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Larry R. Brown, Research Physical Scientist, U.S. Geological Survey, Sacramento, Calif.
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Charles F. Saylor, Biologist, Tennessee Valley Authority, Norris, Tenn.
Stephen B. Smith, Fish and Wildlife Biologist, U.S. Geological Survey, Reston, Va.
James D. Williams, Supervisory Research Fishery Biologist, U.S. Geological Survey, Gainesville, Fla.

Technical Reviewers

Peter M. Ruhl, Hydrologist, U.S. Geological Survey, Raleigh, N.C.
William F. Smith-Vaniz, Research Marine Biologist, U.S. Geological Survey, Gainesville, Fla.

Publications Unit Support

Jeffrey L. Corbett, Scientific Illustrator, U.S. Geological Survey, Raleigh, N.C.
Rebecca J. Deckard, Supervisory Writer/Editor, U.S. Geological Survey, Raleigh, N.C.
Kay E. Hedrick, Writer/Editor, U.S. Geological Survey, Raleigh, N.C.

Photography

Noel M. Burkhead, Research Fishery Biologist, U.S. Geological Survey, Gainesville, Fla.

Approving Official

Larry J. Slack, Supervisory Hydrologist, Pearl, Miss.

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Guidelines for Quality Assurance and Quality Control of Fish Taxonomic Data Collected as Part of the National Water-Quality Assessment Program

By STEPHEN J. WALSH and MICHAEL R. MEADOR

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For additional information write to:

District Chief
U.S. Geological Survey
3916 Sunset Ridge Road
Raleigh, NC 27607

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U.S. Geological Survey
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FOREWORD

The mission of the U.S. Geological Survey (USGS) is to assess the quantity and quality of the natural resources of the Nation and to provide information that will assist resource managers and policymakers at Federal, State, and local levels in making sound decisions. Assessment of water-quality conditions and trends is an important part of this overall mission.

One of the greatest challenges faced by water-resources scientists is acquiring reliable information that will guide the use and protection of the Nation's water resources. That challenge is being addressed by Federal, State, interstate, and local water-resource agencies and by many academic institutions. These organizations are collecting water-quality data for a host of purposes that include compliance with permits and water-supply standards; development of remediation plans for a specific contamination problem; operational decisions on industrial, wastewater, or water-supply facilities; and research on factors that affect water quality. An additional need for water-quality information is to provide a basis on which regional and national-level policy decisions can be based. Wise decisions must be based on sound information. As a society we need to know whether certain types of water-quality problems are isolated or ubiquitous, whether there are significant differences in conditions among regions, whether the conditions are changing over time, and why these conditions change from place to place and over time. The information can be used to help determine the efficacy of existing water-quality policies and to help analysts determine the need for, and likely consequences of, new policies.

To address these needs, the Congress appropriated funds in 1986 for the USGS to begin a pilot program in seven project areas to develop and refine the National Water-Quality Assessment (NAWQA) Program. In 1991, the USGS began full implementation of the program. The NAWQA Program builds upon an existing base of water-quality studies of the USGS, as well as those of other Federal, State, and local agencies. The objectives of the NAWQA Program are to

- Describe current water-quality conditions for a large part of the Nation's freshwater streams, rivers, and aquifers.
- Describe how water quality is changing over time.
- Improve understanding of the primary natural and human factors that affect water-quality conditions.

This information will help support the development and evaluation of management, regulatory, and monitoring decisions by other Federal, State, and local agencies to protect, use, and enhance water resources.

The goals of the NAWQA Program are being achieved through ongoing and proposed investigations of 60 of the Nation's most important river basins and aquifer systems, which are referred to as Study Units. These Study Units are distributed throughout the Nation and cover a diversity of hydrogeologic settings. More than two-thirds of the Nation's freshwater use occurs within the 60 Study Units and more than two-thirds of the people served by public water-supply systems live within their boundaries.

National synthesis of data analysis, based on aggregation of comparable information obtained from the Study Units, is a major component of the program. This effort focuses on selected water-quality topics using nationally consistent information. Comparative studies will explain differences and similarities in observed water-quality conditions among study areas and will identify changes and trends and their causes. The first topics addressed by the national synthesis are pesticides, nutrients, volatile organic compounds, and aquatic biology. Discussions on these and other water-quality topics will be published in periodic summaries of the quality of the Nation's ground and surface water as the information becomes available.

This report is an element of the comprehensive body of information developed as part of the NAWQA Program. The program depends heavily on the advice, cooperation, and information from many Federal, State, interstate, Tribal, and local agencies and the public. The assistance and suggestions of all are greatly appreciated.

Robert M. Hirsch
Chief Hydrologist

CONTENTS

Abstract 1
Introduction 1
Strategy for quality assurance and quality control 3
Accurate taxonomic identification 3
Consistent biological nomenclature 3
Quality-assurance and quality-control plans 3
Selecting a fish taxonomic specialist 4
Pre-sampling fish species list 4
Species occurrence 4
Problematic taxonomic issues 5
Protected species 6
Collecting permits 7
Quality-assurance and quality-control procedures 13
Laboratory identifications 13
Independent taxonomic verification 14
Voucher specimens 15
Photographic documentation 15
Tissue samples 16
Archiving of fish specimens 16
Reference collections 16
Voucher collections 16
Ichthyological curation facilities 16
Processing fish samples in the field 19
Guidelines for live specimen handling and animal care 19
Specimen preparation 20
Fixation and preservation 20
Labeling 21
Supplies 22
Shipment procedures 24
Quality assurance and quality control and reports 24
Summary 24
References cited 25
Appendix: Regional taxonomic references 29

FIGURES

1. Map showing approximate numbers of described native freshwater fish species per State in the United States 2
2. Diagram showing examples of diagnostic characters of many fish species that may require close examination in order to make accurate taxonomic identifications 5
3. Map showing numbers of introduced nonindigenous fish species in the United States by USGS 4-digit Hydrologic Unit Codes (USGS Biological Resources Division, Florida Caribbean Science Center) 6
4. Examples of fish photographs used to illustrate diagnostic coloration patterns that may be useful in distinguishing cryptic species (*Etheostoma coosae* and *E. scotti*) 15
5. Map showing major ichthyological curation facilities in the United States 17
6. Suggested field label 21

TABLES

1. State natural resources agencies that are responsible for issuing scientific, private, and commercial collecting permits; 1998 annual fees; and reporting date requirements 8
2. Address information for international, national, regional, and other important ichthyological curation centers in the United States with significant freshwater holdings 18
3. Common supplies used in processing live and preserved fish specimens in the field and laboratory. 22
4. Selected suppliers of gear and equipment that are useful for processing fish samples in the field and laboratory ... 23

CONVERSION FACTORS

Multiply	By	To obtain
	Length	
millimeter (mm)	0.03937	inch
	Volume	
liter (L)	1.0567	quart
	Weight	
gram (g)	0.03527	ounce

GLOSSARY

Caudal peduncle—The relatively narrow part of a fish's body between the base of the anal fin and the base of the tail fin.

Classification—In systematics, the process of arranging taxa into groups and groups into hierarchical categories forming a system.

Congeners—Two or more taxa that belong to the same genus but are recognized as separate species or populations; congener is singular and applied to the individual taxon.

Cryptic taxa—Groups of organisms that closely resemble one another morphologically and that may overlap in their distribution (sometimes referred to as sibling taxa).

Ethanol—Ethyl alcohol. A preservative used in fish collections, usually at concentrations of 70 to 75 percent.

Fish Taxonomic Specialist—As used here, a fish taxonomic specialist is an individual who has professional training and experience in identifying fishes and recognizing taxonomic problems, and who serves as a qualified expert to provide advice, guidance, and assistance for quality-assurance and quality-control of fish taxonomic identifications.

Fixation—The process of hardening and coagulating tissues of organisms into insoluble substances, such as cross-linked proteins, to prevent cellular breakdown and irreversible denaturation of biological macromolecules.

Formaldehyde—As used for a fixative, the term "formaldehyde" is marketed as a saturated water solution of formaldehyde gas (HCHO), representing a concentrated stock solution of formalin (37-percent formaldehyde by weight).

Formalin—The standard chemical for fixation of fish tissues, usually mixed at a concentration of 10 percent by diluting one part of concentrated formalin (stock 37-percent aqueous formaldehyde) with nine parts of water.

Gas bladder—A gas-filled organ present in many fishes that serves to regulate buoyancy (also known as swim bladder or air bladder).

Ichthyology—The broad field of the scientific study of fishes. A systematic ichthyologist specializes in study of the kinds, relationships, classification, and evolution of fishes.

Isopropanol—Isopropyl alcohol. A preservative used in fish collections, usually at concentrations of 40 to 50 percent.

Meristic characters—Traits that are characterized by and involve variation or modification in numbers; for example, counts of fin rays and scales.

Morphological characters—Traits that vary in form and structure; for example, presence or absence of fin spines. As used in biology, morphological characters encompass all diagnostic anatomical features and include those that can be counted (meristic), measured (morphometric), or characterized in other ways.

Morphometric characters—Traits that vary in relative shape and (or) dimensions; for example, body depth.

Nomenclature—The system of applying scientific names to taxonomic units of plants and animals. The Linnean system of nomenclature for species consists of a binomen, using one name for the genus group and another for the species group (the specific epithet).

Operculum—The bony gill cover of a fish.

Preservation—In ichthyology, the long-term maintenance of fish tissues in a fixed state within a fluid medium, usually alcohol.

Reference collection—An assortment of preserved specimens representing selected species and maintained for comparative purposes and instruction in identifying individual specimens.

Systematics—The study of the kinds and diversity of organisms, including their distinction, classification, interrelationships, and evolution. As a science, systematics encompasses both taxonomy and classification.

Taxa—Natural taxonomic groups or units of plants, animals, and other organisms that occur in nature; singular is taxon.

Taxonomy—The theory and practice of recognizing and describing organisms and biological diversity, including the handling, identification, and study of specimens, and the analysis and publication of data revealing information about variations among specimens.

Voucher collection—An assortment of preserved specimens representing selected species and maintained for validating taxonomic identifications, documenting spatial and temporal distributions, and other purposes.

Guidelines for Quality Assurance and Quality Control of Fish Taxonomic Data Collected as Part of the National Water-Quality Assessment Program

By Stephen J. Walsh and Michael R. Meador

ABSTRACT

Fish community structure is characterized by the U.S. Geological Survey's National Water-Quality Assessment (NAWQA) Program as part of a perennial, multidisciplinary approach to evaluating the physical, chemical, and biological conditions of the Nation's water resources. The objective of quality assurance and quality control of fish taxonomic data that are collected as part of the NAWQA Program is to establish uniform guidelines and protocols for the identification, processing, and archiving of fish specimens to ensure that accurate and reliable data are collected.

Study unit biologists, collaborating with regional biologists and fish taxonomic specialists, prepare a pre-sampling study plan that includes a preliminary faunal list and identification of an ichthyological curation center for receiving preserved fish specimens. Problematic taxonomic issues and protected taxa also are identified in the study plan, and collecting permits are obtained in advance of sampling activities. Taxonomic specialists are selected to identify fish specimens in the field and to assist in determining what fish specimens should be sacrificed, fixed, and preserved for laboratory identification, independent taxonomic verification, and long-term storage in reference or voucher collections.

Quantitative and qualitative sampling of fishes follows standard methods previously established for the NAWQA Program. Common ichthyological techniques are used to process samples in the field and prepare fish specimens to be returned to the laboratory or sent to an institutional repository. Taxonomic identifications are reported by using a standardized list of scientific names that provides nomenclatural consistency and uniformity across study units.

INTRODUCTION

The National Water-Quality Assessment (NAWQA) Program of the U.S. Geological Survey (USGS) 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 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 (Gilliom and others, 1995). This national consistency allows an integrated assessment of the status of 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.

Ecological surveys are a major component of the biological part of the NAWQA Program (Gurtz, 1994) and consist of community characterizations of fishes (Meador and others, 1993), algae (Porter and others, 1993), and benthic invertebrates (Cuffney and others, 1993) and detailed stream habitat assessment (Meador, Hupp, and others, 1993; Fitzpatrick and others, 1998). Ecological surveys are a part of all NAWQA Program activities, including occurrence and distribution

assessments; evaluation of long-term trends and changes; and studies of sources, transport, fate, and effects (Gilliom and others, 1995). 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.

Nationally consistent guidelines for sampling biological communities have been developed for the NAWQA Program to ensure that personnel among study units collect comparable data (Cuffney and others, 1993; Meador and others, 1993; Porter and others, 1993; Fitzpatrick and others, 1998). National consistency in processing ecological survey samples is as important as nationally consistent collection methods (Cuffney and others, 1993). Consequently, guidance and standardization are needed to address suitable quality-assurance and quality-control (QA/QC) methods to ensure accuracy of taxonomic identifications of fish. It is especially important to establish consistent guidelines because of the broad taxonomic and geographic diversity of the approximately 800 freshwater fish species in the United States (Page and Burr, 1991; fig. 1).

The purpose of this report is to provide nationally consistent guidelines and criteria for the taxonomic identification of fish samples collected as part of the USGS NAWQA Program. The guidelines presented here also are used to support other Water Resources Division projects that involve the identification of fishes. These guidelines include QA/QC procedures and standards for specimen

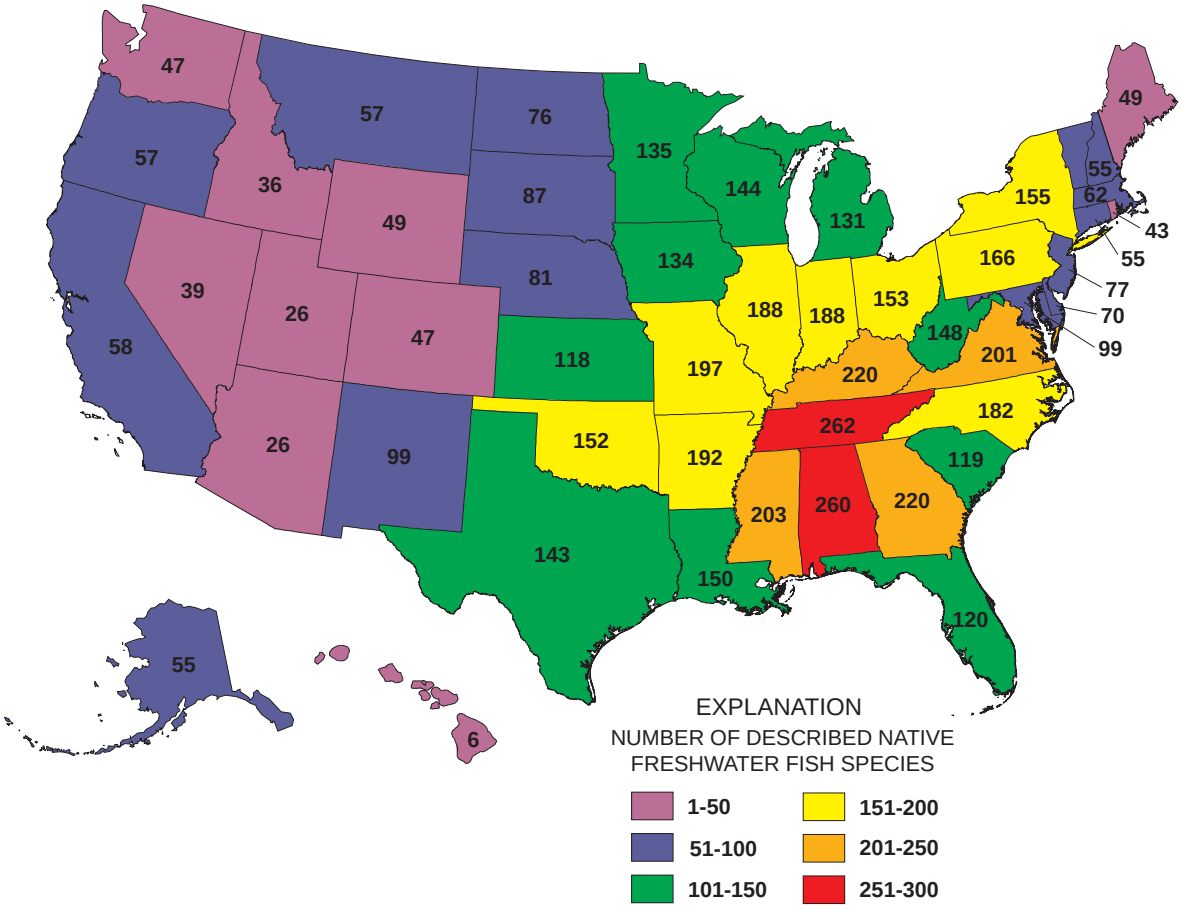

Figure 1. Approximate numbers of described native freshwater fish species per State in the United States (modified from Warren and Burr, 1994).

handling, sub-sampling procedures, selection of voucher specimens and specimens for independent taxonomic verification, labeling and shipping samples, and reporting of taxonomic information. In addition, criteria are provided for selecting fish taxonomic specialists who can make accurate taxonomic identifications.

STRATEGY FOR QUALITY ASSURANCE AND QUALITY CONTROL

The strategy for QA/QC of fish taxonomy in the NAWQA Program is designed to ensure accurate taxonomic identification of individual fish specimens and correct use of biological nomenclature. Accurate taxonomic identification of fish specimens is dependent upon the knowledge, skills, and abilities of the taxonomic specialist who identifies fish specimens in the field and in the laboratory. Consistent and correct nomenclature is based on a standardized list of taxonomic names. QA/QC of fish taxonomy in NAWQA study units is ultimately the responsibility of the study unit biologist.

Accurate Taxonomic Identification

The most controlled approach to ensuring accurate taxonomic identification of fish specimens would be to remove all fish specimens from the field and to determine species' identifications in a laboratory setting. However, it is not legal, ethical, or necessary to remove all fish specimens from the field. Federal and State laws protect many fish species. For example, collection of fishes listed as endangered or threatened requires special permits, and such species can rarely be removed from the field. In addition, some fish species, such as popular sportfishes, are easily recognizable, and it is unnecessary to remove them from the field in order to identify them. Because not all fish specimens can or should be removed from the field, at least some specimens must be identified in the field and returned to the water immediately. Thus, the accuracy of taxonomic identifications is dependent upon the fish taxonomic specialist making these determinations.

Fish taxonomic specialists, also referred to as systematic ichthyologists, are individuals trained in the taxonomic identification of fish species. Not all biologists collecting fishes are adequately trained to provide accurate and reliable taxonomic identifications of sportfish and non-sportfish species. The study unit biologist has overall responsibility for selecting one or more taxonomic specialists to assist in preparing for, collecting, and processing fish samples. With the assistance of fish taxonomic specialists, study unit biologists are responsible for identifying and quantifying specimens according to Meador and others (1993). Cooperating fish taxonomic specialists work with study unit and regional biologists to (1) develop study unit-specific QA/QC procedures, (2) process fish samples according to these QA/QC procedures, (3) produce data in standardized paper and electronic formats, and (4) prepare study unit reference collections as needed.

Consistent Biological Nomenclature

Biological nomenclature used to document fish species collected in the NAWQA Program follows a standardized list of common and scientific names of fishes published by the American Fisheries Society (AFS) Committee on Names of Fishes (Robins and others, 1991). Use of the AFS list of common and scientific names is critical to maintaining a nationally consistent data base. New fish species are occasionally discovered, and systematic studies frequently lead to changes in fish taxonomy and nomenclature. Thus, the AFS list of common and scientific names of fishes is continuously being revised and is published approximately every 10 years. The most recent edition should be used.

QUALITY-ASSURANCE AND QUALITY-CONTROL PLANS

The study unit biologist, working with a regional biologist and a fish taxonomic specialist, develops a plan that stipulates QA/QC procedures to be followed within the study unit. Regional differences in fish faunal diversity, the taxonomic status of certain fish species, and availability of taxonomic specialists across the Nation preclude a single, uniform QA/QC plan for all study units. Instead, plans must be developed that are flexible enough to address fish

taxonomic issues within each study unit. Regional biologists should approve QA/QC plans and maintain copies of the plans. Each plan should address at least five topics: (1) the selection of a fish taxonomic specialist, (2) a pre-sampling fish species list, (3) collecting permits, (4) QA/QC procedures to be used, and (5) the archiving of fish specimens.

Selecting a Fish Taxonomic Specialist

The first step in developing a QA/QC plan for each study unit is the selection of a skilled taxonomic specialist to identify fish species. Universities, museums, and natural resources agencies employ many well-qualified experts that can accurately identify fishes. Such specialists, however, demonstrate a wide range of skills and competence relating to fish taxonomy, systematics, and species-level identification. The following criteria should be considered in evaluating and selecting individuals to conduct identifications:

1. Individuals should have completed a graduate degree that includes coursework or research in fish taxonomy and systematics.
2. Individuals should have a demonstrated proficiency in taxonomic identification of freshwater fishes within the watershed or general region, as evidenced by prior research, publications, and work experience.
3. Individuals should be capable of recognizing, in the field, potentially important new distribution records and specimens of special taxonomic value. In addition, individuals should be experienced or trained to make judicious decisions about which fishes can be released and which fish specimens should be preserved.
4. Individuals should be fully knowledgeable about State and Federal laws governing the collection of protected species or specimens for scientific study. This should include a full awareness of State and Federal lists of protected species that are known to occur in the area of any given study unit.

Each study unit biologist should coordinate efforts with the regional biologist to identify and select a fish taxonomic specialist. A first step in identifying a potential cooperating fish taxonomic specialist could be to review publications related to the taxonomy and identification of fishes within the study unit area. Guides to the taxonomic identification of fishes have been published for many States. A list of authors of these publications is a useful source of information for identifying, selecting, or consulting a taxonomic specialist based on demonstrated knowledge of fish taxonomy (see Appendix).

Other sources of information to assist in selecting a fish taxonomic specialist are directories of professional societies or compiled lists of expertise. The membership directory of the American Society of Ichthyologists and Herpetologists (ASIH) provides names of people, summarized by State, that help to identify potential cooperating fish taxonomic specialists. Another source of information for identifying fish taxonomic specialists is the Interagency Taxonomic Resources and Expertise Directory (TRED), a directory of taxonomic specialists for the biota of the United States and Canada. The TRED can be accessed on the World Wide Web at <http://www.nbii.gov/tred> (Association of Systematics Collections and U.S. Geological Survey, 1998). It should be noted that inclusion in a directory does not guarantee that an individual has attained a certain level of skill and expertise in fish taxonomy.

Pre-Sampling Fish Species List

The study unit QA/QC plan must include a pre-sampling list of fish species, prepared in consultation with the cooperating taxonomic specialist. This list identifies all fish species known or expected to occur in the study unit, including those of problematic taxonomy (for example, known or suspected undescribed species) and any protected species that require immediate identification in the field.

Species Occurrence

Extensive information about the distribution of fish species is available for most parts of the United States. This information can be obtained from regional publications of fish species (see Appendix) and historical data available from ichthyological museums (Collette and Lachner, 1976), State and Federal agencies, or other organizations. General distribution maps for each species (for example, as contained in Lee and others, 1980) can provide additional information about known locations within a study unit. Electronic versions of general distribution maps for many freshwater fish species in North America are available through the Texas Memorial Museum's Natural

History Collection at <http://www.utexas.edu/depts/tnhc/.www/fish/tnhc/na/naindex.html> (University of Texas, 1998).

Problematic Taxonomic Issues

In preparing a pre-sampling list of fish species known or expected to occur within a study unit, it is important to determine which fish taxa may present problematic taxonomic issues relating to accurate species-level identifications. A fish taxonomic specialist can assist in making these determinations. Identifying relevant fish taxonomic issues prior to sampling provides the opportunity to develop QA/QC measures that are designed to address these issues.

Hybridization represents a situation that can provide challenges to proper taxonomic identification. Many freshwater fish hybrids occur commonly in streams and lakes, especially in disturbed habitats. With few exceptions, hybrid fishes are generally not given common names. Instead, scientists routinely refer to hybrids by the scientific names of the parental species. For example, *Luxilus cornutus* x *Notropis rubellus* is the taxonomic designation given to a relatively common natural hybrid of two minnow species. In many cases, identifying the parental species of a hybrid is a difficult and challenging task.

Although a fish taxonomic specialist should be able to identify most fish species in the field, determining the distinguishing characteristics of some fish species may require microscopic examination. Proper identification of such fish species may require that these specimens be removed from the field for identification in the laboratory, where scale or fin-ray counts may have to be made, or other morphological characters examined (fig. 2). Prior to sampling, it is important to note the species that may require laboratory identification so that special attention can be given to them during field processing.

Although most native fish species of North America are already known, discoveries and descriptions of new species continue to occur; such findings of undescribed fish species are a relatively uncommon event. However, some new fish taxa have been discovered recently in the United States but their taxonomic nomenclature has not yet been fully determined. Any new fish taxa that may be expected to occur in a study unit should be noted on the pre-sampling fish species list.

Introduced fish species can present unique challenges in making taxonomic identifications and in accurately determining distributions. Fishes that are native to the United States, including many game species, have been and continue to be extensively transported across drainages and even within watersheds to areas where they do not naturally occur (fig. 3). Recognition or identification of these taxa often does not present a major problem, but a fish taxonomic specialist should be consulted to confirm identification of unfamiliar species or any that are suspected to be introduced. In addition to the many North American fishes that have been transplanted,

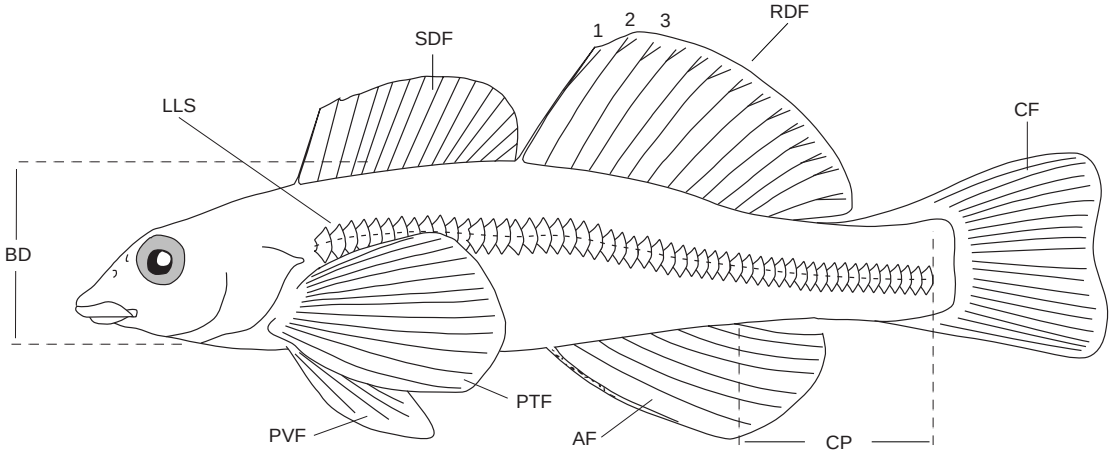

Figure 2. Examples of diagnostic characters of many fish species that may require close examination in order to make accurate taxonomic identifications (AF, anal fin; BD, body depth; CF, caudal fin; CP, caudal peduncle; LLS, lateral line scales; PTF, pectoral fin; PVF, pelvic fin; RDF, rayed dorsal fin; SDF, spinous dorsal fin; modified from Jenkins and Burkhead, 1993).

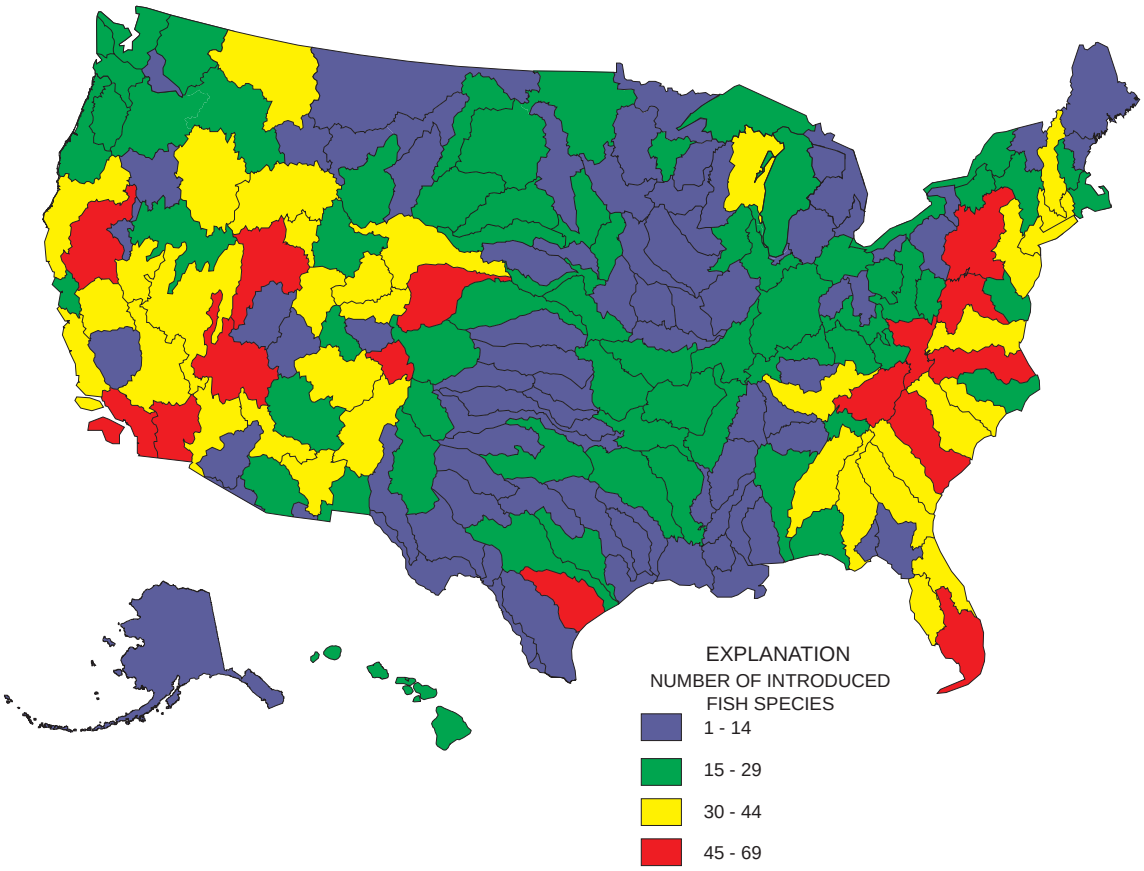

Figure 3. Numbers of introduced nonindigenous fish species in the United States by USGS 4-digit Hydrologic Unit Codes (USGS Biological Resources Division, Florida Caribbean Science Center).

numerous species from foreign countries appear in inland waters on a regular basis, largely resulting from the release of aquarium pets, escape from aquaculture facilities, or by other means. Many of these are isolated records (Fuller and others, in press), but some species have established populations and may continue to expand their nonnative ranges or are repeatedly introduced to new areas. Some foreign species that are introduced can be especially difficult to identify because of unresolved taxonomic status, nomenclatural instability, and other factors. In many of these cases it is important for the study unit biologist and fish taxonomic specialist to consult with additional systematists in order to make the most accurate taxonomic determination(s).

Protected Species

It is also important to note the occurrence of protected species on the pre-sampling fish species list. Protected species are provided some degree of legal security under legislation by one or more regulatory agencies (Johnson, 1987). The category of protected species includes those listed as threatened or endangered under the Federal Endangered Species Act and similar legislation by States. Additionally, special-concern species are recognized by agencies as declining in numbers or distribution, but there may be inadequate information to propose listing these species as threatened or endangered. Many States maintain independent lists of protected taxa that may include species that currently are not provided Federal protection.

The list of fish taxa that are provided protection under the Federal Endangered Species Act is revised periodically. Individual fish species are reviewed and listed by the U.S. Fish and Wildlife Service (FWS) and the National Marine Fisheries Service (NMFS) on an ongoing basis. Because the comprehensive list only

appears periodically, it is incumbent upon study unit biologists, fish taxonomic specialists, and regional biologists to be informed of listing activities within the region of the study unit; this is best accomplished through direct communication with the nearest Ecological Services office of the FWS. Copies of the Federal list of endangered and threatened species can be obtained from Ecological Services offices or from the FWS, Division of Endangered Species, MS 452, 4401 N. Fairfax Drive, Arlington, VA 22203. The list is also available electronically at <http://www.fws.gov> (U.S. Fish and Wildlife Service, 1998). Lists of State-protected species are typically distributed by State natural resource agencies. In addition, the AFS has produced lists of imperiled fishes (Deacon and others, 1979; Williams and others, 1989).

Federal and (or) State laws govern the collection of endangered or threatened species. As required by law, the collection or killing of Federally endangered species is strictly regulated by the FWS and NMFS and is generally not allowed. Collection of threatened species, species of special concern, and similarly designated taxa for the purposes of serving as voucher material for NAWQA studies is unnecessary and should not be considered unless there are significant taxonomic problems associated with a given taxon (or related taxa), such specimens represent extremely important new distribution records, or other special conditions prevail. Even under these unusual circumstances, preservation of protected species must be done in strict compliance with Federal and State laws and under the supervision of a qualified fish taxonomic specialist who is capable of rendering accurate identifications. Specimens of protected taxa that are unintentionally captured during NAWQA sampling procedures should be carefully handled, identified, recorded, and released alive. Prior to sampling, coordination with a fish taxonomic specialist will enable the study unit biologist to ascertain the likelihood of encountering protected taxa, and will ensure that appropriate measures are taken to avoid mortality of endangered or threatened fishes during sampling and specimen processing in the field.

Collecting Permits

The study unit biologist is responsible for securing appropriate collecting permits. A description of the required collecting permits that must be obtained should be included in the study unit’s QA/QC plan. The collection of fish within the waters of a State is regulated and permitted by the State. Additional permits may be required to collect fish within certain local areas, for example, municipal parks, or on Federal lands.

Application procedures, fees, and reporting requirements for State collecting permits vary widely. In some cases, a research plan must be filed prior to sampling, and (or) the species targeted for collection must be specified. Most permits are issued annually and are nontransferable. The permit renewal date varies by State, and in some cases, permits may extend beyond 1 year. Some States require possession of a valid recreational sportfishing license in addition to a scientific collector's permit when conducting fish faunal surveys. Moreover, many States require that regional fisheries management and wildlife law enforcement offices be contacted prior to sampling. A list of species and specimens that were collected generally must be reported, usually at the end of the permit period, and may have to be submitted on a specific form distributed by the permitting agency. Study unit biologists are responsible for reviewing permit terms and applying sufficiently in advance of collecting activities to ensure full compliance with applicable State laws. Addresses and other information pertaining to scientific collecting permits are summarized in table 1.

Table 1. State natural resources agencies that are responsible for issuing scientific, private, and commercial collecting permits; 1998 annual fees; and reporting date requirements

State	State natural resources agency	1998 annual fees	Reporting date requirements
Alabama	Alabama Department of Natural Resources Game and Fish Division Scientific Collecting Permits 64 N. Union Street Montgomery, AL 36130 (334) 242-3469	$1.00	Report due within 10 days of expiration.
Alaska	Alaska Department of Fish and Game Division of Sport Fish P.O. Box 25526 Juneau, AK 99802-5526 (907) 465-4180	No fee	Report due within 30 days of expiration.
Arizona	Arizona Game and Fish Department Nongame Branch 2221 West Greenway Road Phoenix, AZ 85023-4399 (602) 789-3504	No fee	Report due within 30 days of expiration.
Arkansas	Arkansas Game and Fish Commission Fisheries Division 2 Natural Resources Drive Little Rock, AR 72205 (501) 223-6371	No fee	Report due within 30 days of expiration.
California	California Department of Fish and Game License and Revenue Branch 3211 S Street Sacramento, CA 95816-7088 (916) 227-2225	Fee varies; government exemption	Report due within 30 days of expiration unless waived.
Colorado	Colorado Department of Natural Resources Division of Wildlife 6060 Broadway Denver, CO 81601-1000 (970) 945-4717	$20.00; government exemption	Report due within 30 days of expiration.
Connecticut	Connecticut Department of Environmental Protection Fisheries Division 79 Elm Street Hartford, CT 06106-5127 (860) 424-3474	$10.00	Report due at expiration.
Delaware	Delaware Department of Natural Resources and Environmental Control Division of Fish and Wildlife P.O. Box 1401 Dover, DE 19903 (302) 739-3441	No fee	Report due within 30 days of expiration.
Florida	Florida Game and Fresh Water Fish Commission[1] Division of Fisheries Farris Bryant Building 620 South Meridian Street Tallahassee, FL 32399-1600 (904) 488-1600	No fee	Report due at expiration or 30 days prior to renewal.

Table 1. State natural resources agencies that are responsible for issuing scientific, private, and commercial collecting permits; 1998 annual fees; and reporting date requirements—Continued

State	State natural resources agency	1998 annual fees	Reporting date requirements
Georgia	Georgia Department of Natural Resources Wildlife Resources Division Special Permit Office 2109 U.S. Highway 278 S.E. Social Circle, GA 30025 (770) 761-3044	$50.00	Report due at expiration.
Hawaii	Hawaii Department of Land and Natural Resources Division of Aquatic Resources 1151 Punchbowl Street, Room 330 Honolulu, HI 96813 (808) 587-0097	No fee	Report due within 30 days of expiration.
Idaho	Idaho Department of Fish and Game[1] P.O. Box 25 Boise, ID 83707 (208) 334-3791	No fee	Report due at end of calendar year.
Illinois	Illinois Department of Natural Resources Division of Fisheries Office of Resource Conservation 524 S. 2nd Street Springfield, IL 62701-1787 (217) 524-8285	No fee	Report due at end of February.
Indiana	Indiana Department of Natural Resources Division of Fish and Wildlife Commercial License Clerk 402 West Washington Street, Room 273 Indianapolis, IN 46204 (317) 232-4080	$10.00	Report due within 15 days of expiration.
Iowa	Iowa Department of Natural Resources License Bureau Wallace State Office Building Des Moines, IA 50319-0035 (515) 281-8688	$5.00	Report due by January 10.
Kansas	Kansas Department of Wildlife and Parks Fish and Wildlife Division 512 S.E. 25th Avenue Pratt, KS 67124-8174 (316) 672-5911	$10.50	Report due by January 31.
Kentucky	Kentucky Department of Fish and Wildlife Resources Division of Fisheries 1 Game Farm Road Frankfort, KY 40601 (502) 564-3596	$10.00 government; $200.00 private	Report due by January 31.
Louisiana	Louisiana Department of Wildlife and Fisheries Inland Fisheries Division P.O. Box 98000 Baton Rouge, LA 70898 (504) 765-2865	No fee	Report due within 60 days of permit expiration.

Table 1. State natural resources agencies that are responsible for issuing scientific, private, and commercial collecting permits; 1998 annual fees; and reporting date requirements—Continued

State	State natural resources agency	1998 annual fees	Reporting date requirements
Maine	Maine Department of Inland Fisheries and Wildlife Fisheries Research and Management Division 284 State Street 41 State House Station Augusta, ME 04333 (207) 287-5263	No fee	Report due at end of calendar year.
Maryland	Maryland Department of Natural Resources Fisheries Service Tawes State Office Building 580 Taylor Avenue, B-2 Annapolis, MD 21401 (410) 260-8323	$25.00	Report due by January 31.
Massachusetts	Massachusetts Division of Fisheries and Wildlife Executive Office of Environmental Affairs Permit Office 100 Cambridge Street Boston, MA 02202 (617) 727-9800 ext. 327	$1.00	Report due at end of calendar year[2].
Michigan	Michigan Department of Natural Resources Fisheries Division P.O. Box 30028 Lansing, MI 48909 (517) 373-1280	No fee	Report due at end of calendar year.
Minnesota	Minnesota Department of Natural Resources Division of Fisheries 500 Lafayette Road St. Paul, MN 55155-4012 (612) 296-3325	No fee	Report due at end of calendar year.
Mississippi	Mississippi Department of Wildlife, Fisheries, and Parks Division of Wildlife and Fisheries P.O. Box 451 Jackson, MS 39205 (601) 354-7303	$1.00; government and educational exemption	Report due within 15 days of expiration.
Missouri	Missouri Department of Conservation Wildlife Division P.O. Box 180 Jefferson City, MO 65102 (573) 751-4115 ext. 167	No fee	Report due within 1 year of expiration date.
Montana	Montana Fish, Wildlife, and Parks 1420 East 6th Avenue P.O. Box 200701 Helena, MT 59620-0701 (406) 444-2449	$50.00; government and academic exemption	Report due March 1.
Nebraska	Nebraska Game and Parks Commission Wildlife Division 2200 N. 33rd Street P.O. Box 30370 Lincoln, NE 68503-0370 (402) 471-0641	No fee	Permit due by February 1.

Table 1. State natural resources agencies that are responsible for issuing scientific, private, and commercial collecting permits; 1998 annual fees; and reporting date requirements—Continued

State	State natural resources agency	1998 annual fees	Reporting date requirements
Nevada	Nevada Department of Conservation and Natural Resources Division of Wildlife P.O. Box 10678 Reno, NV 89520 (702) 688-1549	$5.00	Report due within 30 days of expiration.
New Hampshire	New Hampshire Fish and Game Department Fisheries Division 2 Hazen Drive Concord, NH 03301 (603) 271-1139	$20.00	Report due by January 31.
New Jersey	New Jersey Department of Environmental Protection Division of Fish, Game, and Wildlife CN 400 Trenton, NJ 08625-0400 (609) 292-8642	$22.00	Report due within 30 days of expiration.
New Mexico	New Mexico Department of Game and Fish Villagra Building P.O. Box 25112 Santa Fe, NM 87504 (505) 827-9904	$15.00; government and educational exemption	Report due by January 31.
New York	New York Department of Environmental Conservation Division of Fish and Wildlife Special Licenses Unit 50 Wolf Road Albany, NY 12233-4752 (518) 457-0689	$10.00	Report due at expiration.
North Carolina	North Carolina Wildlife Resources Commission Division of Boating and Inland Fisheries Archdale Building 512 N. Salisbury Street Raleigh, NC 27604-1188 (919) 733-3633	$5.00; government and educational exemption	Report due quarterly.
North Dakota	North Dakota Game and Fish Department Licensing Division 100 N. Bismarck Expressway Bismarck, ND 58501-5095 (701) 328-6300	$10.00	Report due at expiration.
Ohio	Ohio Department of Natural Resources Division of Wildlife Fountain Square 1840 Belcher Drive Columbus, OH 43224-1329 (614) 265-6666	$10.00	Report due at expiration.
Oklahoma	Oklahoma Department of Wildlife Conservation 1801 North Lincoln P.O. Box 53465 Oklahoma City, OK 73152 (405) 521-3721	$5.00	Report due by January 31.

Table 1. State natural resources agencies that are responsible for issuing scientific, private, and commercial collecting permits; 1998 annual fees; and reporting date requirements—Continued

State	State natural resources agency	1998 annual fees	Reporting date requirements
Oregon	Oregon Department of Fish and Wildlife Fish Division 2501 S.W. First Avenue P.O. Box 59 Portland, OR 97207 (503) 872-5252	No fee	Report due at expiration.
Pennsylvania	Pennsylvania Fish and Boat Commission Nongame and Endangered Species Unit 450 Robinson Lane Bellefonte, PA 16823-9616 (814) 359-5113	No fee (State, Federal government); $10.00 (nonprofit research, educational); $50.00 (private, commercial)	Report due by January 31.
Rhode Island	Department of Environmental Management Rhode Island Division of Fish and Wildlife 4808 Tower Hill Road Wakefield, RI 02879-3075 (401) 222-3075	$25.00	Report due at expiration.
South Carolina	South Carolina Department of Natural Resources Freshwater Fisheries P.O. Box 167 1000 Assembly Street Columbia, SC 29202 (803) 734-3943	$10.00	Report due annually within 120 days of termination of sampling.
South Dakota	South Dakota Department of Game, Fish, and Parks Scientific Collector's Permits 523 East Capitol Avenue Pierre, SD 57501-3182 (605) 773-4191	No fee	Report due by January 31.
Tennessee	Tennessee Wildlife Resources Agency Ellington Agricultural Center P.O. Box 40747 Nashville, TN 37204 (615) 781-6575	No fee	Report due at expiration.
Texas	Texas Parks and Wildlife Department Permits Section 4200 Smith School Road Austin, TX 78744 (512) 389-4491	$50.00; government and educational exemption	Report due at expiration.
Utah	Utah Department of Natural Resources Division of Wildlife Resources 1594 West North Temple, Suite 2110 P.O. Box 146301 Salt Lake City, UT 84114-6301 (801) 538-4781	$55.00 (personal); $105.00 (commercial)[2]	Report due within 30 days of expiration.
Vermont	Vermont Agency of Natural Resources Fish and Wildlife Department 103 S. Main Street, 10 South Waterbury, VT 05676 (802) 241-3708	No fee	Report due within 30 days of expiration.

Table 1. State natural resources agencies that are responsible for issuing scientific, private, and commercial collecting permits; 1998 annual fees; and reporting date requirements—Continued

State	State natural resources agency	1998 annual fees	Reporting date requirements
Virginia	Virginia Department of Game and Inland Fisheries Wildlife Information and Enhancement Division 4010 West Broad Street Richmond, VA 23230-1104 (804) 367-1185	$20.00	Report due by July 31.
Washington	Washington Department of Fish and Wildlife Enforcement Program 600 Capitol Way North Olympia, WA 98501-1091 (360) 902-2380	$12.00	Report due by January 31.
West Virginia	West Virginia Division of Natural Resources Wildlife Resources Section Scientific Collecting Permits P.O. Box 67 Elkins, WV 26241 (304) 637-0245	No fee	Report due within 30 days of expiration.
Wisconsin	Wisconsin Department of Natural Resources Division of Fisheries South Central Regional Headquarters 3911 Fish Hatchery Road Fitchburg, WI 53711 (608) 275-3242	No fee	Report due by January 10.
Wyoming	Wyoming Game and Fish Department Wildlife Division 5400 Bishop Boulevard Cheyenne, WY 82006 (307) 777-4559	No fee	Report due by December 31.

[1]Applications are processed through regional offices; contact agency for more information.
[2]Additional fees are possible; contact agency for more information.

Quality-Assurance and Quality-Control Procedures

The study unit QA/QC plan also must address the specific procedures that will be implemented to address QA/QC of fish taxonomy within the study unit. These procedures will vary, depending on such factors as the expertise of the taxonomic specialist who is selected and problematic taxonomic issues that are preliminarily identified. For example, if a study unit contains relatively few fish species, all or most of which are easily identifiable, no problematic taxonomic issues exist, and the fish taxonomic specialist has more than adequate expertise as evidenced by prior publications or other criteria, then no specific QA/QC procedures may be necessary. In contrast, detailed QA/QC procedures may be required if a study unit contains a large number of fish species that are difficult to identify in the field, problematic taxonomic issues exist, and (or) personnel of the study unit are relying on an individual whose taxonomic expertise or qualifications are limited. Various QA/QC procedures are available to address issues that apply to different study units. These include identification of certain fish species in the laboratory only, independent verification of taxonomic identifications of representative preserved specimens, preserving additional fish as voucher specimens, photographic documentation, and collection of tissue samples. Determination of which QA/QC procedures to include should be made by the study unit biologist in consultation with the regional biologist and a fish taxonomic specialist.

Laboratory Identifications

The selection of subsamples to be fixed, preserved, and identified in the laboratory depends on anticipated taxa that may be difficult to accurately

identify during field processing. In the field, a fish taxonomic specialist can quickly select fish to be returned to the water alive and those to be preserved. The following guidelines are minimum recommendations to consider when selecting subsamples to fix, preserve, and subsequently identify in the laboratory:

1. Species that cannot be positively or reliably identified in the field by the fish taxonomic specialist. Difficulties in making identifications in the field may result from a number of factors, including fish size and age; smaller fish may be more difficult to positively identify in the field than larger fish. Examples of small-sized fish that may require close examination in the laboratory to identify include many of the clupeids (herrings and shads), cyprinodontids (topminnows), poeciliids (livebearers), cyprinids (minnows), catostomids (suckers), percids (darters), and cottids (sculpins). A complete size range of specimens should be preserved unless there is suspicion that the species may be protected or rare, in which case photographic documentation should be considered.

2. Specimens that are to be archived in voucher or reference collections, or intentionally sought for independent taxonomic verification. A small sample should be taken upon consultation with the regional biologist or fish taxonomic specialist, based on suggested need for archiving or when an independent opinion is required.

3. Suspected or known undescribed taxa of which there is a known paucity of museum material, or of which specimens are otherwise taxonomically valuable (for example, for the purpose of comparing morphological variation), and that are available in reasonable numbers and are not known to be imperiled.

4. A species that cannot be easily separated, without careful examination, from one or more congeners or related taxa occurring within the same general range if a significant possibility exists that more than one form could be represented (for example, a species not native to the drainage but that could appear there, either by natural distribution, dispersal, or transplanted by humans). An adequate series of specimens should be collected to verify field identification.

5. Cryptic taxa or two or more species that co-occur in the same drainage and that cannot be easily separated without closer examination of critical characters, especially those requiring use of a microscope. Unless a procedure is adopted and the time is taken to confidently separate such taxa in the field, it will be necessary to preserve all samples for subsequent identification.

6. New drainage records. Any specimen, or a subset of an entire sample, that is recognized as representing new drainage records or significant range extensions within a drainage should be preserved and identified in the laboratory. It is especially important to save samples of preserved specimens of any fishes that are suspected as being introduced, in order to confirm taxonomic identifications and to document new distributional records.

7. Samples of common species (for example, mosquitofish, *Gambusia affinis* or *G. holbrooki*) that are collected in large numbers and that cannot be processed fully in the field may have to be preserved in their entirety or as a subset.

8. Samples that provide important life history specimens. The taxonomic specialist can provide advice if a sample yields valuable specimens that are of interest to ecologists or may otherwise be worth preserving and archiving for future research purposes.

9. Hybrids or unusual anomalies. Specimens that are suspected of being hybrids, or that demonstrate obvious morphological anomalies or injuries that suggest developmental or environmentally induced trauma, should be preserved for identification and additional examination in the laboratory. In the case of large specimens that exhibit anomalies, it may be necessary only to save the affected tissue(s) and any portion(s) of the fish that may be required to confirm taxonomic identification.

Although the above guidelines are general recommendations, each sample requires independent consideration regarding specimens to preserve for identification in the laboratory.

Independent Taxonomic Verification

Independent verification of taxonomic determinations is a mechanism that is used to confirm fish identifications when problematic taxonomic issues exist and the individual making the identifications has limited expertise in dealing with these issues. Depending on the nature of the problematic taxonomic issues pertaining to a study unit, the study unit biologist, in consultation with the regional biologist and fish taxonomic specialist, should select an additional fish taxonomic specialist to independently verify questionable identifications. Regardless of

independent taxonomic verification, voucher specimens should be retained to provide a substantiated record of identifications.

Voucher Specimens

Voucher specimens provide a critical QA/QC function by providing a documented, permanent record of taxonomic identifications. No set standards exist for selecting taxa and numbers of individual fish to serve as voucher specimens. Determination of specimens to be preserved to substantiate taxonomic identifications must be made by the study unit biologist in careful consultation with fish taxonomic specialists and the regional biologist. Study unit biologists are encouraged to preserve one lot of each species from each sampling site. Lots consist of a jar or other appropriate vessel containing one or more specimens of a single species collected together at one place and time. It may be important to preserve more than one lot if taxonomic problems exist or if cryptic species are within the study area and require close examination to determine accurate identifications.

Additionally, it is generally unnecessary to preserve specimens of sportfish species as vouchers unless identifications are questionable. Study unit biologists are encouraged to consult closely with the fish taxonomic specialist in advance of sampling to determine the necessity of preserving protected or sportfish species as voucher material.

Photographic Documentation

Photographic documentation of fish specimens can be an important means of validating field identifications and may be the only means of verifying taxonomic identifications of protected species. Photographs can include 35-mm color slides, black-and-white negatives, or prints by using relatively inexpensive camera equipment. Color slides are favored, especially for cryptic species and (or) groups such as darters (Percidae) that have useful pigmentary diagnostic features (fig. 4). High-resolution digital and video cameras are excellent options for documentation and have potential advantages for storage and long-term archiving. For 35-mm slides, a relatively high-speed film (for example, ASA 200) works well when used properly with light meters and flash attachments or natural sunlight.

Figure 4. Examples of fish photographs used to illustrate diagnostic coloration patterns that may be useful in distinguishing cryptic species (*Etheostoma coosae* and *E. scotti*, photographs by N.M. Burkhead).

Live (anesthetized) or fixed specimens should be photographed by using a macro- or close-up lens. Best results are obtained if specimens are placed against a light background (gray, white, or light blue) of foamboard, plastic, or some other semi-waterproof material. Conventionally, the left side of a fish is photographed, and relative size can be determined by including a small ruler in the photograph. The value of photographs for identification purposes can sometimes be improved by spreading and pinning the fins of fixed specimens, thus revealing diagnostic characters. Several references that provide additional detail regarding photographic techniques include Randall (1961), Emery and Winterbottom (1980), Flescher (1983), Strauss and Bond (1990), and Jenkins and Burkhead (1993). Detailed information on locality, field identification, and other data should be recorded to accompany photographs or digital images.

Study unit biologists also are encouraged to take photographs of representative habitats at selected sampling sites. Photographs of habitats have the benefit of documenting historical changes and localized conditions on a temporal scale.

Tissue Samples

Small samples of tissue taken from fresh fish may be used to distinguish species based on patterns of genetic variation. An increasing trend in systematic ichthyology is to supplement identifications based on morphological, morphometric, and meristic characteristics with biochemical approaches to detecting genetic divergence (Leary and Booke, 1990). These approaches include the use of starch gel electrophoresis, mitochondrial DNA, and nuclear genomes. Removal of tissues for detecting genetic variation is not a routine activity of NAWQA sampling to determine taxonomic identifications, but a demonstrated need may exist for collection of tissues for taxonomic verification or systematic study based on interactions between study unit personnel and other researchers. The type of tissue and the way in which it is processed depend on the type of analysis for which the tissue is intended. The study unit biologist is advised to consult with the fish taxonomic specialist, regional biologist, and other researchers to determine the proper methods for the collection of tissue samples if they are needed for further scientific study.

Archiving of Fish Specimens

The archiving of fish specimens into scientific collections can provide valuable resource material for many purposes, including instruction in fish taxonomy and a permanent record of fish taxonomic identifications (Crossman, 1980; Haedrich, 1983). Collections that are maintained to provide specimens for local instruction in fish taxonomy are often referred to as reference collections, whereas collections of specimens maintained primarily for research purposes and to substantiate taxonomic identifications are known as voucher collections. Many natural history museums and universities throughout the United States maintain permanent fish collections (Collette and Lachner, 1976). The QA/QC plan for each study unit must provide a general indication of the specimens that will be archived as part of a collection and the location of the facility where the collection will be maintained.

Reference Collections

Reference collections are not essential to the QA/QC of taxonomic identifications, but they may be helpful in educating study unit personnel and cooperators about the identification of fish specimens. A fish taxonomic specialist should examine all specimens in the reference collection to confirm that scientific and common names are correct and that locality and other collection data are valid. Because reference specimens are often handled frequently during instruction in distinguishing characteristics, reference specimens are generally not in ideal condition for use as voucher specimens. Thus, archival of a reference collection is for a comparatively short period of time and responsibility for its maintenance lies within the study unit, whereas a voucher collection is permanently archived at an ichthyological curation facility.

Voucher Collections

Voucher collections are essential to prevent ambiguities in data among study units and to provide a means of checking possible erroneous identifications. Moreover, voucher specimens within the collections are important for unequivocally verifying new distribution records, for providing historical documentation of fish faunal composition, and for providing critical material for scientific research purposes. As with reference collections, a fish taxonomic specialist should examine all voucher specimens to confirm that scientific and common names are correct and to validate locality and other collection information. Once the information has been confirmed, voucher specimens should be shipped to an appropriate ichthyological curation facility with an established and reputable history of permanent archiving of fish collections.

Ichthyological Curation Facilities

Leviton and others (1985) and Leviton and Gibbs (1988) compiled comprehensive lists of most ichthyological curation facilities worldwide. The ASIH conducted a survey of fish collections and identified ichthyological curation facilities as international, national, or regional curation centers, as well as important nonpermanent and other collections, based on a number of factors that included the number of specimens maintained and their historical importance (Collette and Lachner, 1976). Subsequently, Poss and Collette (1995) re-evaluated the status of North American fish curation facilities to evaluate and identify new issues relevant to long-term curation of collections (fig. 5). The second survey revealed that significant changes have occurred at some facilities

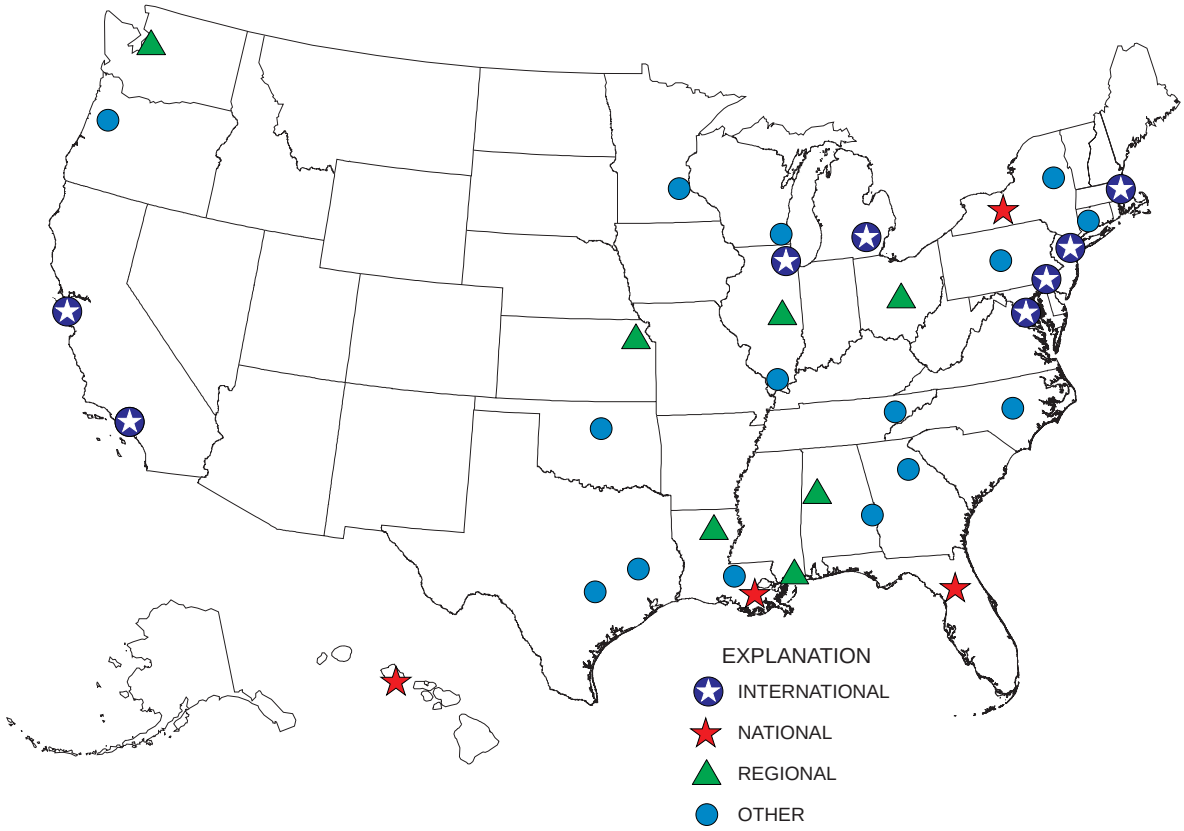

Figure 5. Major ichthyological curation facilities in the United States (Poss and Collette, 1995).

that have affected the long-term curation of collections. Thus, the study unit biologist must consider several factors when selecting an ichthyological curation facility to archive a voucher collection.

The study unit biologist, in consultation with a fish taxonomic specialist and the regional biologist, should make the selection of an ichthyological curation facility. Voucher collections should be deposited in reputable, permanent curation facilities that are willing to accept, catalog, and curate specimens. Preference should be given to an established permanent curation center that is within the general region of the study unit. Address information for international, national, regional, and other important curation centers is provided in table 2. Curators at these centers may be able to provide study unit biologists with additional information and assistance in the development and oversight of QA/QC of fish taxonomy.

For voucher collections, a two-way exchange of information must occur between the study unit and the curation center. First, the study unit biologist is responsible for providing the repository center with collection information, including sample locations, taxonomic identifications made by the fish taxonomic specialist, and the name of the fish taxonomic specialist who made those identifications. After specimens are catalogued, curation center personnel should provide the study unit biologist with catalogue numbers for voucher specimens. In cases where collections are computerized, it is preferable to have the curation center provide catalogue information in an electronic file so that data accuracy can be assessed by the study unit biologist.

Table 2. Address information for international, national, regional, and other important ichthyological curation centers in the United States with significant freshwater fish holdings. Abbreviation codes in parentheses are standard codes for curation centers (Leviton and others, 1985)

Ichthyological curation centers

International

Academy of Natural Sciences of Philadelphia (ANSP)
Department of Ichthyology
19th and The Parkway
Philadelphia, PA 19103

American Museum of Natural History (AMNH)
Department of Ichthyology and Herpetology
79th Street and Central Park West
New York, NY 10024

California Academy of Sciences (CAS)
Department of Ichthyology
Golden Gate Park
San Francisco, CA 94118

Field Museum of Natural History (FMNH)
Division of Fishes
Roosevelt Road at Lake Shore Drive
Chicago, IL 60605

Museum of Comparative Zoology (MCZ)
Harvard University
26 Oxford Street
Cambridge, MA 02138

National Museum of Natural History (USNM)
Division of Fishes
Smithsonian Institution
Washington, DC 20560

Natural History Museum of Los Angeles County (LACM)
Ichthyology Section
900 Exposition Boulevard
Los Angeles, CA 90007

University of Michigan Museum of Zoology (UMMZ)
Division of Fishes
Ann Arbor, MI 48109-1079

National

Bernice P. Bishop Museum (BPBM)
Ichthyology Collection
P.O. Box 19000-A
1355 Kalihi Street
Honolulu, HI 96817-0916

Cornell University (CU)
Ichthyology Collection
83 Brown Road
Research Park, Building 3
Ithaca, NY 14850

National (Continued)

Tulane University Museum of Natural History (TU)
Ichthyological Collection
Riverside Research Laboratories
Route 1, Box 46-B
Belle Chase, LA 70037

University of Florida (UF)
Florida Museum of Natural History
Gainesville, FL 32611

Regional

Gulf Coast Research Laboratory Museum (GCRL)
P.O. Box 7000
Ocean Spring, MS 39564-7000

Illinois Natural History Survey (INHS)
607 E. Peabody Drive
Champaign, IL 61820

Northeast Louisiana University (NLU)
Museum of Zoology
Collection of Fishes
Monroe, LA 71209

Ohio State University (OSM)
Museum of Zoology
Division of Fishes
1813 N. High Street
Columbus, OH 43210

University of Alabama Ichthyological Collection (UAIC)
Museum of Natural History
Box 870344
University, AL 35487-0344

University of Kansas (KU)
Museum of Natural History
Dyche Hall
Lawrence, KS 66045-2454

University of Washington (UW)
Fish Collection
FTR Building HF-15
Seattle, WA 98195

Other important collections

Auburn University (AU)
Museum Fish Collection
Department of Zoology and Wildlife Science
101 Cory Hall
Auburn, AL 36849

James Ford Bell Museum of Natural History (JFBM)
University of Minnesota
Minneapolis, MN 55455

Table 2. Address information for international, national, regional, and other important ichthyological curation centers in the United States with significant freshwater fish holdings. Abbreviation codes in parentheses are standard codes for curation centers (Leviton and others, 1985)—Continued

Ichthyological curation centers	
Other important collections (Continued)	
James Ford Bell Museum of Natural History (JFBM) University of Minnesota Minneapolis, MN 55455	Southern Illinois University at Carbondale (SIUC) Ichthyology Collection Department of Zoology Carbondale, IL 62901-6501
Louisiana State University Museum of Zoology (LSUMZ) Division of Fishes Baton Rouge, LA 70803	Texas Cooperative Wildlife Collection (TCWC) Texas A&M University College Station, TX 77843
Milwaukee Public Museum (MPM) Vertebrate Zoology 800 W. Wells Street Milwaukee, WI 53233	Texas Natural History Collection (TNHC) Texas Memorial Museum University of Texas 2400 Trinity Austin, TX 78705
New York State Museum (NYSM) CEC 3140 Albany, NY 12230	University of Georgia Museum of Natural History (UGAMNH) Ichthyological Collection Athens, GA 30602
North Carolina State Museum of Natural History (NCSM) P.O. Box 27647 102 N. Salisbury Street Raleigh, NC 27611	University of Tennessee (UT) Fish Collection Department of Zoology Knoxville, TN 37996-0810
Oklahoma State University (OSUS) Department of Zoology Collection of Vertebrates Stillwater, OK 74078	Yale University Peabody Museum (YPM) 170 Whitney Avenue New Haven, CT 06520
Oregon State University (OS) Museum of Natural History Corvallis, OR 97331	
Pennsylvania State University (PSU) Fish Museum School of Forestry University Park, PA 16802	

PROCESSING FISH SAMPLES IN THE FIELD

Guidelines for processing fish samples in the field as part of an assessment of the fish community are provided by Meador and others (1993). The completed study unit QA/QC plan will provide additional guidance for the processing of fish samples in the field to ensure the quality of fish taxonomic identifications. In addition to the study unit plan, consideration should be given to factors related to processing fish samples in the field, such as live specimen handling, specimen preparation, fixation and preservation, labeling, supplies needed, and shipment procedures. General information about methods that are used in processing fish samples is available in Schreck and Moyle (1990) and Murphy and Willis (1996).

Guidelines for Live Specimen Handling and Animal Care

Proper handling of live fish maximizes their survival following release back into the water and will ensure that data can be collected efficiently and expeditiously. Fish can be held temporarily in insulated

coolers or similar containers with ambient water during processing. Care must be taken to prevent temperature or dissolved oxygen from reaching lethal levels by frequently aerating and (or) changing the water. Examples of types of fish that may be difficult to temporarily hold alive include clupeids (shads and herrings), atherinids (silversides), and certain other species that are sensitive to handling stress and intolerant of hot or hypoxic conditions and, thus, may easily succumb during sample processing. Live fish must be handled very carefully and for the least amount of time necessary to determine species, weigh and measure, and inspect for diseases and parasites.

Use of surgical gloves may enable better handling of active fish, help prevent injury to the fish during processing, and reduce loss of protective mucus, thereby minimizing the likelihood of bacterial or other infections in fish following their release. Study unit biologists are advised to review animal care guidelines and are expected to adhere to safe, ethical, and humane handling procedures. Published reviews of acceptable animal welfare guidelines for fishes include those of the American Society of Ichthyologists and Herpetologists (1987; reprinted in Kelsch and Shields, 1996), Schaeffer and others (1992), and DeTolla and others (1995).

Specimen Preparation

Specimens should be anesthetized and euthanized prior to fixation in a formalin solution. Additionally, some specimens that are to be returned to the water alive may require sedation to minimize the potential for injury to the fish during sample processing. A variety of chemicals have been used to anesthetize fish (Summerfelt and Smith, 1990; DeTolla and others, 1995). However, use of many of these chemicals is restricted. For example, tricaine methanosulfonate (MS-222) is prohibited for use in fish that are to be returned to the water within a 21-day period (Kelsch and Shields, 1996). In addition, certain substances occasionally used as anesthetics may be carcinogenic or dangerous to humans. Carbon dioxide is recommended for use as a fish anesthetic in the NAWQA Program (Meador and others, 1993) because it is readily available, inexpensive, unrestricted, and safe to handle. Anesthesia can be accomplished by using sodium bicarbonate (142 to 642 parts per million for 5 minutes), carbonated water, or gaseous carbon dioxide.

Fixation and Preservation

Proper fixation and preservation of specimens is required for fish that must be returned to the laboratory for identification or saved as voucher or reference specimens. Formalin is a hazardous material with noxious fumes, and it must be handled with extreme care. Special types of gear are necessary, and many precautions must be used when handling formalin, alcohol, and other substances used to fix and preserve fish specimens; a fish taxonomic specialist can assist in reviewing all safety procedures that should be exercised. Detailed instructions for mixing and safely handling the correct formalin solution, sacrificing and fixing fish, and labeling specimens is provided in Haedrich (1983), Meador and others (1993), and in many general ichthyological references. A 10-percent solution of formalin is typically used to fix most fish specimens, but higher concentrations may be desirable if specimens are large or will be crowded in the container in which they are fixed. Quantities of fixative are prepared by diluting concentrated formalin stock (37-percent aqueous formaldehyde); a 10-percent solution of formalin is made by adding nine parts of water per one part of concentrated stock solution. Three grams of borax are added per liter of 10-percent formalin to act as a buffer, neutralizing the pH of the fixative, retarding tissue shrinkage, and preventing decalcification of the tissues (Meador and others, 1993). A general rule is to have no more than 40-percent biomass per container of formalin during fixation.

When working with formalin, surgical or rubber gloves should be used to prevent excessive contact with the skin, and protective eyewear should be worn. Respiratory masks are available to prevent inhalation of dangerous fumes. Formalin should be stored in plastic bottles with tight-fitting lids; avoid storing formalin in glass containers that might result in spills from breakage. Large specimens (>100 to 150 millimeters standard length) should either be injected with formalin by using a syringe and hypodermic needle, or by making a small incision on the right side of the abdomen to allow fixative to penetrate internal tissues. If a syringe is used, it is preferable to use a screw-on or locking hypodermic needle to prevent separation of the needle and possible splashing of formalin toward the face or skin. When injecting large fish, it is important to puncture or penetrate the gas bladder and gut in order to allow for proper fixation. Following fixation for at least several days and soaking

in water for 24 hours or longer, specimens should be preserved in 70-percent ethanol or 50-percent isopropanol for long-term storage. Ethanol is the preferred long-term storage medium for fish specimens because it causes less pigment loss, clearing, and tissue degradation than isopropanol. To prepare one liter of 70-percent ethanol, dilute 700 milliliters of 95-percent ethanol with 150 milliliters of water. A concentration of 50-percent isopropanol is prepared by a 50:50 dilution of 100-percent isopropanol. When transferring specimens from formalin to alcohol, it is best to use 2 to 3 steps to minimize specimen shrinkage (for example, sequential transfers for one day or more each in concentrations of 30-percent and 50-percent ethanol before final transfer to 70-percent ethanol). The concentration of large quantities can be determined with an appropriate hydrometer and adjusted accordingly. Specimens and samples should be properly labeled at all stages of fixation and preservation. Waste formalin and alcohol are hazardous substances and must be properly discarded according to State and local guidelines.

Labeling

Proper labeling of preserved specimens is critical through every step of processing to avoid lost or transposed data and other problems. For specimens placed into small jars or bottles, a label should always be placed inside the vessel; labels affixed to the outside, using tape or other adhesive materials, may peel or fall off, leaving the researcher without any means for identifying collection information. Transposed data can occur if labels are placed on lids that subsequently become switched between jars. Internal labels should be on heavy rag waterproof paper that will not deteriorate in fluid preservatives. Information on the label should be written with an indelible-ink pen or pencil, never with ballpoint pens or other media that will fade in alcohol or other solvents. Biologists are strongly encouraged to use labels that are preprinted with waterproof ink to ensure that all relevant data are included (fig. 6). Many investigators also use an embossed label with a field number as a backup should the hand-written label be destroyed or lost.

For large fish of different species that must be placed together in larger containers because of size, each specimen must be individually tagged with a unique identifying label. Tags should be durable paper, cloth, or similar material and must be firmly attached to the specimen with strong thread, string, or other means. Tags can be tied around the caudal peduncle or, in some species, threaded through the mouth and gills, and should be knotted close to the body so that loose loops are not snagged or broken. For added precaution, some field workers also place a folded label inside the mouth, body cavity, or under the operculum.

For specimens that have been identified to species, a handwritten label with the scientific name, date examined, and name of the fish taxonomic specialist who identified the fish also should accompany the specimen. In cases where identifications have been changed, it is helpful to keep all labels together so that a complete history of identification of the specimen(s) can be tracked.

U.S. GEOLOGICAL SURVEY
NAWQA FISH COLLECTION

Study Unit:_____ **Station Name:**_____
Station ID Number:_____ **State:**_____
Sampling Gear:_____ **County:**_____
Collected By:_____ **Date:**_____
Reference Location:_____
USGS Quad:_____

Figure 6. Suggested field label.

Supplies

Sampling of fish specimens requires a moderate amount of supplies for field procedures as well as for methods used in the laboratory to process preserved specimens. A list of supplies typically required to process fish samples is presented in table 3. Many of these supplies (for example, plastic bags and rulers) can be purchased inexpensively from local retailers. Other items, such as Nalgene bottles and surgical gloves, must be purchased from companies that specialize in scientific equipment. A list of some companies that supply many of the specialized items is provided in table 4. The Curation Committee of the ASIH maintains electronic copies of its newsletters on the society's World Wide Web homepage; these newsletters are another source of useful information about specialized supplies, such as waterproof paper and specimen tags.

Table 3. Common supplies used in processing live and preserved fish specimens in the field and laboratory

Plastic jars
Glass jars with polypropylene lids
Large specimen forceps or tongs
Fine-tip forceps
Scalpels and blades
Clipboards
Measuring board, tape measures, rulers
Calipers
Syringes with locking hypodermic needles
Cheesecloth
Resealable plastic bags (mixed sizes)
Large plastic bags
Tape (duct, cellophane, flagging, etc.)
Surgical gloves
Chemical splash goggles
Respiratory mask with formaldehyde filter
Specimen labels
Waterproof paper
Data sheets
Tags
Heavyweight thread or string
Permanent marking pens and pencils
Utility knife
Insect pins
Foamboard
Dissecting pans
Anesthetic (carbon dioxide)
Stock (37-percent) formaldehyde
Ethanol
Carrying crates
Insulated coolers
Aerators
Batteries
Buckets, carboys, and liquipaks
Graduated cylinder
Hanging scales
Portable weighing balance

Table 4. Selected suppliers of gear and equipment that are useful for processing fish samples in the field and laboratory

Allen-Bailey Tag & Label, Inc.
One Main Street
Whitinsville, MA 01588
(800) 724-1069
(800) 836-4074 fax

Aquatic Eco-Systems
1767 Benbow Court
Apopka, FL 32703
(800) 422-3939
(407) 886-6787 fax

Argent Chemical Laboratories
8702 152nd Avenue, N.E.
Redmond, WA 98052
(206) 885-3777

Ben Meadows Company
3589 Broad Street
Atlanta, GA 30341
(800) 241-6401
(800) 628-2068

Cabela's
One Cabela Drive
Sidney, NE 69160
(800) 237-4444
(800) 496-6329 fax

Consolidated Plastics Company, Inc.
8181 Darrow Road
Twinsburg, OH 44087
(800) 362-1000
(216) 425-3333 fax

Daigger & Company, Inc.
675 Heathrow Drive
Lincolnshire, IL 60069-4206
(800) 621-7193
(800) 320-7200 fax

E & B Discount Marine
P.O. Box 50070
Watsonville, CA 95077-0070
(800) 262-8464
(408) 761-4421 fax

Fisher Scientific
711 Forbes Avenue
Pittsburgh, PA 15219
(800) 766-7000
(800) 926-1166 fax

Forestry Suppliers, Inc.
P.O. Box 8397
Jackson, MS 39284
(800) 647-5368
(800) 543-4203 fax

Global Equipment Company
1070 Northbrook Parkway, Dept. CF
Suwanee, GA 30174
(800) 645-1232
(800) 336-3818 fax

Memphis Net and Twine Co., Inc.
2481 Matthews Avenue
P.O. Box 8331
Memphis, TN 38108
(901) 458-2656
(901) 458-1601 fax

MMI-Federal Marketing Service
P.O. Box 241367
Montgomery, AL 36124-1367
(800) 826-0446
(800) 205-9661 fax

National Bag Company, Inc.
2233 Old Mill Road
Hudson, OH 44236
(800) 247-6000
(216) 425-9800 fax

Nylon Net Company
845 N. Main Street
P.O. Box 592
Memphis, TN 38101-0592
(800) 238-7529
(901) 526-6538 fax

Uline Shipping Supply Specialists
2200 S. Lakeside Drive
Waukegan, IL 60085
(800) 295-5510

United States Plastic Corporation
1390 Neubrecht Road
Lima, OH 45801
(800) 537-9724
(419) 228-5034 fax

West Marine
P.O. Box 50070
Watsonville, CA 95077-0070
(800) 538-0075
(408) 761-4421 fax

Shipment Procedures

In cases where study unit personnel are conveniently located near an ichthyological curation center that has arranged to accept voucher specimens, it is preferable to hand-carry preserved specimens to the repository institution. In other instances, specimens must be shipped to museums or to specialists requested to provide verification of identifications. Shipment of preserved specimens is controlled, in part, by postal, airline, private courier, and other legal regulations governing transportation of goods. Individual carriers have specific requirements for packaging and shipment of hazardous materials. Thus, it is necessary to check closely with the carrier for packaging requirements and shipping limitations prior to preparing specimens for shipment. For some carriers, such as Federal Express or United Parcel Service, it may be necessary to use specific packaging and labeling materials, and package contents must be clearly indicated. Shipment of specimens of protected species may require complete documentation and approval permits; additional information can be obtained from the FWS, 4401 N. Fairfax Drive, Arlington, VA 22203.

Fish to be shipped are fixed in formalin and rinsed well or transferred to long-term preservative prior to shipping. Specimens should be carefully wrapped in cheesecloth, lightly moistened with alcohol, labeled, and double-sealed inside completely leak-proof plastic bags. It is necessary to include only enough alcohol to keep specimens moist during transit. Bags of specimens are then placed in sturdy containers and cushioned by using appropriate protective packaging materials. Prior to sealing a package, individuals are advised to place an invoice of the contents and a notice inside each package requesting the shipper to moisten and re-wrap specimens in plastic, in the rare event that a package is opened for inspection or requires repackaging during shipment. Packages should then be securely sealed and taped in a manner that will prevent damage during shipping. Liquipaks are large, plastic containers with tight-fitting lids, and are ideal for transporting or holding bulky specimens and large volumes of material.

QUALITY ASSURANCE AND QUALITY CONTROL AND REPORTS

The elements of the study unit QA/QC plan for fish taxonomic data should be included in appropriate reports. For example, summary reports should include the name and affiliation of the fish taxonomic specialist who identified the specimens, any problematic taxonomic issues that existed in the study unit, and how these issues were addressed. This also should include the name(s) of the fish taxonomic specialist(s) who conducted independent taxonomic verifications, if needed. Also, information about voucher specimens and where they are located should be included in the report.

As previously indicated, taxonomic nomenclature that is used to describe fish species collected in the NAWQA Program follows the most recent AFS list of common and scientific names of fishes. Reports should note that fish taxonomy follows this standardized list, and data bases must follow the nomenclature used in the AFS list. However, the taxonomic nomenclature of some fishes may change within the 10-year period between revisions of the standardized list of fish names. Study unit biologists may choose to reference a fish species in a report by a recently updated scientific name. If so, report authors should note the names used that are exceptions to the AFS list and provide citations to support the changes in taxonomy. Sources such as Mayden and others (1992), Eschmeyer (1998), and Gilbert (1998) may recognize taxonomic nomenclature that has changed since the publication of Robins and others (1991). Because of the dynamic nature of systematics, study unit biologists are urged to consult fish taxonomic specialists to ensure that reports reflect the most current information on scientific names for taxa within the area of study.

SUMMARY

Qualitative and quantitative samples of fishes are collected as part of the NAWQA Program. These samples are part of a multidisciplinary approach for evaluating fish community characteristics and other physical, chemical, and biological factors as part of an integrated assessment of the status and trends of water-quality conditions in the United States. The long-term and broad geographic scope of the NAWQA Program requires that uniform, repeatable procedures be used for the collection, processing, identification, and quantification of biological samples in order to facilitate the production of consistent and accurate data that meet local, regional, and national needs.

Fish samples are collected and processed by local study unit teams using standardized procedures.

Field processing involves the capture, holding, identification, and examination of fish specimens. Quantitative and qualitative data are collected to characterize the diversity, abundance, and general health of fish populations in each sampled stream reach. These data are accompanied by length and weight measurements of individual fish, together with information about parasites, diseases, and the presence or absence of other health indicators. Some specimens are returned alive to the aquatic habitat and other specimens are preserved for subsequent identification and inspection in the laboratory, and (or) to serve as voucher or reference specimens.

Personnel at each study unit collaborate closely with regional biologists and fish taxonomic specialists to develop a pre-sampling QA/QC study plan. Using a specified set of criteria, the initial step is to identify and select a fish taxonomic specialist to assist in developing the study plan, generate a pre-sampling species list, and aid in field and laboratory identifications of fish. A provisional list of the fish taxa expected to occur in the study unit is generated from previously published literature and other available sources of distributional data. Potential problematic taxonomic issues, to the maximum extent known, are identified and included in the study plan prior to sampling. Protected species also are determined and any required scientific collecting permits are obtained sufficiently in advance of collecting activities.

To ensure adequate QA/QC procedures, fish specimens are identified in the field by a taxonomic specialist trained to accurately identify individual fish species. Information is recorded on a standardized data sheet and includes the name of the person making the identifications. Scientific names are used that follow the AFS list of common and scientific names of fishes. In the event that field identifications are tentative or questionable, or taxa cannot clearly be distinguished or identified, samples are to be fixed and preserved for subsequent examination in the laboratory. In addition, some specimens are selected to serve as vouchers, reference material, or for the taxonomic identifications to be independently verified. Photographic documentation and (or) removal of tissues also may be done to augment determination of species based on field examination of external characters.

Selection of subsamples to be preserved for examination in the laboratory is based on a suggested list of criteria that relate to a number of issues, including problematic taxonomic and voucher specimens. Subsamples to be removed from the field are processed following commonly used ichthyological methods for specimen handling, preparation, fixation, and preservation. Fish are fixed in a 10-percent buffered formalin solution and eventually transferred to 70-percent ethanol or 50-percent isopropanol for storage. Safety precautions should be used during specimen preparation, and adherence to published animal-care guidelines during processing of live fish is recommended for ensuring humane and ethical treatment of fish.

Preserved subsamples are returned to the laboratory for closer examination and identification by the fish taxonomic specialist. Selected specimens may be sent to other taxonomic specialists for independent taxonomic verification. A reference collection with accurate documentation can be assembled for each study unit for instructional purposes and to serve as comparative material. Specimens are sent to reputable ichthyological curation centers to serve as permanent voucher material, based on advice from taxonomic specialists, the regional biologist, and scientific need or importance. Repository institutions are identified in the pre-sampling study plan and are contacted prior to the collection of samples. Labeling and shipping of specimens is done according to commonly used curation practices. Collection information is carefully recorded and accompanies specimens during all phases of processing.

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