Section 11
Fish Tissue Contaminants

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In addition to gathering data on the aquatic vertebrate assemblage (Section 10), fish are retained for analysis of tissue contaminants. In general, the focus is on fish species that commonly and occur throughout the region of interest, and that are sufficiently abundant within a sampling reach. The fish tissue contaminants indicator, which measures bioaccumulation of persistent toxics, is used to estimate regional risks of consumption to fish predators, either wildlife or human. Various studies that have been done on fish tissue contaminants have focused on different parts of the fish: whole fish, fillets, livers. EMAP-SW will focus on whole fish because of its emphasis on the ecological health of the whole stream (as opposed to a focus on human health concerns). Whole fish are a good ecological indicator and a better indicator of risk to piscivorous wildlife than fillets. It is hoped to also be able to say something about risks to human health by analyzing whole fish. Whole fish also present fewer logistical problems for field crews (no gutting required in the field) and the analytical lab (no filleting necessary).

For the fish contaminants indicator in EMAP-SW STREAMS, an attempt was made to collect two fish samples at as many sites as possible. One sample, of **Primary Target Species**, was stream fish whose adults are small (in Mid-Atlantic streams examples are: dace, chub, sculpins, stonerollers, shiners, and darters). The second sample, where available, of a **Secondary Target Species**, was a species whose adults are of larger size (In Mid-Atlantic streams examples are: bass, trout, sunfish, suckers, carp). In addition to being more ubiquitous than the larger fish (and therefore more likely to be present in sufficient numbers to composite), small fish have other ad-

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vantages over large fish. Most importantly, it may be possible to get a more representative sample of the contaminant load in that stream section (although it would be at a lower expected level of bioaccumulation) by compositing say, in the range of 20 to 200 small fish individuals than by compositing 3 to 5 large fish. Small fish may be a more appropriate indicator for assessing ecological risk, as they might be expected to be prey for a larger number of fish-eating animals (the majority of which will be piscivorous birds and small mammals). The major advantage that larger fish could potentially offer, whether predators (piscivores) or bottom feeders, is a higher level of bioaccumulation and thus greater sensitivity to detect contaminants. The relative bioaccumulation of contaminants by large and small stream fish is not known, thus the reason for having Primary and Secondary Target Species in this study.

In trying to answer these questions, the field crews' efforts to apply the protocol for sampling, handling and shipping in a **consistent manner** are **critical**. The diligence of the field crews in following the protocols is especially important in a status and trends study such as EMAP-SW where it is critical to get a **standard sample** from each site so that there is confidence that differences seen over time and between sites represents variations in the ecosystems and not differences in sampling and handling between the crews. Suggestions from field crew members on how the protocol can be improved are welcomed and will be incorporated to improve them, but protocols should be followed as written until official changes are made.

11.1 Selecting Fish Tissue Specimens

If possible, obtain one sample each, of the desired **weight** or **number** (see below) of **similarly sized*** individuals, from the **Primary** and **Secondary** target species lists (**2 composite samples** total). To judge if the proper amount of a target species is present in the fish catch, **weight** will be used for **primary** target species and **number of individuals** of sufficient size will be used for **secondary** target species.

I. Primary Target Species

Small adult fish (in priority order)	Weight
1) Blacknose Dace	50** - 400 g
2) Another Dace species	50** - 400 g
3) Creek Chub or Fallfish	50** - 400 g
4) Slimy Sculpin/Mottled Sculpin	50** - 400 g
5) Stoneroller	50** - 400 g
6) A Darter species	50** - 400 g
7) A Shiner species	50** - 400 g

A) Choose the **highest priority target species** from the above list, 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).

B) If **less than the desired weight of any primary target species** is collected, send individuals of a small **nontarget** species if 50 g or more are available.

* - The general rule-of-thumb for similar size is that the smallest individual in the sample should be at least 75% of the length of the largest individual. This rule applies to both primary and secondary target species. Crews just need to keep this criterion in mind while selecting the final sample. Any obviously small or large individuals should not be kept if there is a sufficient sample to return

without them. If there is a conflict between criteria, getting a sufficient sample is a higher priority than getting similar-sized individuals.

** - This weight represents the **minimum amount** of tissue needed for laboratory analysis. Crews **should 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.

II. Secondary Target Species

Collect and save a sample of secondary target species if such a sample of desired number of individuals of desired size is available. Collect **similar sized individuals** if enough are present.

Larger adult fish (in priority order)	Desired Size	Desired Number
1) A Bass species	120 mm	5
2) A Trout species*	120 mm	5
3) A Sunfish species	120 mm	5
4) Catfish	120 mm	5
5) White sucker	120 mm	5
6) Hogsucker	120 mm	5
7) Carp	120 mm	5

* - Collect only those trout that appear not to be recently stocked.

A) If **fewer than the desired number** of secondary target species individuals **of desired size** are collected, add smaller individuals of the same species, if available, to achieve the desired number (5).

B) If **fewer than 5 fish** of **any size** are available, you may send **as few as 3 fish** that are at least at or near the minimum desired size (120 mm).

C) 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 or secondary species sample that meets these criteria is available, use your best judgement in sending some type of fish sample.

11.2 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, but getting a sufficient sample is a higher priority than getting similar-sized individuals.

Prepare a primary sample as described in Table 11-1. Choose a species that has at least enough individuals to attain the minimum weight (50 g). Send as many fish as possible up to the 400 g weight goal. If there is no single species with enough individuals available, prepare the sample using individuals of multiple species.

Prepare a secondary sample as described in Table 11-1. Choose a species that has 5 similar-sized individuals (minimum total length = 120 mm) available. 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 neither a primary nor secondary sample is available, use your best judgement to obtain some type of fish tissue sample from the available species collected. Use the procedure for either primary or secondary spe-

Table 11-1. Procedure to Prepare Fish Tissue Samples.

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

Primary Sample (P)

After all voucher specimens have been prepared, choose a primary species that has enough similarly sized individuals to weigh to 400 g (smallest to largest should not differ by more than 25% in length).

Secondary Sample (S)

After all voucher specimens have been prepared, select a large secondary species that has at least 5 individuals 120 mm. Include similar sized individuals if available (smallest to largest should not differ by more than 25% in length).

1. Place the fish into a bucket with two carbon dioxide tablets (e.g., "Alka Seltzer®") and a small volume of water. After they have been anaesthetized, use clean hands to transfer them to aluminum foil.
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 repellant, etc.)

3-P. For primary samples, record the common name (from a standardized list) of the species, its species code (if required), and the number of individuals in the sample in the appropriate fields on line "P1" of the Sample Collection Form (Figure 11-1).

3-S. Measure the total length (TL) of each secondary 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 composite samples were collected from throughout the sampling reach, place an "X" in the "Yes" box in the 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-P. Wrap all primary fish together in a single piece of aluminum foil, making sure the **dull side of the aluminum foil is in contact with the fish**. Place the sample in a self-sealing plastic bag.

5-S. Wrap each fish of the secondary sample 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.

6. Expel excess air and seal the bag. Wrap clear tape around the bag to seal and make a surface for each sample label..

7-P. Prepare two Fish Tissue sample labels (each having the same sample ID number [Figure 11-2]) 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.

7-S. Prepare two Fish Tissue sample labels (each having the same sample ID number [Figure 11-2]) 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.

8. Attach the appropriate label to the tape surface of the bag. Cover the label with a strip of clear tape. Place the labeled bag into a second self-sealing plastic bag. Seal the bag and attach the second label to the outside of the appropriate bag. Cover the label with a strip of clear tape.
9. Place the double-bagged sample into a cooler containing bags of ice until shipment. Keep the sample **frozen** until shipment.

cies, 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 11-1. 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 (Figure 11-2). Make sure the sample ID numbers (barcodes) recorded on the collection form match those on the sample labels.

Tissue samples are stored in 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 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).

11.3 Equipment and Supplies

Figure 11-3 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 river 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.

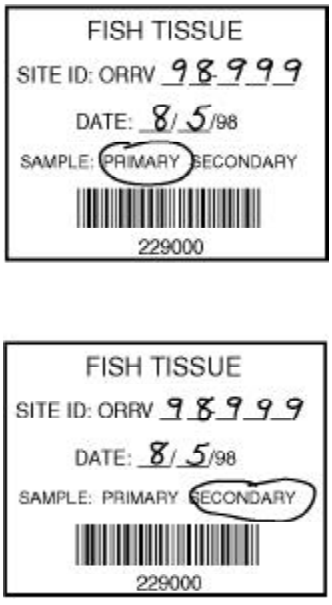


Figure 11-1. Completed sample labels for fish tissue contaminants.

Reviewed by (initial) DKA

SAMPLE COLLECTION FORM - RIVERS (continued)

SITE NAME: BEAVER RIVER DATE: 8 / 5 / 98 VISIT ☐1 ☒2 ☐3

SITE ID: ORRV 98-999 TEAM ID (X): ☒1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8

CHEMISTRY AND MICROBIAL WATER SAMPLE (Chem: 4-L Cubitainer and 2 Syringes, Micro: Glass Bottle)

	SAMPLE ID (BARCODE)	TRANSECT	FLAG	COMMENTS
CHEMISTRY	243012	K		
MICROBIAL	243113	K		

SEDIMENT TOXICITY SAMPLES

SAMPLE ID (BARCODE)	FLAG	COMMENTS

FISH TISSUE SAMPLES - PRIMARY SAMPLE (min. 50g total wgt)

SAMPLE ID (BARCODE) → 243223

LINE	SPECIES CODE	COMMON NAME	NUMBER OF INDIVIDUALS	FLAG
P1	COTTZZZZ	UNKNOWN COTTIA	11	

IS COMPOSITE SAMPLE COMPOSED OF INDIVIDUALS COLLECTED FROM THROUGHOUT REACH? (X) → ☐YES ☒NO

IF NO, EXPLAIN: COLLECTED AT ONE PROFILE

FISH TISSUE SAMPLES - SECONDARY SAMPLE (where available; 5 individuals)

SAMPLE ID (BARCODE) → 243224

LINE	SPECIES CODE	COMMON NAME	TOTAL LENGTH (MM)	FLAG
S1	PTYCOREG	NORTHERN SQUAWFISH	220	
S2	PTYCOREG	NORTHERN SQUAWFISH	220	
S3	PTYCOREG	NORTHERN SQUAWFISH	265	
S4	PTYCOREG	NORTHERN SQUAWFISH	290	
S5	PTYCOREG	NORTHERN SQUAWFISH	215	

IS COMPOSITE SAMPLE COMPOSED OF INDIVIDUALS COLLECTED FROM THROUGHOUT REACH? (X) → ☒YES ☐NO

IF NO, EXPLAIN:

LINE	COMMENT OR FLAG EXPLANATION FOR FISH TISSUE

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

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SAMPLE COLLECTION FORM - RIVERS - 2

Figure 11-2. Sample Collection Form, showing information recorded for fish tissue samples.

Equipment And Supplies For Fish Tissue Contaminants		
Qty.	Item	
1	Plastic bucket for anesthetization	
4	Carbon dioxide tablets (Alka-Seltzer® or equivalent)	
1 roll	Clear tape for sealing tissue sample bags	
1	Pesola® portable scale, precision ±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	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 11-3. Equipment and supplies checklist for fish tissue contaminants.