

**ENVIRONMENTAL MONITORING AND ASSESSMENT PROGRAM-  
SURFACE WATERS:**

**FIELD OPERATIONS AND METHODS FOR MEASURING THE  
ECOLOGICAL CONDITION OF WADEABLE STREAMS**

Edited by

James M. Lazorchak<sup>1</sup>, Donald J. Klemm<sup>1</sup>, and David V. Peck<sup>2</sup>

<sup>1</sup> U.S. Environmental Protection Agency  
Ecosystems Research Branch  
Ecological Exposure Research Division  
National Exposure Research Laboratory  
Cincinnati, OH 45268

<sup>2</sup> U.S. Environmental Protection Agency  
Regional Ecology Branch  
Western Ecology Division  
National Health and Environmental Effects Research Laboratory  
Corvallis, OR 97333

NATIONAL EXPOSURE RESEARCH LABORATORY  
OFFICE OF RESEARCH AND DEVELOPMENT  
U.S. ENVIRONMENTAL PROTECTION AGENCY  
RESEARCH TRIANGLE PARK, NC 27711

NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH LABORATORY  
OFFICE OF RESEARCH AND DEVELOPMENT  
U.S. ENVIRONMENTAL PROTECTION AGENCY  
RESEARCH TRIANGLE PARK, NC 27711

## SECTION 13

### FISH TISSUE CONTAMINANTS

by

Roger B. Yeardley<sup>1</sup>, James M. Lazorchak<sup>2</sup>, and Frank H. McCormick<sup>2</sup>

In addition to gathering data on the aquatic vertebrate assemblage (Section 12), certain specimens of fish are retained for analysis of fish tissue contaminants. In general, the focus is on fish species that commonly occur throughout the region of interest, and that are sufficiently abundant within a sampling reach. Two types of composite samples of fish are prepared at each site (if possible). One composite sample is prepared using individuals of a *Primary Target Species*. Primary target species include species of fish whose adults are small (e.g., small minnows, sculpins, or darters). The second composite sample is prepared using individuals of a *Secondary Target Species*. Secondary target species include species whose adults are of larger size (e.g., suckers, bass, trout, sunfish, carp).

#### 13.1 PREPARING COMPOSITE SAMPLES FOR PRIMARY AND SECONDARY TARGET SPECIES

To determine the proper quantity for each composite sample, weight is used for the primary target species and the number of individuals of sufficient size is used for the secondary target species. Prepare each composite sample using similar sized individuals if possible. The general rule-of-thumb for “similar size” is that the smallest individual in the sample should be at least 75% of the total length of the largest individual. Keep this criterion in mind while selecting the final samples. Do not include any obviously small or large individuals if there is a sufficient sample (weight or number of individuals) without them. If there is a conflict between criteria, getting a sufficient sample is a higher priority than getting similar-sized individuals.

---

<sup>1</sup> SoBran Environmental, Inc., c/o. U.S. EPA, 26 W. Martin Luther King Dr., Cincinnati, OH 45268.

<sup>2</sup> U.S. EPA, National Exposure Research Laboratory, Ecological Exposure Research Division, 26 W. Martin Luther King Dr., Cincinnati, OH 45268.

Prepare a composite sample of a primary target species, as described in Table 13-1. For the primary species composite sample, choose the highest priority target species that has at least enough individuals to attain the minimum weight (50 g). Get as much weight of fish as possible within the desired weight range (50-400 g). Do not settle for the minimum amount (weight) if more fish are present, but instead send as many fish as possible up to the 400 g weight goal. If there are no primary species with enough individuals available to meet the desired weight goal, prepare the primary composite sample using individuals of a small nontarget species for which there are enough individuals available (after vouchering) to prepare a sample of at least 50 g.

Prepare a composite sample of a secondary target species as described in Table 13-2. For the secondary species composite sample, choose the highest priority target species that has the desired number (5) of similar-sized individuals (minimum total length=120 mm) available. If, for any secondary target species, you did not collect 5 individuals of the desired size, prepare the composite sample from a species having 5 individuals available (including smaller sized individuals). If fewer than 5 fish of any size for any secondary species are available, prepare the composite sample using as few as 3 fish that are at least at or near the minimum desired size. If an acceptable secondary target species sample (by the above criteria) is not available, send only the primary target species sample.

If neither a primary nor secondary species sample that meets these criteria is available, use your best judgement in preparing some type of fish tissue sample from the available species collected. Use the procedure for either primary or secondary species, depending upon the species used and the size range of individuals selected.

Individuals comprising the primary composite sample are wrapped together in aluminum foil and placed into a single plastic bag. Each individual comprising the secondary composite sample is wrapped separately, but all individuals are placed into a single plastic bag. Each composite sample is labeled as shown in Figure 13-1. Prepare two identical labels for each composite sample. Double-bag each sample, and place a label on each bag. Record information about each composite sample on page 2 of the Sample Collection Form as shown in Figure 13-2. Make sure the sample ID numbers (barcodes) recorded on the collection form match those on the sample labels.

Tissue samples are stored frozen, using either a portable freezer, a container with dry ice, or a cooler with several bags of ice. When using ice, double bag the ice and tape the last bag shut to prevent contamination of samples by melting ice. Store tissue samples

**TABLE 13-1. PROCEDURE TO PREPARE THE PRIMARY COMPOSITE SAMPLE FOR FISH TISSUE CONTAMINANTS**

**NOTE: If neither a primary nor secondary species sample is available, use your best judgment in sending some type of composite fish tissue sample.**

1. After all voucher specimens have been prepared, choose the highest priority primary target species from the list below that has at least enough individuals to attain the minimum weight (50 g). Include as many individuals as possible to attain a maximum sample weight of 400 g.

**PRIMARY TARGET SPECIES (small adult fish)\***

- |   |  |
|---|--|
| 1) The most common minnow species in the region (e.g., blacknose dace)                    | 5) Another common minnow species (e.g., stoneroller)   |
| 2) Another dace species   | 6) A darter species  |
| 3) Another common minnow (e.g., creek chub or fallfish)                                   | 7) A shiner species  |
| 4) The most common sculpin species in the region (e.g., Slimy sculpin or mottled sculpin) | 8) If less than the desired weight of <u>any</u> primary target species is collected, send individuals of a small nontarget species if 50 g or more are available. |

\* The smallest individual in the sample should be at least 75% of the length of the largest individual. If there is a conflict between criteria, getting a sufficient sample is a higher priority than getting similar-sized individuals.

2. Prepare a clean work surface to prepare the primary composite sample. Keep hands, work surfaces, and wrapping materials clean and free of potential contaminants (mud, fuel, formalin, sun screen, insect repellent, etc.)
3. Rinse the teflon weighing beaker (to be used ONLY for weighing fish) with deionized water or stream water. Line the beaker with a sufficiently large piece of aluminum foil. Place the dull side of the foil toward the inside so it will be in contact with the fish. Place the beaker with foil on the scale and tare it.
4. If not done previously during the preparation of voucher specimens, place the individuals for the primary composite sample (Step 1) into a bucket with two carbon dioxide tablets (e.g., "Alka Seltzer®") and a small volume of water. After the individuals have been anaesthetized, use clean hands to transfer them into the beaker with foil.
5. Measure the total weight to the nearest 5g. Record the common name (from a standardized list) of the primary target species, its species code (if required), and the number of individuals in the sample in the appropriate fields on line "P1" in the primary tissue sample section of the Sample Collection Form. Enter an "F" series flag in the "Flag" field. Record the total weight of the sample in the comment/flag explanation section of the Sample Collection Form (if necessary).

**(continued)**

**TABLE 13-1 (Continued)**

---

6. If the individuals included in the composite sample were collected from throughout the sampling reach, place an "X" in the "YES" box in the primary sample section of the Sample Collection Form. If the individuals were only collected from a limited segment of the sampling reach, place an "X" in the "NO" box and explain in the "EXPLAIN" field on the form.
  7. Wrap the fish in the aluminum foil from the beaker. Make sure the dull side of the aluminum foil is in contact with the fish.
  8. Place the sample in a self-sealing plastic bag. Expel excess air and seal the bag(s). Wrap clear tape around the bag to seal and make a surface for each sample label.
  9. Prepare two Fish Tissue sample labels (each having the same sample ID number) by filling in the stream ID and the date of collection. Circle "PRIMARY" on each label. Record the sample ID number (barcode) in the primary sample section of the Sample Collection Form. Attach one label to the tape surface of the bag. Cover the label with a strip of clear tape.
  10. Place the labeled bag into a second self-sealing plastic bag. Seal and attach the second label to the outside of the bag. Cover the label with a strip of clear tape.
  11. Place the double-bagged sample into a portable freezer, into a container with dry ice, or into a cooler containing bags of ice until shipment. Keep the sample **frozen** until shipment.
-

**TABLE 13-2. PROCEDURE TO PREPARE THE SECONDARY COMPOSITE SAMPLE FOR FISH TISSUE CONTAMINANTS**

**NOTE: If neither a primary nor secondary species sample is available, use your best judgement in sending some type of composite fish tissue sample.**

1. After all voucher specimens have been prepared, select the highest priority secondary target species from the list below that has at least 5 individuals of the desired size (≥120 mm) is available. Include similar sized individuals if available.

**SECONDARY TARGET SPECIES (Larger adult fish)**

- |  |  |
|--|--|
| 1) A regionally common bottom feeder (e.g., white sucker)        | 7) If fewer than 5 individuals of the desired size are collected for any target species, select a species having 5 individuals, even if some individuals are smaller than the desired size.        |
| 2) Another regionally common bottom feeder (e.g., hogsucker)     |  |
| 3) A regionally common piscivore (e.g., a bass species)          | 8) If fewer than 5 individuals of any size are available for any target species, prepare a composite sample using as few as 3 fish that are at least at or near the minimum desired size (120 mm). |
| 4) Another regionally common piscivore (e.g., a trout species)   |  |
| 5) Another regionally common piscivore (e.g., a sunfish species) | 9) If an acceptable secondary target species sample (by the above criteria) is not available, send only the primary target species sample.   |
| 6) Carp  |  |

\* The smallest individual in the sample should be at least 75% of the length of the largest individual. If there is a conflict between criteria, getting a sufficient sample is a higher priority than getting similar-sized individuals.

2. Prepare a clean work surface to prepare the secondary composite sample. Keep hands, work surfaces, and wrapping materials clean and free of potential contaminants (mud, fuel, formalin, sun screen, insect repellent, etc.)
3. Measure the total length (TL) of each individual. Record the common name (from a standardized list) of the secondary target species, its species code (if required), and the total length for each individual on lines S1 through S5 in the secondary sample section of the Sample Collection Form.
4. If the individuals included in the composite sample were collected from throughout the sampling reach, place an "X" in the "YES" box in the secondary sample section of the Sample Collection Form. If the individuals were only collected from a limited segment of the sampling reach, place an "X" in the "No" box and explain in the "EXPLAIN" field on the form.
5. Wrap each individual separately in aluminum foil, with the dull side of the foil in contact with the fish. Place all the wrapped individuals into a single self-sealing plastic bag.

**(continued)**

**TABLE 13-2 (Continued)**

---


6. Expel excess air and seal the bag. Wrap clear tape around the bag to seal and make a surface for the sample label.
  7. Prepare two Fish Tissue sample labels (each having the same sample ID number) by filling in the stream ID and the date of collection. Circle "SECONDARY" on each label. Record the sample ID number (barcode) in the secondary sample section of the Sample Collection Form. Attach one label to the tape surface of the bag. Cover the label with a strip of clear tape.
  8. Place the labeled bag into a second self-sealing plastic bag. Seal and attach the second label to the outside of the bag. Cover the label with a strip of clear tape.
  9. Place the double-bagged sample into a portable freezer, a container with dry ice, or a cooler containing ice bags until shipment. Keep the sample **frozen** until shipment.
-

**FISH TISSUE**

SITE ID: MAIA 97-999

DATE: 7/15/98

SAMPLE: PRIMARY SECONDARY




229013

**FISH TISSUE**

SITE ID: MAIA 97-999

DATE: 7/15/98

SAMPLE: PRIMARY SECONDARY



229014

**Figure 13-1. Completed sample labels for fish tissue contaminants.**

frozen until they can be shipped (Section 3). Tissue samples can be stored and shipped with other samples requiring freezing ( periphyton chlorophyll, periphyton biomass, periphyton APA, and sediment metabolism samples). If shipping on dry ice, special containers and shipping forms will be required.

## 13.2 EQUIPMENT AND SUPPLIES

Figure 13-3 is a checklist of equipment and supplies required to conduct protocols described in this section. This checklist may differ from the checklists presented in Appendix A, which are used at a base site to ensure that all equipment and supplies are brought to and are available at the stream site. Field teams are required to use the checklist presented in this section to ensure that equipment and supplies are organized and available to conduct the protocols efficiently.



Reviewed by (initial): SP

SAMPLE COLLECTION FORM - STREAMS (continued)				
SITE NAME: <u>MILL CREEK</u>		DATE: <u>7/15/97</u> VISIT: <input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2		
SITE ID: <u>MAIA 97-999</u>		TEAM ID (X): <input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4 <input type="checkbox"/> 5 <input type="checkbox"/> 6 <input type="checkbox"/> 7 <input type="checkbox"/> 8		
CHEMISTRY AND MICROBIAL WATER SAMPLE (Chem: 4-L Cubitainer and 2 Syringes, Micro: Glass Bottle)				
	SAMPLE ID (BARCODE)	TRANSECT	FLAG	COMMENTS
CHEMISTRY	<u>229015</u>	<u>X</u>		
MICROBIAL				
SEDIMENT TOXICITY SAMPLES				
	SAMPLE ID (BARCODE)	FLAG	COMMENTS	
	<u>229011</u>			
FISH TISSUE SAMPLES - PRIMARY SAMPLE (min. 50g total wgt)				
SAMPLE ID (BARCODE) -		<u>229013</u>		
LINE	SPECIES CODE	COMMON NAME	NUMBER OF INDIVIDUALS	FLAG
<u>P1</u>	<u>NOCOLE</u>	<u>Bluehead chub</u>	<u>16</u>	<u>F1</u>
IS COMPOSITE SAMPLE COMPOSED OF INDIVIDUALS COLLECTED FROM THROUGHOUT REACH? (X) -			<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
IF NO, EXPLAIN:				
FISH TISSUE SAMPLES - SECONDARY SAMPLE (where available; 5 individuals)				
SAMPLE ID (BARCODE) -		<u>229012</u>		
LINE	SPECIES CODE	COMMON NAME	TOTAL LENGTH (MM)	FLAG
<u>S1</u>	<u>CATOCO</u>	<u>White sucker</u>	<u>128</u>	
<u>S2</u>	<u>CATOCO</u>	<u>White sucker</u>	<u>125</u>	
<u>S3</u>	<u>CATOCO</u>	<u>White sucker</u>	<u>134</u>	
<u>S4</u>	<u>CATOCO</u>	<u>White sucker</u>	<u>128</u>	
<u>S5</u>	<u>CATOCO</u>	<u>White sucker</u>	<u>125</u>	
IS COMPOSITE SAMPLE COMPOSED OF INDIVIDUALS COLLECTED FROM THROUGHOUT REACH? (X) -			<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
IF NO, EXPLAIN:				
LINE	COMMENT OR FLAG EXPLANATION FOR FISH TISSUE			
<u>P1</u>	<u>F1 = 16 individuals weighed 60 g.</u>			

Flag codes: K= Sample not collected; U= Suspect sample; F1, F2, etc.= misc. flag assigned by field crew. Explain all flags in Comments sections.

Figure 13-2. Sample Collection Form , showing information recorded for fish tissue samples.

**EQUIPMENT AND SUPPLIES FOR FISH TISSUE CONTAMINANTS**

<b>QTY.</b>	<b>Item</b>	
1	Plastic bucket for anesthetization	
4	Carbon dioxide tablets (Alka-Seltzer® or equivalent)	
1 roll	Clear tape for sealing tissue sample bags	
1	Teflon beaker for weighing primary tissue sample	
1	Portable scale, precision $\pm 5g$	
1 roll	Aluminum foil	
4	1-gallon self-sealing plastic bags	
1	Sample Collection Form	
2 sets	Fish tissue sample labels (each set with a different sample ID number [barcode])	
1 pkg.	Clear tape strips	
	Soft (#2) lead pencils to record data	
	Fine-point indelible markers to fill out labels	
1	Portable freezer, OR container with dry ice, OR cooler with ice (double-bagged and taped)	
1 copy	Field operations and methods manual	
1 set	Laminated sheets of procedure tables and/or quick reference guides for fish tissue contaminants	

**Figure 13-3. Equipment and supplies checklist for fish tissue contaminants.**