

2016 RMP Sturgeon Derby Study

Sampling and Analysis Plan

I. Introduction

A second year of Sturgeon Derby monitoring will be conducted in collaboration with the 32nd annual Original Sturgeon Derby held by the Foundation Sportsman's Club out of Bay Point, CA. The sturgeon derby and tissue sampling will occur on February 6-7, 2016. Tissue samples from female white sturgeon will be collected, and samples from up to fifteen sturgeon will be analyzed.

The Derby offers an opportunity to collect a variety of tissue samples from fish caught for the competition that are otherwise unavailable. This will allow for comparison between selenium concentrations measured in tissues that are easy to obtain non-lethally (muscle plugs, fin rays) and those that are not, but may be of greater interest toxicologically (ovaries, liver) or analyzed for microchemistry (otoliths, for comparison with fin rays). If approved, additional collection of muscle fillet samples will also contribute to a better understanding of the relationship between muscle fillet and muscle plug selenium concentrations. Analysis of tissue samples for stable isotopes will also provide information on diet and habitat use by the sturgeon. Otolith microchemistry will be conducted to support development of a method for fin rays, which can be collected from sturgeon non-lethally during future monitoring efforts.

This study will be performed as a collaboration between SFEI (with RMP funding), USFWS, USGS, UC Davis, and Stantec.

The purpose of this Sampling and Analysis Plan is to outline the details for how the samples will be collected, handled, and analyzed by the various parties involved with the study. Additional pathology samples taken by USFWS will not be included in this document, except where needed to inform the collection of other samples.

II. Key Personnel and Approvals

The personnel and work assignments for this study are shown in Table 1. These key personnel have indicated their approval of the Sampling and Analysis Plan by adding their initials and date in the far right column.

Table 1: Key Personnel for 2016 RMP Sturgeon Derby Study

Name	Affiliation	Duties	Phone	Email & Shipping Address (if needed)	Initial and Date to Indicate Approval of Plan
Jay Davis	SFEI	RMP Lead Scientist	530-304-2308	jay@sfei.org	JD 1/27/16
Phil Trowbridge	SFEI	RMP Program Manager	603-340-5220 510-746-7345	philt@sfei.org	PT 1/26/16
Jennifer Sun	SFEI	RMP Project Manager	949-202-6671 510-746-7393	Jennifers@sfei.org 4111 Central Ave. Richmond, CA 94804	JS 2/1/16
Amy Franz	SFEI	RMP Data Manager	510-282-5012	amy@sfei.org	AF 1/26/16
Don Yee	SFEI	RMP Quality Assurance Officer	510-508-2995	donald@sfei.org	DY 2/1/16
Patrick Kim	SFEI	Field Staff	510-685-0574	patrickk@sfei.org	
Carolyn Doehring	SFEI	Field Staff	815- 922-5973	carolynd@sfei.org	
Vince Palace	Stantec	Microchemistry analyses of fin rays and otoliths	204-226-8313 204-928- 7618	Vince.Palace@stantec.com 500-311 Portage Avenue Winnipeg MB Canada R3B 2B9	VP 1/4/16
Lisa Peters	Stantec	Gonad Hstiology	204 291-7714 204 928-7629	Lisa.Peters@stantec.com 500-311 Portage Avenue Winnipeg MB Canada R3B 2B9	LP 1/4/16
Robin Stewart	USGS	Selenium analyses of muscle plug, muscle fillet (if approved), ovary, and liver samples	650-329-4550	arstewart@usgs.gov Water Resources Division 345 Middlefield Rd. MS496 Menlo Park, CA 94025	ARS 12/31/15
Zachary Jackson	USFWS Lodi CA	Field sample collection	209-403-1457 209-334-2968 x 408	zachary_jackson@fws.gov Anadromous Fish Restoration Program Lodi Fish and Wildlife Office 850 S Guild Ave., Suite 105 Lodi, CA 95240	

Ken Nichols	USFWS	Field pathology analysis & sample collection	530-365-4271	ken_nichols@fws.gov CA-NV Fish Health Center	
Emily Schick	UC Davis	Isotope analyses of muscle plug and ovary samples	530-752-8100	ekngo@ucdavis.edu UCD Stable Isotope Facility Dept of Plant Sciences Rm 1210 PES One Shields Ave. Davis, CA 95616	
Molly Web	USFWS Bozeman, MT	Blood plasma analyses	406-994-9907	molly_webb@fws.gov Bozeman Fish Technology Center 4050 Bridger Canyon Road Bozeman, MT 59715	
Jerry Davis	Foundation Sportsman's Club	Derby Organizer	925-382-7521		

III. Sampling Design

Sampling will take place on February 6-7, 2016 at the 32nd annual Original Sturgeon Derby held at the following location:

McAvoy Harbor
1001 Mc Avoy Rd
Bay Point, CA 94565

Tissues will be collected by USFWS, Stantec and SFEI staff from up to 15 female white sturgeon caught by Derby anglers, with angler permission. Possession of sturgeon tissues has been approved under an existing USFWS Scientific Collection Permit. If more than 15 female white sturgeon are caught, the field team will continue to collect samples. The RMP Lead Scientist and project partners will decide later which 15 of the samples to analyze or if additional funds should be sought to analyze all the samples.

Attachment 1 outlines the target samples, analyses to be performed, sample handling, responsible agencies for each type of sample, and whether samples are being collected for the RMP or external projects.

The following tissue types will be collected from each fish:

- Blood plasma
- Muscle Plugs
- Ovaries

- Liver
- Muscle Fillet
- Fin rays
- Otoliths

Additional tissues will be collected for the USFWS from male white sturgeon:

- Blood plasma
- Fin rays (Left)
- Testis

Additional pathology samples will be collected by Ken Nichols of USFWS from all sturgeon:

- Kidney (bacteriology swab, virology sample)
- Spleen (virology sample)
- Skin (virology sample, parasites)
- Gills (virology sample, parasites)

The Derby spans two days. Anglers can bring in fish at any time from 7 am on February 6 and 1 pm on February 7, including the middle of the night. SFEI staff will be present to collect samples on February 6 between 10 am and 12 am and on February 7 between 10 am and 3 pm. Staff from other agencies may be present at other times during the Derby. Table 2 lists the tentative field staff schedule.

Table 2: Field Schedule for 2016 RMP Sturgeon Derby Study

Name	Affiliation	Hours
Jennifer Sun	SFEI	on call (tentative field hours below) Sat: 7 am - 12 pm Sun: 7 am - event conclusion
Carolyn Doehring	SFEI	Sat: 9 am - 4 pm Sun: 7 am - event conclusion
Patrick Kim	SFEI	Sat: 2 pm - 12 am Sun: 10 am - event conclusion
Jay Davis	SFEI	on call
Vince Palace	Stantec	Sat: 7 am - 12 am Sun: 12 am - event conclusion
Lisa Palace	Stantec	Sat: 7 am - 12 am Sun: 12 am - event conclusion
Ken Nichols	USFWS	Sat: 3 pm - 12 am Sun: 6 am - event conclusion
Zachary Jackson	USFWS	Sat: 7 am - 12 am Sun: 6 am - event conclusion
Laura Heronimus		

Crystal Castle		
Nathan Cullen		

IV. Field Data Sheets

The fish number, tissue type, station code, date and time of fish collection, total length (cm), fork length (cm), fish sex, and number of samples of each tissue type collected will be recorded on field data sheets (Attachment 2). If blood plasma and fin ray samples are collected by the USFWS, the USFWS IDs for these samples will be recorded in the comments section of the field sheet.

Organism ID

Each sturgeon from which samples are collected will be assigned a unique Organism ID

16SD-## where:

- 16SD is the project ID (2016 Sturgeon Derby study) and will be consistent for every sample.
- ## is a unique, 2-digit number corresponding with the consecutive number of the fish collected, starting with 01.

Sample ID

Sample containers (as provided by SFEI unless otherwise previously stated) will be labeled with the Sample ID, which is a concatenation of the project ID (16SD), fish number (01, 02, 03...), the tissue type code, and the sample replicate number (if the number of replicates of a tissue type is greater than 1), where the tissue type codes are:

- BP is blood plasma
- MP is muscle plug
- MF is muscle fillet
- LI is liver
- OV is ovary
- OH is ovary for histology analysis
- TH is testis for histology analysis
- OT is otolith
- FL is fin ray left
- FR is fin ray right

16SD-## XX-YY where:

- 16SD = project ID
- ## = fish number
- XX = tissue type codes

- YY= number of field replicates for that tissue type

Example: 16SD-01-BP-01

If USFWS uses a different sample ID, it should be recorded in the notes section of the Field Data Sheet.

V. Sample Collection Methods

a. Blood plasma

Blood plasma will be collected by USFWS according to their protocols. Blood plasma is not part of the RMP study. However, sex steroids measured in blood plasma collected by USFWS may be compared with the reproductive staging and ovary histological analysis conducted by Stantec in order to better understand the ability of sex steroids to predict sex and sexual maturity in female white sturgeon in San Francisco Bay.

b. Muscle plugs

Three or four muscle plugs will be taken from each female fish using a disposable 5 mm biopsy punch. Plugs should be taken from the epaxial muscle near or slightly in front of the dorsal fin, offset from the midline (Figure 1). The sturgeon skin will be rinsed with DI water prior to sampling. The biopsy punch should be inserted into the muscle tissue using a twisting motion, rotated several times to cut through tissue, and removed with a scooping motion. Thin forceps should be used to remove the tissue plug from the biopsy as completely as possible and place it in a 2 mL long-term storage cryovial. Forceps will be rinsed with DI water and wiped with a kimwipe between use on samples from different fish. A new 5 mm biopsy punch will be used for each fish.

Samples will be collected and stored in the field with the skin on. All plugs taken from the same fish can be stored in the same cryovial. At the end of each sampling day, prior to freezing the samples, samples will be removed from their vials and the skin removed using dissection scissors or a scalpel. Dissection equipment will be rinsed with DI water between samples.

Cryovials will be stored and transported on wet ice in the field and frozen at -20 C in the lab until shipping to USGS in Menlo Park. Sample will be stored at -80 C at USGS.



Figure 1. Location of muscle plug collection

c. Muscle Fillet

Muscle fillet samples will be collected from each female using a filleting knife. To collect the sample, a single section of muscle (approximately 5 cm x 2 cm x 3 cm of fillet below the skin, or about 4-5 times the volume of liver and ovary collected) will be removed with the skin on from a region immediately adjacent to the area where the muscle plugs were taken. The skin will later be removed from the fillet in the field as time allows.

The filleting knife and collection surface will be rinsed with DI water between the collection of tissues from different fish. About 25 g of muscle will be removed with the skin on. Samples will be stored on wet ice until the skin can be removed between sample collections in the field, after which they will be stored on wet ice. Pre-cleaned sample jars from VWR will be used to store samples.

d. Liver

Liver samples will be collected from each female using a filleting knife. To collect this sample, one full lobe will be removed from the liver by making a clean cut at the confluence of the lobes. The same lobe will be removed from each fish collected. Samples will be collected from 3 different sections of the liver into a single collection vial. The collection vial will be stored and transported on wet ice in the field and frozen at -20 C in the lab until shipping to USGS in Menlo Park, where samples will be stored at -80 C.

The filleting knife and collection surface will be rinsed with DI water between the collection of different tissue types or tissues from different fish. Enough sample mass will be collected to fill a scintillation vial about three-quarters full of tissue. Pre-cleaned sample vials will be provided by USGS.

e. Ovary

Ovary samples will be collected from each female using a filleting knife. Samples will be collected from 3 different sections of the ovary, corresponding with the locations of histology

sample collection, into a single collection vial. The collection vial will be stored and transported on wet ice in the field and frozen at -20 C in the lab until shipping to USGS in Menlo Park, where samples will be stored at -80 C.

The filleting knife and collection surface will be rinsed with DI water between the collection of different tissue types or tissues from different fish. Enough sample mass will be collected to fill a scintillation vial about three-quarters full of tissue. Pre-cleaned sample vials will be provided by USGS.

f. Ovary - histology samples

Additional ovary samples will be collected from each female for histological analysis. For this analysis, samples (approximately 0.1 to 0.5 g) will be obtained from 3 different sections of the ovary using a clean scalpel. Each section will be handled with clean forceps and placed in a separate and labeled histology cassette and fixed in 10% neutral buffered formalin for 48 hours. After 48 hours of fixing, tissues will be rinsed with ethanol and stored in 70% ethanol for shipping. It is recommended that both the storage and shipping containers be 1.0 L widemouth Nalgene® bottles. Instruments will be rinsed with ethanol between each fish.

g. Testis - histology analyses

Testicular tissue samples will be collected from each male fish that is sampled. Blood plasma will be collected by USFWS according their protocols and is not part of the RMP study. Tissues will be stored in 20-mL pre-filled 10% formalin containers.

h. Otoliths

Sagittal otoliths will be collected from each female and male fish that is sampled. Otoliths will be dissected by splitting the skull in the sagittal plane using either a hacksaw or reciprocating power saw with a fine toothed blade. The paired otoliths will be removed using forceps, rinsed in distilled water and placed into paper coin envelopes for drying, storage and shipping. All sampling equipment will be cleaned and rinsed with DI water between fish.



i. Fin rays

The entire left and right pectoral fin rays will be collected from each male and female fish that is sampled. The fin rays will be cut as proximal to the body wall as practical using a penny cutter. Each fin ray will be rinsed in distilled water and stored for shipping in a paper envelope. All sampling equipment will be cleaned and rinsed with DI water between fish. Right fin rays will be analyzed by Stantec, and left fin rays will be analyzed by USGS.

j. Pathology samples

A kidney swab for bacteriology analysis and visual inspection for parasites and other clinical signs of infection will be conducted immediately after dissection of the body cavity, prior to the collection of RMP samples. Additional kidney, spleen, skin, and gill samples will be collected by USFWS for virology analyses.

VI. Sample Handling and Storage

Sample handling, storage, and shipping instructions are shown in Attachment 1.

Liver, ovary, blood plasma, and muscle tissues will be stored in the field on wet ice. Muscle plugs and fillets will be collected with the skin on. The skin will be removed from the muscle plugs and fillets prior to storage at -20 C. At the end of each sampling day (or more frequently), muscle plug, liver, ovary, and blood plasma samples will be transported to SFEI for storage at -20 C. Muscle plug, ovary and liver samples can be stored at -20 C until shipped to USGS (up to 3 months), and will be stored at -80 C at USGS. Blood plasma samples can be stored at -20 C for up to 2 years.

Once frozen, samples must be maintained at -20 C during transport to the respective laboratories (ship overnight on dry ice). Any deviation should be noted and reported to RMP staff.

Fin ray and otolith samples will be stored in the field at room temperature. Histology samples will be fixed in formalin for 48 hours before being stored in 70% ethanol. Histology cassettes will be stored in a 1 L nalgene bottle of 10% formalin in the field. 48 hours after the end of the event, SFEI staff drain the bottle of formalin, rinse the samples with 70% ethanol, and refill the bottle with 70% ethanol for storage. Samples stored at room temperature in the field can be shipped at room temperature.

All samples will be accompanied by a chain of custody form (COC) provided by SFEI. The COC form will include the Sample ID, collection date, sample type, analysis required, and other remarks. Shipping information is provided in Table 1 and Attachment 1.

Project samples will not be disposed of until all analyses are complete and analytical and QC results have been reviewed and approved by the RMP Project Manager and QA Officer.

VI. RMP Sample Archiving

RMP samples will be collected from all female white sturgeon caught during the Derby, but all samples may not be analyzed. Samples that are not analyzed will be archived according to the storage instructions and archive locations shown in Attachment 1.

Samples will not be archived according to the standard RMP archiving protocol at either the short- or long-term archiving locations (Schaffer's Meats and Cold Storage in Oakland, CA or NIST in Charleston, SC). Instead, excess samples will be stored by the agencies conducting sample analysis.

Blood plasma samples that are not analyzed will be stored at USGS at -20 C for up to two years.

Muscle plug, ovary, and liver samples that are not analyzed will be stored at USGS at -80 C for at least 5 years. Fin ray, otolith, and histology samples that are not analyzed will be stored at Stantec at room temperature for at least 5 years.

VII. Equipment Lists

Safety (Quantity)

- Work gloves (3)
- Cut resistant gloves (3)
- Eye protection (3)
- Nitrile gloves – 1 box each med and large
- Safety bag
- Sunscreen
- Hand sanitizer
- Field binder with contacts and hospital map

Sample storage and tracking

- Coolers with wet ice
- Box to keep otolith, fin ray, and ovary histology samples in
- Labels and sharpies
- Laptop w datasheets
- Backup hard copies of datasheets

Otoliths

- Power tools, etc – Zac will bring
- Vials/coin envelopes - SFEI

Muscle plugs (SFEI)

- DI water
- Biopsy punch
- Forceps
- Vials
- Sharps container

Fin ray

- Fixed blade sturdy knife
- Penny cutter scissors
- Envelopes - SFEI

Dissection

- Bone shears
- Buck knife
- Scalpels
- Dissection scissors
- Forceps

Gonads

- Dissection supplies - Zac
- Vials - Robin

- Histology cassettes - Vince/Zac
- Formalin (for histology) - Zac

Liver

- Dissection supplies - Stantec
- Ziploc Bags - SFEI

Blood

- Blood collection supplies - USFWS
- Centrifuge - USFWS
- Plasma vials – USGS

Non SFEI Supplied Equipment Summary

USGS

Ovary Vials
Plasma Vials

Stantec

Otolith and fin ray dissection tools
Ovary histology dissection supplies
Endolymph Containers

USFWS

Power tools
Fin ray collection supplies
Blood collection supplies
Centrifuge
Large Whirlpak
Sturgeon cradle, table, hose, garbage cans, etc.