



# Selenium in Sturgeon Muscle Plugs: 2014

By:

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## **Executive Summary**

The San Francisco Bay Regional Water Quality Control Board recently approved a selenium TMDL for North San Francisco Bay, which established a target selenium concentration of 11.3 ug/g dry weight in white sturgeon muscle tissue. This study was the first year of a planned three-year effort to develop a non-lethal tissue sampling technique using muscle plugs to assess attainment of the TMDL target. Plug sampling will allow for the collection of larger numbers of white sturgeon tissue samples than would lethal sampling, while minimizing impact to the sturgeon population. In September and October 2014, RMP staff collected muscle plug samples from nine live white sturgeon in Suisun Bay in coordination with the California Department of Fish and Wildlife's sturgeon tagging study. Selenium concentrations measured in the muscle plugs were within the range of concentrations previously measured in muscle plugs and fillets collected from white sturgeon in San Francisco Bay. Isotope analyses showed that most of the fish had been feeding primarily on invertebrates in the North Bay.

This pilot study demonstrated that muscle plug sampling is a viable non-lethal technique for collecting muscle tissue for selenium analysis. The second year of this study was completed in 2015, and a third year of sampling is planned for 2017. Based on this first year of sampling in 2014, refinements to the field collection and laboratory analysis techniques were made during the second year of this study in 2015 to further develop the muscle plug monitoring protocol. Further evaluation and analysis will be conducted after the 2015 and 2017 muscle plug sampling events.

## Introduction

Since 1998, San Francisco Bay has been identified as impaired by selenium under the Clean Water Act. Selenium has been measured at levels of potential concern in fish and other wildlife, including in white sturgeon (*Acipenser transmontanus*). While selenium is an essential element, it has a tendency to bioaccumulate and can become toxic at concentrations just an order of magnitude greater than those required for biological function (Baginska 2015).

Selenium contamination in the Bay-Delta originates from two major sources: agricultural drainage from the Central Valley and effluent from oil refineries (Presser and Luoma 2006). Efforts have been made to reduce loads from refinery effluent and agricultural runoff. However, although ambient selenium concentrations in water are well below the National Toxics Rule chronic freshwater criterion for selenium of 5 ug/L, concentrations measured in a small proportion of sturgeon muscle samples have exceeded a target of 11.3 ug/g dry weight (dw) in muscle established by the TMDL for the North Bay (Baginska 2015). Studies have shown that the primary pathway of concern for selenium toxicity in wildlife is bioaccumulation rather than direct exposure in the water column, and especially in systems with mollusk-based food webs (USEPA 2015). The San Francisco Estuary is particularly vulnerable to selenium impacts on wildlife because a dominant benthic organism, the invasive overbite clam (*Corbula amurensis*), has been shown to be up to 10 times slower at releasing accumulated selenium compared to other prey (Stewart et al. 2004).

In an effort to assess and reduce potential impairment due to selenium, the San Francisco Bay Regional Water Quality Control Board began the development of a selenium TMDL for North San Francisco Bay in 2007. The TMDL established a target concentration of 11.3 ug/g dw in white sturgeon muscle tissue as the basis for evaluating impairment (Baginska 2015). White sturgeon is a bottom-feeding species that is considered to be at particular risk for selenium exposure in the Bay because its diet consists primarily of the selenium-rich overbite clam (Beckon and Maurer 2008; Stewart et al. 2004). Although white sturgeon can be found from South San Francisco Bay to the upper reaches of the Sacramento and San Joaquin River systems, where they are known to spawn, the San Francisco Bay white sturgeon population predominantly feeds in North San Francisco Bay, which hosts a large population of overbite clam. In addition to their benthic feeding habits, other high-risk factors for white sturgeon include their longevity (lifespan of over 100 years) and long egg maturation times (several years) (Beckon and Maurer 2008).

White sturgeon were routinely sampled every three years by RMP sport fish monitoring from 1997-2009. However, the number of fish collected in each round of sampling has been small (12 fish per sampling event), and future sampling events are now scheduled on a five-year cycle. Identifying a means to obtain a larger number of white sturgeon muscle samples on a more frequent basis has been identified as a high priority by the RMP's Selenium Workgroup, in order to assess attainment of the North Bay selenium TMDL and other regulatory thresholds.

Muscle plug sampling provides a non-lethal method for monitoring contaminants in sport fish that has been successfully used to monitor mercury and selenium concentrations fish, including threatened fish species (Baker et al. 2004; Waddell & May 1995). During the 2009 RMP sport

fish sampling event, an effort began to establish a non-lethal and efficient method of collecting sturgeon muscle tissue using plugs. Another round of evaluation of selenium concentration correlation was completed with plug and fillet samples from the 12 sturgeon collected during the 2014 RMP sport fish sampling event.

The RMP Selenium Workgroup proposed a special study to use muscle plug sampling in coordination with California Department of Fish and Wildlife (CDFW) monitoring as a low-cost monitoring element that could provide high-value information in support of policy development and decision-making. The study was funded by the RMP and implemented during the fall of 2014. The ultimate objective of this monitoring element is to obtain a relatively large number of sturgeon muscle samples to develop a monitoring method that can be used to assess attainment of the North Bay selenium TMDL and other regulatory thresholds. These data can also be used to establish a more precise understanding of impairment, and to begin to track inter-annual trends. This study addresses key questions identified by the Selenium Strategy and the RMP (Table 1).

**Table 1.** Key Selenium Strategy and RMP management questions addressed by this study

<b>RMP Management Questions addressed</b>
<ol style="list-style-type: none"> <li>1. Are chemical concentrations in the Estuary at levels of potential concern and are associated impacts likely?               <ol style="list-style-type: none"> <li>b. What potential for impacts on humans and aquatic life exists due to contaminants in the Estuary ecosystem?</li> </ol> </li> </ol>
<ol style="list-style-type: none"> <li>4. Have the concentrations, masses, and associated impacts of contaminants in the Estuary increased or decreased?               <ol style="list-style-type: none"> <li>b. What are the effects of management actions on the potential for adverse impacts on humans and aquatic life due to Bay contamination?</li> </ol> </li> </ol>
<b>Selenium Strategy questions addressed</b>
<ol style="list-style-type: none"> <li>2. Are the beneficial uses of San Francisco Bay impaired by selenium?</li> </ol>
<ol style="list-style-type: none"> <li>4. How do selenium concentrations and loadings change over time?</li> </ol>

This study completed the first year of a three-year effort to develop the muscle plug sampling method for measuring Se in white sturgeon. The study was repeated in the fall of 2015, and is planned to continue for a third year in 2017. Several modifications to the field collection and laboratory analysis technique were made in 2015 based on lessons learned during this initial study. This document is a preliminary report on the first year of sampling. Further evaluation and analysis will be conducted after the 2015 and 2017 muscle plug sampling events.

## Methods

### Field collection

CDFW conducts annual white sturgeon tagging to track population trends. From August to October, white sturgeon in San Pablo Bay and Suisun Bay are caught using nets set from research vessels and tagged with plastic disk-tags. CDFW then monitors the ratio of tagged to untagged fish in subsequent surveys, and collects information on where tags are recovered by anglers to estimate sturgeon abundance and survival rate (DuBois and Harris 2013; more information at <http://www.dfg.ca.gov/delta/data/sturgeon/bibliography.asp>).

RMP staff collaborated with CDFW by taking muscle plug samples from a subset of the sturgeon collected during the tagging surveys. Muscle plug samples were taken using a 5-mm biopsy punch. For each fish, two muscle plugs were collected from the region laterally adjacent to the dorsal fin. The area was rinsed with DI water prior to sample collection. Fish fork length, collection time, and collection location were recorded for each fish sampled. Plugs were stored in labeled vials and kept on ice in the field, then transferred to a -20 C freezer until they could be shipped to the USGS analytical lab. Samples were shipped on dry ice.

RMP staff planned for 1-2 days of field work to train CDFW staff to perform sampling independently. The plan was for CDFW to then collect samples from up to 60 fish (30 samples to analyze this year and 30 to archive). However, due to initial difficulties with the sampling technique and logistical difficulties of freezing and storing the samples, it was not feasible for CDFW to sample independently in 2014. CDFW staff typically do not return to their office between sampling days, crews change daily, and staff rotate between boats on different days, which complicated the storing of samples and restocking of ice and other field supplies. Therefore, RMP staff collected all of the muscle plug samples in 2014.

RMP staff went out on CDFW tagging vessels in Suisun Bay for 3 days in September and October (September 15, September 24, and October 22) and took samples from a total of 9 fish. On the first day, one fish was captured; however, attempts to take muscle plug samples from that fish were unsuccessful. Subsequent sampling efforts were made more successful by applying a firm twisting motion when inserting the biopsy punch, using a scooping motion when removing the biopsy punch, and using fine-tipped forceps to help remove the tissue. Five samples were collected (out of six fish on which sampling was attempted) on the second day of sampling and four samples on the third day (out of four attempts). At the end of each day samples were transported to a -20 C freezer at SFEI for storage prior to transport to the analytical lab.

### Laboratory analysis

The USGS analyzed muscle plug samples (skin off) for selenium using an HG-ID-ICP-MS method. C, N, and S stable isotope analyses were conducted using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (EA-IRMS). Isotope analyses had been planned for all samples, but were run on only a subset of samples with enough sample mass

for these analyses. Duplicate samples could not be run for verification due to insufficient sample mass. Samples were analyzed with the skin removed.

### Quality Assurance and Data Management

Samples collected in October 2014 were improperly kept refrigerated for two weeks before being frozen. The range of selenium concentrations in these samples (3.2-13 ug/g dw) was consistent with the range of concentrations measured in samples that were frozen right away (1.8-25 ug/g dw). Therefore, data from these samples were still considered useable but have been flagged with a footnote in Table 2.

Selenium and moisture were analyzed in 2 lab batches (samples collected in September and October). Four method blanks (two per batch) and 10 certified reference materials (CRMs; 3 run in replicate in one batch and 2 run in replicate in the other batch) were also analyzed. All data were reported blank corrected. Accuracy was evaluated using the CRMs with certified values for selenium, and precision was evaluated using other samples.

C, N, and S isotopes for a subset of the samples were analyzed in one lab batch, together with a subset of the 8 samples collected for the 2015 Sturgeon Derby Special Study. One lab replicate was run for C and N isotopes on a Derby sample (C and N isotopes were run concurrently), but not enough sample mass was available to run a lab replicate for S isotopes. QC analyses included four certified reference materials for C and N isotopes, 7 laboratory control materials (LCMs) for C and N percent masses, over 30 LCM results for C and N isotopes, and 24 LCMs for S isotopes. No method blanks were analyzed.

## **Results and Discussion**

### Field Sample Collection

Muscle plug samples were successfully collected from nine white sturgeon and analyzed for selenium. This study demonstrated the viability of muscle plug sampling as a means of non-lethally monitoring selenium concentrations in sturgeon. No immediate indications of increased mortality or significant harm to the sampled fish were detected. While mortality or sublethal effects were not evaluated as part of this study, several previous studies have demonstrated low mortality and sublethal impacts to fish as a result of handling and muscle plug collection in both laboratory and field settings (based on recapture ratios and qualitative evaluation of recaptured fish) (Baker et al. 2004; Waddell and May 1995; Hamilton et al. 2002).

Although logistical constraints substantially limited the sample size, the samples that were obtained were of sufficient mass to analyze for selenium while meeting method detection limit objectives and yielding quantitative detection in all samples. After the sampling technique was improved in October, the method yielded sufficient mass to analyze for both selenium and isotopes.



### Quality Assurance Results

Selenium was detected in all composite samples. Recoveries and relative standard deviations were within target method quality objectives. Selenium was reported in the two method blanks for one of the batches at an average level that was 0.18% of the average selenium concentration measured in the composite samples. In that particular batch, the standard deviation of the method blank results was less than the average of the method blank MDLs, so no qualifiers were needed.

No isotope values were reported non-detect, but the sulfur isotope result reported for sample ST-0924-01-MP was below the limit of quantitation. This sample was analyzed with less than the required sample mass, and the result is considered dubious by the analytical laboratory. The standard deviation among replicates was less than 1‰ for all isotopes, while the relative standard deviations for carbon, nitrogen, and sulfur mass in replicates were < 25%.

### Selenium Results

Selenium concentrations measured in this study ranged from 1.8 – 25 ug/g dw (Table 2). The sample group had a median concentration of 4.4 ug/g dw and a mean concentration of 7.3 ug/g dw, with a standard error of 2.4 ug/g dw. The concentrations measured in this study were generally within the range of concentrations measured previously in sturgeon muscle plugs collected by the RMP Status and Trends program in 2009 and 2014 (2009: median = 4.9 ug/g dw, mean = 5.8 +/- 1.1 ug/g dw; 2014: median = 5.8 ug/g dw, mean = 6.0 +/- 1.4 ug/g dw; combined 2009 and 2014: median = 5.4 ug/g dw mean = 5.9 +/- 0.7 ug/g dw; range = 1.5 – 16.5 ug/g dw; Figure 1). Unlike this study, Status and Trends monitoring included sturgeon collected throughout all sections of the Bay from late April to early August. Although the mean selenium concentration measured in this study was higher than the previously measured averages, the median concentration was lower than those previously measured. The high average concentration in this study was largely driven by one particularly high selenium measurement (25 ug/g dw).

The muscle plug selenium concentrations measured in this study were also within the range that has been measured in muscle fillets by the RMP (median = 5.4 ug/gdw; mean = 6.4 +/- 0.4 ug/g dw, range = 1.5 – 18.8 ug/g dw, 1997-2014) and in other studies of selenium in sturgeon muscle in the Estuary: 7-15 ug/g dw in sturgeon sampled between 1986 and 1990 (DFG 1991), and 7.1+/-0.52 in 2003-2005 (median = 5.8 ug/g dw; Linares-Casenave et al 2015).

Substantial variability in sturgeon tissue selenium concentrations has been observed in the past, and could be attributed to a number of factors. Recent work by Linares-Casenave et al. (2015) found significantly higher concentrations of gonad and muscle selenium in vitellogenic females compared to pre-vitellogenic females (for muscle: 10.2 +/- 1.9 ug/g compared to 5.5 +/-0.64 ug/g). This pattern may explain the elevated selenium concentrations measured in one fish in this study (25 ug/g dw). Although typically selenium has not been shown to increase with age, Linares-Casenave et al. (2015) found that selenium concentrations were higher in older fish of both sexes (> 8 year old fish), and speculated that this could be due to differences in foraging with age.

### Isotope Results

C, N, and S isotopes values were measured in a subset of the muscle plug samples with enough sample mass for analysis (Table 2), and may provide information about habitat use and foraging. The measured isotope values generally fell within the range of values previously observed in white sturgeon collected in the North Bay (Stewart et al. 2004).  $\delta^{15}\text{N}$  values ranged from 14.8 to 18.5‰, suggesting mixed foraging patterns. Fish with lower  $\delta^{15}\text{N}$  values are likely foraging more heavily on invertebrates, while fish with higher  $\delta^{15}\text{N}$  are likely piscivorous.  $\delta^{13}\text{C}$  values ranged from -21.3 to -25.7‰, which is consistent with expected values for sturgeon caught in Suisun Bay.  $\delta^{34}\text{S}$  values ranged from 13.0 to 16.2‰, which similarly suggest that foraging is primarily occurring in the Bay, as indicated by  $\delta^{34}\text{S}$  values above 12‰. Further analysis of the isotope data for these samples will be presented in the 2015 RMP Sturgeon Derby Special Study report, together with isotope data for muscle plugs collected during the 2015 Annual Original Sturgeon Derby (Sun et al. 2016).

### Future Studies

Sturgeon age and sex data were not collected during this study. Sturgeon sex cannot be differentiated using external morphology, and no reliable, non-invasive techniques for identifying sex are commonly used. However, fish sex can be determined post-hoc from blood plasma hormone levels. Non-invasive techniques for identifying sturgeon sex and maturity are being investigated for future sampling events.

USFWS collected fin ray and blood plasma samples from several of these fish within defined size ranges, and will be estimating fish age from the fin ray samples. However, fin rays cannot be collected from fish within the legal slot limit (101.6-152.4 cm), and the size range of fish sampled for the USFWS did not consistently overlap with the fish sampled for muscle plugs. In future studies, additional coordination with USFWS may allow for an increased number of samples for which both muscle plug and age data are available.

## **Conclusion**

This sampling event represents the first effort by the RMP to collect muscle plug samples from live sturgeon. The small mass of several of the initial samples collected in September 2014 made skin removal and homogenization during sample processing somewhat challenging, and could have affected the quality of the results. However, several modifications to both the field and laboratory techniques were made during the 2014 and 2015 field seasons to optimize for the collection of samples from live fish and analysis using samples with small mass.

Data from related studies conducted in recent years will also help support a more robust interpretation of these results. Results from the 2014 RMP sport fish sampling event will provide additional data on the correlation between selenium concentrations in muscle plugs and fillets, improving our ability to interpret muscle plug data and analyze these results within the larger context of historically collected sturgeon selenium data. Results from the 2015 RMP Sturgeon

Derby study will also provide information about the correlation between muscle plug and ovary selenium concentrations, which the US Environmental Protection Agency has identified as the preferred endpoint most directly tied to adverse effects (USEPA 2015). Laboratory analysis of samples collected during the 2015 Sturgeon Derby study has been completed, and will be published in the 2015 RMP Sturgeon Derby Special Study report (Sun et al. 2016). A second round of Sturgeon Derby sampling is scheduled to occur in 2016.

Overall, sampling in 2014 demonstrated that the muscle plug technique is viable. This technique currently continues to present the best opportunity to non-lethally measure selenium concentrations in a large number of sturgeon, and continued improvements in the sample collection and processing techniques will increase the consistency and reliability of results in future field seasons. Another round of sturgeon plug monitoring in 2015 helped to refine the logistics and methods. Results from the 2015 effort will expand the muscle plug dataset to build towards a more complete picture of status and trends. A third round of plug sampling is also planned for 2017.

**Table 2.** Selenium and isotope results and fish lengths for the 9 muscle plug samples collected

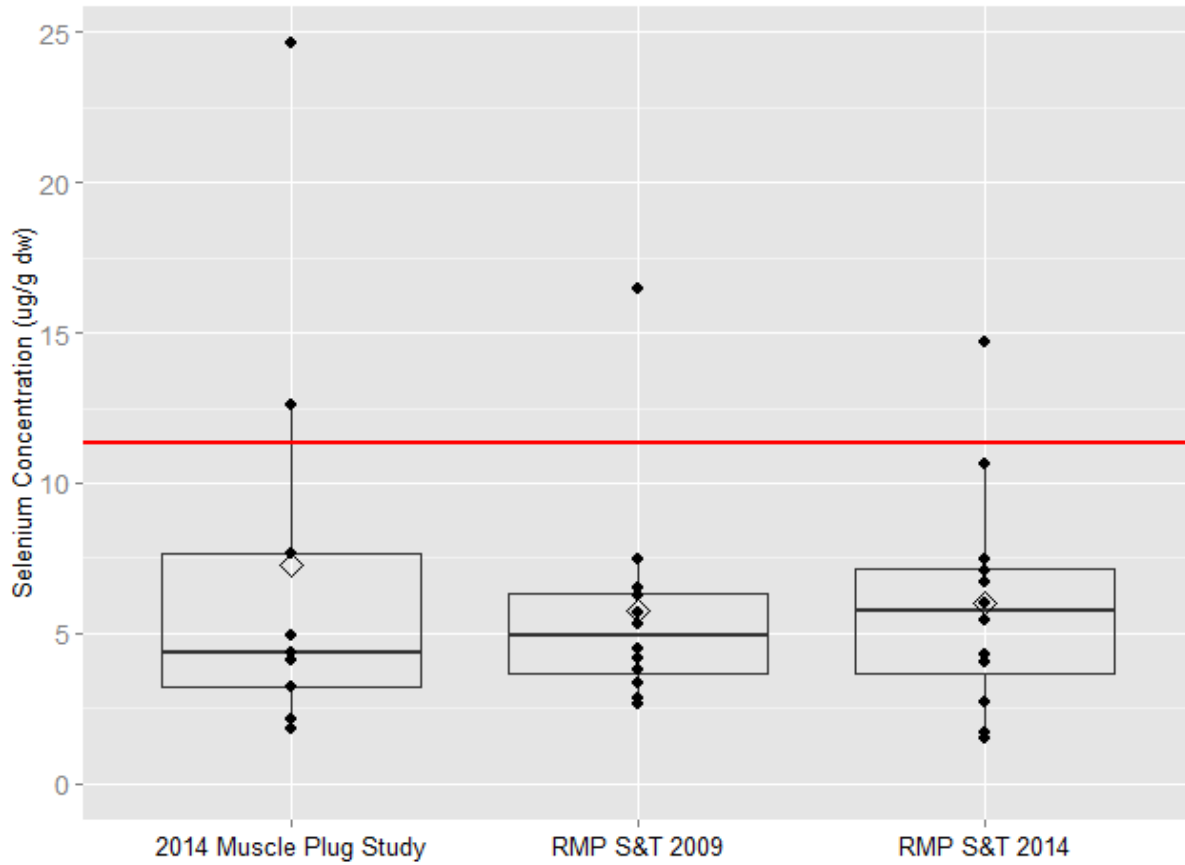
Sample Date	Sample ID	Se (ug/g dw)	% Moisture	Fork length (cm)	Sample mass (mg dw)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{34}\text{S}$ (‰)
9/24/14	ST-0924-01-MP <sup>3</sup>	1.8	97	112	12.7	-21.8	14.8	
9/24/14	ST-0924-02-MP	4.4 <sup>1</sup>	98	91	8.3			
9/24/14	ST-0924-03-MP	2.2	98	140	9.1			
9/24/14	ST-0924-04-MP	25	89	94	23.5	-25.7	15.2	13.0
9/24/14	ST-0924-05-MP	4.9	93	83	6.9	-25.0	17.0	
10/22/14	ST-1022-01-MP	7.6 <sup>2</sup>	82	80	13.6	-21.3	18.1	16.2
10/22/14	ST-1022-02-MP	3.2 <sup>2</sup>	79	76	11.9	-21.9	18.5	
10/22/14	ST-1022-03-MP	13 <sup>2</sup>	78	72	12.8	-24.0	16.1	16.2
10/22/14	ST-1022-04-MP	4.1 <sup>2</sup>	49	98	106.3	-24.2	16.8	14.6

1-Fish skin could not be fully removed in this sample.

2-Tissue samples collected on 10/22/14 were stored in a refrigerator (instead of being frozen as they should have been) for two weeks before being transported to the analytical lab. The range of selenium concentrations in these samples (3.2-13 ug/g dw) was consistent with the range of concentrations in samples that were frozen right away (1.8-25 ug/g dw), so these data are still considered useable.

3- The analytical lab reported a sulfur isotope value of 9.5, below the limit of quantitation. This sample was analyzed with less than the required sample mass and the result is considered dubious.

**Figure 1.** Selenium concentrations in muscle plugs collected during the 2014 Muscle Plug Special Study, in comparison with muscle plugs collected during RMP Status and Trends monitoring in 2009 and 2014. The red line is the North Bay TMDL tissue target for white sturgeon (11.3 ug/g dw). The bottom, middle, and top lines of each box are the 25<sup>th</sup>, 50<sup>th</sup>, and 75<sup>th</sup> percentiles of the data (i.e., the middle line represents the median). Whiskers extend from the top and bottom of the boxes to the highest and lowest values that are within 1.5 times the interquartile range (distance between the 25<sup>th</sup> and 75<sup>th</sup> percentiles) of the upper or lower hinge (25<sup>th</sup> or 75<sup>th</sup> percentile). The black dots are measured values. The open diamonds show the sample means.



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