

2015 RMP Selenium in Sturgeon Muscle Plug Study Sampling Plan

Approved by the RMP Lead Scientist

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Introduction

The San Francisco Bay Regional Water Quality Control Board has released for public review a draft TMDL for selenium in North Bay, which establishes a target selenium concentration in white sturgeon muscle tissue as a basis for evaluating impairment. It is a priority of the Regional Board to establish a non-lethal sturgeon tissue monitoring protocol that will allow for the efficient collection of large numbers of tissue samples for measurement of impairment.

In 2009, the Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) began an effort to establish an efficient, non-lethal method of collecting sturgeon muscle tissue through the use of muscle biopsy plugs. Concentrations in plugs were found to correlate well with concentrations in muscle fillets for the 12 fish sampled. Another round of evaluation of this correlation will occur using data from 2014. This correlation has opened the door to an opportunity to obtain a large number of sturgeon muscle plug samples through a collaboration with the California Department of Fish and Wildlife's (CDFW) annual white sturgeon tagging program that is tracking population trends, and a US Fish and Wildlife Service (USFWS) study on fish movement patterns.

For this study, the CDFW and USFWS will assist the RMP in developing the non-lethal sturgeon tissue monitoring protocol. The CDFW annual sturgeon tagging effort currently offers the best opportunity to regularly access a large number of white sturgeon to measure for attainment of the TMDL. The USFWS collects fin ray and blood samples from sturgeon collected during this tagging effort, and will be available to assist the RMP in collection additional tissues in 2015.

Samples will be collected by USFWS staff on CDFW boats in San Pablo and Suisun Bays during the August-October 2015 field season. The target samples to be collected for the RMP study are muscle tissue plugs and blood. Muscle plugs will be sent to USGS for selenium analysis, and UC Davis for C, N, and S isotope analysis. Blood plasma samples will be sent to the Bozeman Fish Technology Center for testosterone and estradiol analysis, in order to determine sex of the fish. The results of this study will be included in a 2015 Selenium in Sturgeon Muscle Plug report that will be prepared by the RMP by June 2016.

The purpose of this Sampling and Analysis Plan is to clearly outline the sampling design, sample collection and target analyses to make it easier for project partners to coordinate.

Sampling Design

White sturgeon tissue samples will be collected by USFWS staff during the CDFW Annual Sturgeon tagging effort. Sturgeon will be collected aboard the *New Alosa* and *Striper II* in San Pablo and Suisun Bays between August and October 2015. The objective of the RMP study is to collect from 60 white sturgeon the following samples for analyses:

1. 2 muscle plugs - minimum target 120 mg wet weight per plug with skin
 - a. Selenium
 - b. C, N, and S isotopes (if sufficient tissue mass is available)
2. 2 mL whole blood samples - minimum target 1 mL blood plasma for analysis
 - a. Testosterone
 - b. Estradiol

Muscle plugs and blood plasma samples from 30 fish will be analyzed immediately, and samples from 30 fish will be archived. However, these target numbers may vary based on the number of fish caught and the number of blood samples shared between the USFWS and RMP. The number of samples to be analyzed and archived will be revised after the end of the field season.

Table 1. Target samples collected. On October 1 the catch relative to these targets will be evaluated. If it seems unlikely that the target of exactly 10 fish in each size range will be attained, the overall target number of 60 will be reached by obtaining more than 10 in the more abundant size ranges.

Target Size Range (cm, fork length)	Target # of Fish	Target Tissue Collected	
		Muscle Plugs (# of 5 mm plugs)	Whole Blood (mL)
101-110	10	2	2
111-120	10	2	2
121-130	10	2	2
131-140	10	2	2
141-150	10	2	2
151-160	10	2	2

Sampling Locations and Schedule

California Department of Fish and Wildlife boats will leave from **Antioch and Martinez**. One or two boats will sample each week between approximately the second week of August and the third week of October for no more than 4 days.

Table 2. Field Contacts

Name	Affiliation	Cell Phone #
Jennifer Sun	RMP	(949) 202-6671
Patrick Kim	RMP	(510) 685-0574
Laura Heironimus	USFWS	
Garrett Giannetta	USFWS	
Zac Jackson	USFWS	(209) 403-1457
CDFW Staff 1	CDFW	
CDFW Staff 2	CDFW	
Marty Gingras	CDFW	(831) 372-2581

Sample Collection Method

Field sheets

The Organism ID, station code, date and time of fish collection, USFWS blood sample ID, CDFW disc tag number, total length (cm), fork length (cm), and number of muscle plugs collected will be recorded on field data sheets in coordination with the USFWS (Attachment 1). The date of collection, boat name, and disc tag number will be used to cross-reference additional information about sampling location (latitude and longitude) and field sampling conditions recorded on CDFW field data sheets.

Each sturgeon from which samples are collected will be assigned a unique Organism ID 15MP-XX-## where:

15MP is the project ID (2015 Muscle Plug study)

XX is the Boat ID (NA for New Alosa, ST for Striper II)

is a unique number corresponding with the consecutive number of the sample collected on that boat (ie. 01, 02, 03....60)

Muscle plug and blood sample containers will be labeled with this unique Organism ID.

The USFWS will periodically send SFEI copies of the field sheets during the field season. The sampling design may be adjusted at the beginning of October to based on the samples collected by that time.

Muscle plugs

Two muscle plugs will be taken from each 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 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.

All plugs taken from the same fish can be stored in the same cryovial. Cryovials pre-labeled with the Organism ID will be provided by RMP staff. Cryovials will be stored and transported on wet ice in the field and frozen at -20 C in the lab until shipping.



Figure 1. Location of muscle plug collection

Blood plasma

At least 2 mL (typically 6-8 mL) of whole blood will be drawn from each fish behind the anal fin using a syringe and sealed vacutainer. Whole blood samples will be stored on wet ice and centrifuged at the end of the day. After centrifuging, at least 1 mL (typically 3-4 mL) of blood plasma will be drawn off and pipetted between 2 microcentrifuge tubes, which will be frozen until shipping. One sample tube will serve as a backup sample in case of shipping and handling error.

The USFWS will be collecting blood samples for the same sex steroid analyses as the RMP, but will be targeting a different size distribution of sturgeon. A single blood sample will be taken from fish that are targeted by both the USFWS and RMP, and a waterproof label on the sample vacutainer will be labeled with the USFWS Fish ID. Plasma samples from these fish will be labeled with the USFWS Fish ID and a replicate number. At the end of the field season, the USFWS Fish ID will be cross-referenced with the Organism ID using the field sheets. Pairs of these samples will be distributed between USFWS and RMP for shipping and analysis at the

Bozeman Fish Technology Center. Samples shipped by the RMP will be relabeled with the Organism ID.

Blood and blood plasma samples collected from fish for the RMP only will be labeled with the Organism ID and a replicate number.

Sample Handling & Storage

Both muscle plug and blood samples will be double-bagged in Ziploc freezer bags and stored on wet ice in the field. Both muscle plug and blood plasma samples will then be stored at -20 C until the end of the sampling season. When shipped, samples will be transported overnight in coolers on dry ice.

At the end of the field season, RMP staff will collect all muscle plug and blood plasma samples collected for the RMP only, as well as some blood plasma samples from which data will be shared between USFWS and the RMP. Samples will be transported in coolers on dry ice and stored at -20 C until samples are shipped for analysis and archiving. The number of muscle plug and blood plasma samples the RMP ships for analysis will be based on the number of blood plasma samples collected and analyzed by the USFWS.

Muscle plug samples should be stored at -80 C whenever possible, and should not be stored at -20 C for longer than 3 months. Blood plasma samples can be stored for 2 years at -20 C. Blood plasma samples not analyzed for sex steroids will be stored at -20 C at Schafer's Meats & Cold Storage in Oakland, CA. Muscle plug samples not analyzed for selenium or isotopes will be stored at -80 C at the USGS in Menlo Park, CA.

All samples will be accompanied by a chain of custody form (COC) provided by SFEI. The COC form will include the Organism ID, site name, collection date, sample type, analysis required, and other remarks. Shipping information is provided in Table 3.

Sample Analysis

Muscle plug samples will be shipped to the USGS in Menlo Park, CA for selenium analysis. USGS will process plug samples, including skin tissue removal and homogenization. USGS will prepare samples for isotope analysis, and send samples to UC Davis in Davis, CA for C, N, and S isotope analysis as tissue as sample mass is available. Blood plasma samples will be shipped to the Bozeman Fish Technology Center in Bozeman, MT for testosterone and estradiol analysis.

All samples must be maintained at -20C during transport to the respective laboratories. Any deviation should be noted and reported to RMP staff. 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.

A summary of the target sample analyses, required sample mass, and reporting parameters are listed in Table 4.

Table 3. Shipping Contacts

Name	Affiliation	Function	Phone	Email	Shipping Address
Jennifer Sun	SFEI	RMP Project Manager	510-746-7393	jennifers@sfei.org	San Francisco Estuary Institute 4111 Central Ave. Richmond, CA 94804
Robin Stewart	USGS	Muscle plug processing, Se analysis, isotope sample preparation	650-329-4550	arstewar@sfei.org	U.S. Geological Survey Water Resources Division 345 Middlefield Rd. MS496 Menlo Park, CA 94025
Emily Schick	UC Davis	C, N, S isotope analyses	530-752-8100	sif@ucdavis.edu	UC Davis Stable Isotope Facility Dept of Plant Sciences – Rm 1210 PES One Shields Ave. Davis, CA 95616 USA
Molly Webb	Bozeman Fish Technology Center	Sex steroid analyses	406-994-9907	molly_webb@fws.gov	Bozeman Fish Technology Center 4050 Bridger Canyon Road Bozeman, MT 59715
Paul Salop	AMS	Coordinate RMP short term storage archives	925-373-7142	salop@amarine.com	Applied Marine Sciences 4749 Bennett Dr., Ste. L Livermore, CA 94551

Table 4. Reportable Parameters

Tissue	Minimum Total Sample Required	Number of Samples ¹				
		Se	C & N isotopes	S isotope	Testosterone	Estradiol
Muscle tissue plug	25 mg dw	30	30	30		
Blood plasma	1 mL				30	30
Laboratory & Reporting Parameters						
Analytical Lab	--	USGS	UC Davis	UC Davis	Bozeman Fish Technology Center	Bozeman Fish Technology Center
Minimum Sample Mass Required	--	16 mg dw	1.5 mg dw (3 mg dw with dups)	3 mg dw (6 mg dw with dups)	0.5 mL	0.5 mL
Reporting Units	--	ug/g dw	delta units	delta units	ng/mL	ng/mL
Analysis Method	--	HG-ID-IC P-MS	EA-IRMS	EA-IRMS		
Sample Container	--	2 mL cryovial			2 mL microfuge tube	2 mL microfuge tube

1 - The number of samples will vary based on the number of fish caught and the number of blood samples shared between USFWS and the RMP. The number of samples requested will be revised after the end of the field season.

