Standard Operating Procedure

Laboratory Procedures for Fish Processing and Taxonomic Identification

Commonwealth of Kentucky
Energy and Environment Cabinet
Department for Environmental Protection
Division of Water

Version 3.0

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Version 3.0 January 2022	Entire Document	New language and updates to methods. Fish data entry application methodology added. Taxa list removed. Suggested taxonomic reference list updated.	David Cravens Davy Black Jacob Culp
Version 2.1 January 1, 2010	Definitions Methods	Add definitions. Reduce redundancy, better define seine method and increase maximum sample reach. General editing	Rodney Pierce Sue Bruenderman Eric Eisiminger John Brumley
Version 2.0 January 1, 2009	Section 8. Fish Community Structure	**Standard Methods for Assessing Biological Integrity of Surface Waters in Kentucky** Laboratory Methods for Fish in Wadeable Waters was separated from preceding document and revised/updated for general content regarding laboratory methods.	Rodney Pierce Sue Bruenderman Eric Eisiminger John Brumley
Version 1.0 March 13, 2008		**Standard Methods for Assessing Biological Integrity of Surface Waters in Kentucky** General Content Document was re-formatted for maintaining headers, section titles, etc. in a consistent style. All references to detailed water chemistry sampling were removed, and a reference inserted directing the reader to the Standard Operating Procedures for Sampling and Monitoring Surface Waters for Kentucky', in draft.	
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1.0 SCOPE & APPLICABILITY

This procedure has been developed by the Kentucky Division of Water (DOW) as guidance for the uniform and accurate processing, taxonomic identification, and quality assurance/quality control (QA/QC) of fish samples collected from the surface waters of Kentucky (DOW 2022). The procedures defined herein are required for all fish processing, taxonomic identification, and QA/QC activities resulting in information used for the Integrated Report to Congress on the Condition of Water Resources in Kentucky (305(b) and 303(d) Reports) and other data needs of the DOW up to the point where data are ready for entry into the Kentucky Water Assessment Data for Environmental Monitoring (K-WADE) database.

A multi-metric index, the Kentucky Index of Biotic Integrity (KIBI), is used to assess stream health by examining fish community structure (Compton et al. 2003). Advantages of using fish as biological indicators include their 1) widespread distribution from small streams to all but the most polluted waters; 2) utilization of a variety of trophic levels; 3) stable populations during summer months; and 4) the availability of extensive life history information (Karr et al. 1986). The methods used for laboratory processing and identification of fish samples for use in the KIBI are outlined in this document.

Any data submitted to DOW for review will undergo QA/QC and those identified as not following the methods set forth in this document will be flagged as not suitable for the Integrated Report to Congress on the Condition of Water Resources in Kentucky (305(b) and 303(d) Reports)**.** These data may be retained in DOW files for other data purposes.

2.0 SUMMARY OF METHOD

This document summarizes the laboratory procedures for processing and identification of fish samples taken during water quality monitoring activities by DOW along with the required QA/QC checks. Personnel qualifications for identification of fishes are outlined along with procedures detailing the proper logging of samples once they enter the taxonomy laboratory and subsequent disposal or long-term preservation of samples.

3.0 DEFINITIONS & ACRONYMS

303(d) – Section 303(d) of the Clean Water Act (33 USC §1313(d))
305(b) – Section 305(b) of the Clean Water Act (33 USC §1315(b))
DOW – Kentucky Division of Water
EtOH – Ethanol
FDEA – Fish Data Entry Application
KIBI – Kentucky Index of Biotic Integrity
K-WADE – Kentucky Water Assessment Data for Environmental Monitoring
SDS – Safety Data Sheet
PPE – Personal Protective Equipment

PTD – Percent Taxonomic Disagreement
QA – Quality Assurance
QC – Quality Control

4.0 HEALTH & SAFETY STATEMENT

All staff should review the *Worksite Hazard Assessment Guidance Document* (DOW 2017). In addition, each employee will be individually trained by his/her supervisor, or designee, to perform assigned job tasks safely, prior to his/her performing the task.

Proper personal protective equipment (PPE) shall be worn by all DOW personnel while processing samples and handling chemicals. Refer to the appropriate SDS sheet for the correct PPE while handling chemicals. It is strongly recommended that lab coats also be worn to protect clothing from spillage. Protective eyewear should be worn when the potential exists for particulate, vapor, liquid or foreign objects to become lodged in the eye. Forceps should be used to remove specimens from collecting jars to reduce the risk of exposure from chemical preservatives.

When working with chemicals that cause harmful fumes, personnel are required to use a fume hood to reduce the threat of inhalation exposure to them and their fellow coworkers when indoors. A fume hood is mandatory when utilizing any carcinogenic compound (e.g. formalin). Fume hoods shall also be used when changing preservatives in collection jars to reduce the potential for adverse effects to staff.

Toxic or caustic materials should be stored in the chemical storage cabinet. Excess toxic or caustic materials should be stored in the flammable cabinets found in the 150 building or in the hazardous material cages in the basement of the 100 lab building. Two or more personnel should be involved in retrieving materials from the hazardous materials cage.

When a chemical spill (e.g. a broken mercury thermometer, broken large containers of acids or preservatives, etc.) occurs, the first line supervisor will be notified. The first line supervisor will notify the second line supervisor and the Division safety officer. The Division safety officer will then notify the Department safety officer. Do not attempt to clean-up a chemical spill if inhalation exposure or skin, throat, or eye irritation is a threat.

If injury or exposure occurs within the laboratory facilities, proper first aid attention will be administered by other certified lab personnel as soon as possible. If the condition is serious, the victim should be transported to a medical facility as soon as possible. For chemical exposures, refer to the appropriate SDS sheet for first aid treatment. SDS sheets shall be maintained in a readily accessible location in the lab for each chemical stored or used in the lab. If any exposure occurs while in the laboratory, an IA-1 exposure or injury form needs to be submitted to the Department of Workers' Claims within 72 hours of exposure or injury.

5.0 CAUTIONS & INTERFERENCES

Several cautions exist with regard to activities and negligence that could possibly cause equipment damage, degradation of the sample, and possible invalidation of the results. The first is care and maintenance of the optical equipment (i.e. stereomicroscopes). Microscopes should be maintained by the manufacturer or a professional service center.

In addition, samples stored in containers using ethanol (EtOH) are not always airtight. In consequence, liquid may evaporate leading to desiccation of the sample. Stored samples must be kept in a well ventilated room. Staff should check EtOH levels periodically to ensure that levels are adequate (at least monthly) and add EtOH as needed. This should be a common practice not only before the sample is identified, but for the duration of the project after identification to satisfy retention time requirements. Additionally, samples stored as the DOW reference collection should be checked periodically by a designated employee to ensure that adequate EtOH levels are maintained to avoid desiccation. To help minimize amount of EtOH loss due to evaporation staff can line bottle lids with parafilm or use thread tape on jar lids to create better seals. However, each time the storage vessel is opened the parafilm or thread tape will likely need to be replaced.

6.0 PERSONNEL QUALIFICATIONS

All fish identifications will be performed by approved personnel. Personnel performing fish identifications will be trained in taxonomic identification of fish by formal academic instruction, which can include courses in ichthyology or workshops focused on the identification of fishes. Additionally, personnel that have not had formal academic instruction in collection and identification of fish will be deemed technically competent based on their knowledge, skills, and abilities by DOW management. Taxonomic education will continue with on-the-job training, interaction with experienced taxonomists, and continued outside training when education opportunities become available.

7.0 EQUIPMENT & SUPPLIES

The following is a list of common equipment and supplies typically employed by DOW fish taxonomists when identifying specimens.

- Stereomicroscope (Dissecting) w/ 10X ocular and appropriate zoom magnification
- Plastic pans (assorted sizes)
- Forceps (specimen and fine-tip)
- Probes
- Compressed air
- Disposable gloves
- Counter
- Fume hood

- 70% EtOH
- Glass specimen jars (assorted sizes)
- FDEA (Fish Data Entry Application)
- Sample log
- Taxonomic literature (Appendix A)

8.0 STEP-BY-STEP PROCEDURE

The following sections describe the methods that are to be followed when processing, identifying, and recording fish samples in the laboratory.

8.1 Logging Samples

All samples shall be logged into the electronic sample control log upon returning from the field and stored in an approved refrigeration unit. Information included in the logbook (or file) must include DOW Station ID, stream name, latitude and longitude, date collected, collectors, type of sample, number of samples, date-to-lab, date rinsed in tap water, and date transferred to EtOH (DOW 2022). Additionally when a fish sample is returned to the lab, the sampling effort must be entered into K-WADE as a field activity during data entry for the field station visit. For fish, the sample information must be entered into K-WADE up to the Fish Sample Effort Information page for each sampling effort included in the sample.

8.2 Preservation

Following initial field collection, fish <150mm need to be stored in the buffered (borax) formalin (10-15%) solution for a minimum of 3 days. Fish >150mm should be allowed longer time for proper fixation and must either be injected with formalin via needle and syringe, or have an incision made into their abdominal cavity (with care made to avoid piercing organs) to allow formalin quicker access to tissue. After initial preservation in formalin, samples are triple rinsed and soaked in tap water for 1-3 days, with a change of water each day. Used formalin must be poured into an approved, designated waste container found in the chemical storage cage. After rinsing in tap water, fish are then transferred to 70% EtOH for long-term preservation and storage in glass jars. All preservation work conducted in the laboratory must be performed under a fume hood.

8.3 Labeling

If a label was not placed in the container while in the field, a label will be placed in the collection jar when the sample is returned to the laboratory or before being identified. The label must be waterproof and include the site number, stream name, location, county, date sampled, collectors' initials, and collection method. Additional information can be added including shock time, seine time, and distance sampled but is not required.

8.4 Laboratory Methods

Before taxonomic identification, fish are triple rinsed in tap water and placed in sorting pans with tap water in the pan. If fish have not been transferred to EtOH before identification, they must be tripled rinsed and soaked for 1-3 days in tap water before identification.

All individuals in the sample are identified and enumerated using all available taxonomic keys and distribution records. All taxonomic identifications are made to the lowest practical level using the most current taxonomic references available (Appendix A). A current master taxa list can be found on the network drive located at V:\DOWWQB\Biology\Fish\Fish Taxa List Updates. In rare instances, FDEA and K-WADE might not have the most up-to-date taxonomical names. In those instances the current taxonomy will be noted in the comment section and prior taxonomy used for data entry. Independent taxonomic verifications of problematic specimens are obtained as needed from recognized experts, either DOW co-workers or staff from other agencies (e.g. Kentucky State Nature Preserves Commission). The occurrence of species that are listed as rare, endangered, or threatened by federal or state agencies is also documented and the appropriate agencies are notified with the Threatened/Endangered Species Report Form (Appendix B).

After identification, fish are returned to glass containers with 70% EtOH for storage. Individuals from seining and electrofishing must be kept separate but can be combined in an appropriately sized glass jar. When combining, individuals from seining effort are put into a Ziploc bag that has had holes poked in it allowing for soaking in EtOH.

As staff come across new taxa that they have not identified during their tenure with the DOW, they must pull representative specimens of the taxa and add them to their personal reference collection maintained by each staff member. These individual species will be placed in a specimen vial labeled and eventually turned in to a 2nd party taxonomist for verification. When individuals are removed from the sample for purposes of reference collection it must be annotated on the bench sheet.

At the conclusion of a projects established sample retention time, samples are either donated to other institutions for further study or dried and discarded. Samples may be retained for longer periods of time for special studies.

9.0 DATA & RECORDS MANAGEMENT

The bench sheet for recording all ichthyofaunal sample collection, processing, and identification data is in the form of a Microsoft Excel spreadsheet application. The FDEA will also complete all KIBI metric calculations automatically. When laboratory processing of the sample is initiated, a copy of the master FDEA is placed in the appropriate project folder on the network drive. Once placed, the copy must be renamed as the Station ID for the sample site. In cases where sample duplicates are taken at a later time the Station ID will have the suffix “-dup” added to it. In the rare case that multiple fish collections are conducted throughout the year at the same site,

then sequential replicate numbers (starting with 1) will be appended to the filename after a dash (-) (e.g. –rep1, rep2, etc.). Additional instructions for completing data entry using the FDEA application are found within the FDEA via the "read me" tab. For occasions when the FDEA may not be available for use, a paper bench sheet (Appendix C) may be used in lieu of, or transferred over to the FDEA, at a later time.

At the conclusion of a projects established sample retention time, samples are either donated to other institutions for further study or dried and discarded. Samples may be retained for longer periods of time for special studies.

All records, including hardcopy and electronic files, that are collected by DOW staff or that are collected for the explicit use of DOW must be kept according to DEP record retention policy (KDLA 2006).

10.0 QUALITY CONTROL & QUALITY ASSURANCE

All new taxonomists will have their first five headwater and first five wadeable samples re-identified by another trained staff taxonomist. When being checked by a qualified taxonomist the new taxonomist FDEA filename will be labeled as station ID-Training. The trained taxonomist will utilize a separate FDEA with the filename station ID-QA.

Staff are also required to maintain a personnel reference collection of fish species they have identified. Collections will be verified by 2nd party taxonomist. New additions to collections should be verified on a yearly basis. Once verified, collections can be stored in the taxonomy lab reference collection.

Additionally, to assure quality data and assessments, QA/QC will be performed on a percentage of all samples collected to verify consistent collection, identification, and data entry as detailed in applicable PMP's or summarized in section 2.5 of the QAPP.

A minimum of five percent of all identified samples will be re-identified by a second taxonomist. Samples selected for re-identification are chosen randomly using a random numbers table, or other random selection methodology, and are identified in house, by a second trained taxonomist. The trained taxonomist will utilize a separate FDEA with the filename station ID-QA.

Percent taxonomic precision is measured by percent taxonomic disagreement (PTD) (Stribling et al. 2003 and 2008) as follows:

$$PTD = [1-(N_{Pos}/N_{Tot})]*100$$

Where:

N_{Pos} = number of agreements, and

N_{Tot} = total number of specimens in the larger of the two counts.

PTD values $\leq$ 10% is the target criteria. If the sample fails, taxonomy must be reconciled by both taxonomists and a third taxonomist if deemed necessary.

11.0 REFERENCES

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Appendix A. Suggested Taxonomic References

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Appendix B. Threatened/Endangered Species Report Form

THREATENED/ENDANGERED SPECIES REPORT FORM		
Species Collected:	Date Collected:	
# Collected:	Released ☐Yes ☐No	
If No, Explain:		
Stream:	Site #:	Basin:
Location:	County:	
Quad:	Collected/Identified by:	
Collection method:		
Comments:		

Appendix C. Fish Bench Sheet

Site #: _____ Collect Date: _____

Stream Name _____ EF Time: _____

County: _____ Seine Time: _____

Collectors: _____ Distance: _____

Identified By: _____ ID Date: _____ Date Entered KWADE: _____

Species	Electrofishing Counts Field	Electrofishing Counts Voucher	Electrofishing Number Diseased	Seining Counts Field	Seining Counts Voucher	Seining Number Diseased	Notes

Species	Electrofishing Counts Field	Electrofishing Counts Voucher	Electrofishing Number Diseased	Seining Counts Field	Seining Counts Voucher	Seining Number Diseased	Notes