SAMPLING And ANALYSIS PLAN
HUDSON RIVER FISH HEALTH ASSESSMENT
PHASE I: FIELD SAMPLING, NECROPSY, HISTOPATHOLOGY, DISEASE, FISH AGE (FIELD VERSION)

HUDSON RIVER NATURAL RESOURCE DAMAGE ASSESSMENT

Hudson River Natural Resource Trustees

State of New York

U.S. Department of Commerce

U.S. Department of the Interior

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*Names of individuals and affiliations have been removed for purposes of litigation

Sampling and Analysis Plan

Hudson River Fish Health Assessment

Phase I: Field Sampling, Necropsy, Histopathology, Disease, Fish Age

(Field Version)

Prepared for:

Hudson River Trustee Council

Prepared by:

redacted

with assistance from:

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October 3, 2001

Sampling and Analysis Plan Approval

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Date: _____

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Date: _____

Table of Contents

Tables

Figures

Acronyms and Units

Acronyms

ASTM	American Society for Testing and Materials
BB	brown bullhead
BGF	blue gill fin
BHIA	brain heart infusion agar
CCO	catfish ovary
CHSE	chinook salmon embryo
CPE	cytopathic effect
CPR	cardiopulmonary resuscitation
DC	direct current
DOI	U.S. Department of the Interior
DOJ	U.S. Department of Justice
EPA	U.S. Environmental Protection Agency
EPC	epithelio-papilloma of carp
FHM	fathead minnow
GLP	Good Laboratory Practice
GPS	global positioning system
H&E	hematoxylin and eosin staining (a method of dyeing tissue samples for examination under a microscope)
HASP	health and safety plan
HBSS	Hank's balanced salt solution
HPLC	High Performance Liquid Chromatography
HPTs	Histopathology Prevalence Tables
HSO	Health and Safety Officer
IDLH	immediately dangerous to life and health
LC50	concentration which is lethal to 50% of a sample population
MSDSs	material safety data sheets
NIOSH	National Institute for Occupational Safety and Health
NOAA	National Oceanic and Atmospheric Administration
NRDA	natural resource damage assessment
NYSDEC	New York State Department of Environmental Conservation
OSHA	Occupational Safety and Health Administration
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PCR	polymerase chain reaction
PEL	permissible exposure limit
PPE	personal protective equipment

QA	quality assurance
QAP	Quality Assurance Plan
QC	quality control
RI/FS	remedial investigation/feasibility study
SAP	sampling and analysis plan
SOP	standard operating procedure
SPT	Summary Prevalence Table
TLV	threshold limit value
TSA	tryptic soy agar
TWA	time weighted average
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey
UTM	Universal Transverse Mercator
WGS 84	World Geodetic System 1984

Units

EC	degrees centigrade
cm	centimeter
g	gram
kg	kilogram
L	liter
m	meter
mg	milligram
mL	milliliter
mm	millimeter
µg	microgram
ng	nanogram
oz	ounce
ppm	parts per million
V	volts

1. Introduction

This sampling and analysis plan (SAP) describes the field collection and health assessment of Hudson River fish scheduled for fall 2001. Phase I of the study includes the field collection, necropsy assessment, tissue collection, histopathologic examination of tissues, analysis of tissue disease status, and determination of fish age and sex. Phase II is the chemical and biochemical, analyses of tissue residues from fish collected during Phase I. The decision to implement Phase II will be made following completion of Phase I. If Phase II is deemed necessary, a Phase II analysis plan will be developed. This fish health assessment is being conducted for the Hudson River Trustee Council in support of the Hudson River Natural Resource Damage Assessment (NRDA). The Hudson River Trustee Council includes the State of New York, the National Oceanic and Atmospheric Administration (NOAA), and the U.S. Fish and Wildlife Service (USFWS).

The objectives of the Phase I study described here are to:

- Compare the prevalence of toxicopathologic lesions between fish from areas of the Hudson River downstream of polychlorinated biphenyl (PCB) releases from General Electric facilities near Glens Falls, New York (henceforth, the "assessment area") and fish from relatively less contaminated reference areas (Feeder Dam Pool and Oneida Lake).

- Compare bacterial and viral infection prevalence and severity between fish from assessment and from reference areas.

- Collect and archive tissues for Phase II biochemical analysis, chemical residue analysis, and/or chemical metabolite analysis (PAH metabolites) as deemed necessary following evaluation of the results of the Phase I fish health assessment.

This SAP is organized as follows:

- Section 2 lists personnel involved with the field collections and the sampling schedule.

- Section 3 describes sampling locations and numbers, including target fish species.

- Section 4 describes the fish collection procedures.

- Section 5 describes the fish field processing procedures, including external and internal examinations, sample collection, sampling containers, sample preservation, and chain of custody.

- Section 6 describes the Phase I laboratory analyses that will be performed on samples.
- Section 7 describes quality assurance/quality control procedures that will be used in the study.
- Section 8 lists references cited.

In addition, this SAP contains the following appendices:

- Appendix A is the Health and Safety Plan for the field sampling effort.
- Appendix B describes physical characteristics and habitat requirements for each fish species to be sampled.
- Appendix C provides lists of field equipment required for the study.
- Appendix D contains standard operating procedures (SOPs) referenced in the SAP.
- Appendix E includes maps and global positioning system (GPS) coordinates of fish collection locations.

2. Sampling Schedule and Personnel Organization

Sampling will be conducted in the fall of 2001. The sampling schedule is subject to change due to inclement weather, equipment problems, scheduling difficulties, or necessary modifications as directed by the Field Team Coordinator.

The field sampling team will be divided into four crews. Two crews will collect the fish and two crews will process the fish and collect tissue samples from the fish. Each of the crews will have an assigned crew chief responsible for execution of the crew's work pursuant to the SAP, as well as for the safety of the crew. Each crew chief will coordinate with the Field Team Coordinator, who has overall responsibility for the execution of the sampling. Table 1 describes the responsibilities of each crew, the number of members for each crew, and the anticipated source of the crewmembers. In addition to the crewmembers identified in Table 1, the Field Team Coordinator will provide oversight of all field activities and provide assistance to crews as necessary.

Table 1. Field crew descriptions

Crew	Responsibility of crew	Minimum number of crew members	Potential crew source
Fish collection (2 crews)	Collect and tag fish and record information in field notebook	3	NYSDEC, USFWS, NOAA
Fish processing Station 1 (1 crew)	Measure length and weight, take blood sample, record information on fish processing forms	2	USGS, NYSDEC, USFWS, NOAA
Fish processing Station 2 (2 crews)	Conduct external and internal necropsy, collect organ samples, record information on fish processing forms	3	*redacted*
Fish processing Station 3 (1 crew)	Collect scale, spine, and fillet samples, and record information on fish processing forms	2	NYSDEC, USFWS, NOAA

The key members of the overall Phase I sampling effort identified to date are as follows:

- Field Team Coordinator: *redacted*.
- Lead pathologist and crew chief for fish processing crew #1: *redacted*.
- Fish collection supervisor and crew chief for fish collection crew #1: *redacted* (NYSDEC).

Additional information on project management structure is provided in Section 7.1.

Members of the field sampling team must read the SAP and the Health and Safety Plan (Appendix A) before conducting fieldwork. In addition, immediately prior to the start of sample collection, the field crews will engage in a "dry run" field exercise in which the entire procedures of each crew are carefully worked through and evaluated. The dry run exercise will be conducted just prior to the start of the sampling program, and will be monitored and evaluated by the Quality Assurance Officer. The dry run exercise will be evaluated and discussed by the Quality Assurance Officer, Field Team Coordinator, lead pathologist, and fish collection supervisor to determine whether any changes to the Sampling and Analysis Plan are required. If any changes are necessary, the changes will be fully documented and justified, and communicated to the entire field crew.

3. Sampling Locations and Numbers

Fish health sampling will be performed at two Hudson River assessment areas and two reference locations (Figure 1). The assessment areas are the Thompson Island Pool, which runs from approximately Fort Edward (river mile 195) downstream to the Thompson Island Dam (river mile 189), and the downstream half of the Stillwater Pool, which runs from approximately Lock 5 (river mile 182) downstream to the Stillwater Dam (river mile 168). NYSDEC data indicate that fish in these two areas have historically contained elevated PCB concentrations, and some species from these areas have been shown to have increased prevalence of adverse pathology (Kim et al., 1989; Bowser et al., 1990). The two reference sampling locations are the Hudson River upstream of the Feeder Dam near Fernwood (approximately at river mile 205) and in Oneida Lake near Syracuse, New York. The specific sampling areas within each of these sampling locations will be selected based on available habitat and on the professional judgment of the fish collection crews. Smallmouth bass tend to be located in rockier, less vegetated areas, while yellow perch and brown bullhead may be found in a variety of habitats. Most of the surface area of the selected Hudson River pools are likely to be covered at least once during the sampling.

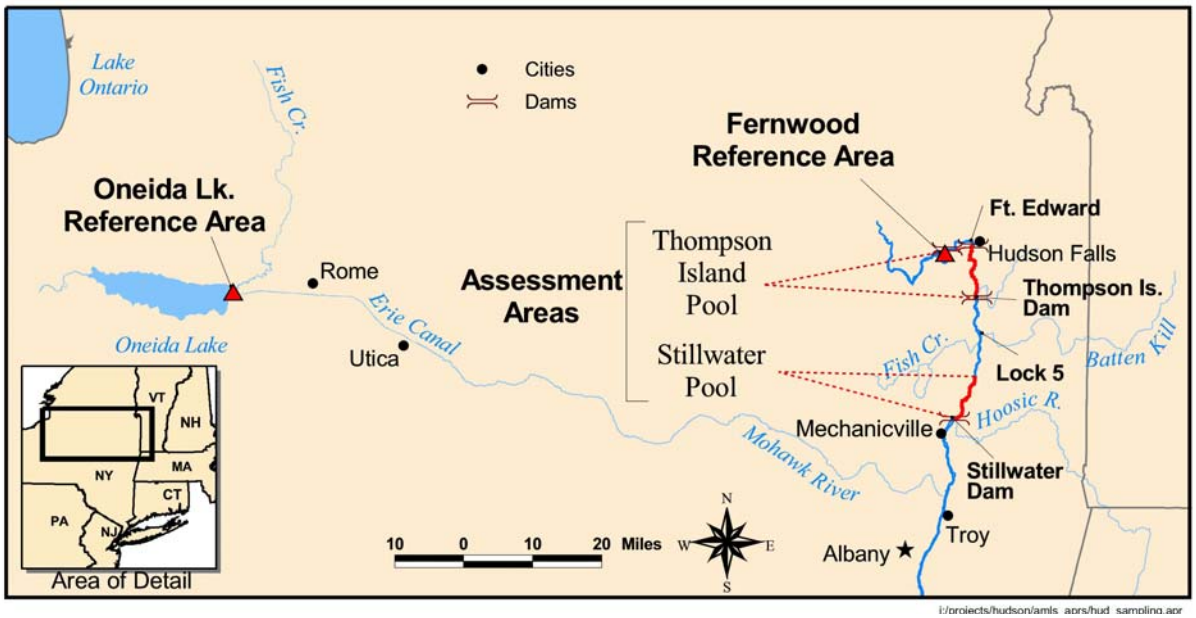

Figure 1. Approximate sampling locations.

Three fish species are targeted in this study: brown bullhead (*Ameiurus nebulosus*), smallmouth bass (*Micropterus dolomieui*), and yellow perch (*Perca flavescens*) (see Appendix B for species descriptions). These three species were selected for study for the following reasons:

- It should be possible to obtain sufficient sample sizes of these species, based on the extensive fish sampling experience of *redacted*, fish collection supervisor.
- Brown bullheads are a potential indicator of PCB-induced injuries in the Hudson River (Kim et al., 1989; Bowser et al., 1990).
- Smallmouth bass are top predators and important game fish, and they have been shown to accumulate relatively high concentrations of PCBs in their tissues in the Hudson River.
- Yellow perch are relatively abundant in the river, and may be fairly susceptible to PCB-induced injuries given their high sensitivity to dioxin (i.e., relatively low LC50 in juveniles; Monosson, 2000). In addition, yellow perch are in the same family as walleye, which have been the subject of PCB-caused injury studies at other sites (e.g., Barron et al., 2000).

Target sample numbers, fish size (length) criteria, and fish sex criteria are shown in Table 2. The target sample numbers were determined from a power analysis using data from a previous study on the prevalence of foci of alteration and tumors in walleye livers in PCB-contaminated and reference sites (Barron et al., 2000). In the Barron et al. (2000) study, abnormal livers were observed in approximately 30% of fish in the assessment areas, and in approximately 5% of fish from the reference areas. Assuming the same prevalence rates and unequal variance between sample sites, sample sizes of 50 fish from the assessment area and 30 fish from the reference area provide a greater than 90% chance of detecting any real difference between assessment areas and reference areas.

The size criteria are intended to target mature individuals, and are based on information presented in Smith (1985) and professional judgment. The sampling will target a sex ratio of 1:1 for each species at each location, and fish of a given species and sex may be rejected without processing once 30 (of 50) fish at assessment area locations, and 20 (of 30) fish at reference area locations, have been collected and processed for that given species and sex. The sample collection targets listed in Table 2 will be achieved to the extent practically and reasonably possible, with the total sample size for each location and species being the primary objective.

Table 2. Sample collection goals

Sampling location	Target species	Target length	Sample size
Stillwater Pool near Stillwater Dam (assessment area)	Yellow perch	20 cm (~8 in.)	50
	Brown bullhead	25 cm (~10 in.)	50
	Smallmouth bass	30 cm (~12 in.)	50
Thompson Island Pool (assessment area)	Yellow perch	20 cm (~8 in.)	50
	Brown bullhead	25 cm (~10 in.)	50
	Smallmouth bass	30 cm (~12 in.)	50
Feeder Dam Pool (reference area)	Yellow perch	20 cm (~8 in.)	30
	Brown bullhead	25 cm (~10 in.)	30
	Smallmouth bass	30 cm (~12 in.)	30
Oneida Lake (reference area)	Yellow perch	20 cm (~8 in.)	30
	Brown bullhead	25 cm (~10 in.)	30
	Smallmouth bass	30 cm (~12 in.)	30

4. Fish Collection Procedures

Fish will be collected using electroshocking (described in Section 4.1) and trap netting (described in Section 4.2). Hook and line angling may be used to supplement collections if weather interferes with boats, or if sufficient numbers of species can not be located by electrofishing and trap netting. The fish collected that meet the sampling requirements for species and sizes will be transferred to the fish processing crews according to the procedure described in Section 4.3. Trap netting will be employed initially only at Oneida Lake.

4.1 Electroshocking Collection Procedures

Equipment

The equipment necessary for the electroshocking collection procedure is listed in Appendix C.

Preparation

1. Locate appropriate fish habitat (see Appendix B) within each general sampling location (see Figure 1).

Collection Procedures

1. The electroshocking must be conducted in accordance with the health and safety requirements described in the Health and Safety Plan (Appendix A) and in accordance with applicable collection permits of the State of New York. All members of the electroshocking crew must carefully review the Health and Safety Plan and must be given the opportunity to ask any questions regarding the health and safety requirements before electroshocking begins.

2. Begin shocking the selected area using a pulse DC setting and voltage appropriate for the conditions (to be determined by the fish collection supervisor).

3. Net any fish that may be longer than 20 cm (approximately 8 in.) in total length. Do not net smaller fish.

4. Identify any target species using the species descriptions in Appendix B. If the species is one of the target species for that location (see Table 2), retain and measure the total length of the fish according to SOP 1.

5. If the fish falls within the target length for that species (Table 2) and the target sample number for that species has not yet been collected from that sampling location (Table 2), place a Floy tag on the fish according to SOP 2. Place the tagged fish in a live well.

6. Transfer fish from the live well to an in-river holding pen or hatchery flow-through tank throughout the sampling day. The maximum holding time in the collection boat live well will be approximately one hour. Fish that cease opercular movement after electrofishing will be disposed of without processing.

Documentation

Each electroshocking collection crew will have a dedicated, bound field notebook in which all necessary information will be recorded (see Field Notebooks). The notebook will also include portions of this SAP and all SOPs that are relevant to the electroshocking collection crews.

The general information that will be recorded includes page number, the location being sampled, location number as per assembled maps, and time of the start and end of each electrofishing run, weather, habitat, sampling crew, and recorder's initials (including blank pages). Information specific to each collected fish will be recorded on shore and will include species and Floy tag number. The fate of each Floy tagged fish (used for sample collection, screened out from sample collection, died in holding, etc.) will be documented. All reviewed pages, including blanks, will be underlined and initialed.

A single member of each fish collection crew will be designated as the field recorder. Entries in the field notebooks will be made in waterproof ink, and any necessary corrections will be made with a single line through the error accompanied by the correction date and corrector's initials. Each field recorder will date, initial, and draw a line through any pages or entries not filled out. After the completion of each day's field activities, the notes will be reviewed for completeness and accuracy by the field recorder and the fish collection crew chief of each fish collection crew, and any necessary corrections will be made. Any corrections made to data sheets at the end of each day's collection activities will be explained in detail. Field notebooks will be photocopied periodically to lessen loss of data in the event that a field notebook is lost.

4.2 Trap Net Collection Procedures

Equipment

The equipment necessary for the trap net collection procedure is listed in Appendix C.

Preparation

1. Locate appropriate habitat (see Appendix B) within each general sampling location (see Figure 1).

Collection Procedures

1. Place the trap nets on the afternoon or evening before collections are to be initiated at the sample location.

2. Place the nets in optimum habitat for each species to be sampled at the sample location. Securely fasten the nets in place and mark the nets so they are easily visible to boaters (including the electroshocking crews.

3. Every morning following net placement, the fish transport crew will check each net. Identify and remove any undesirable animal inadvertently captured by the trap net. Turtles, snakes, and large predatory fish (e.g., pike) may be encountered.

4. Identify any target species using the species descriptions in Appendix B. If the species is one of the target species for that location (see Table 2), measure the total length of the fish according to SOP 1.

5. If the fish falls within the target length for that species (Table 2) and the target sample number for that species has not yet been collected from that sampling location (Table 2 place the fish in the live well for placement and recording of Floy tags and numbers. The maximum holding time in the live well is approximately 1 hour, after which time the fish must be transferred to the in-river holding pen, hatchery flow-through tank, or 50 gallon aerated tank.

Documentation

The trap net placement and fish collection details will be recorded in the fish collection crew's field notebook. The notebook will also include portions of this SAP and all SOPs that are relevant to the trap net collection procedure.

The general information that will be recorded (see field notebooks) includes page numbers, the location being sampled, GPS coordinates and time of the trap net placement and re-visit, weather, habitat, sampling crew names and affiliations, and recorder's initials for all pages (including blanks). Information on the status of each net upon arrival to check for fish will also be recorded. Information specific to each collected fish will be recorded once a fish has been Floy tagged, and will include species and Floy tag number. The fate of each Floy tagged fish

(used for sample collection, screened out from sample collection, died in holding, etc.) will be documented.

A single member of the fish collection crew will be designated as the field recorder. Entries in the field notebook will be made in waterproof ink, and any necessary corrections will be made with a single line through the error accompanied by the correction date and corrector's initials. Each field recorder will date, initial, and draw a line through any pages and/or entries not filled out. After the completion of each day's field activities, the notes will be reviewed for completeness and accuracy by the field recorder and the crew chief, and any necessary corrections will be made. Any corrections made to data sheets at the end of each day's collection activities will be explained in detail. Field notebooks will be photocopied periodically to lessen loss of data in the event that a field notebook is lost.

4.3 Fish Transport to Processing Crews

After collection and Floy tagging, fish will be held in an in-stream holding pen in the river or hatchery flow-through tanks until processing begins. In-stream holding cages (approximately 64 cubic feet, with flow-through netting) will be placed in the ambient river water near the shore. Fish will then be transferred in small groups from the in-stream holding pen to a 50-gallon aerated tank in the fish processing area. Fish will be held in the aerated tank for no more than 4 hours, and aeration will be checked every hour. Fish in hatchery flow-through tanks will be taken for processing, as needed, without an intermediate transfer to an aerated tank. The collection crews will help coordinate timing and effort to ensure that the capabilities of the fish processing team are not overwhelmed. The fate of each fish obtained (transfer to processing crews, died in transit, etc.) will also be recorded.

5. Field Examination and Tissue Collection Procedures

This section describes the procedures that will be used by the fish processing crews to conduct external and internal examinations and to collect tissues for chemical, histopathological, or other analyses. This section is organized as follows:

- Section 5.1 describes the procedures for holding the fish until they are processed.
- Section 5.2 describes blood collection and length and weight measurements
- Section 5.3 describes the external examination procedures.
- Section 5.4 describes the internal examination procedures.
- Section 5.5 describes procedures for collecting tissue samples for preservation and shipment to laboratories for additional analysis.
- Section 5.6 describes procedures for collecting spines, scales, and fillet samples.
- Section 5.7 describes procedures for collecting blank samples.
- Section 5.8 describes the requirements for sample containers, preservation, and storage.
- Section 5.9 describes sample-labeling procedures.
- Section 5.10 describes sample chain of custody procedures.

The general steps in the external examination, internal examination, and tissue collection procedures are shown in Figure 2.

Equipment

The equipment that is required by the fish processing crews to conduct this work is listed in Appendix C.

Documentation

- A project management notebook will track the capture location and holding time for each fish, the tally of fish numbers by sex and species, calibration notes for balances, shipping document numbers, general notes on sites and conditions, and procedural changes.

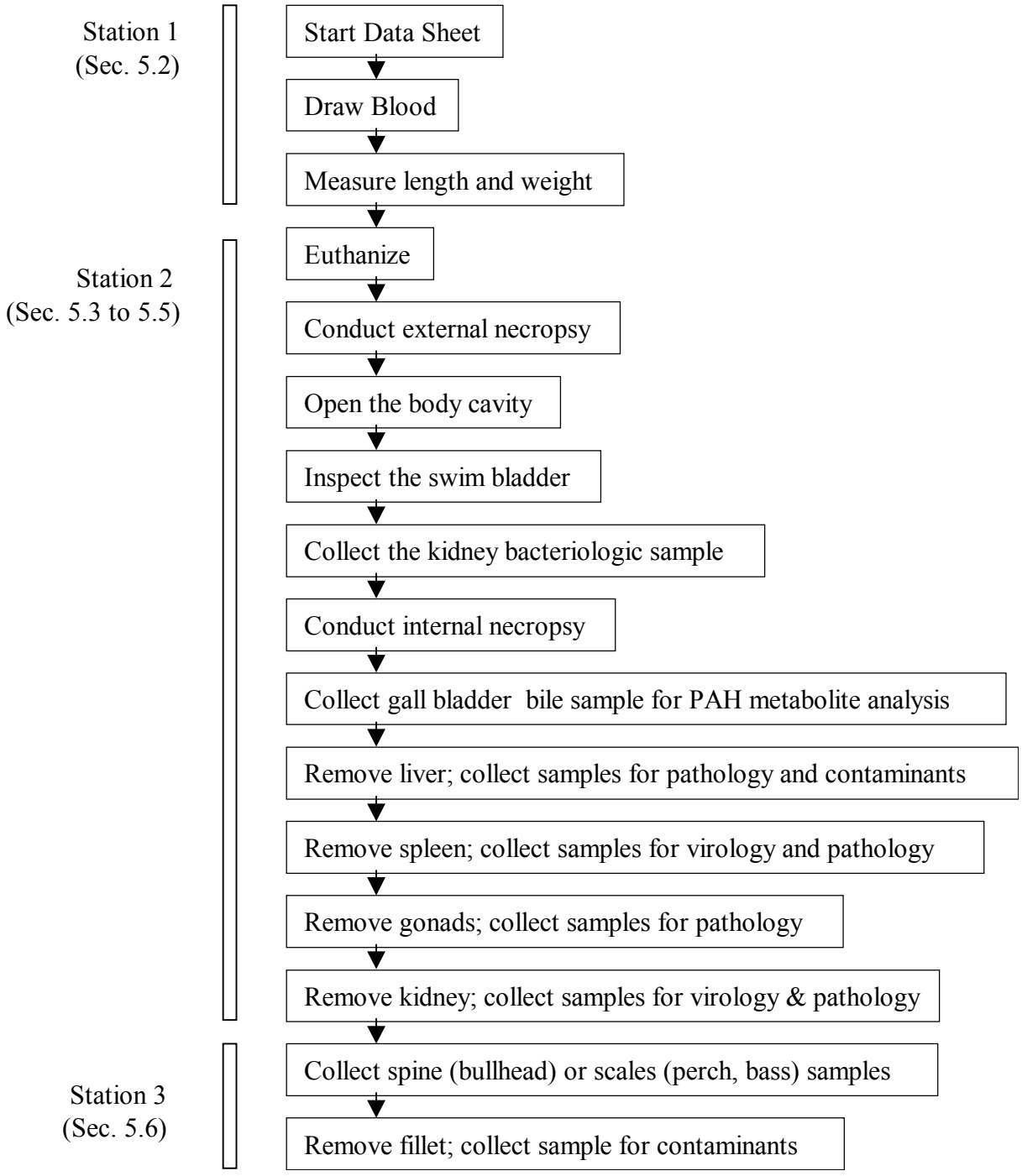

Figure 2. Generalized steps in external examination, internal examination, and sample collection procedures.

- Individual Fish Data Sheets (including blank, formatted pages) will accompany each fish through the entire collection process. These data sheets will be collected into notebooks sorted by species and location.

Photographic documentation will be used to record the necropsies. Abnormal tissues designated by the pathologist will be photographed, as will several normal tissues for photographic comparison to abnormal tissues. Photographic documentation will be conducted using a digital camera, and each photograph will be taken against a solid color background sheet with a sample identification label clearly visible in the photograph. The disk will be changed daily and sequentially numbered (month, day, disk number). Disks will be archived as original data.

One member of each fish processing crew will be the designated recorder. Entries on the individual fish data sheets will be made in waterproof ink, and any necessary corrections will be made with a single line through the error accompanied by the correction date and corrector's initials. Each field recorder will date, initial, and draw a line through any pages and/or entries not filled out. After the completion of each day's field activities, the notes will be reviewed for completeness and accuracy by the field recorder and the crew chief, and any necessary corrections will be made. Any corrections made to data sheets at the end of each day's collection activities will be explained in detail. Individual fish data sheets will be photocopied periodically to lessen loss of data. Copies of relevant data sheets will be provided to laboratories for further analyses (histopathology, aging, disease screen).

5.1 Fish Holding

Procedure

Fish will be held at the processing station according to the following procedure:

1. Place each fish from the fish collection crew into an in-stream holding cage or hatchery flow-through holding tank. Record the species, Floy tag number, and time of transfer into the cooler for each fish.

2. Fish will be removed from the holding pen to Station 1 as fish are needed by Station 2.

3. After blood has been drawn and the fish weight and length have been determined (see Section 5.2), transfer the fish to a 50 gallon aerated tank. Place fresh water in the aerated tank at least twice per day. Aeration will consist of at least two aeration stones to maintain dissolved oxygen.

4. Remove any dead fish from the cooler immediately, and record their Floy tag number and their death. Dispose without further processing.

5. Remove fish for processing in the approximate order that they were collected from the river by keeping group sizes small and processing the entire group before adding fish. Fish will be processed as soon as possible after receiving the fish from the fish collection crews with priority given to the more sensitive species (yellow perch and smallmouth bass). If any fish remain at the end of the processing day, remaining fish may be held overnight in the in-stream holding cage or hatchery flow-through holding tank.

Fish holding information will be documented in a separate field notebook.

5.2 Blood Collection and Length and Weight Measurement (Station 1)

Procedure

Blood samples will be collected according to the following procedures:

1. Put on a fresh pair of nitrile gloves.

2. Take a fish from the holding pen or hatchery flow-though tank and measure and record the total length (to the nearest millimeter) and weight (to the nearest gram) of the fish according to SOP 1.

3. Draw blood sample from the posterior caudal artery or vein according to Sections 6.1 and 7.0 of Schmitt et al. (1999).

4. Record the Floy tag number, species, and site location on an individual fish data sheet.

5. Place fish in a holding tank.

5.3 External Examination (Station 2)

The results of the external examination are to be recorded using the field forms, which also serve as a guide for the examination.

Preparation

1. Prepare a label for each fish with the Floy tag number on it that will be easily visible in photographs of the fish, and label bottles for tissue collections

2. Prepare a clean surface for necropsies and tissue collections, and use clean instruments.

Procedure

1. Put on a fresh pair of nitrile gloves.

2. Remove the fish from the holding tank, acquire the corresponding individual fish data sheet and place the fish in a cooler of ice water to subdue the fish.

3. Euthanize the fish by cervical dislocation.

4. Conduct the external necropsy, using the field form to record the results:

    a. *Body shape and appearance.* Record any abnormalities such as spinal curvature, swollen abdomen, protruding eyes, scars, lesions, parasites, tumors, or any other abnormalities.

    b. *Head appearance.* Record any head abnormalities, such as tumors, lesions, scars, or parasites.

    c. *Eye appearance.* Record fish with protruding or missing eyes, or other eye abnormalities.

    d. *Fin clips and tags.* Record and describe fin clips and/or tags (other than the Floy tag inserted by the collection crew).

    e. *Operculum.* Observe and record the condition of the operculum.

    f. *Gills.* Record and photograph (if possible) any obvious parasitism or morphological abnormalities, including color and integrity.

    g. *Other.* Record and photograph (as appropriate) any other distinct physical anomalies.

5. Take photographs of anomalies as determined by the lead pathologist.

6. Place the fish on a new sheet of aluminum foil for internal necropsy and tissue collection.

5.4 Internal Examination (Station 2 — cont.)

The results of the internal examination are to be recorded using the field forms, which also serve as a guide for the examination. Refer to Figure 3 for a generalized diagram of fish anatomy.

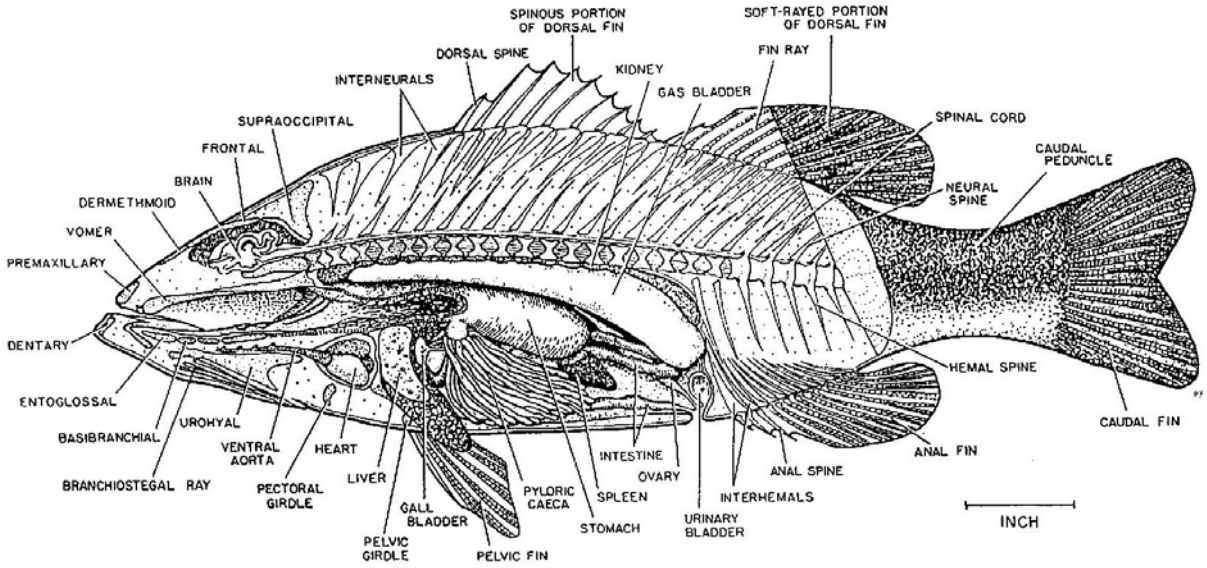

Figure 3. Generalized diagram of the anatomy of spiny-rayed fish.

Preparation

1. Decontaminate all re-useable sampling equipment according to SOP 4. Place the equipment on a new, dry Kimwipe® or other suitable clean surface.

First Procedure: Open the Body Cavity and Collect the Kidney Disease Screen Sample

1. Verify sample container labels against fish species and Floy tag number. Verify that all equipment is new (one-time use equipment) or decontaminated and sterilized (re-useable equipment).

2. Position the fish with the left side up, ventral side closest to the examiner.

3. Wipe the entire left side of the fish with a disposable towel saturated with methanol.

4. Using freshly decontaminated and sterilized dissection scissors and forceps, make the following cuts (using care not to puncture any internal organs):

    a. Cut a small vertical (perpendicular to the backbone) incision approximately 1 cm anterior to the vent at the ventral axis.

b. Cut from this vertical incision to the base of the operculum along the ventral axis.

c. Cut from both ends of the ventral axis incision to the lateral line.

d. Cut along the lateral line and remove the left side of the musculature of the fish to fully expose the internal organs.

5. Record any abnormalities on the peritoneal side of the musculature removed from the fish.

6. Determine the sex of the fish. If the fish is of the sex still needed, record the sex and proceed. Otherwise, discard the fish and move to the next fish.

7. For yellow perch and smallmouth bass, record the condition of the swim bladder before proceeding.

8. Collect the kidney disease screen sample as soon as possible after the incision, using the following procedure:

a. Using the dull side of a newly sterilized scalpel blade (or a new disposable sterilized scalpel), (as a probe), pull the swim bladder away from the kidney located just posterior, and attached, to the spine.

b. Observe, and record any abnormal kidney appearance or structures (lesions, tumors, hemorrhages, discoloration, etc.).

c. Cut a small incision in the center of the kidney with the tip of the sterile scalpel blade. Note: The center of the kidney is sampled to avoid tissues to be sampled for histopathology.

d. Stab the incision with a new sterile loop to collect the sample.

e. Streak the sample onto the labeled BHIA slant and discard the loop.

f. Collect a duplicate sample at a frequency of 1 in 20 samples. Use a new sterile loop (see Section 5.7 for duplicate sample labeling procedures).

g. Store the sample according to the procedures in Section 5.6.

h. Photograph any kidney lesions, as appropriate.

Second Procedure: Conduct Internal Necropsy

1. The internal necropsy can be performed using the forceps and dull side of the scalpel from the previous procedure.

2. Carefully inspect the internal surfaces of the peritoneal cavity for hemorrhages, discoloration, or other abnormalities.

3. Carefully inspect the gonads to determine the sex of the fish, and note any abnormalities or unique features.

4. Carefully inspect the liver for any abnormalities, including discoloration, abnormal shape, tumors, disfiguring features, and hemorrhages.

5. Carefully inspect the spleen for any abnormalities, including discoloration, abnormal shape, tumors, disfiguring features, and hemorrhages.

6. Carefully inspect the gall bladder for any abnormalities, including discoloration and relative amount of fluid.

7. Carefully inspect the pyloric caeca for any abnormalities, including discoloration, abnormal shape, tumors, disfiguring features, hemorrhages, and relative size in relation to the stomach size.

8. Carefully inspect the intestine for any abnormalities, including discoloration, abnormal shape, tumors, disfiguring features, and hemorrhages.

5.5 Tissue Collection

Note: The procedure for collecting scales or spines for age analysis and fillets for contaminants analysis is described in Section 5.6, and the procedure for collecting a kidney disease screen sample is described in Section 5.4.

This section describes the procedures for collecting fish tissue samples for storage and shipment to laboratories for analysis. All histopathological samples will be placed in labeled cassettes. A single, labeled bottle of fixative will be used for the histopathological samples from each fish. Other tissues will be stored in the containers indicated in Table 3. The following tissues will be collected from each fish:

- gall bladder bile (analysis of PAH metabolites)
- spleen (histopathological, bacteriological, and virological analysis)

- liver (histopathological analysis and contaminants)
- gonad (histopathological analysis)
- head kidney (histopathological analysis)
- trunk kidney (histopathological and bacteriological analysis)
- fillet (contaminant analysis).

Preparation

1. Check balance calibration using a standard weight and re-calibrate if necessary.

2. Prepare all sample containers and labels. Sample container requirements are described in Section 5.8, and sample labeling procedures are described in Section 5.9.

3. Decontaminate all re-useable sampling equipment according to SOP 4. Wrap in clean aluminum foil.

Procedures

1. Collect gall bladder bile (if sufficient bile is present):

    a. Use newly decontaminated dissecting equipment to remove the liver by cutting the connective tissue and vasculature that attaches the liver to the viscera and place the liver on a cutting board covered with clean aluminum foil prior to dissection.

    b. Carefully separate the gall bladder from the liver and grasp the cystic duct with a hemostat. Cut the cystic duct between the hemostat and the liver taking care not to touch the liver with the scissors. Care should also be taken not to spill any bile onto the liver.

    c. Hold the freed gall bladder over the mouth of the amber vial designated for bile collection.

    d. With a clean scalpel blade puncture the gallbladder and allow the bile to drip down the tip of the blade into the vial.

    e. Preserve and store the sample according to the procedures in Section 5.8.

    f. At a frequency of 1 in 20 samples, a duplicate sample of bile will be collected for analysis. The bile collected from the designated fish will have to be divided into two amber vials that are appropriately labeled.

    g. Decontaminate the hemostat and scissors before using again.

2. Continue to process the liver:

   a. Remove any non-liver tissue from the surface of the liver and carefully inspect the liver for any lesions or abnormalities.

   b. Place the liver in an aluminum weigh boat and weigh (to the nearest 0.01 g).

   c. If there are no gross lesions visible, samples for histopathology will be taken from three different areas of the liver. The samples should not exceed one centimeter in thickness and they should be removed from the right side of the liver, from the center, and from the left side of the liver (see Figure 4). If possible these samples should be identified as to their origin (right, center or left) and placed in cassettes or gauze bags as appropriate to the size of the sample. These samples should be placed in a container with Dietrich's fixative for histopathology (along with other tissues from the same fish for histopathology).

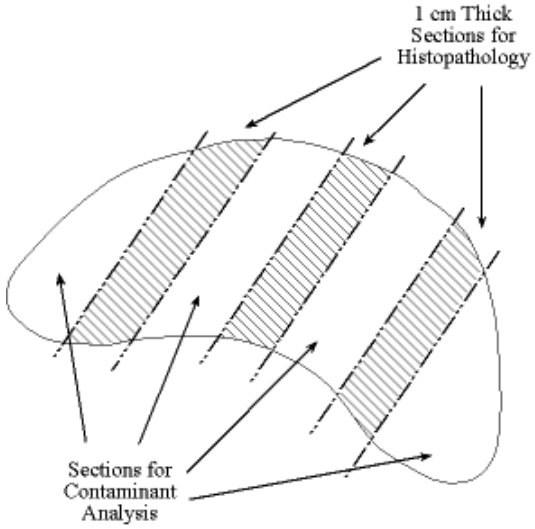

Figure 4. Liver dissection.

   d. Place the remainder of the liver in an appropriate sample bottle for contaminant analysis.

   e. If there are gross lesions visible in the liver, sampling will have to be adapted to the site of the gross lesion or lesions. It is difficult to make firm rules for sampling in this case. As a general rule try to sample the same areas as are sampled in the liver with no gross lesions. For example, if a discrete gross lesion is present on the right side of

the liver, sample the gross lesion and then take sections from the left side and center of the liver in addition for consistency. Guidelines for sampling tissue when gross lesion(s) are present are as follows. If there is more than one lesion and the lesions are discrete, sample each lesion taking care to leave an amount of "normal" liver issue around the lesion. Estimate and record the size and location of each lesion along with other discernible features (color, texture, shape). Number the lesions in order with the suffix "EH1," "EH2," etc., after the species identifier and Floy tag number identifier of the sample identification code (see Section 5.7.2). Place each lesion in a separate cassette or gauze pouch as appropriate to size and label that container with the assigned letter. Place these cassettes or pouches in a sample bottle that contains Dietrich's fixative (along with the other tissues from the same fish for histopathology). Any liver that remains after the gross lesions and any appropriate section of "normal" liver are sampled will be placed in the appropriate sample bottle for contaminant analysis.

If the liver has a gross lesion that is not discrete and involves over 50% of the liver, describe the lesion as stated above with the location and size as well as observations of color, texture, and any other unique features. Remove the entire lesion and some adjacent "normal" liver for histopathology if this sampling can be done without compromising the amount of liver needed for contaminant analysis. With a sharp scalpel make incomplete slices into the liver lesion perpendicular to the "normal" liver at one centimeter intervals to assure adequate fixation of the tissue. Place the liver lesion in a sample bottle that contains Dietrich's fixative (along with other tissues from the same fish for histopathology). The remainder of the liver can be divided for placement in the appropriate container for contaminant analysis.

If the liver has a lesion which involves the entire liver diffusely, then sample the liver as described for a liver with no gross lesions. In this case the description of the appearance should be as detailed as possible. If the liver exhibits a variety of features, try to include as many of the different features as possible in the samples. Place each sample in a separate cassette or gauze pouch appropriately labeled as to the location of the sample (right, center, or left). If necessary, take additional samples to include all the various features of the lesion and label them appropriately. Place these specimens in a sample bottle that contains Dietrich's fixative (along with other tissues from the same fish for histopathology). The remainder of the liver can be divided for placement in the appropriate container for contaminant analysis.

3. Collect the spleen samples:

   a. Use newly sterilized dissecting equipment (scalpel, razor blade, forceps), or sterile disposable equipment.

b. Remove the spleen from the visceral mass using a scalpel and forceps.

c. Place the spleen on a new piece of weigh paper, blot dry with a Kimwipe®, and weigh (to nearest 0.01 g).

d. Cut the spleen into two equal sections along the longitudinal axis.

e. Remove any gross histopathological lesions and place in a sample bottle that contains Dietrich's fixative (for histopathological analysis).

f. Place one half of the spleen in the 5.5 ml snap-cap tube containing HBSS[1] (for virological analysis).

g. At a frequency of 1 in 20 samples, collect a duplicate sample for virological analysis by slicing the spleen half in two and placing the two smaller sections in two separate snap-cap tubes (see Section 5.7 for duplicate sample labeling procedures).

h. Place the other half in an appropriate sample bottle that contains Dietrich's fixative (for histopathological analysis).

i. Store samples according to the procedures in Section 5.8.

4. Collect the gonad samples:

a. The dissecting equipment used for the previous procedure can be re-used for this procedure. Wipe the equipment clean before proceeding.

b. Remove the gonads from the visceral mass.

c. Place the gonads in an aluminum weigh boat and weigh.

d. Remove any gross histopathological lesions and place in a sample bottle that contains Dietrich's fixative (for histopathological analysis).

e. Place the gonads in a sample bottle with Dietrich's fixative (for histopathological analysis).

f. Store the sample according to the procedures in Section 5.6.

1. Procedures for preparation of HBSS and buffered formalin preservative solutions are described in SOP 5.

5. Collect the head and trunk kidney samples:

   a. Use newly sterilized dissecting equipment (scalpel, razor blade, forceps), or sterile disposable equipment.

   b. If possible, remove sufficient trunk kidney for the virology sample using a scalpel and forceps. Otherwise, use head kidney for the virology sample.

   c. Place the kidney virology sample in the same 5.5 ml snap-cap tube containing HBSS[2] as the spleen virology sample (for virological analysis).

   d. Remove any gross histopathological lesions and place in a sample bottle that contains Dietrich's fixative (for histopathological analysis).

   e. Remove 1 cm of the anterior-most portion of the head kidney.

   f. Place the 1 cm section of head kidney in a sample bottle with Dietrich's fixative (for histopathological analysis).

   g. Remove 1 cm of the posterior-most portion of the trunk kidney. *Do not remove any tissue that may have been involved with the kidney disease screen sample (Section 5.3)*.

   h. Place the 1 cm section of trunk kidney in a sample bottle with Dietrich's fixative (for histopathological analysis).

   i. Store the samples according to the procedure in Section 5.6.

   j. Pass fish on to station 3 with the data sheet and the jar of tissues with fixative.

2. Procedures for preparation of HBSS and buffered formalin preservative solutions are described in SOP 5.

5.6 Spines, Scales, and Fillet Collection (Station 3)

1. Check Floy tag against data sheet for agreement

2. Remove the left pectoral fin spine from brown bullheads:

   a. Apply gentle pressure, then twist the spine, dislocating it from the socket, and complete the operation by tearing the disarticulated spine from the skin. (Try to dislocate the spine, not break it.)

   b. Place the spine in a properly labeled scale envelope, and store it according to the procedure in Section 5.6.

   c. For duplicate spine samples, remove the right pectoral fin spine and place in a second scale envelope with the duplicate sample label.

3. Remove at least ten scales from yellow perch and smallmouth bass:

   a. Scrape at least five scales from the skin of the fish just posterior to the longest point of the pectoral fin, below the dorsal line. Place the scales into a properly labeled scale envelope and store it according to the procedure in Section 5.6.

   b. For duplicate scale samples, add a duplicate sample label to the scale envelope and indicate that a second reading should be taken.

4. Collect the fillet sample:

5. Use newly decontaminated equipment (scalpel, fillet knife, forceps) for this procedure.

6. Turn the fish over so the right side is up.

7. Wipe the outside of the fish with a methanol soaked disposable towel.

8. For brown bullheads, remove skin from the area to be filleted. For yellow perch and smallmouth bass, remove scales from the area to be filleted.

9. Make a cut along the ventral midline of the fish from the vent to the base of the jaw.

10. Make a diagonal cut from base of cranium following just behind gill to the ventral side just behind pectoral fin.

11. Remove the flesh and ribcage from one-half of the fish by cutting from the cranium along the spine and dorsal rays to the caudal fin. The ribs should remain on the fillet.

12. Place fillet in a labeled glass jar.

13. Store the samples according to the procedure in Section 5.8.

14. Place the Floy tag in the histopathology sample jar and seal the jar with parafilm.

15. Stockpile all used chemicals and all used tissues for disposal through NYSDEC at the Hale Creek Laboratory or other state facilities.

5.7 Equipment and Rinsate Blank Sample Collection

Field blank samples will be collected at a frequency of once per day or every 20 samples, whichever is more frequent. The following types of blank samples will be collected:

- disease screen equipment blank (kidney bacteriology and spleen/kidney virology)
- contaminant rinsate blank (liver and fillet)
- bottle blank (vials and jars for bile, liver, fillet).

Equipment and rinsate blank samples will be collected using material and procedures similar to those used for actual samples, and will provide information on the possibility that samples are being contaminated in the field. (See also Section 7.2 below.)

Preparation for equipment and rinsate blanks:

1. Prepare sample containers and labels for the equipment or rinsate blank samples. Sample container requirements are described in Section 5.8, and sample labeling procedures are described in Section 5.9.

2. Decontaminate all re-useable sampling equipment according to SOP 4. Place the clean equipment in clean aluminum foil.

Procedure for equipment and rinsate blanks (every 20th fish):

1. After the excision of tissues (liver and fillets) during sample processing, the instruments used in the excision (forceps, scissors, scalers, fillet knives, etc.) are to be decontaminated following the procedures outlined in SOP 4.

2. Collect into an appropriately labeled jar a contaminant rinsate blank by rinsing the cleaned dissecting tools that are used to collect liver or fillet samples with pesticide grade methanol. After rinsing with methanol, one Kimwipe® will be used to swab each tool. This Kimwipe® will be placed in the jar along with the methanol rinsate. This step will be repeated for each tool used during tissue excision with the Kimwipes® and rinsate for all utilized tools collected in a single field blank jar. Separate rinsate blanks will be taken for fillet excision

3. Several Kimwipes® that have not been used to clean any instruments will be placed in an appropriately labeled sample jar and will be submitted for analysis along with the rinsate blanks.

4. Collect a kidney disease screen blank sample by streaking a new sterile loop onto a BHIA slant.

5. Store the samples according to the procedure in Section 5.8.

In addition to equipment and rinsate blanks, one bottle from each lot of bottles used for tissue collection will also be reserved as a bottle blank and stored for possible analysis of contaminants.

5.8 Sample Containers, Preservation, and Holding Times

Table 3 lists the sample containers, preservative solutions, storage temperature requirements, and holding times for each type of sample being collected. All bottles for PAH metabolites and contaminant samples will be pre-cleaned and a bottle blank will be taken for each new lot of bottles used, or one per day, whichever is more frequent. All sample containers will be packaged to prevent breakage during shipment, including wrapping with bubble wrap, placement in styrofoam or plastic vial holders, placement in taped zip-lock bags, or other techniques to cushion containers and prevent their movement within shipping coolers.

5.9 Sample Labeling Procedures

All sample containers will be labeled with a unique numbering system that identifies the fish species, required analysis, and Floy tag number of each fish captured and sampled.

Table 3. Sample container and preservation requirements

Tissue[a]	Analysis	Container	Preservative solution[b]	Field storage and shipping temperature	Holding time/storage temperature
Blood plasma	Endocrine biomarkers	Cryo-vial	None	Liquid nitrogen (-190ºC)	Indefinite/stored over liquid nitrogen (-190ºC)
Kidney	Disease screen	BHIA slant culture tube	None	Wet ice	7 days/-20ºC
Gall bladder bile	PAH metabolites	4 ml amber-colored screw cap glass vial w/Teflon-lined caps, pre-cleaned	None	Dry ice	2 years/-70ºC
Spleen	Histopathology	1 to 2 L Nalgene jar[c]	Dietrich's fixative	Ambient	3 mos. for embedding, indefinite thereafter/ambient
	Virology	5.5 ml snap-cap bullet tube	HBSS	Wet ice	7 days/-20ºC
Liver	Histopathology	1 to 2 L Nalgene jar [c]	Dietrich's fixative	Ambient	3 mos. for embedding, indefinite thereafter/ambient
	Contaminants	20 ml vials, with Teflon-lined caps; or larger glass jars with Teflon-lined caps	None	Dry ice	2 years/-70ºC
Gonad	Histopathology	1 to 2 L Nalgene jar [c]	Dietrich's fixative	Ambient	3 mos. for embedding, indefinite thereafter/ambient
Head kidney	Histopathology Virology (if insufficient trunk kidney)	1 to 2 L Nalgene jar [c]	Dietrich's fixative	Ambient	3 mos. for embedding, indefinite thereafter/ambient
Trunk kidney	Histopathology Virology (if sufficient sample)	1 to 2 L Nalgene jar [c]	Dietrich's fixative	Ambient	3 mos. for embedding, indefinite thereafter/ambient
Scales/spines	Age	Scale envelope	None	Ambient	Indefinite/ambient
Fillet	Contaminants	Glass jars with Teflon-lined caps	None	Dry ice	2 years/-70ºC

a. Tissues are listed in the order of their collection, as specified in Sections 5.2 through 56.
b. Procedures for preparation of preservative solutions are described in SOP 5.
c. All histopathological samples will be placed in appropriately sized, labeled Surgipath "cassettes." One labeled bottle will be used for each fish.

5.9.1 Sample labels

- Sample labels will be filled out using permanent markers and affixed to the sample containers as follows: For blood plasma samples, label cryovials with the species code and Floy tag number using only cryomarkers.
- For scale envelopes (scales and spine samples), fold over, but do not seal, the scale envelope and affix the completed label to the outside of the envelope.
- For most other sample bottles, jars, and tubes, affix the sample label to the outside of the sample jar and cover it with clear packing tape.

5.9.2 Sample identification code

The following sample identification code will be used:

SP- NUM- AN-C

where:

SP = a two-letter code designating the species collected or QC sample type:

- BB = brown bullhead
- SB = smallmouth bass
- YP = yellow perch
- QC = QC samples.

NUM = a unique three-digit numerical code that corresponds to the Floy tag number (or the QC sample number, starting with 001). (NUM codes for bottle blanks will begin with 901 and increase sequentially for each bottle blank sample.)

AN = a unique two-letter code that designates the analysis to be performed:

- Age analysis:
  - **SA** = scales or spines for determining the age of the fish
- Blood plasma
  - **BP =** blood plasma for endocrine biomarkers
- Disease screen:
  - **KD** = kidney disease screen sample
  - **SD** = spleen disease screen sample
- Contaminants:
  - **BR** = bile for PAH metabolites
  - **LR** = liver for residues
  - **FR =** fillet for residues
  - **BB =** bottle blanks
  - **KB** = Kimwipe® blank
- Histopathology:
  - **SH** = spleen for pathology
  - **LH** = liver for pathology
  - **GH** = gonad for pathology
  - **HH** = head kidney for pathology
  - **TH** = trunk kidney for pathology
  - **EH** = gross lesion for pathology.

C = a code for additional identification of samples:

- **S** = single sample only
- **D** = duplicate sample (residue and biochemical analysis, KD samples)
- **R =** liver histopathological sample from the right half of the liver
- **C =** liver histopathological sample from the center of the liver
- **L =** liver histopathological sample from the left of the liver
- **1, 2, 3 . . . =** the gross lesion number collected from a given fish.

Compact disks with photographs from the digital cameras will also be assigned a five to six digit sample number according to the following :

mm-dd-D-#

- mm = one to two digits for the month
- dd = date
- D = identifier for photo disc
- # = number for each photo disc.

5.10 Chain of Custody Procedures

All samples collected during this study will be maintained under strict chain of custody, which is the documentation of a sample's history from time of collection through sample analysis to final disposal. A chain of custody record will be maintained for each fish starting at the time it is labeled with a Floy tag, and for each tissue sample starting at the time of sample extraction from a fish.

The field recorder of each crew is personally responsible for the care and custody of the samples that are in that crew's possession. A sample is in custody of the field recorder if any of the following occur:

- The sample is in the individual's possession.
- The sample is within view after being in possession.
- The sample is in a locked or sealed container that prevents tampering after being in possession.
- The sample is in a designated secure area.

A chain of custody transfer occurs when the sample's custody is transferred from one crew to another (e.g., from fish collection crew to fish processing crew), or when the samples are shipped to and received by the laboratory or storage facility. Chain of custody transfers that occur in the field (e.g., from the fish collection crew to the fish processing crew) will be documented in the field notebooks of each crew. The date and time of transfer will be recorded in the field notebooks.

When the samples are packed in coolers or other containers for shipment to the laboratory or storage facility, the samples will be accompanied by completed chain of custody records. The chain of custody record will contain the following information:

- project name
- sample identification (unique for each sample)
- date and time of sample collection
- sample matrix (e.g., liver, kidney)
- analysis required for each sample
- name and signature of individual relinquishing custody
- inclusive dates and times of possession for each person
- sample shipping date and mode.

Each shipping container containing samples will be accompanied by an original chain of custody record (in a plastic sealable bag to keep it dry) and be sealed with custody seals after making a copy to keep with the air bill. Custody seals are used to detect unauthorized tampering with samples after sample collection until the time of use or analysis. Signed and dated gummed paper seals may be used for this purpose. The seals will be attached so that they must be broken to open the shipping container.

Coolers or other containers containing samples will be opened at the analytical laboratories or archiving facility only by a person authorized to receive the samples. The containers will first be inspected for integrity of the chain of custody seals or other signs of tampering. The receipt of each sample in the coolers or containers will be verified on the chain of custody forms. The signed chain of custody forms will be photocopied, and the photocopy will be mailed to the sending party. Samples will be stored in a secure area according to procedures documented for each analytical facility.

6. Sample Analysis

This section describes the laboratory procedures that will be used in Phase I to analyze the samples. The following types of analyses will be conducted in Phase I[3]:

- histopathological examination of tissues from liver, gonads, spleen, head kidney, trunk kidney, and any gross lesions sampled in the field
- virological and disease screen, of spleen and kidney, respectively
- fish age determination of scales or spines.

Samples will also be collected for potential contaminant analyses in liver, bile, and fillet, and PAH metabolites in bile (Phase II of the study). The purpose of the Phase II analysis will be to evaluate the potential relationship between contaminant concentrations and any tissue abnormalities observed in Phase I. The decision of whether to conduct Phase II analyses, as well as the number and types of analyses if they are to be done, will be made by the Hudson River Trustee Council. If the decision is made to conduct Phase II analyses, Phase II analysis plans will be prepared at that time.

6.1 Histopathological Analysis

All samples will be hand delivered to *redacted* to avoid preservative leaks during commercial shipment.

redacted will conduct the histopathological analysis of tissue from fish species collected during the survey. Liver, gonad, spleen, head kidney, trunk kidney, and gross lesions will be evaluated in all fish that are collected and necropsied. The histology and histopathological evaluation will be conducted in accordance with the SOPs and protocols of *redacted*, as summarized below. The Quality Assurance Unit of *redacted* will review all of the paperwork for the study from the receipt of specimens to the issuing of the final pathology report to assure that all phases of the operation are conducted in accordance with Good Laboratory Practice (GLP) regulations. In addition, the specific procedures used by *redacted* will be carefully reviewed by an independent histologist before beginning the study to ensure that the specific methods are appropriate for the needs of this study.

3. Note that blood plasma samples are to be collected by the USGS concurrent with this study according to the procedures described in Schmitt et al., 1999.

6.1.1 Inventory

Wet tissues in fixative from individual fish shipped to *redacted* for histopathology will be inventoried upon arrival. When the inventory is complete, an internal document called the Project Sheet will be prepared to provide directive for the processing of the tissues to slides and the evaluation of the histologic sections by the pathologist. The Project Sheet will be in the form of a protocol and any additional instructions necessary to achieve the required sampling of tissues. Once the Project Sheet is prepared, the study is entered into *redacted*'s computer system such that each animal in the study is assigned a unique *redacted* accession number. An Individual Animal Work Sheet will be prepared that will follow the animal through all phases of slide preparation and slide evaluation. Also, a list of gross lesions and their respective descriptions will be prepared to ensure that these lesions are recognized at gross trimming and during microtomy.

6.1.2 Gross trimming

Tissues will be trimmed to a size that is appropriate for adequate processing, no greater than 4 mm in thickness and no longer than 2.5 cm.

Tissues that require decalcification, such as the trunk kidney if it is attached to the vertebral column or skin with scales, will be placed in a decalcifying solution for an appropriate length of time depending upon the size and type of specimen (e.g., skin may require less time for decalcification than the vertebral column). After decalcification the tissue will be trimmed to the appropriate size for processing.

6.1.3 Tissue processing

Tissues from fish of the size that will be collected in this project will be processed according to *redacted*'s Tissue Processing Program One in a VIP automatic tissue processor. In this program tissues are dehydrated through a graded series of ethyl alcohol (40 minutes in each of seven alcohol baths), cleared in Clear Rite 3 (60 minutes in each of 2 baths) and infiltrated with paraffin (60 minutes in each of three baths).

6.1.4 Embedding

Processed tissues will be embedded in paraffin in a plane of section appropriate to the tissue (e.g., skin would be embedded on edge in order to capture all levels from epidermis to the subcutaneous tissue).

6.1.5 Microtomy

The initial phase of microtomy for each tissue block is "facing" the block to remove any artifacts created on the cut surface of the tissue during gross trimming. This is accomplished by cutting through the block until the full face of the tissue is exposed. Care will be taken during this rough trimming phase not to cut through any gross lesions that have been described in the tissue being microtomed.

The final section or sections, as required, will be cut at 4-5 microns and mounted on an appropriate number of slides.

6.1.6 Staining

The tissues on slides will be stained with hematoxylin and eosin in an automatic stainer (Hacker linear stainer) and will be coverslipped (Hacker RCM-3660 Robot Coverslipping Machine).

6.1.7 Precheck

During the precheck procedure each slide will be evaluated for quality of coverslipping. In addition, the slides and blocks will be properly matched as to tissue and to number, and any discrepancies will be identified and corrected. The precheck technician will compare the slides and blocks for each animal with the information on the Individual Animal Work Sheet to be sure that none are missing.

6.1.8 Checkout

During the checkout procedure, slides will be examined and labeled in preparation for evaluation by the *redacted* pathologist. The quality of each slide will be assessed (tissue thickness, presence of sectioning artifacts, poor staining, etc.) and any quality recuts that are necessary will be requested. All tissues required by the Individual Animal Work Sheet will be accounted for by the checkout technician. The slides will be arranged appropriately in boxes and will be delivered to the pathologist who will evaluate them.

6.1.9 Pathology

The pathologist will determine the maturity of the gonadal tissue through visual observation. Rate the sexual maturity as follows:

1. Males

    a. Stage 0 — undeveloped: little or no spermatogenic activity in germinal epithelium; testicular tissue contained exclusively immature stages of spermatogenesis with no spermatozoa observed.

    b. Stage 1 — Early spermatogenic: mostly thin germinal epithelium with scattered spermatogenic activity; immature stages (spermatocytes to spermatids) predominate, but spermatozoa may also be observed.

    c. Stage 2 — Mid-spermatogenic: germinal epithelia are of moderate thickness; a mix of spermatocytes, spermatids, and spermatozoa are present in roughly equal proportions.

    d. Stage 3 — Late spermatogenic: thick germinal epithelium;

        i. Stage 3A — All stages observed, however, mature sperm predominate; immature spermatogenic stages are still consistently present throughout the testis.

        ii. Stage 3B — All stages observed, however, mature sperm predominate; immature spermatogenic stages are either completely absent or restricted to scattered, small nests of cells.

2. Females

    a. Stage 0 — undeveloped: pre-vitellogenic oocytes observed exclusively; oocyte diameter <250 μg; cytoplasm stains basophilic with H&E.

    b. Stage 1 — early development: >90% pre-vitellogenic, remaining oocytes early to mid-vitellogenic; oocytes slightly larger (up to 300 μg); late perinucleolus through cortical alveolar stages.

    c. Stage 2 — mid-development: majority of observed follicles are early and mid-vitellogenic; oocytes larger, 300-600 μg diameter, and containing peripheral yolk vesicles; globular and uniformly thick chorion; cytoplasm is basophilic, yolk globules eosinophillic.

    d. Stage 3 — late development: majority of developing follicles are late vitellogenic; oocyte diameter is 600-1000 μg; eosinophillic yolk globules distributed throughout the cytoplasm.

    e. Stage 4 — late development/hydrated: majority of developing follicles are late vitellogenic; follicles are much larger (>1,000 μg).

f. Stage 5 — post-ovulatory: spent follicles, remnants of the theca externa and granulose.

The pathologist will examine the tissues (liver, gonads, spleen, head kidney, trunk kidney) from each fish and will dictate any abnormalities found in the tissue(s) into a Lanier Voicewriter 210 recording device. Tapes will be archived at *redacted*. Table 4 presents a list of possible histopathological lesions that may be observed. The taped dictation of the pathologist will be transcribed by a pathology data technician into *redacted*'s computer based pathology data system. The diagnoses made for each tissue from each fish will be printed out on an Individual Animal Record. The tapes recorded by the pathologist will be listened to by a second pathology data technician, and the diagnoses will be checked against the Individual Animal Record to assure that the pathologist's data has been correctly entered into the system. After the diagnoses from all the fishes in the study have been entered into the database, Histopathology Prevalence Tables (HPTs) and Summary Prevalence Tables (SPTs) will be generated and given to the pathologist.

Table 4. List of possible histopathological conditions in tissues

Tissue	Possible histopathology
Liver	Tumors, foci of cellular alteration, glycogen depletion, macrophage aggregates, leukocytes/lymphocytes, megalocytosis/karyomegaly, hemorrhaging, cellular degeneration, altered cell structure
Kidney	Lesions, tumors, hemorrhaging, cell degeneration, altered cell structure including renal corpuscles, tubular epithelial cell necrosis, atrophy, hypertrophy, hyperplasia, edema, macrophage aggregates, inflammation, developing tubules
Gonad	Lesions, tumors, intersex, altered cell structure, inflammation, reduced spermatogenic elements, abnormal spermatozoa, hemorrhaging, abnormal tissue structure, atretic eggs, abnormal yolk development
Spleen	Tumors, macrophage aggregates, abnormalities, cellular necrosis, vascular necrosis

The pathologist will review the data in the tables and will write a narrative summary, which includes the objective(s) of the study, the study design, a summary of the histology methods, results of the histopathologic evaluation of the tissues, and conclusions. The narrative summary and appropriate data tables, including HPTs and SPTs, will be submitted to the sponsor in draft form.

As part of the Quality Assurance/Quality Control procedures, a random subset (10% of all samples) of tissue samples will be examined independently by two other pathologists. The three pathologists will review and discuss the results of their examinations and reach consensus on any differences. If the initial interpretations of the three pathologists are different on more than 10% of the samples examined by all three, then a second pathologist will examine all abnormal tissues, and the two pathologists will reach consensus on any differences in results. If the initial interpretations of the three pathologists are different on 10% or fewer of the samples, the

pathologists will determine whether additional tissue review is necessary, and the type and extent of review if it is deemed necessary.

6.2 Disease Screen

The disease screen (bacterial screen of kidney tissue and viral screen of spleen tissue) will be conducted according to the American Fisheries Society Fish Health Section Blue Book (Thoesen, 1994) disease assessment protocols. These protocols are used by the U.S. Fish and Wildlife Service as part of the National Wild Fish Health Survey.

Specifics of the analysis for this study are outlined in SOP 7. The specific analytical procedures of the selected laboratory will be carefully reviewed and evaluated for adherence to the Quality Assurance Plan (QAP) and the Phase I study's objectives.

6.3 Age Analysis of Scales and Spines

Scales from all yellow perch and smallmouth bass collected in this study and spines from all brown bullhead will be analyzed to determine the age of each fish. Age analysis will follow standard methods commonly used in fishery science outlined in SOP 6. The specific analytical procedures of the selected laboratory will be carefully reviewed and evaluated for adherence to the QAP and the Phase I study's objectives.

6.4 Data Analysis

The data obtained in the Phase I study will be evaluated quantitatively to determine whether fish from the assessment areas within the Hudson River differ in the prevalence and/or severity of the endpoints being examined in the study compared to fish from the reference areas. The conclusions regarding whether differences exist between assessment area and reference area fish will be made based on statistical testing.

The specific statistical procedures that will be used to compare assessment area and reference area fish will depend on the characteristics of the endpoint measurements. Endpoint measurements may be categorical (histopathological scores), frequency based (e.g., presence/ absence of disease), ordinal (e.g., age), or continuous (e.g., weight), with various possible underlying data distributions. For each endpoint, the data type and distributions will be carefully examined by an experienced statistician, who will then make recommendations to the Field Team Coordinator as to the specific statistical procedures that should be used to analyze each endpoint.

7. Quality Assurance Plan

This study is being conducted in accordance with the Quality Assurance Plan (QAP) for the Trustee's Hudson River NRDA. As described in the QAP, four general elements of quality assurance/quality control (QA/QC) must be addressed:

- project management
- data generation and acquisition
- assessment and oversight
- data validation and usability.

This section describes the Quality Assurance Plan for the Phase I fish health study, based on these four general elements.

7.1 Project Management

The organization of the study team for the Phase I fish health investigation is shown in Figure 5. The study personnel are organized in such a way as to provide clear lines and areas of responsibility and to ensure good communication within the study personnel. Field personnel are first organized by field task, with a separate crew being assigned to each type of task (electroshocking and fish processing). Personnel will remain on the same crews throughout the study (to the extent possible). Each crew has a crew leader, who is responsible for all activities of the crew. All of the fish collection crews are subsequently under the direction and supervision of the fish collection supervisor (*redacted* of NYSDEC), who is responsible for all fish collection activities in the study. Similarly, the two fish processing crews are under the direction of *redacted*, who is the lead pathologist for the study.

The fish collection supervisor and the lead pathologist coordinate with the field team coordinator (*redacted*). The field team coordinator is responsible for resolving any issues raised by the fish collection supervisor or the lead pathologist and will serve as the final decision-maker when issues are not resolved by the crew chiefs, fish collection supervisor, or lead pathologist. The field team coordinator will also coordinate the project teams from each of the analytical laboratories. The field team coordinator reports to the Trustee Council representatives for the Phase I fish health study (*redacted*, U.S. FWS and *redacted*, NOAA), as well as to the Quality Assurance Officer (*redacted*). As described in the QAP, the Quality Assurance Officer will assist the field team coordinator in ensuring that the Phase I fish health study is conducted in accordance with the QAP.

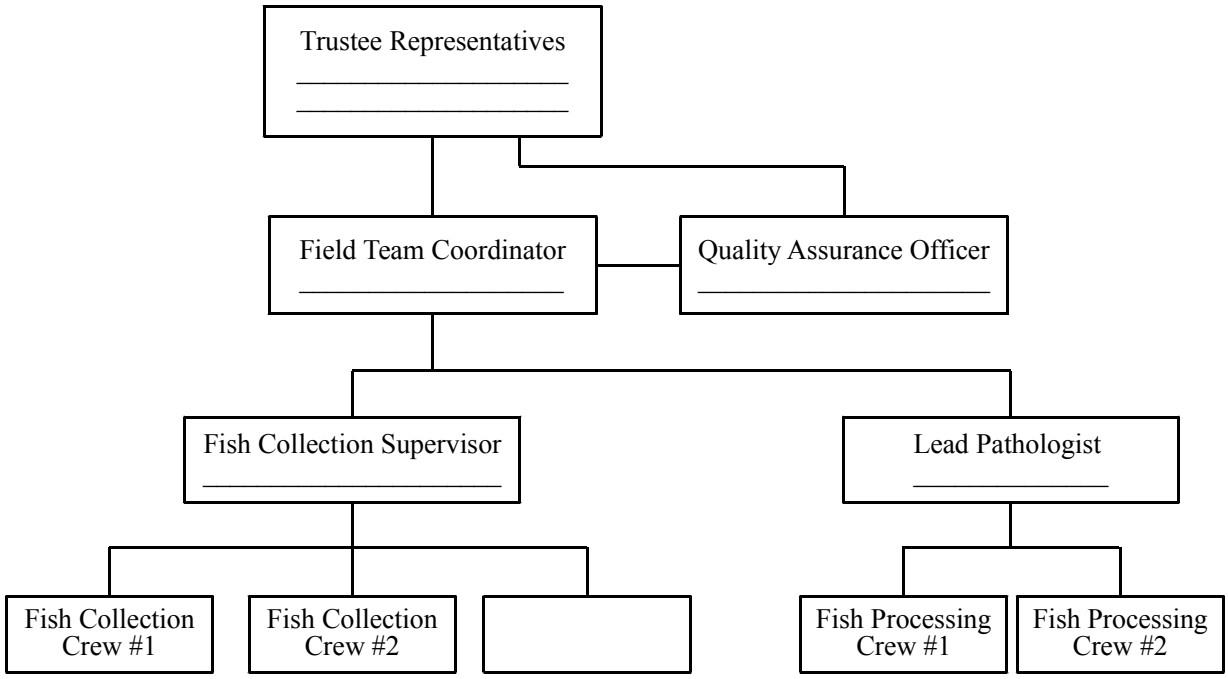

Figure 5. Project management structure.

This SAP for the Phase I health assessment of Hudson River fish was developed to provide detailed and explicit instructions for the field crews to follow in collecting the study data. The SAP has been reviewed and commented on by key parties to the study before the beginning of sample collection. Reliance on a detailed, explicit, and fully reviewed SAP ensures that:

- Study objectives, methods, procedures, and details are completely thought out before sampling.
- Data will be collected in a systematic and consistent way throughout the study.

It is the responsibility of every member of the study team to adhere to the requirements of the SAP. Each field team member is required to sign a statement that they have read the SAP and understand it. In particular, the field crew leaders must make sure that their crews adhere to the SAP.

Nevertheless, the procedures specified in the SAP must be considered somewhat flexible by the field study team. Many events can arise during field data collection that require changes to the procedures being used. In these circumstances, deviations from the SAP will be conducted only after consultation between the field crew chiefs, the fish collection supervisor or the lead pathologist, and the Field Team Coordinator. Any SAP deviations will be carefully documented, including an explanation as to why the deviations are necessary.

7.2 QA/QC Samples

QA/QC samples can include samples such as blanks or duplicates that can be used to assess the degree to which the sampling program and analytical measurements meet data quality requirements. QA/QC samples can include those collected in the field (e.g., blanks or duplicates) or those generated within the laboratory as part of analysis (blanks, duplicates, standards).

Table 5 lists the field QA/QC samples that will be collected. Field duplicate samples will be collected for age analysis of fish scales/spines, kidney disease screen, and the spleen virology screen. Duplicate samples will be collected at a frequency of 1 in 20 samples. Equipment blank samples will be collected for the kidney and spleen disease screen analysis at a frequency of 1 per 20 samples or 1 per day, whichever is more frequent. Bottle blanks will be collected one per lot or one per day, whichever is more frequent. One rinsate blank for contaminants analysis will be collected at the end of each sampling day. Procedures for collecting the duplicate, equipment, and rinsate blank samples are included in the specific sampling procedures in Section 5.

Table 5. Field QA/QC samples

Type of analysis	Field duplicates	Equipment blanks	Bottle blanks	Rinsate blanks
Age (scales/spine)	One in 20 samples (two scales from same fish)	Not applicable	Not applicable	Not applicable
Blood Plasma	One in 20 samples	Not applicable	One/day	Not applicable
Disease screen (kidney)	One in 20 samples (two swipes from the same tissue)	Once/day or once/20 samples	Not applicable	Not applicable
PAH metabolites	One in 20 samples	Not applicable	One/lot	Not applicable
Histopathology	Not applicable	Not applicable	Not applicable	Not applicable
Liver contaminants	Not applicable	Not applicable	One/lot	One per day
Fillet contaminants	Not applicable	Not applicable	One/lot	One per day

Laboratory QA/QC samples will vary depending on the type of analysis being conducted and will be specified in the laboratory analytical and/or Quality Assurance Plans. Laboratory QA/QC samples may include replicates, blanks, calibration standards, or standard reference materials.

7.2.1 Study documentation

All study activities will be documented in bound, waterproof, and paginated notebooks. To the extent possible, information will be recorded on pre-formatted data sheets. The use of pre-formatted data sheets is a QA/QC measure that is designed to:

- ensure that all necessary and relevant information is recorded for each sample and each sampling activity
- serve as a checklist for the field crews to help ensure completeness of the data collection effort
- assist the field crews by making data recording more efficient
- minimize the problem of illegible field notebook entries.

Each field crew will have a single field data recorder who is responsible for documenting all information in the field notebooks or on the forms. Assigning this responsibility to a single person will help ensure that documentation is complete and consistent throughout the sampling event. The field data recorder is also responsible for the care, custody, and disposition of the field notebook.

Field notebook entries will be made in waterproof ink, and corrections will be made with a single line through the error accompanied by the correction date and corrector's initials. Each completed data sheet will be reviewed, corrected (if necessary), and initialed by the field data recorder and the appropriate field crew leader. Following completion of the study, field notebook originals will be stored at the *redacted*.

7.2.2 Chain of custody procedures

Strict chain of custody procedures will be used throughout the study. The chain of custody procedures will begin when a sampled fish is Floy tagged, indicating that it may be used in the study. Chain of custody will continue until samples obtained from the fish are analyzed or discarded (or the fish is released before samples are obtained).

7.2.3 Personnel experience and training

The field sampling crews will receive explicit instructions in the execution of this SAP. The field crews will be instructed in the field before beginning any sampling, and the instructions will be repeated or refreshed during the sampling period as necessary.

Immediately prior to the start of sample collection, the field crews will engage in a "dry run" field exercise in which the entire procedures of each crew is carefully worked through and evaluated. The dry run exercise will be conducted just prior to the start of the sampling program, and will be monitored and evaluated by the Quality Assurance Officer. The dry run exercise will be evaluated and discussed by the Quality Assurance Officer, Field Team Coordinator, lead pathologist, and fish collection supervisor to determine whether any changes to the Sampling and Analysis Plan are required. If any changes are necessary, the changes will be fully documented and justified, and communicated to the entire field crew.

7.3 Assessment and Oversight

The QAP specifies that studies that generate data will be audited to ensure that the project-specific plans are being properly implemented. Several mechanisms for internal audits of the data generation process will be used in the Phase I fish health assessment. These mechanisms include the following:

- A project management structure that defines clear lines of responsibility and ensures communication between field crews and with the Field Team Coordinator, fish collection supervisor, and lead pathologist. Clear responsibilities and communication can serve as a means of providing internal audits of the sample collection process as it proceeds.

- A requirement that field notebooks be reviewed daily by data recorders and field crew leaders.

- The use of pre-formatted data sheets that serve as a checklist for sampling procedures, thereby helping to ensure that sampling is complete.

The Quality Assurance Officer (*redacted*) or a delegate (*redacted*) will attend, observe, and evaluate the "dry run" field exercise that will be conducted immediately prior to the start of the study, and will provide immediate feedback to the Field Team Coordinator as to whether approaches or procedures should be modified prior to the start of sampling. The sampling will not begin until approval is received from the Quality Assurance Officer or her delegate. The Quality Assurance Officer or her delegate will remain in the field at the start of the sampling effort to ensure that the work is being conducted in accordance with Quality Assurance

requirements for the project. In addition, the Quality Assurance Officer will conduct a field audit of procedures and documentation of the study.

7.4 Data Validation and Usability

The SAP for the Phase I fish health assessment has been extensively reviewed for the adequacy of the sampling design and methods. The original filed notebooks will be maintained. Final reports can then be reviewed against the sampling records to ensure that the data presented in the reports represent complete and accurate information.

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