APPENDIX 1:

FISH TISSUE MONITORING PROGRAM STANDARD OPERATING PROCEDURES

STANDARD OPERATING PROCEDURES

FISH TISSUE ASSESSMENTS

NORTH CAROLINA
DEPARTMENT OF ENVIRONMENT
and NATURAL RESOURCES
Division of Water Quality
Environmental Sciences Section
Biological Assessment Unit

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Standard Operating Procedures
Fish Tissue Assessments

Environmental Sciences Section
Biological Assessment Unit

NORTH CAROLINA DEPARTMENT OF ENVIRONMENT
AND NATURAL RESOURCES
Division of Water Quality

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NC DWQ STANDARD OPERATING PROCEDURES FISH TISSUE ASSESSMENTS REVISION LOG

Date Edited	Editor	Version Edited	Section Edited	Changes/updates
12/01/2011	Jeff DeBerardinis	Version 1 June, 2006	Introduction	Prior approval version date changed from January 1997 to June 2006. Deleted statement about prior SOP version containing both fish and benthic macroinvertebrates.
12/01/2011	Jeff DeBerardinis	Version 1 June, 2006	1.0	Rewording of statements concerning unsafe sampling conditions.
12/01/2011	Jeff DeBerardinis	Version 1 June, 2006	3.6	NC Health director selenium limit updated from 5µg/g to 10 µg/g. (limit changed in 2007)
12/01/2011	Jeff DeBerardinis	Version 1 June, 2006	Table 1.	EPA screening value for mercury updated from 0.4 ppt to 0.3 ppt. NC Health director selenium limit updated from 5µg/g to 10 µg/g. (limit changed in 2007)
12/01/2011	Jeff DeBerardinis	Version 1 June, 2006	Appendix 1.	Fish tissue raw data sheet replaced with new version.
12/01/2011	Jeff DeBerardinis	Version 1 June, 2006	Appendix 2.	Fish tissue survey form replaced with new version.

INTRODUCTION

It is the purpose of this manual to provide details on standard operating procedures of the Biological Assessment Unit of the Division of Water Quality (Division) for the collection and analysis of fish tissue data. Consistency in data collection and analysis is the cornerstone for evaluating biological integrity. The procedures provided in this manual are a synthesis of widely used methods and methods developed from the experience of personnel within the unit. These methods have been shown to provide repeatable and useful data for water quality evaluation.

This manual will be reviewed regularly and revised as necessary. The prior approved version of this manual was dated June 2006. All current employees and new employees within the unit will be provided with this manual to serve as a guideline of the unit's activities, methods, and procedures. Revisions of this manual will be provided to each employee and it will be the responsibility of the employee to keep his or her manual current.

The standard operating procedures (SOP) and quality control procedures (QC) in this manual will be the basis for all fish tissue monitoring in the waters of North Carolina, and the subsequent data provided in memoranda and reports prepared by the Biological Assessment Unit. Deviations from these procedures for unusual sampling situations shall be documented in the appropriate report or memorandum.

1.0 SAFETY PROGRAM

The Biological Assessment Unit is required to sample throughout North Carolina at times and places where medical facilities may not be readily available. It is imperative that all employees are instructed in and follow safety precautions when using sampling equipment and hazardous materials. The Environmental Sciences Section has a Safety Committee which is responsible for maintenance and development of current safety procedures. The Committee also maintains the safety standard operating procedures document with which all personnel should be familiar. In addition, all personnel involved in electrofishing activities should be trained in First Aid and CPR and should be familiar with standard electrofishing safety procedures.

Sampling conditions are the primary safety factor to be considered for field work. If any field conditions present an unsafe situation, such as high flows or thunderstorms, then sampling should be postponed or rescheduled. Decisions should always be made with the safety of personnel of prime concern. This same concern for safety of staff must be of primary importance when scheduling the amount of time to be spent in the field. Long days combined with strenuous effort increase the probability of accidents occurring. "**Safety first**" must always be the rule.

Employees should promptly report on-the-job accidents to their supervisor. If an accident occurs during field operations, the first responsibility of the team leader is to get first aid treatment for the injured employee; their second responsibility is to promptly notify their supervisor. The Safety Committee maintains a written record of accidents.

2.0 STUDY PLANS

All investigations conducted by the Biological Assessment Unit will follow a written study plan including but not limited to the:

- **Introduction** - Will identify the nature and history of the area being investigated and the person or agency requesting the study.

- **Objectives** - The purpose of the investigation and expected accomplishments.
- **Sampling Location Selection** - Locating sampling points is of extreme importance in the initiation of fish tissue monitoring. The variables in watersheds are many and should be considered in as much detail as possible before sites are selected to monitor any body of water. Land use (*i.e*., urban, rural, forested, agricultural, and industrial) should be considered when locating sample sites, because man-made activities significantly affect the amount of sedimentation, nutrients, and organic or inorganic compounds entering a given segment of a river, lake, or stream. The location of permitted dischargers should be reviewed, using the database provided by the Division's NPDES Unit. Discussion of the proposed study with regional office personnel can also provide additional information useful for determining sampling locations. Pre-study planning of this nature will enhance data interpretation once collections and analyses begin.

- **Methods** - Sampling techniques should be listed with reference to those described in this manual. Any deviation from these standard methods must be noted and described.

- **Analytical Requirements** - All water chemistry and quality parameters to be collected, and analyses that will be required, should be noted.

- **Logistics** - Shall include estimates of manpower requirements, equipment needed, time requirements, methods of sample transport to laboratories, *etc*. The study plan must be submitted and approved by the employee's supervisor prior to conducting the investigation.

A study is complete when a written memorandum is sent to and approved by the appropriate level of management (typically the Environmental Sciences Section head) within the Division. Each memorandum should contain these sections: an **Introduction or Background**, **Sampling Sites**, **Methods**, **Results and Discussion,** and **Summary or Recommendations**. Any figures, maps, and photographs needed to allow a reader to easily locate the sampling sites should also be included. When the report or memorandum is approved, a Biological Assessment Unit file number is assigned. Finally, the report or memorandum is filed in a Projects File that is organized by basin and subbasin.

3.0 FISH TISSUE

Because fish spend their entire lives in the aquatic environment, they incorporate chemicals from this environment into their body tissues. Contamination of aquatic resources have been documented for heavy metals, pesticides, and other complex organic compounds. Once these contaminants reach surface waters, they may be available for bioaccumulation, either directly or through aquatic food webs, and may accumulate in fish and shellfish tissues. Results from fish tissue monitoring can serve as an important indicator of further contamination of sediments and surface water.

This procedure is used by the Division to collect and process fish tissue samples to be analyzed for chemical contaminants. These procedures are based on established guidelines described in USEPA (2000). The procedure does not include procedures used by the Division's Analytical Chemistry Laboratory.

3.1 Study Design

A detailed sampling plan should be developed by the primary researcher and approved by the Biological Assessment Unit supervisor prior to initiating any studies. At minimum, a study should involve a two tiered approach:

- Screening studies should identify sites where commonly consumed fish species are contaminated with target analytes and may pose a risk to human health.
- Intensive studies should characterize the magnitude and geographical extent of contamination in harvestable fish at sites identified in screening studies. Intensive studies should also be designed to verify results of screening studies.

Further information on study objectives and sampling design may be found in USEPA (2000).

3.2 Sample Collection

In most cases the Division will employ electrofishing as the primary means of fish collection. Collections on lakes and non-wadeable streams are usually accomplished using a boat mounted electrofisher powered by a 2.5 watt or a 7.5 watt generator. Collections on wadeable streams are accomplished using back pack electrofishing techniques [refer to the Fish Community Assessment SOP (NCDWQ, 2006a) for details on this method].

During fish tissue sampling, a standard measured distance is not sampled, rather sampling is conducted until the required number of fish are collected. All personnel involved should be familiar with standard electrofishing operational and safety procedures (Reynolds, 1996).

In certain cases electrofishing may not be effective especially when targeting Ictalurids (catfishes) and other benthic species. In these cases, trot lines, traps, or gillnets may be used (Hubert, 1996).

Certain studies may require that fish be collected by other agencies or that fish be purchased from commercial fishermen. Division personnel should provide quality control measures necessary to ensure that samples are collected and handled properly with minimal contamination and that sampling sites are verified.

At each sampling station personnel should fill out a Fish Tissue Survey Form (Appendix 2) to provide additional information regarding the site visit. The form allows field staff to document access conditions, all species observed during electrofishing, water quality measurements, disease information, and any comments about the station.

3.3 Sample Shipment and Handling

Fish collected for analyses must be shipped to the processing laboratory in such a manner as to prevent decomposition or contamination. Fish should be removed from live wells, holding tanks, or buckets, rinsed with ambient water to remove foreign matter, and placed on a contaminant free surface for sorting. Skins on fish selected for analysis should be examined for breaks or lacerations from sampling gear - a possible source of contamination. Fish samples should be sorted by species before packaging for shipment.

Fish selected for metals analysis are placed by species in polyethylene bags. After removing as much air as possible, the bags are sealed and tagged with the date, time, station name, species, and collector(s).

Fish selected only for organics analyses, including dioxins, are wrapped whole in clean aluminum foil with the dull side of the foil against the skin of the animal. Large spines on any fish should be sheared to minimize puncturing of the foil. Wrapped fish are sorted by species and placed in tagged polyethylene bags as described for metals samples.

Packaged fish are placed immediately on wet ice and chilled to 4°C for transport back to the laboratory. Samples shipped on wet ice should reach the processing laboratory within 24 hours of collection to allow sufficient time for processing. Samples to be filleted should be processed no later than 48 hours after collection.

If samples cannot be processed within this time frame then they should be frozen as whole fish, delivered to the laboratory as soon as possible, and stored at -20 ^{o}C until processing can be performed. **Freezing samples should be avoided whenever possible due to the possibility of rupturing internal organs and contaminating fillet tissue.** If fish are frozen they should not be allowed to thaw during transport. Prior to processing, frozen fish samples should only be partially thawed before filleting (ice crystals should still be visible in the fillet tissue).

3.4 Laboratory Procedures

All laboratory personnel performing sample processing procedures should be trained or supervised by an experienced biologist.

Individual fish received for filleting should be unwrapped and inspected carefully to ensure that they have not been compromised in any way (i.e., not properly preserved during shipment). Any specimen deemed unsuitable for further processing and analysis should be discarded and identified on the sample processing record.

A wet weight is determined for each fish to the nearest 0.1 gram and recorded on the Fish Tissue Raw Datasheet (Appendix 1). All samples should be weighed on balances that are properly calibrated and of adequate accuracy and precision to meet program data quality objectives. Balance calibration should be checked at the beginning of each weighing session.

A total length is determined for each fish to the nearest centimeter using a length board such as the Wildco® Model 118 and recorded on the Fish Tissue Raw Datasheet (Appendix 1).

Individual fish are identified to species under the supervision of an experienced biologist familiar with North Carolina fish fauna. Fish are first identified using current, regional identification manuals and other appropriate taxonomic literature (ie:Menhinick, E. F. 1991). If questions occur, identifications are verified by other taxonomists in the Biological Assessment Unit or by personnel from the North Carolina Museum of Natural Science.

Processing Equipment: Equipment used in processing samples for metals analysis should be made of stainless steel, glass, or plastic. Chromium and nickel contamination can occur from the use of stainless

steel. Therefore, if these metals are of concern, other materials should be used during sample processing. Equipment used in processing samples for organics analysis should be made of stainless steel, glass, or anodized aluminum.

Prior to preparing metals samples, all surfaces in the processing laboratory are washed with a detergent and rinsed with a metal free water (treated by reverse osmosis). Utensils and containers should be cleaned thoroughly with a detergent solution, rinsed with tap water, soaked in 50 percent HNO3 , for 12 to 24 hours at room temperature, and then rinsed with organics- and metal-free water. Note: Chromic acid should not be used for cleaning any materials. Acids used should be at least reagent grade. Stainless steel parts may be cleaned using this recommended procedure with the acid soaking step method omitted (Stober, 1991).

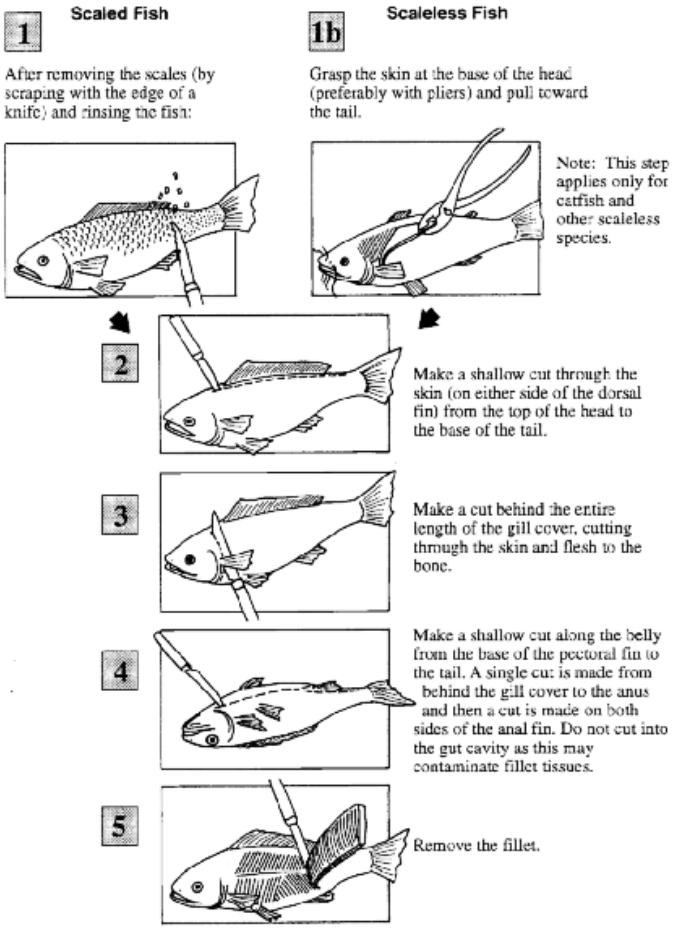
Equipment used in processing samples for organics analysis should be of stainless steel, anodized aluminum, borosilicate glass, polytetrafluoroethylene (PTFE), ceramic, or quartz. Polypropylene and polyethylene (plastic) surfaces, implements, gloves, and containers are a potential source of contamination by organics and should not be used. If a laboratory chooses to use these materials, there should be clear documentation that they are not a source of contamination. Filleting should be done on glass or PTFE cutting boards that are cleaned properly between fish or on cutting boards covered with heavy duty aluminum foil that is changed after each filleting. Tissue should be removed with clean, high-quality, corrosion-resistant stainless steel or quartz instruments or with knives with titanium blades and PTFE handles (Lowenstein and Young, 1986). Fillets or tissue homogenates may be stored in borosilicate glass, quartz, or PTFE containers with PTFE-lined lids or in heavy duty aluminum foil (see Table 7-1). Prior to preparing each composite sample, utensils and containers should be washed with detergent solution, rinsed with tap water, soaked in pesticide-grade isopropanol or acetone, and rinsed with organic-free, distilled, deionized water. Work surfaces should be cleaned with pesticide-grade isopropanol or acetone, washed with distilled water, and allowed to dry completely. Knives, fish scalers, measurement boards, etc., should be cleaned with pesticide-grade isopropanol or acetone followed by a rinse with contaminant-free distilled water between each fish sample (Stober, 1991). The total length of each fish is determined to the nearest millimeter and the wet weight of each fish is determined to the nearest gram. Fish are weighed on foil lined trays and the foil is changed between each species. All data are recorded on the laboratory data sheet (Appendix 1).

Scaling: Scaling is performed on cleaned stainless steel or plastic surfaces covered in heavy duty aluminum foil. Separate cutting boards and utensils are used for scaling and skinning to prevent cross contamination of tissues. Fish are scaled prior to filleting using an automatic rotary scaler and rinsed with water filtered *via* reverse osmosis (R.O.) to remove slime and foreign matter. The scaling surfaces are also rinsed between fish to prevent contamination. Scaleless fish (catfish) are skinned prior to filleting.

Fillets: Filleting is performed on plastic or stainless steel surfaces covered with heavy duty aluminum foil. Aluminum foil is rinsed with R.O. water between fish from the same station and changed completely between stations. Filleting is performed using cleaned bare hands or talc free disposable gloves. Hands or gloves should be rinsed between samples to prevent cross contamination. Fillets are ressected using high grade stainless steel knives cleaned according to the above directions. Knives are rinsed with R.O. water between fish from the same station and recleaned or changed between stations.

Fillets should be ressected according to the general procedure (Figure 1). Fillets should be removed from the lateral area of the fish behind the head and pectoral fin and should include the belly flap. **Care should be taken not to cut into the gut cavity as it may contaminate the fillet tissue.**

Figure 1. Procedure for filleting fish.



Source: U.S. EPA, 1991d.

Fillets are ground and homogenized prior to analysis to ensure equal distribution of contaminants throughout the sample. Fillets are ground using a glass and stainless steel high speed blender or Hobart® Model 8145 commercial grinder. Samples are ground until they appear homogenous. Samples processed in the Hobart® Model 8145 commercial grinder are removed from the grinder and further mixed by hand. Hand mixing is accomplished by dividing the sample into quarters, mixing opposite quarters, and then mixing the remaining halves. Composite samples are prepared from at least 4 but no more than 10 individuals of the same species and should be of the same general size class. **Individuals of different species are never mixed to form composite samples.**

Final Sample: The final individual or composite samples should be composed of at least 100 g of tissue to ensure an adequate amount of material for analysis. Metals samples are placed in foil cups with foil lined lids, and labeled. Organics samples are wrapped in aluminum foil, dull side against the tissue, then wrapped in plastic to prevent desiccation.

All samples are then sent either directly to the Division's Analytical Chemistry Laboratory (or other analytical laboratory), or frozen immediately and stored at -20 ^{o}C for later analysis.

3.5 Quality Assurance/Quality Control

To assess total variability, duplicate samples will be prepared from at least 10% of the fish samples. Duplicates are prepared using tissue from the same fillet or composite homogenate. Duplicates are

assigned a "dummy" sample identification which is recorded in the processing laboratory log. The analytical laboratory does not receive this information.

During intensive or Tier 2 studies, portions of at least 10% of the prepared homogenates will be frozen at -20 ^{o}C and archived at the Division's Water Quality Laboratory for a period of at least 6 months after completion of the study. This is done in case of analytical problems or the need for future references.

To assess interlaboratory variability, the Division will attempt to split sample homogenates with other laboratories for analysis at least twice per year. Numbers of splits will depend on time and resource constraints of participating laboratories. Results from splits are tallied and plotted using descriptive statistics. Laboratory variability is considered acceptable if it is within two standard deviations of the mean for all measurements.

3.6 Data Analysis and Reporting

Data reported from the analytical laboratory below the method quantitation limit (MQL) are assigned a value of one-half the MQL. Data reported at or above the MQL are used as reported. The following statistics are calculated for each sampled species at each site:

- Range of target analyte concentrations;
- Arithmetic mean of target analytes;
- Standard deviation of mean target analyte concentrations; and
- Number of samples

Comparisons are performed using the Student t-Test (parametric) or the Sign Test (nonparametric).

In evaluating fish tissue analysis results, several criteria are used. Human health concerns related to fish consumption are screened by comparing results with federal Food and Drug Administration (FDA) action levels (USFDA 1980), Environmental Protection Agency (EPA) recommended screening values, and criteria adopted by the state Health Director (Table 1). Results which seem to be of potential human health concern are evaluated by the N.C. Division of Occupational and Environmental Epidemiology by request from the DWQ.

The FDA levels were developed to protect people from the chronic effects of toxic substances consumed in foodstuffs and thus employ a "safe level" approach to fish consumption. Presently, the FDA has developed metals criteria only for mercury.

The EPA has recommended screening values for target analytes formulated from a risk assessment procedure (USEPA 2000). These are the concentrations of analytes in edible fish tissue that are of potential public health concern. The DWQ compares fish tissue results with EPA screening values to evaluate the need for further intensive site specific monitoring.

The North Carolina State Health Director has adopted a selenium limit of 10 µg/g for issuing an advisory. Although the USEPA has suggested a screening value of 0.7 ppt (pg/g) for dioxins, the State of North Carolina currently uses a value of 4.0 ppt in issuing an advisory.

Table 1. Fish tissue criteria. All wet weight concentrations are reported in parts per million (ppm, µg/g), except for dioxin which is in parts per trillion (ppt, pg/g).

Contaminant	FDA Action Levels	US EPA Screening Values Recreational Fishermen	US EPA Screening Values	NC Health Director
Metals				
Arsenic (Inorganic)		1.2	0.00327	
Cadmium		4.0	0.491	
Mercury	1.0	0.3	0.049	0.4
Selenium		20	2.457	10.0
Tributyltin		1.2	0.147	
Organics				
Aldrin	0.3			
Chlorpyrifos		1.2	0.147	
Total chlordane		0.114	0.014	
Cis-chlordane	0.3			
Trans-chlordane	0.3			
Total DDT[1]		0.117	0.0144	
o, p DDD	5.0			
p, p DDD	5.0			
o, p DDE	5.0			
p, p DDE	5.0			
o, p DDT	5.0			
p, p DDT	5.0			
Diazinon		2.8	0.344	
Dicofol		1.6	0.196	
Dieldrin		0.0025	3.07×10^{-4}	
Dioxins (total)		2.56×10^{-7}	3.15×10^{-8}	4.0 (ppt)
Disulfoton		0.16	0.019	
Endosulfan (I and II)		24	2.949	
Endrin	0.3	1.2	0.147	
Ethion		2.0	0.245	
Heptachlorepoxide		0.00439	5.40×10^{-4}	
Hexachlorobenzene		0.025	0.00307	
Lindane		0.0307	0.00378	
Mirex		0.8	0.098	
Oxyfluorfen		0.546	0.0671	
Total PCBs		0.02	0.00245	0.05
PCB-1254	2.0			
Terbufos		0.08	0.009	
Toxaphene		0.0363	0.00446	

[1] Total DDT includes the sum of all its isomers and metabolites (i.e. p, p DDT, o, p DDT, DDE, and DDD).

[2] Total chlordane includes the sum of cis-and trans- isomers as well as nonachlor and oxychlordane.

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Appendix 1. Fish tissue raw data sheet.

Station _____ **County** _____ **Subbasin** _____ **8 Digit HUC** _____

Latitude _____ **Longitude** _____ **Collection Date** _____

Processing Date _____ **Date To Lab** _____

Station Comments _____

Species Code	Total Length (mm)	Weight (g)	DWQ Number	Comments

Appendix 2: Fish Tissue Survey

Fish Tissue Survey Information

Waterbody: _____ **Location** _____ **Study Site #** _____

Latitude _____**Longitude**_____ **Nearest City/Town/Landmark**_____

County _____ **Subbasin** _____ **8 digit HUC** _____

Tributaries:_____

Survey Date/Time:_____ **Survey Duration: Hours** _____**Min**_____

Staff: _____

Survey Method : Big boat ☐ Small Boat ☐ Back Pack ☐ Other ☐ Describe:_____

Ramp Info: Wildlife ☐ Marina ☐ Public ☐ Private ☐ Other ☐ Describe:_____

Ramp Condition: Paved ☐ Sand ☐ Gravel ☐ Earth ☐ Slide in ☐ Comments:_____

Water Quality Measurements: Temp:_____ **pH:**_____ **D.O.:** _____ **Cond:**_____ **Salinity:**_____

Species Observed:

__ AMERICAN EEL
__ BLACK BULLHEAD
__ BLACK CRAPPIE
__ BLUEGILL SUNFISH
__ BLUE CATFISH
__ BLUEHEAD CHUB
__ BOWFIN
__ BROOK TROUT
__ BROWN BULLHEAD
__ BROWN TROUT
__ CARP
__ CHAIN PICKEREL
__ CHANNEL CATFISH
__ CREEK CHUBSUCKER
__ FLAT BULLHEAD
__ FLATHEAD CATFISH
__ FLIER
__ GIZZARD SHAD
__ GOLDEN SHINER
__ GREEN SUNFISH
__ LARGEMOUTH BASS
__ LONGNOSE GAR
__ NORTHERN HOG SUCKER
__ PINFISH
__ PUMPKINSEED
__ QUILLBACK
__ RAINBOW TROUT

__ REDBREAST SUNFISH
__ REDEAR SUNFISH
__ REDFIN PICKEREL
__ REDHORSE SUCKER
__ GOLDEN REDHORSE
__ NOTCHLIP REDHORSE
__SHORTHEADREDHORSE
__ ROCK BASS
__ SMALLMOUTH BASS
__ SMALLMOUTHBUFFALO
__ SNAIL BULLHEAD
__ SPOTTED BASS
__ SPOTTED SUCKER
__ SPOTTED SUNFISH
__ STRIPED BASS
__ STRIPED KILLIFISH
__ STRIPED MULLET
__ WALLEYE
__ WARMOUTH
__ WHITE BASS
__ WHITE CATFISH
__ WHITE CRAPPIE
__ WHITE PERCH
__ WHITE SUCKER
__ YELLOW BULLHEAD
__ YELLOW PERCH

OTHER SPECIES:

Disease Observed: Lesions/Sores [ ] Injuries [ ] Flared Gills [ ] Excessive mucus [ ] Tumors [ ] Visible Parasites [ ]
Other[ ] Describe_____

Species Collected:

Station Comments/Notes: