comments upon receipt of samples. (2) Save form. Fax or mail copy to Study Unit contact.
Study Unit (SU) notes to lab:	
Analyses requested: **Stable isotopes (delta-13C, delta-15N, %C, %N, all)**	**Medium Code: C**
Lab notes / condition of samples:	
Sample data	

STAID	**DATES**	**TIMES** *(24 h)*	**# Individuals in composite**	**Sample wet wt, field (g)**	**Species Latin name**	**Species Common name**	**Sample ID**	**Comments**

Appendix 1. Forms and labels used for sample collection and processing of invertebrates and fish—Continued.

Appendix 1c. Sample submission and tracking forms—Continued.

1c–3. Request for total mercury and methylmercury analysis for fish tissue samples.
This form is available for download at http://pubs.usgs.gov/ofr/2008/1208/

Fish: total mercury and methylmercury analysis submission form

SAMPLE SUBMISSION/TRACKING FORM	**Page ___of ___**
USGS Mercury studies--Fish tissue samples	**Shipper instructions:**
Filename: HgStudy_TERL_SUID_YYYYMMDD.xls	(1) Save a COPY of this file in the format: HgStudy_TERL_SUID_YYYYMMDD.xls
Study Unit contact:	(2) Enter sample data into this spreadsheet, electronically, exactly as in NWIS.
Fax number:	(3) Sign, date, and ship hard copy of this completed form with samples.
Shipped by:	(4) Email electronic copy to laboratory to facilitate lab log-in.
Shipping date:	**Lab Instructions:**
Received by:	(1) Sign, date, and note comments upon receipt of samples.
Received date:	(2) Save form. Fax or mail copy to Study Unit contact.

Study Unit (SU) notes to lab: *Please send splits of freeze-dried/ground tissue to L. Chasar, USGS-FISC, 2010 Levy Avenue, Tallahassee, FL 32310. Include copy of spreadsheet with samples when shipped. Please email/call L. Chasar prior to shipping.*

Analyses requested: *Total Mercury* | **Medium Code:** C

Lab notes / condition of samples:

Sample data

STAID	DATES	TIMES *(24 h)*	Species (latin name)	Fish length, total (cm)	Fish wt (g)	Sample Cut	Sample wt (g)	Gender (M/F/I)	Sample ID	Comments

Appendix 1. Forms and labels used for sample collection and processing of invertebrates and fish—Continued.

Appendix 1c. Sample submission and tracking forms—Continued.

1c–4. Request for mercury isotope analysis for fish tissue samples.
This form is available for download at http://pubs.usgs.gov/ofr/2008/1208/

Fish: stable isotopes analysis submission form

SAMPLE SUBMISSION/TRACKING FORM	**Page ___of ___**
USGS Mercury studies--Fish tissue samples	**Shipper instructions:**
Filename: HgStudy_XXXX_SUID_YYYYMMDD.xls	(1) Save a COPY of this file in the format: HgStudy_XXXX(LAB)_SUID_YYYYMMDD.xls
Study Unit contact:	(2) Enter sample data into this spreadsheet, electronically, exactly as in NWIS.
Fax number:	(3) Sign, date, and ship hard copy of this completed form with samples.
Shipped by:	(4) Email electronic copy to laboratory to facilitate lab log-in.
Shipping date:	**Lab Instructions:**
Received by:	(1) Sign, date, and note comments upon receipt of samples.
Received date:	(2) Save form. Fax or mail copy to Study Unit contact.
Study Unit (SU) notes to lab:	
Analyses requested: Stable isotopes (delta-13C, delta-15N, %C, %N, all)	**Medium Code: C**
Lab notes / condition of samples:	

Sample data

STAID	DATES	TIMES *(24 h)*	Species (latin name)	Fish length, total (cm)	Fish wt (g)	Sample Cut	Sample wt (g)	Gender (M/F/I)	Sample ID	Comments

Appendix 1. Forms and labels used for sample collection and processing of invertebrates and fish—Continued.

Appendix 1c. Sample submission and tracking forms—Continued.

1c–5. Request for age analysis for fish tissue samples.
This form is available for download at http://pubs.usgs.gov/ofr/2008/1208/

Fish: age analysis submission form

SAMPLE SUBMISSION/TRACKING FORM **Page ___of ___**

USGS Mercury studies--Fish tissue samples	**Shipper instructions:**
Filename: HgStudy_XXXX_SUID_YYYYMMDD.xls	(1) Save a COPY of this file in the format: HgStudy_XXXX(LAB)_SUID_YYYYMMDD.xls
Study Unit Contact:	(2) Enter sample data into this spreadsheet, electronically, exactly as in NWIS.
Fax number:	(3) Sign, date, and ship hard copy of this completed form with samples.
Shipped by:	(4) Email electronic copy to laboratory to facilitate lab log-in.
Shipping date:	**Lab Instructions:**
Received by:	(1) Sign, date, and note comments upon receipt of samples.
Received date:	(2) Save form. Fax or mail copy to Study Unit contact.

Study Unit (SU) notes to lab:

Analyses requested: Ageing (otolith, scale, spine) **Medium Code: C**

Lab notes / condition of samples:

Sample data

STAID	**DATES**	**TIMES** *(24 h)*	**Species (latin name)**	**Fish length, total (cm)**	**Fish wt (g)**	**Sample Cut**	**Sample wt (g)**	**Gender (M/F/I)**	**Sample ID**	**Comments**

Appendix 1. Forms and labels used for sample collection and processing of invertebrates and fish—Continued.

Appendix 1d. Wisconsin Mercury Research Laboratory (WMRL) forms.

1d–1. WMRL request for analysis.

This form is available for download at http://pubs.usgs.gov/ofr/2008/1208/

Wisconsin Mercury Research Laboratory request for analysis form

WISCONSIN MERCURY RESEARCH LABORATORY REQUEST FOR ANALYSIS

SITE NAME:_____ SITE NUMBER:_____

PROJECT NAME:_____ PROJECT NUMBER:_____

DATE:_____ TIME:_____ DEPTH (M):_____ REPLICATE:_____

container identification	sample medium	analysis type	filter type	volume filtered	<----------preservative----------> type	identification	volume

COMMENTS: _____

SPECIAL REQUESTS

LABORATORY FILTERING			LABORATORY SPLITTING	
container identification	analysis types	filter type	container identification	analysis types

LABORATORY USE ONLY

LOGIN DATE: _____ ANALYST: _____

COMMENTS: _____

PAGE 1 OF ____

WMRL FORM F03 REVISION 4 , 7/25/07

Appendix 1. Forms and labels used for sample collection and processing of invertebrates and fish—Continued.

Appendix 1d. Wisconsin Mercury Research Laboratory (WMRL) forms.

1d–2. WMRL cooler inventory form.

This form is available for download at http://pubs.usgs.gov/ofr/2008/1208/

Wisconsin Mercury Research Laboratory cooler inventory form

COOLER INVENTORY FORM

page _____ of _____

USGS -- WISCONSIN MERCURY RESEARCH LABORATORY
8505 RESEARCH WAY
MIDDLETON, WI 53562

PROJECT:

SAMPLERS:

SITE NAME	DATE	TIME	DEPTH	SAMPLE MEDIA	TOTAL NUMBER OF CONTAINERS	125 ML TEFLON	250 ML TEFLON	500 ML TEFLON	1 L TEFLON	2 L TEFLON	5 L TEFLON	PETRI DISHES	40 ML GLASS	SEDIMENT VIAL			LABORATORY CHECK-IN COMMENTS

Relinquished by:	date/time:	Received by:	date/time:	COMMENTS:
Relinquished by:	date/time:	Received by:	date/time:	
Relinquished by:	date/time:	Received by:	date/time:	

WMRL F02 REVISION 4 JULY 25, 2007

Appendix 2. Equipment and Supplies used for U.S. Geological Survey Mercury Studies of Invertebrates and Fish

Appendix 2. Equipment and supplies used for U.S. Geological Survey mercury studies of invertebrates and fish—Continued. This form is available for download at http://pubs.usgs.gov/ofr/2008/1208/

[HCl, hydrochloric acid; %, percent; v/v, volume per volume; DI, deionized; lbs, pounds; mL, milliliters; mm, millimeter; L, liter; PETE, polyethylene terephthalate; g, gram; PP, polypropylene]

GENERAL

- ☐ Acid, HCl Omni Trace, 5% v/v in DI water
- ☐ Barcodes with duplicates (samples, field sheets)
- ☐ Batteries (9-volt, AA, C, D)
- ☐ Carboys, 5-gallon (2, tap; 2, DI water; 1, 5% HCl)
- ☐ Camera, digital
- ☐ Chairs, folding
- ☐ Clipboard (wooden or plastic)
- ☐ Coolers (4, wet ice; 2, dry ice)
- ☐ Collection permits/licenses
- ☐ Detergent, phosphate-free (e.g., Liquinox®)
- ☐ Field data sheets (pre-printed, water-resistant paper)
- ☐ Field log sheets (pre-printed, water-resistant paper)
- ☐ First aid kit
- ☐ Flashlights and headlamps
- ☐ Formalin (100%, buffered to pH = 7)
- ☐ Garbage bags, large, plastic
- ☐ Gloves, powder-free (nitrile, vinyl, other non-latex)
- ☐ Gloves, heavy duty rubber (reusable)
- ☐ Goggles, safety/chemical
- ☐ Global Positioning System (GPS) unit
- ☐ Head nets
- ☐ Multi-parameter sampler for water quality
- ☐ Multi-parameter sampler calibration standards
- ☐ Ice, dry (approximately15 lbs per day of trip per cooler plus extra)
- ☐ Ice, regular, “wet”
- ☐ Insect repellent
- ☐ Keys, identification, for invertebrates and fishes
- ☐ Labels, (waterproof, pre-printed)
- ☐ Maps
- ☐ Markers, fine-tip, alcohol/solvent resistant
- ☐ Markers, wide-tip, alcohol/solvent resistant
- ☐ Personal Flotation Device
- ☐ Phone
- ☐ Phone numbers, emergency
- ☐ Protocol (on water-resistant paper)
- ☐ Shipping paperwork (instructions, lab sumission forms, pre-printed FedEx labels, dry ice stickers)
- ☐ Signs (road/safety)
- ☐ Sunscreen
- ☐ Table, folding
- ☐ Tape, flagging, biodegradable
- ☐ Tax exempt forms
- ☐ Tape, clear, 2", for shipping
- ☐ Travel authorization
- ☐ Tray or tub, large (for dishwashing/storage)
- ☐ Trays, plastic, large (for cleaning equipment)
- ☐ Trays, plastic, shallow (for weighing fish)
- ☐ Towels, lint-free, clean-room grade
- ☐ Towels, paper rolls
- ☐ Traffic cones
- ☐ Trash bags, heavy duty
- ☐ Waders
- ☐ Wader repair kit
- ☐ Wash Bottles (500 mL, Teflon®; 1, tap water; 1, DI water; 1, dilute HCl; 1,dilute detergent)
- ☐ Water, high-purity DI
- ☐ Water, tap

Appendix 2. Equipment and supplies used for U.S. Geological Survey mercury studies of invertebrates and fish—Continued. This form is available for download at http://pubs.usgs.gov/ofr/2008/1208/

[HCl, hydrochloric acid; %, percent; v/v, volume per volume; DI, deionized; lbs, pounds; mL, milliliters; mm, millimeter; L, liter; PETE, polyethylene terephthalate; g, gram; PP, polypropylene]

INVERTEBRATES

Sampling Gear

- ☐ Dipnet, D-frame
- ☐ Forceps, plastic
- ☐ Gloves, powder-free (nitrile, vinyl, other non-latex)
- ☐ Sieves, plastic, 12-inch (coarse, 6-mm mesh)
- ☐ Sieves, plastic, 12-inch (500-micron mesh)

Sample Processing

- ☐ Bags, plastic, heavy-weight, zipper-seal, 1 L
- ☐ Counters, hand-held
- ☐ Forceps, plastic
- ☐ Gloves, powder-free (nitrile, vinyl, other non-latex)
- ☐ Ice cube trays (4), for sorting invertebrates
- ☐ Vials with caps, plastic, 20 mL (e.g., PETE or PP)
- ☐ Magnifying glass/hand lens
- ☐ Scale, top-loading, accuracy to 0.01 g
- ☐ Trays, plastic, shallow (for picking invertebrates)
- ☐ Table cloths, plastic, medium weight

FISH

Sampling Gear

- ☐ Aerators, battery-operated
- ☐ Batteries or fuel for electrofishing unit
- ☐ Buckets, 10 gallon
- ☐ Electrofishing unit (backpack/towed/boat)
- ☐ Gloves, rubber, safety (insulated)
- ☐ Polarizing sunglasses
- ☐ Live well or net
- ☐ Nets, large dipnet
- ☐ Nets, small
- ☐ Seine (1/4 to 1/2-inch maximum mesh)

Sample Processing

- ☐ Anesthetic CO_2 tablets (e.g., AlkaSeltzer®)
- ☐ Bags, plastic, heavy-weight, zipper-seal, 1 L
- ☐ Bags, plastic, heavy-weight, zipper-seal, 4–8 L
- ☐ Cutting sheet/mat/board, plastic
- ☐ Forceps, plastic
- ☐ Gloves, powder-free (nitrile, vinyl, other non-latex)
- ☐ Knives, filleting
- ☐ Measuring board (non-metallic)
- ☐ Pliers, needle-nose
- ☐ Rulers, 6-inch plastic
- ☐ Scale, top-loading, accuracy to 0.01 g
- ☐ Scale, top-loading, accuracy to 0.1 g
- ☐ Scale, hanging or hook, for large fish
- ☐ Scalpels, high-grade stainless
- ☐ Scissors, dissecting, high-grade stainless steel
- ☐ Tin snips, small, for otolith extraction
- ☐ Vials with caps, plastic, 20 mL, PETE or PP

Appendix 3. U.S. Geological Survey (USGS) National Water Information System (NWIS) Coding of Biological Tissue Samples for USGS National Water-Quality Assessment Program (NAWQA) Mercury Studies.

All biological tissue results are to be stored in the USGS National Water Information System (NWIS) with all other water-quality data. For general NAWQA guidance on coding regular and QA/QC biological samples, refer to: http://nm.water.usgs.gov/nawqa_natsyn/sample-coding/outline.bst.html. Also refer to NWIS documentation (http://wwwnwis.er.usgs.gov/nwisdocs4_4/qw/QW.user.book.html), especially Appendix A for code descriptions (http://wwwnwis.er.usgs.gov/nwis-docs4_4/qw/QW-AppxA.pdf).

Required fields for all samples in NWIS are site or station identification number, sample date, time, and medium code (NWIS alpha parameter codes “STAID,” “DATES,” “TIMES”, and “MEDIM,” respectively); purpose of site visit (P50280) and sample purpose (P7199) are required for all NAWQA samples.

A. Routine parameters for all biological samples:

1. Station identification number (STAID)
2. Sample date (DATES)
3. Sample time (TIMES)
4. Medium codes (MEDIM):
    a. Animal tissue, including fish and benthic invertebrates:
        i. Medium C
        ii. Medium X (QC samples, such as replicates and field-submitted [single-blind] standard reference materials)
    b. Plant tissue, including periphyton:
        i. Medium D
        ii. Medium Y (QC samples, such as replicates and field-submitted [single-blind] standard reference materials)
5. Purpose of Site Visit (POSV)(P50280):
    a. Hg synoptic: 3003 (NAWQA synoptic survey)
    b. Hg topical biota sampling: 5099 (other)
6. Sample purpose (P71999) = 15 (NAWQA)
7. Taxonomic identification (TAXON): Code to lowest known taxon (typically genus or species). NWIS stores taxonomic data using codes from the Integrated Taxonomic Information System (ITIS). During sample login, the user is prompted for entry of the ITIS code at field 26 of the login frame.
    a. If a code is not known, search NWIS for ITIS codes that have been defined within NWIS by typing a ‘?’ and then the first several letters of the scientific (Latin) name of the genus.
    b. If a code is not defined in NWIS, search the ITIS data base at: http://www.itis.usda.gov/. If a code exists in ITIS, but not in NWIS, the user must request that the missing ITIS code be incorporated into the NWIS system.
8. Body part codes (BDPRT). During sample login, enter the body part code in field 27 of the login frame. Common codes used in mercury studies are:
    a. Whole organisms and composites of whole organisms: 59
        i. Used for invertebrates
        ii. Used for whole forage fish with head and guts intact
    b. Skinless fillet tissue (also known as axial muscle): 86
        i. Used for Hg synoptic sampling of game fish
        ii. Used for Hg topical studies of game fish
    c. Headless, eviscerated organism: 95
        i. Used for forage fish with head and gut removed
    d. Whole organism except shell or carapace—for example, soft tissues of clams, snails, or similar hard-shelled organisms that have had shell removed: 119

Appendix 3. U.S. Geological Survey (USGS) National Water Information System (NWIS) coding of biological tissue samples for USGS National Water-Quality Assessment Program (NAWQA) mercury studies—Continued.

B. Composite samples

These are parameter codes used for composites of more than one individual organism (codes in parentheses). Examples are composite fish samples for 1998 and 2002 National Synoptic Study and composite invertebrate samples for Mercury Topical Studies. For information about samples of one individual organism, skip to the next section.

1. Length data for composite samples, measured in centimeters (cm). Lengths are total length of organism.
    a. Length of organisms, average (arithmetic mean) for composite, cm (P01371)
    b. Length of organism, minimum in composite sample, cm (P72146)
    c. Length of organism, maximum in composite sample, cm (P72140)
    d. Length of organisms, standard deviation, composite sample, cm (P72141)
2. Weight data for composite samples, measured in grams:
    a. Weight of organisms, average (arithmetic mean) for composite, grams (P01373)
    b. Weight of organism, minimum in composite sample, grams (P72142)
    c. Weight of organism, maximum in composite sample, grams (P72143)
    d. Weight of organisms, standard deviation, in composite sample, grams (P72144)
3. Age data for composite samples:
    a. Mean age of organisms in composite sample, years (P62886)
        Method code A: age determined from scale sample(s)
        Method code B: age determined from otolith sample(s)
        Method code C: age determined from pectoral spine sample(s)
        Method code D: age determined from dorsal spine sample(s)
        Method code E: age determined from cleithrum sample(s)
        Method code F: age determined from more than one anatomical structure sample
        Method code G: age estimated from length-frequency distribution
    b. Minimum age of organisms in composite sample, years (P62887)
    d. Maximum age of organisms in composite sample, years (P62888)
4. Number of organisms in composite:
    a. Number of individuals in sample (P81614)
    b. Number of males in sample (P47463)
    c. Number of females in sample (P47462)

C. Individual-organism samples

1. Length of organism (total length), cm (P91106)
2. Weight of organism, fresh weight in field, grams (P91104)
3. Weight of sampled tissue from organism, fresh weight in field, grams (P91105)
4. Age of organism, years (P84015)
    a. Method code A: age determined from scale sample(s)
    b. Method code B: age determined from otolith sample(s)
    c. Method code C: age determined from pectoral spine sample(s)
    d. Method code D: age determined from dorsal spine sample(s)
    e. Method code E: age determined from cleithrum sample(s)
    f. Method code F: age determined from more than one anatomical structure sample
    g. Method code G: age estimated from length-frequency distribution
5. Number of individuals in sample (P81614) = 1
6. Number of males or females (for samples of individuals, values for these parameter codes should be either 0 or 1):
    a. Number of males in sample (P47463)
    b. Number of females in sample (P47462)

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For more information concerning this publication, contact:
Chief, USGS National Water Quality Assessment Program
MS 413 National Center
Reston, VA 20192
(703) 648–5716
Or visit the National Water Quality Assessment Program site at:
http://water.usgs.gov/nawqa/