RMP
REGIONAL MONITORING
PROGRAM FOR WATER QUALITY
IN SAN FRANCISCO BAY

sfei.org/rmp

Selenium in White Sturgeon from North San Francisco Bay: The 2015-2017 Sturgeon Derby Study

Prepared by:

Jennifer Sun and Jay A. Davis
San Francisco Estuary Institute

Robin Stewart
United States Geological Survey

Vince Palace
International Institute for Sustainable Development

San Francisco Estuary Institute • 4911 Central Ave., Richmond, CA • www.sfei.org

Selenium in White Sturgeon from North San Francisco Bay: The 2015-2017 Sturgeon Derby Study

Jennifer Sun and Jay A. Davis
San Francisco Estuary Institute

Robin Stewart
United States Geological Survey, Menlo Park, CA

Vince Palace
International Institute for Sustainable Development – Experimental Lakes Area,
Winnipeg, Manitoba

Table of Contents

APPENDIX A – Individual Results

APPENDIX B – QA/QC summary (detailed results of % precision, % recovery, batch details, etc.)

APPENDIX C – 2017 Sampling and Analysis Plan

Executive Summary

This report presents the findings from a study evaluating selenium concentrations in white sturgeon (*Acipenser transmontanus*) tissues collected during the 2015-2017 Sturgeon Derby events in North San Francisco Bay. The goal of this study was to investigate the distribution of selenium among sturgeon tissues to inform the toxicological and regulatory interpretation of selenium measured in non-lethally collected tissues, including muscle plugs and fin rays. This technical report provides documentation of the study and presents its major findings.

The Original Sturgeon Derby is an annual fishing derby held at McAvoy Harbor in Bay Point, CA on Super Bowl weekend in late January or early February. This event presents a unique opportunity to collect and analyze selenium in a variety of tissue samples from angler-harvested white sturgeon. Tissue samples were collected from 9 females in 2015, 8 females in 2016, and 4 females and 9 males in 2017. The primary tissues collected and analyzed for selenium included muscle (plugs and fillets [2016-2017 only]), liver, ovary, fin rays, and otoliths. This study addressed the following three main objectives:

1. evaluate plugs as proxies for fillets to monitor white sturgeon muscle tissue selenium concentrations;
2. evaluate relationships between selenium concentrations in non-lethally collected tissues (muscle plugs, fin rays) and those of greater toxicological relevance (ovary, liver); and
3. evaluate the use of microchemical analyses of fin rays to evaluate long-term selenium trends.

Objective #1 – Selenium concentrations in muscle plugs and fillets were found to be strongly correlated, indicating that plugs can be used as reliable proxies for fillets to non-lethally monitor selenium in sturgeon. However, differences in the slope of the plug-fillet regression between the current study and previous studies indicate that further analysis is needed to interpret plug selenium results. Future analyses should specifically link muscle plugs sampled using the method established for the Muscle Plug Study and muscle fillets sampled using the method used for Status and Trends monitoring.

Objective #2 – Selenium concentrations in muscle plugs were positively related to concentrations in ovary and liver, both in the current study and within a larger dataset (including data from the Selenium Verification Study [SVS: CSWRCB 1987, 1988, 1989, 1991], RMP Status and Trends, Linares-Casenave et al. [2015a,b], and Stewart et al. [2004]). However, low to moderate R^2 values indicate that selenium concentrations in muscle tissue are not precise predictors of ovary or liver selenium concentrations. Still, statistically significant relationships indicate that elevated selenium concentrations in muscle tissue samples generally would suggest elevated selenium in ovary or liver as well. Fin ray data will be published in a separate report (Palace et al. in preparation).

Objective #3 – Fin ray microchemistry data will be published in a separate report (Palace et al. in preparation).

Data collected during this study also provide information about the current status and recent trends in selenium concentrations in white sturgeon in North Bay, where most fish were caught. Measured muscle, liver, and ovary concentrations fell within the ranges of previous

observations, but with a high proportion of values toward the upper end of the historic distribution. Selenium concentrations in sturgeon muscle plugs exceeded the North Bay TMDL target (11.3 µg/g dw) in 38%, 44%, and 54% of samples in 2015, 2016, and 2017, respectively. Mean concentrations were near the TMDL target (11, 11, and 12 µg/g dw in 2015, 2016, and 2017, respectively), but only exceeded it in 2017. Ovary selenium, the most direct indicator of potential reproductive impairment, exceeded the USEPA freshwater selenium criterion in 75%, 22%, and 50% of samples each year from 2015 to 2017. Liver selenium exceeded a published toxicity threshold (15 µg/g dw) even more frequently, in 88%, 54% and 56% of samples each year from 2015 to 2017.

Several factors may have contributed to the elevated selenium concentrations observed. Sturgeon sampled in this study were collected during the winter pre-spawning season immediately following critically dry years, periods when selenium concentrations are elevated in clams (Stewart et al. 2013, Stewart et al. in preparation). Additionally, most samples were collected in Suisun Bay, where selenium concentrations have been elevated relative to other regions of the Bay-Delta (Sun et al. 2017a). Further analysis and discussion of these and other factors affecting sturgeon selenium concentrations is presented in the 2015-2017 Muscle Plug report (Sun et al. 2019).

Overall, these results support the use of muscle plugs in long-term monitoring of selenium in sturgeon. Muscle plug selenium can be considered a good proxy for muscle fillet selenium when making comparisons against historical measurements and regulatory thresholds. Muscle plug selenium can also be used as a general indicator of potential reproductive toxicity risks. While muscle plug selenium should not be considered a strong predictor of liver or ovary selenium concentrations, elevated muscle plug selenium are generally indicative of increased liver and ovary concentrations, and accordingly increased risk of reproductive impairment. In this study, elevated selenium concentrations in muscle, ovary, and liver were found, compared to previously measured values in the Bay as well as regulatory and non-regulatory thresholds. Further discussion of the drivers and implication of these elevated concentrations is presented in the 2015-2017 Muscle Plug report (Sun et al. 2019).

Introduction

Selenium is an essential micronutrient that can bioaccumulate and become toxic at concentrations just an order of magnitude greater than those required for biological function (SFBRWQCB 2015). Since 1998, San Francisco Bay has been identified as impaired by selenium under the Clean Water Act, with levels of potential concern in diving ducks and fish, including white sturgeon (*Acipenser transmontanus*). The primary source of selenium loading into North Bay is inflow from Central Valley watersheds through the Delta, including agricultural return flows from regions in which selenium is naturally occurring in soils. Petroleum refineries and runoff from local tributaries contribute additional inputs of selenium; minor sources include other industrial and municipal dischargers and atmospheric deposition (SFBRWQCB 2015). Despite significant selenium load reductions from both Central Valley flows and petroleum refineries since the 1990s, selenium concentrations in wildlife have continued to occasionally exceed toxicity thresholds or regulatory guidelines (Presser and Luoma 2013, SFBRWQCB 2015).

To address selenium impairment, the San Francisco Bay Regional Water Quality Control Board initiated development of a selenium Total Maximum Daily Load (TMDL) for North San Francisco Bay in 2007. The TMDL that was formally approved in 2016 established numerical fish tissue targets for muscle and whole body samples (11.3 and 8.0 µg/g dw, respectively), which were subsequently adopted as numeric targets for the North Bay in the Basin Plan (SFBRWCB 2015; CSWRCB 2016). The North Bay TMDL and the numeric targets established within it apply to the region extending from Suisun Bay to the Bay Bridge in Central Bay. In June 2016, the U.S. Environmental Protection Agency (USEPA) also released draft revised Clean Water Act criteria for fish tissue in the entire San Francisco Bay-Delta (USEPA 2016a). The criteria proposed for muscle and whole body fish tissue (11.3 and 8.5 µg/g dw) for the protection of wildlife were similar to the targets in the North Bay TMDL. These criteria were proposed as instantaneous measurements not to be exceeded. In contrast, the draft USEPA monitoring implementation guidance for USEPA's freshwater selenium criteria recommends using a t-test to statistically compare the mean of all fish tissue data for a single species to fish criteria (USEPA 2016b). The draft implementation guidelines also recommend that states each determine the statistical tests most suited to their systems. In this report, both mean concentrations and the percent of individual samples exceeding the TMDL targets are reported.

White sturgeon was identified in the North Bay TMDL as the key indicator species to be monitored to measure attainment of the TMDL muscle tissue target. White sturgeon is a bottom-feeding species that is considered particularly vulnerable to selenium exposure in the Bay because its diet consists primarily of the selenium-rich overbite clam (*Potamocorbula amurensis*) (Stewart et al. 2004; Beckon and Maurer 2008; Zeug et al. 2014). Studies suggest that this invasive clam species is up to 10 times slower at releasing accumulated selenium compared to other sturgeon prey species (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 spawn, the San Francisco Bay white sturgeon population predominantly resides and feeds in North San Francisco Bay, which hosts a large population of overbite clams. Attainment of the TMDL target in white sturgeon is expected to be protective of other species in the Bay as well, including green sturgeon (*Acipenser medirostris*), which are currently listed as a threatened species.

In 2009, the Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) began developing a non-lethal sturgeon muscle tissue monitoring method using muscle plugs to facilitate the collection of a large number of samples to assess attainment of the regulatory thresholds while minimizing impacts to the white sturgeon population. Previously, selenium was measured in white sturgeon muscle fillets every three years between 1997 and 2009 and every five years after 2009 as part of the RMP Status and Trends sport fish monitoring element. Only 12 individual sturgeon were sampled during each monitoring event, with an even smaller subset collected in North Bay. In 2009 and 2014, paired muscle plug and muscle fillet samples were collected as part of this monitoring effort, to begin assessing whether plugs could be used as proxies for fillets in selenium monitoring.

Following the establishment of the RMP Selenium Workgroup in 2014, two additional special studies were conducted to further develop muscle plug and other non-lethal sturgeon sampling methods. The first, the Muscle Plug Study, was piloted in the fall of 2014 and conducted annually thereafter through 2017, tested the muscle plug monitoring method on live white sturgeon. Results from the pilot monitoring effort in 2014 have been published (Sun et al. 2016), and a synthesis of results from 2014-2017 was published in 2019 (Sun et al. 2019). Long-term muscle plug monitoring is planned to continue, following a similar study design.

The second study was the Sturgeon Derby Study, a three-year effort initiated in 2015. The goal of this study was to investigate the distribution of selenium among sturgeon tissues to inform the toxicological and regulatory interpretation of selenium measured in non-lethally collected tissues, including muscle plugs as well as fin rays. This three-year RMP study was implemented by the San Francisco Estuary Institute (SFEI) in collaboration with the US Fish and Wildlife Service (USFWS), the US Geological Survey (USGS), and Vince Palace at the International Institute of Sustainable Development (IISD; formerly at Stantec, Inc.) (Table 1). This report presents results and conclusions from the Sturgeon Derby Study.

The Study addressed the following three main objectives:

1. evaluate plugs as proxies for fillets used to measure white sturgeon muscle tissue selenium concentrations;
2. evaluate relationships between selenium concentrations in non-lethally collected tissues (muscle plugs, fin rays) and those of greater toxicological relevance (ovary, liver); and
3. evaluate the use of microchemistry analyses of fin rays to evaluate long-term selenium trends.

Objective #1. Evaluation of non-lethal muscle plug monitoring began during the RMP 2009 and 2014 Status and Trends sport fish sampling events, during which 12 paired muscle plug and fillet samples were collected each year at locations throughout the Bay. These data showed a positive correlation between selenium concentrations measured in muscle plugs and muscle fillets (linear regression, $R^2=0.88$), but with plug selenium concentrations consistently lower than fillet concentrations (Sun et al. 2017a). However, during these studies, muscle plugs were collected using different methods than those used in the field on live sturgeon (see "Plug-Fillet Regression" section in the Results and Discussion). Additional muscle plug and fillet samples collected in the Derby Study contributed more samples to this analysis, and included muscle plugs collected using the live sturgeon sampling method (i.e., 5-mm diameter plugs collected from the epaxial muscle through the skin, near or slightly in front of the dorsal fin).

Objective #2. The second objective of the Sturgeon Derby Study was to examine correlations between tissues that can be monitored non-lethally (muscle plugs and fin rays) and tissues that are more directly tied to adverse reproductive effects (ovaries and liver). The primary pathway of selenium exposure in white sturgeon is uptake as dietary seleno-methionine and maternal transfer to the eggs, where elevated selenium concentrations can cause developmental deformities or mortality. Seleno-methionine is first incorporated into the egg yolk precursor protein vitellogenin in the liver, which is subsequently transformed into egg yolk proteins in the ovaries (SFBRWQCB 2015, Linville 2006). Correlations between selenium concentrations measured in sturgeon muscle tissue and those measured in liver or ovary tissues would indicate that muscle tissue selenium can provide an indirect indication of reproductive impairment risk. In other species, correlations have been established between muscle plug and ovary selenium concentrations (Osmundson and Skorupa 2011).

Objective #3. Fin ray microchemical analysis is a second potential method for non-lethal sturgeon monitoring for selenium. Fin rays can be taken as a non-lethal clip, are easy to collect by non-specialists, and their removal has been shown to not be harmful to sturgeon (Collins and Smith 1996). Fin ray clips were collected during the Muscle Plug Study in 2014 and 2015. Microchemical analyses in otoliths, which are calcareous bodies located in the inner ear of vertebrates, have previously been used as good predictors of muscle fillet selenium in several other species. Otoliths exhibit annual growth rings that are often used to estimate the age of fish, and are known to be chemically stable. Selenium and other elements can then be measured in each annual ring and assembled into a time series of selenium concentrations (Reash et al. 2014). These data can potentially be used to better understand the temporal relationships between water, prey, and sturgeon tissue concentrations. Dr. Vince Palace and Dr. Norman Halden at the University of Manitoba have developed a method for microchemical analysis of selenium and other trace elements in sturgeon fin rays as a non-lethal alternative to microchemical analysis in otoliths. Because fin rays have a regular annual growth pattern like otoliths, concentrations of selenium can similarly be measured across each annual ring to recreate a history of selenium exposure in each fish. However, unlike otoliths, the chemical stability of fin rays remains to be established. The Derby presented the opportunity to collect both fin rays and otoliths for comparative analysis to assess the chemical stability of fin ray samples.

This report focuses on analyses related to muscle plug method development (objectives #1 and #2), while a separate report prepared by IISD will focus on the fin ray method, including fin ray, otolith, and endolymph data (objectives #2 and #3). These two documents are the final synthesis reports for the three-year Sturgeon Derby Study (2015-2017). Results from the first year of sampling were also separately presented in the 2015 Sturgeon Derby report (Sun et al. 2017b). These detailed technical reports are intended to document the Study and to facilitate technical peer review.

Methods

Field Sample Collection

The Sturgeon Derby is an annual angling competition held by the Foundation Sportsman's Club out of McAvoy Harbor in Bay Point, CA. The Derby provides a rare opportunity to collect tissue samples from white sturgeon harvested by anglers. The USGS

initiated tissue sample collections from the Derby for selenium studies in 2000/2001 (Stewart et al. 2004), while USFWS collected samples more recently, through 2016. USFWS facilitated the collection of additional samples for the RMP during the first two years of this study; IISD provided additional sample collection assistance and led sample collected during the third year of this study.

The Derby awards cash prizes to anglers who bring in fish closest to a chosen target length, which is chosen randomly from within the legal slot limit of 40-60 inches (102-152 cm) fork length. The competition runs between 7:00 am on Saturday and 1:00 pm on Sunday of Super Bowl weekend each year, with cash prizes awarded for the fish measured closest to the target length each day. Fish can be caught anywhere within the San Francisco Bay-Delta, and must be brought alive to McAvoy Harbor to be measured. Most anglers gave permission for SFEI staff and collaborators to sacrifice and process their sturgeon for tissues.

Objectives #2 and #3 were the initial focus of this study. Because the RMP initially was primarily interested in understanding the relationship between selenium in muscle plugs and risk of reproductive toxicity, most tissue samples were collected from female sturgeon during the first two years of the study. The primary tissues sampled to address Objective #2 were muscle plugs, gonads, and liver, which were collected only from females in 2015-2016 and both males and females in 2017. Additional ovary samples were collected during all three years of the study for histological examination, as were testes samples in 2017.

To address Objective #3, fin rays and otoliths were collected and analyzed for both males and females across all three years of this study. In 2016 and 2017, endolymph samples were also collected to characterize selenium transfer from blood plasma to endolymph to otoliths. Analyses of these samples will be presented in a separate report (Palace et al. in preparation).

Objective #1 was added during the second year of the study. Muscle fillets were first collected from only females in 2016, and subsequently both plugs and fillets were collected from both males and females in 2017.

Additional tissue samples that were either archived or collected by collaborating agencies are listed in Table 1. Samples archived by the RMP include blood plasma samples from all three years of the study, as well as testes samples for selenium and histology analyses collected in 2017. Analyses conducted by USFWS in 2015 and 2016 included sex steroids in blood plasma, gonad histology, and fin ray microchemistry. Additional data collected included age (estimated from fin rays and otoliths during microchemistry analysis), fish weight, liver and gonad weight, and qualitative descriptions of fish condition, gonad condition, and gut contents.

Tissue collection methods were kept consistent throughout the study and are described in detail in Appendix A of the 2015 Sturgeon Derby report (Sun et al. 2017b), as well as the Sturgeon Derby Sampling and Analysis Plans. The most effective method for accessing sturgeon otoliths was developed over the course of the study and is described in the 2017 Sturgeon Derby Sampling and Analysis Plan (Appendix C).

Approximate fish catch locations were obtained from angler descriptions for most fish, and are presented in Appendix A. Sturgeon were predominantly caught in the North Bay, although occasionally fish were caught in the South Bay or the Delta. Sturgeon for which location information could not be collected were assumed to have been collected in Suisun Bay,

where most fish with known sampling locations were taken. All female fish in 2015 and 2016 were known or assumed to have been from Suisun Bay. In 2017, a larger proportion of fish were caught farther downstream in Carquinez Strait or San Pablo Bay, likely because of the large volume of freshwater inflow reaching North Bay following heavy winter rains. A map of approximate sampling locations in 2016 and 2017 is provided in Figures 1A-B. However, sturgeon are highly mobile, and sample collection location may not represent feeding locations over prior months. Isotope data presented in this report provide a stronger indication of feeding locations from which selenium may have bioaccumulated.

Laboratory Analysis and QA/QC

Muscle plugs (skin off), muscle fillets (skin off), liver, and ovary tissues were digested and analyzed for total selenium and moisture by USGS, following isotope dilution-hydride generation-inductively coupled plasma-mass spectrometry (ID-HG-ICP-MS) methods described in Kleckner et al. (2017). All tissues were run together in the same lab batch each year, except for a single ovary sample that was rerun in a separate lab batch in 2017. One laboratory replicate for each tissue type was run with each lab batch. Three method blanks and three replicates each of two different certified reference materials (CRMS) were run with each lab batch. Accuracy was evaluated using CRMs with certified values for selenium, and precision was analyzed using duplicate samples, in some cases including duplicates of samples collected from other studies that were run in the same lab batch.

Selenium results in muscle, ovary, and liver tissue are reported as blank-corrected dry-weight concentrations. For laboratory duplicates, the first result measured was reported (not an average of duplicates). All laboratory duplicates met RMP precision standards (RPD or RSD < 35%; Yee et al. 2017). For rerun samples, the first rerun sample result was reported, even if rerun samples did not indicate an error in the original sample run.

Carbon (δ^{13}C), nitrogen (δ^{15}N), and sulfur (δ^{34}S) isotope ratios in muscle plugs were measured by UC Davis with an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (EA-IRMS). Detailed sample preparation and method descriptions are available on the UC Davis Stable Isotope Facility website (https://stableisotopefacility.ucdavis.edu/13cand15n.html; https://stableisotopefacility.ucdavis.edu/34s.html). δ^{13}C and δ^{15}N isotopes were run concurrently, while δ^{34}S isotopes were run separately. At least one lab replicate was run for each isotope except for δ^{34}S isotopes in 2015, when not enough sample mass was available for a δ^{34}S isotope duplicate. QA/QC analyses included CRMs, laboratory control materials (LCMs) for isotope percent masses, and additional LCMs for isotopes. No method blanks were analyzed.

All selenium and isotope results met measurement quality objectives (Yee et al. 2017). Detailed QA/QC results are presented in Appendix B.

Ovary histology examinations, microchemical analyses in fin rays and otoliths, and selenium analyses in endolymph, were conducted by the University of Manitoba in partnership with IISD. Sample preparation, analysis, and QA/QC details for these samples are presented in a separate report along with the results (Palace et al. [in preparation]).

Additional Data Sources

Several analyses in this report include data compiled from the following studies, including both RMP and non-RMP studies available in the literature:

- 1986-1990 Selenium Verification Study (SVS): sturgeon collected in North Bay by the California Department of Fish and Wildlife (CDFW) and State Water Resources Control Board in North Bay (CSWRCB 1987, 1988, 1989, 1991);
- 1999-2001, data published in Stewart et al. (2004): sturgeon collected during prior Sturgeon Derby events;
- 2002-2005, data published in Linares-Casenave et al. (2015): sturgeon collected year-round in the North Bay and Delta by UC Davis, CDFW, and the Bureau of Reclamation
- 1997-2014 RMP Status and Trends studies: sturgeon collected during summer months throughout the Bay (Sun et al. 2017a and prior studies);
- 2012-2014, summary statistics published in Gunderson et al. (2017): sturgeon collected during prior Sturgeon Derby events; and
- 2015-2017 RMP Muscle Plug Study: muscle plugs sampled by the CDFW during their annual fall sturgeon tagging in the North Bay (Sun et al. 2019).

All available muscle, ovary, and liver data from these studies were compiled, including both adult and juvenile sturgeon collected anywhere within the San Francisco Bay-Delta system (15% were juveniles, 17% were located outside of North Bay). However, only data from fish for which muscle selenium data are available were used in this report. Muscle plug results were available for the current study, the RMP Muscle Plug Study and the 2009 and 2014 RMP Status and Trends sampling events. For all other RMP Status and Trends events and all non-RMP studies, only muscle fillet selenium data were collected. For the RMP Sturgeon Derby Study, muscle plug selenium results are reported in time series and tissue-tissue comparison plots when results were available for both muscle plugs and fillets. For the 2009 and 2014 Status and Trends events, muscle fillet data were used for the long-term trend figures (Figure 3), but muscle plug data were used for the tissue correlation figures (Figure 6).

Data Analysis

Mean concentrations are reported as arithmetic means. The relationship between selenium in muscle plugs and muscle fillets, relationships among selenium concentrations in different tissue types, and relationships between selenium concentrations in tissue types and fork length were determined using linear regressions on log-transformed data. All tests were evaluated using alpha=0.05. Data analyses were performed using RStudio (v.1.1.383; 2016).

Periods of high freshwater inflow are generally associated with lower water column selenium concentrations – and consequently wildlife tissue selenium concentrations – due in part to greater freshwater dilution and lower rates of biotransformation associated with lower residence times in the Estuary (Presser and Luoma 2013; Foe et al. 2016). Sturgeon selenium concentrations are discussed in this report in relationship to hydrologic conditions in North Bay (i.e., wet or dry conditions), which in turn reflect relative levels of selenium in the water column. Hydrologic condition is represented using the California Department of Water Resources water year classification indices for the Sacramento and San Joaquin Rivers, the two dominant tributaries to North Bay. Water years are classified as Wet, Above Normal, Below Normal, Dry,

or Critical on each river based on a weighted calculation that takes into account the volume of unimpaired runoff measured at various locations along each river during the given water year, and the previous water year's classification (http://cdec.water.ca.gov/cgi-progs/iodir/WSIHIST). In this report a six-month lag period is used when describing comparisons of sturgeon selenium concentrations to water year types, to account for the lag time between ambient water selenium exposure at the bottom of the food web and uptake into sturgeon tissues (Beckon 2016, Sun et al. 2019)**.** For example, we assume that selenium concentrations in sturgeon caught during the current study in late January or early February reflect ambient water selenium concentrations and associated hydrologic conditions in late July or early August of the previous year. Therefore, selenium concentrations in sturgeon caught in February are associated with hydrologic conditions in August 2016, which occurs in water year 2016, which is classified as a critically dry year for both the Sacramento and San Joaquin Rivers.

Results and Discussion

Selenium concentrations in muscle, ovary, and liver tissue, along with $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ isotope ratios in muscle plugs are presented in Appendix A and are available through the Contaminant Data Display and Download tool (CD3, www.sfei.org/cd3), as well as CEDEN.

Dataset Summary

Selenium concentrations were measured in 8 female sturgeon in 2015, 9 females in 2016, and 4 females and 9 males in 2017, including sturgeon ranging from 112 to 152 cm fork length. Concentration ranges, means, medians and variances for each year and tissue type are presented in Table 2 (all individual results are presented in Appendix A).

Concentrations were highly variable and log-normally distributed, with coefficients of variation ranging from 40% to 87% across matrices and years (Figure 2). Measured muscle, liver, and ovary concentrations fell within the ranges of previous observations, but with a high proportion of values toward the upper end of the historic distribution (Figure 3). Like patterns observed in the historic data, sturgeon with the highest concentrations in the current study were collected during winter pre-spawning season, and predominantly in the North Bay.

Comparison to the TMDL Target and Published Thresholds

Selenium concentrations in sturgeon muscle plugs generally fell within the range of previous concentrations but commonly exceeded the North Bay TMDL target (38-54% of samples each year; Table 2). The North Bay TMDL established a numerical muscle tissue target of 11.3 µg/g dw, which was derived from the USEPA freshwater selenium criteria for fish tissue measured as an instantaneous not-to-exceed maximum (SFBRWQCB 2015, USEPA 2016b). Three of eight (38%), four of nine (44%) and seven of thirteen (54%) plugs measured in 2015, 2016, and 2017, respectively, exceeded this threshold (Table 2, Figure 2). The median and mean concentrations exceeded the numeric criterion in 2017 (12.2 and 12.4 µg/g dw, respectively). In prior studies (1986-2014, not including the 2014 RMP Muscle Plug pilot study), muscle selenium concentrations above the 11.3 µg/g dw threshold were somewhat less frequent (36%).

Concentrations above 20 µg/g dw were less common historically (11%), occurring only in 1988-1989 during the SVS and during the 1999-2001 Sturgeon Derby events, from which sturgeon were collected during the same season (winter pre-spawning) and from the same region (North Bay) as the present study. In the present study, one female per year as well as one male in 2017 had a concentration above 20 µg/g dw. Notably, the SVS was conducted during dry or critically dry water years, like the current study, but the 1999-2001 Sturgeon Derby sampling took place during above normal water years.

Ovary selenium concentrations, which provide the strongest indicator of potential reproductive impairment, were high relative to both the USEPA freshwater selenium criterion and previously measured concentrations in the Bay. The USEPA freshwater selenium criterion for fish eggs/ovary of 15.1 µg/g dw (from which the muscle tissue criterion was derived; USEPA 2016b) was exceeded by 75%, 22%, and 50% of ovary samples collected each year from 2015-2017 (Table 2, Figure 2). The maximum concentration in this study (47 µg/g dw) was the same as the maximum in the Linares-Casenave et al. (2015) study, but the mean (16 µg/g dw) and median (14 µg/g dw) were substantially higher than found by Linares-Casenave et al. (mean=10 µg/g dw; median=3 µg/g dw). Fifty percent of ovary samples analyzed by Linares-Casenave et al. were below the minimum concentration measured in the current Derby Study (3.2 µg/g dw). Additionally, the highest concentrations measured in the Linares-Casenave et al. study were largely from vitellogenic females during a similar winter pre-spawning season; however, most females in the present study were not vitellogenic (Palace et al. in preparation). Similarly, the mean in the present study was substantially higher than the mean from the 2012-2014 Derby Study, both for all females (7.9 µg/g dw) and vitellogenic females (8.9 µg/g dw, converted from wet weight based on a 54% moisture assumption) (Gunderson et al. 2017). The elevated ovary concentrations found during the present Derby Study may be more attributable to higher clam selenium concentrations during the recent time, linked at least in part to extended drought conditions (Stewart et al. 2013, Stewart et al. in preparation), rather than the inclusion of vitellogenic females.

Liver selenium concentrations similarly were frequently toward the upper end of the historic distribution, and compared to muscle and ovary most frequently exceeded published toxicity thresholds (54-88% each year; Table 2, Figure 3A). While no regulatory threshold for selenium in fish liver has been established, Stewart et al. (2004) used a screening threshold of 15 µg/g dw, based on fish (but not sturgeon) data published by Lemly (1997) that showed evidence of teratogenesis and reproductive failure consistently appearing at tissue concentrations above this threshold. Toxicity has been observed in other studies at sturgeon liver selenium concentrations as low as 12 µg/g dw (Tashjian et al. 2006, De Riu et al. 2014), while reproductive toxicity has been observed at sturgeon liver concentrations as low as 10 µg/g dw (Linville 2006). The less conservative 15 µg/g dw threshold was exceeded by 88%, 56%, and 54% of samples in 2015, 2016, and 2017, respectively; mean concentrations also exceeded this threshold every year (Table 2, Figure 3A). In comparison, 68% of samples collected during the 1999-2001 Derby study exceeded this threshold (both males and females), but only 35% of samples from the 2012-2014 Sturgeon Derby Study (females only) did so (Gunderson et al. 2017).

Historically, a similar distribution of liver concentrations was found during the SVS (max=76 µg/g dw; median=15 µg/g dw; mean=24 µg/g dw) and 2015-2017 Derby Study (max=74 µg/g dw, median=21 µg/g dw; mean=26 µg/g dw). In contrast, the mean and median

concentrations measured in the 2015-2017 Derby Study were similar to those measured during the 1999-2001 Derby events (median=21 µg/g dw; mean=22 µg/g dw; Stewart et al. 2004), but the previous maximum concentration (43 µg/g dw) was lower. Both the SVS and the 1999-2001 Derby studies were conducted at a similar time of year and in the same region as the current study, but those prior Derby events took place during wetter years. On the other hand, the mean liver concentration from the present study was also substantially higher than the mean for females sampled during the 2012-2014 Derby events (12.7 µg/g dw for all females, 15.6 µg/g dw for vitellogenic females, converted from wet weight based on a 74% moisture assumption), which took place earlier in the same drought period that ended in 2017. Liver concentrations in this study were also substantially elevated compared to those measured by Linares-Casenave et al. (2015) (max=32 µg/g dw; median=8 µg/g dw; mean=12 µg/g dw) and during the RMP Status and Trends monitoring events, both of which included juveniles as well as sturgeon caught outside of North Bay during summer and fall months.

Plug-Fillet Relationship

Results from this study, together with previous measurements, indicate that muscle plugs can be used as proxies for muscle fillets in selenium monitoring, but that further study is needed to resolve the slope of the relationship. Paired muscle plug and fillet analyses were first conducted during the 2009 and 2014 RMP Status and Trends monitoring effort, with 12 paired samples analyzed each year. These samples showed a significant relationship between selenium measured in sturgeon plugs and fillets, but a substantial amount of variability in this relationship (Figure 4):

2009: log(Fillet) = 0.85 * log(Plug) + 0.17; p=9.8 x 10^{-4}, R^2 =0.65

2014: log(Fillet) = 1.11 * log(Plug) – 0.04; p=1.3 x 10^{-6}, R^2 =0.90

Notably, in both years most fillet samples (10 of 12 in 2009; 8 of 12 in 2014) had higher selenium concentrations than their corresponding plug samples**.** The coefficient of the regression line in 2009 does not indicate that fillets had systematically lower concentrations than plugs in that year, but is rather a reflection of the variability in the data and the poor fit of the regression line.

To further evaluate this relationship, additional paired plug and fillet samples were collected in the Derby Study in 2016 and 2017. These samples, which included a wider range of concentrations, also showed a significant relationship between selenium concentrations measured in plugs and those in fillets (Figure 4). These results showed less variability and greater consistency between years, as well as a near one-to-one relationship:

2016: log(Fillet) = 0.99 * log(Plug) – 0.05, p=1.3 x 10^{-5}, R^2 = 0.94

2017: log(Fillet) = 0.98 * log(Plug) + 0.03, p=8.7 x 10^{-13}, R^2 = 0.99

The differences found between the two studies are likely a result of differences in the tissue sampling method, and potentially differences in the laboratory analysis method as well. In the Status and Trends study (2009 and 2014), tissue samples were collected from a section between the anal vent and caudal fins that was cut and prepared in the field (Figure 5). In the lab, the skin was removed from one half of the section, from which the muscle fillet was collected.

Two or three 8-mm-diameter muscle plugs were collected through the skin (i.e., mimicking sampling on live sturgeon in the field) from the other half of the section, after which the skin was removed. The location on the fish from which the muscle plugs and muscle fillet subsamples were collected and the depth of the fillet samples below the skin were not noted. Greater variability in the location of tissue collection between samples, and between plugs and fillets collected from the same fish, likely contributed to the higher variability in the plug-fillet relationship observed. Samples were processed and analyzed by Moss Landing Marine Laboratories using an ICP-MS method.

In contrast, muscle plugs collected from live white sturgeon in 2016 and 2017 were consistently sampled from the epaxial muscle (dorsal trunk muscle) through the skin, near or slightly in front of the dorsal fin (Figure 5). All muscle plug samples collected for the RMP special studies were taken using this technique, including plugs collected during the Sturgeon Derby (the basis for the 2016 and 2017 regressions). These plugs were smaller than those collected during Status and Trends monitoring (5-mm diameter), resulting in a smaller volume of tissue sample available for laboratory analysis. Muscle fillets also were collected through the skin, as an approximately 5 x 3 x 2 cm section collected from the epaxial muscle directly posterior to the location of muscle plug collection. To accommodate the small muscle plug sample masses, all tissue samples were analyzed by USGS Menlo Park using an ID-HG-ICP-MS method (Kleckner et al. 2017).

Despite differences between the two studies, the strong relationships observed between selenium concentrations in muscle plugs and fillets support the use of non-lethally sampled muscle plugs as a proxy for muscle fillets. However, further analyses would provide a better understanding of the relationship between selenium measured in the epaxial muscle (i.e., Derby Study and Muscle Plug Study samples) compared to the posterior section of fish (i.e., 2009 and 2014 Status and Trends samples), which will help to resolve the differences found in the slopes of the regression lines between the two studies. To do this, paired samples could be collected in future Status and Trends monitoring to compare muscle fillets sampled using methods established for the Status and Trends monitoring effort (i.e., representative of all historically collected RMP data) and fillets sampled using the Derby study method. These samples should also be compared to muscle plugs sampled from the same sturgeon, live in the field, using methods established for the Muscle Plug study (similar to those used in the 2016-2017 Derby Study). Although a strong, nearly one-to-one relationship has already been established between fillets and plugs sampled from the epaxial muscle, these additional samples collected during future Status and Trends monitoring can be collected and analyzed at a minimal additional cost, and can improve our understanding of selenium variability measured in muscle plugs (Sun et al. 2019).

Relationships Among Tissues

Significant positive correlations were found among selenium concentrations measured in sturgeon muscle plug, ovary, and liver tissue, both in the three-year Derby Study dataset and a larger dataset including the SVS (CSWRCB 1987, 1988, 1989, 1991), Stewart et al. (2004), Linares-Casenave et al. (2015), and RMP Status and Trends monitoring (Figures 6A-C). Data from the current study show that the relationship between selenium in ovary and muscle tissue is positive and significant (R^2=0.22, p=0.0188, n=21). The relationship between ovary and liver

tissue was stronger (R^2=0.32, p=4.65x10^{-3}), while the relationship between liver and muscle tissue was strongest (R^2=0.40, p=1.03x10^{-4}, n=30).

The strength of those correlations increased when including data from other studies. This was particularly true of data from Linares-Casenave et al. (2015), which spanned a larger range of concentrations, including low concentrations that were primarily measured in smaller sturgeon collected during the post-spawning season, a subgroup that was not included in the other studies. In the larger dataset, the correlation between selenium in ovary and muscle tissue, and the correlation between selenium in liver and muscle tissue, were similar (ovary versus muscle: R^2=0.43, p=<0.0001; liver versus muscle: R^2=0.45, p=<0.0001 respectively; Figures 6A,B). The relationship between selenium in ovary and liver was stronger, with an R^2 of 0.64 (p=<0.0001; Figure 6C). However, even in the combined datasets, moderate R^2 values indicate a substantial amount of unexplained variability in the correlations, particularly in the correlations with muscle tissue.

These results were generally consistent with expectations. Varying selenium uptake mechanisms and turnover rates in different tissues likely contribute to the lack of a strong relationship between tissues – particularly between selenium in liver versus muscle tissue, or selenium in ovary versus muscle tissue. The primary selenium uptake mechanism is considered to be incorporation of selenium into proteins as seleno-methionine. Given the close linkage between selenium in the ovaries and liver – selenium is incorporated into pre-vitellogenic and other proteins in the liver before being incorporated into egg yolk proteins in the ovaries – selenium concentrations in these two tissues can be expected to be more closely related than the other relationships tested (Linville 2006, SFBRWQCB 2015). In contrast, the linkage between selenium in liver or ovaries and selenium in muscle is less direct. Protein turnover is generally faster in fish liver than muscle: proteins are both synthesized and degraded more quickly in the liver, experiencing poorer retention efficiency under starvation conditions, while protein storage is more significant in muscle (Peragón et al. 1992, 1994). Selenium depuration in splittail (*Pogonichthys macrolepidotus*) was faster in liver tissue compared to muscle tissue (Deng et al. 2007), reflecting expectations based on protein turnover rates, and similar patterns would be expected in other fish species. Temporal mismatches in selenium concentrations between these tissues based on varying dietary selenium uptake and depuration rates may have contributed to the observed variable selenium correlations.

Studies in other fish species have found that different stages in the reproductive cycle could affect the rate of selenium incorporation into egg yolk or egg yolk precursor proteins in the ovaries or liver (Osmundson and Skorupa 2011). Higher rates of selenium incorporation into ovary and liver tissues could be expected in reproductively mature fish in the pre-spawning season. However, few females included in the current study were vitellogenic (Palace et al. *in prep*).

Overall, these results indicate that selenium concentrations in ovary and liver are correlated with concentrations in muscle in sturgeon. Although the relationships among tissues are statistically highly significant, moderate R^2 values indicate that selenium concentrations in muscle tissue are not precise predictors of ovary or liver selenium concentrations. However, elevated selenium concentrations in muscle tissue samples generally would suggest correspondingly elevated selenium in ovary or liver as well. Variation in the lag time between

selenium concentrations in the diet and in different tissues, due to different pathways of selenium uptake, likely contribute to the unexplained variation.

Factors Influencing Selenium Concentrations

Several factors may have contributed to the elevated selenium concentrations found during this study. Sturgeon for this study were all collected in winter pre-spawning season during critically dry years, periods when clam selenium concentrations have been found to be elevated (Stewart et al. 2013, Stewart et al. in preparation). All sturgeon were also estimated to be adults, based on their lengths, and most were collected in North Bay (further discussed below).

Sturgeon length and sampling location were further evaluated as potential contributors to the variability in selenium concentrations. However, due to the nature of the Sturgeon Derby, in which anglers are targeting a specific fish length, fish sampled during this study cover a relatively narrow distribution of lengths and sampling locations.

Further discussion of the effect of these factors on sturgeon selenium concentrations is presented in the 2015-2017 RMP Muscle Plug Study report (Sun et al. 2019). The Muscle Plug Study report includes a more comprehensive analysis of the effects of these factors on variability and trends in sturgeon selenium in a larger dataset compiled from multiple studies conducted since 1986, including the present study. That analysis provides further context and support for the results described below.

Length - Within the limited size range evaluated in this study (112 -152 cm fork length), no significant relationship was found between sturgeon fork length and selenium concentration in muscle tissue, ovary, or liver (Figure 2). While selenium concentrations are not generally expected to correlate with length or age, for which length is considered a proxy, Linares-Casenave et al. (2015) found significantly higher selenium concentrations in the gonads, liver, and muscle in larger sturgeon, based on binned size classifications of sturgeon ranging from 61 to 193 cm total length (or 53 cm to 170 cm fork length) (both total and fork length were measured and are presented in the Supplementary Information). They hypothesized that differences in foraging behavior or locations with age could have contributed to these observed patterns.

Estimates of the approximate age and length of maturation are variable; for the Sacramento-San Joaquin population, females are estimated to mature at around 12-16 years or 95-135 cm fork length (Moyle 2002). Linares-Casenave et al. (2015) classified fish into three size classes (Class I: 61-105 cm, Class II: 106-150 cm, and Class III: 151-193 cm total length; approximately equivalent to Class I: 53-93 cm, Class II: 93-130 cm, and Class III: 131-170 cm fork length). In this classification scheme, female sturgeon in Class II fall within the size range in which Moyle estimates females undergo maturation. Following the Linares-Casenave et al. (2015) size classifications, and conservatively assuming sturgeon in both Class II and Class III are adults, the present study did not include any juveniles.

Sampling Location – Sturgeon caught in Suisun Bay had the highest selenium concentrations of the four regions represented in this study (Delta, Suisun Bay, San Pablo Bay, and South Bay), while the individual fish sampled in the Delta and South Bay had the lowest (Figure 7). Although the limited number of samples collected outside of Suisun Bay prevents a strong statistical

analysis of this spatial pattern, these results are consistent with observations previously made during the 2014 RMP Status and Trends study (Sun et al. 2017a).

Sturgeon sampling location information represents an estimate of recent foraging location, and was collected anecdotally from anglers. White sturgeon are highly mobile, moving between the Bay and the Sacramento and San Joaquin Rivers to spawn, though they are thought to forage primarily in North Bay. It is also thought that the North Bay population moves continuously between Suisun Bay and San Pablo Bay. However, in recent years the population has appeared to focus upon popular foraging areas in Suisun Bay – particularly clam beds in the Grizzly Bay shallows – based on reported capture locations from both the Sturgeon Derby and the California Department of Fish and Wildlife's fall sturgeon tagging events. Additionally, telemetry studies conducted by UC Davis, which include an array of sensors on the Richmond-San Rafael Bridge in Carquinez Strait between Suisun and San Pablo Bays, suggest that some individual sturgeon may be spending months at a time within Suisun Bay (Emily Miller, UC Davis, personal communication).

For the present study, location information was not collected in 2015, but all female sturgeon measured in 2016 were reported to have been caught within Suisun Bay (except for one sturgeon with no location reported). In 2017, 62% of sturgeon were collected in Suisun Bay, while 23% of fish were collected farther downstream in Carquinez Strait or San Pablo Bay, likely due to a large volume of freshwater inflow reaching North Bay following heavy winter rains; one fish was collected in South Bay and one in the Delta.

In 2017, the mean and median plug concentrations measured in Suisun Bay (17 µg/g dw and 14 µg/g dw, n=8) were substantially higher than in San Pablo Bay (6.8 µg/g dw and 3.1 µg/g dw, n=3). The mean concentrations in both North Bay embayments were higher than those measured in the individual fish collected from the Delta (2.2 µg/g dw) and South Bay (3.3 µg/g dw), but the median concentration in San Pablo Bay (3.1 µg/g dw) was within the same range.

Because selenium concentrations in sturgeon are lognormally distributed, there was a higher likelihood of finding high concentrations in regions where more samples were collected. Still, it is notable that the mean concentration in Suisun Bay (17 µg/g dw) was as high as the maximum measured in San Pablo Bay, the region with the second-highest selenium concentrations. Additionally, the minimum concentration measured in Suisun Bay (6.1 µg/g dw) was elevated compared to the minimum concentrations in all other regions, which were similar to each other (Delta: 2.2 µg/g dw; San Pablo Bay: 3.0 µg/g dw; South Bay: 3.9 µg/g dw).

A similar pattern of relatively high concentrations in Suisun Bay sturgeon was found in 2014 in RMP Status and Trends sport fish monitoring (Sun et al. 2017a). In that study, significantly elevated concentrations were found in two sturgeon collected in Suisun Bay, compared to those collected in San Pablo Bay or other embayments. Like this study, however, the sample size was relatively low.

Isotope ratios measured in sturgeon muscle tissue can be used (Appendix A), supported by selenium and isotope data collected in North Bay clams (Stewart et al. unpublished; Sun et al. 2019), to provide further insight into foraging patterns assumed based on sturgeon capture locations. A comparison of δ^{15}N in sturgeon muscle tissue collected during this study and *Potamocorbula amurensis* over the same period (Robin Stewart, USGS, *unpublished data*) suggests that, as expected, bivalves were a major component of the diet of the sturgeon sampled

in this study (Stewart et al. 2004), and therefore a comparison of carbon and sulfur isotopes in sturgeon and *Potamocorbula* can provide a useful estimate of foraging location. Elevated δ^{15}N in three sturgeon captured in South Bay and San Pablo Bay in 2017, however, suggests that these sturgeon were foraging in different locations and/or at a higher trophic level, contributing to the low selenium concentrations observed in these fish (Appendix A).

In the majority of other sturgeon, however, δ^{13}C and δ^{34}S suggest that most sturgeon collected in this study were foraging in North Bay, as expected based on sturgeon capture location. Both δ^{13}C and δ^{34}S in the single sturgeon captured in the Delta (near Prospect Slough) in 2017 was indeed foraging outside of Suisun Bay (-26.1‰ and 2.2‰, respectively). However, these data were not sufficient to conclusively resolve between sturgeon foraging in San Pablo Bay versus Suisun Bay, likely because sturgeon are known to be highly mobile within North Bay. This effect may also contribute to the conflicting findings in previous studies that showed significantly higher selenium concentrations in either Suisun or San Pablo Bay.

Conclusions

The results of this study demonstrate that muscle plugs can be used to monitor selenium concentrations in sturgeon muscle tissue to evaluate status and trends, compare concentrations to regulatory thresholds, and provide an indication of potential reproductive impairment risks. Data from 2016 and 2017, together with data previously collected in 2009 and 2014, show that the relationship between selenium in muscle plugs and fillets is strong and highly significant, indicating that plugs can be used as proxies for fillets to monitor selenium in sturgeon muscle tissue. However, further work would be needed to make results comparable for muscle plugs sampled using the method established for the Muscle Plug Study and muscle fillets sampled using the method historically used for Status and Trends monitoring.

Data from this study also indicate that selenium concentrations in muscle plugs are correlated with, but not a precise predictor of, selenium concentrations in ovary and liver. Relationships among muscle, ovary, and liver selenium were statistically highly significant, but R^2 values were low to moderate (muscle-ovary: 0.22; muscle-liver: 0.40; ovary-liver: 0.32). The strength of these relationships increased when including data from previous studies, which both increased the sample number and the range of concentrations measured, but R^2 values were still below 0.50. However, the significant positive relationships indicate that elevated selenium in muscle tissues would generally suggest that selenium concentrations in ovary and liver are elevated as well.

Selenium concentrations measured during this study commonly fell within the upper end of historic distributions for the San Francisco Bay. Individual samples regularly exceeded the regulatory target for muscle tissue established to protect white sturgeon reproduction, as well as published non-regulatory toxicity thresholds for ovary and liver tissue, although mean concentrations in muscle tissue were slightly below the regulatory target in two out of the three years sampled. Several factors may have contributed to these high concentrations, including elevated clam selenium concentrations due to recent drought conditions, season, fish sex and reproductive status (predominantly female), fish size or age (mature adults), and recent foraging location (assumed to be predominantly in Suisun Bay). Initial results from this study show no significant relationship between fish length and selenium concentration within the 112-152 cm

fork length size range (adults only), as well as substantially higher selenium in fish caught in Suisun Bay compared to other regions of the Bay-Delta. The limited sample size and distribution of samples from this study prevent a more comprehensive analysis of these factors, but a more robust analysis was conducted in the context of all available studies of sturgeon selenium in the Bay as part of the 2015-2017 Muscle Plug Study (Sun et al. 2019).

Fin rays and otoliths also were collected during the study to evaluate the potential to use non-lethal fin ray sampling to evaluate long-term selenium trends, and to serve as a proxy for selenium concentrations in muscle tissue. Results from those analyses will be published in a separate report (Palace et al. in preparation).

Figures

Figures 1A-B. Approximate sturgeon catch locations, 2016 and 2017. The sizes of the circles are roughly proportional to the number of sturgeon caught at each location. Pink circles represent approximate catch locations anecdotally provided by anglers. Purple circles represent samples for which the embayment (Suisun Bay, San Pablo Bay) is the most detailed level of location information available; the catch location within the given embayment is unknown.
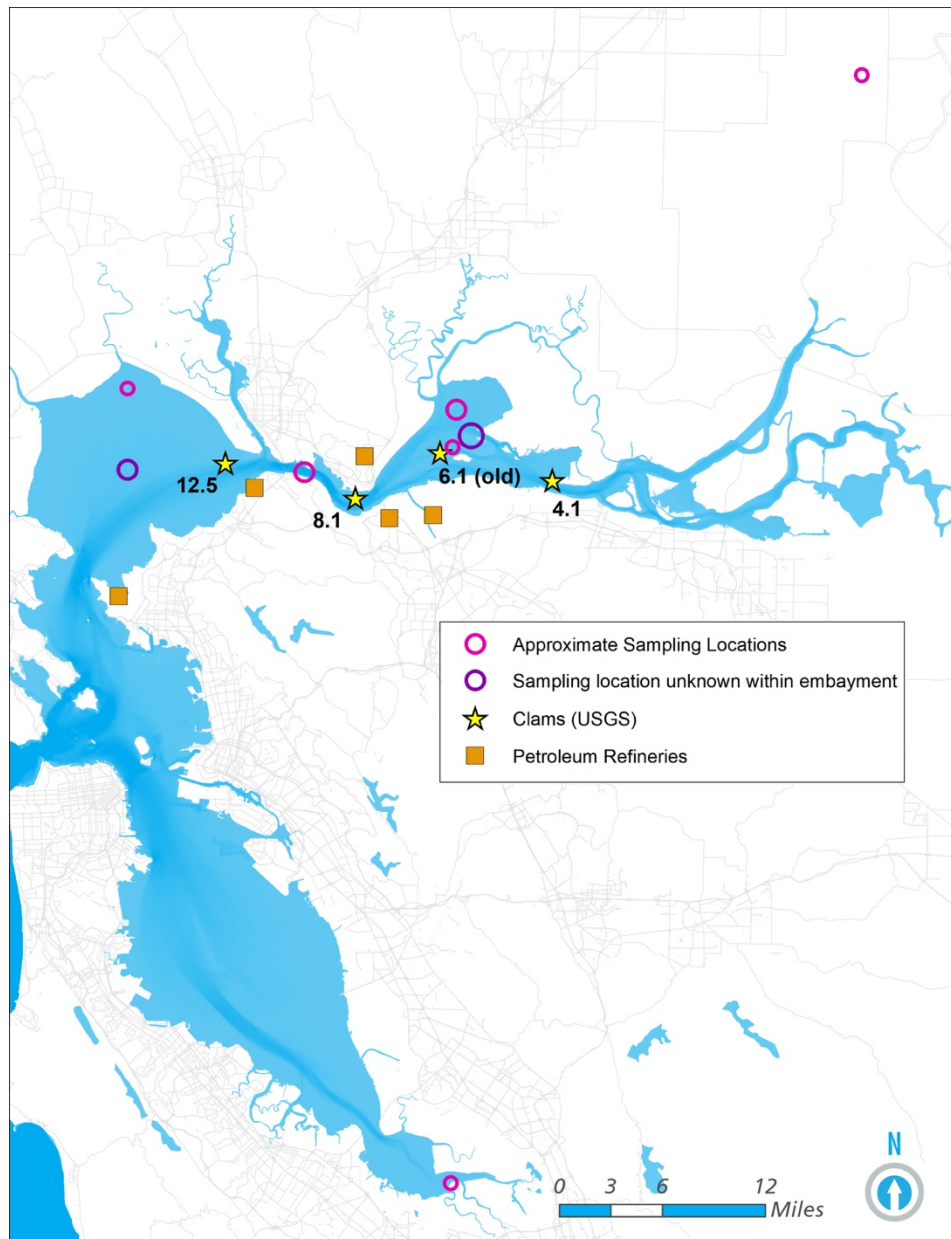
A. Samples from 2016
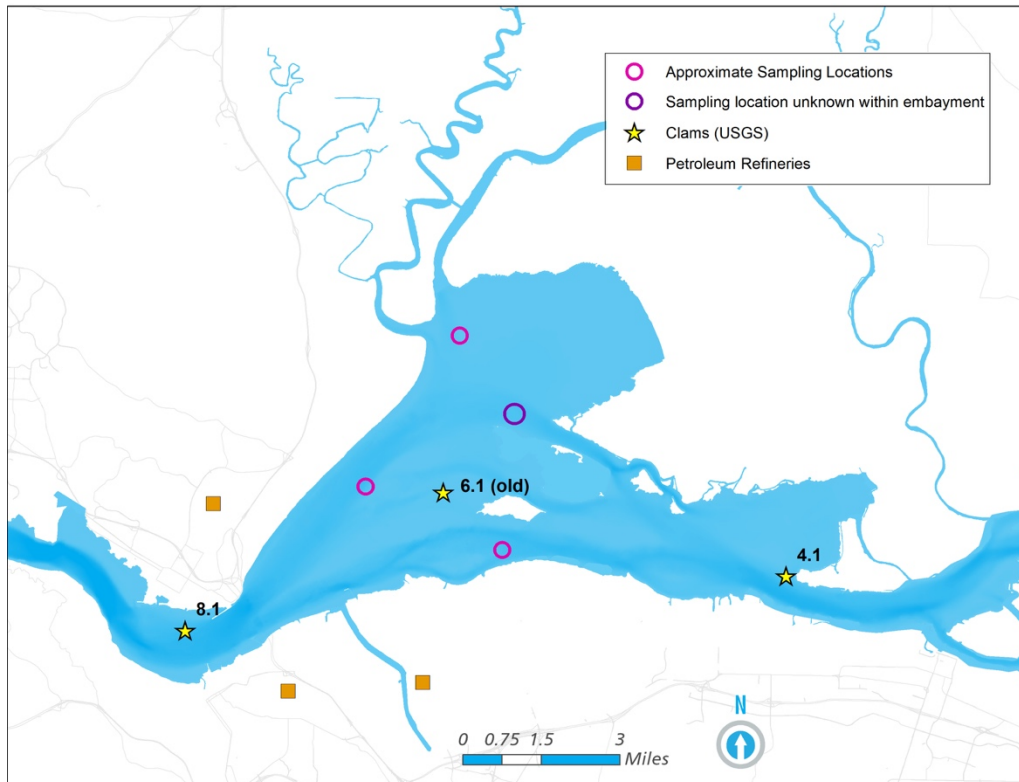
B. Samples from 2017

Figure 2. Fork length (cm) versus selenium concentrations (µg/g dw) in muscle plug, ovary, and liver tissues collected in 2015-2017. No statistically significant relationships were found.
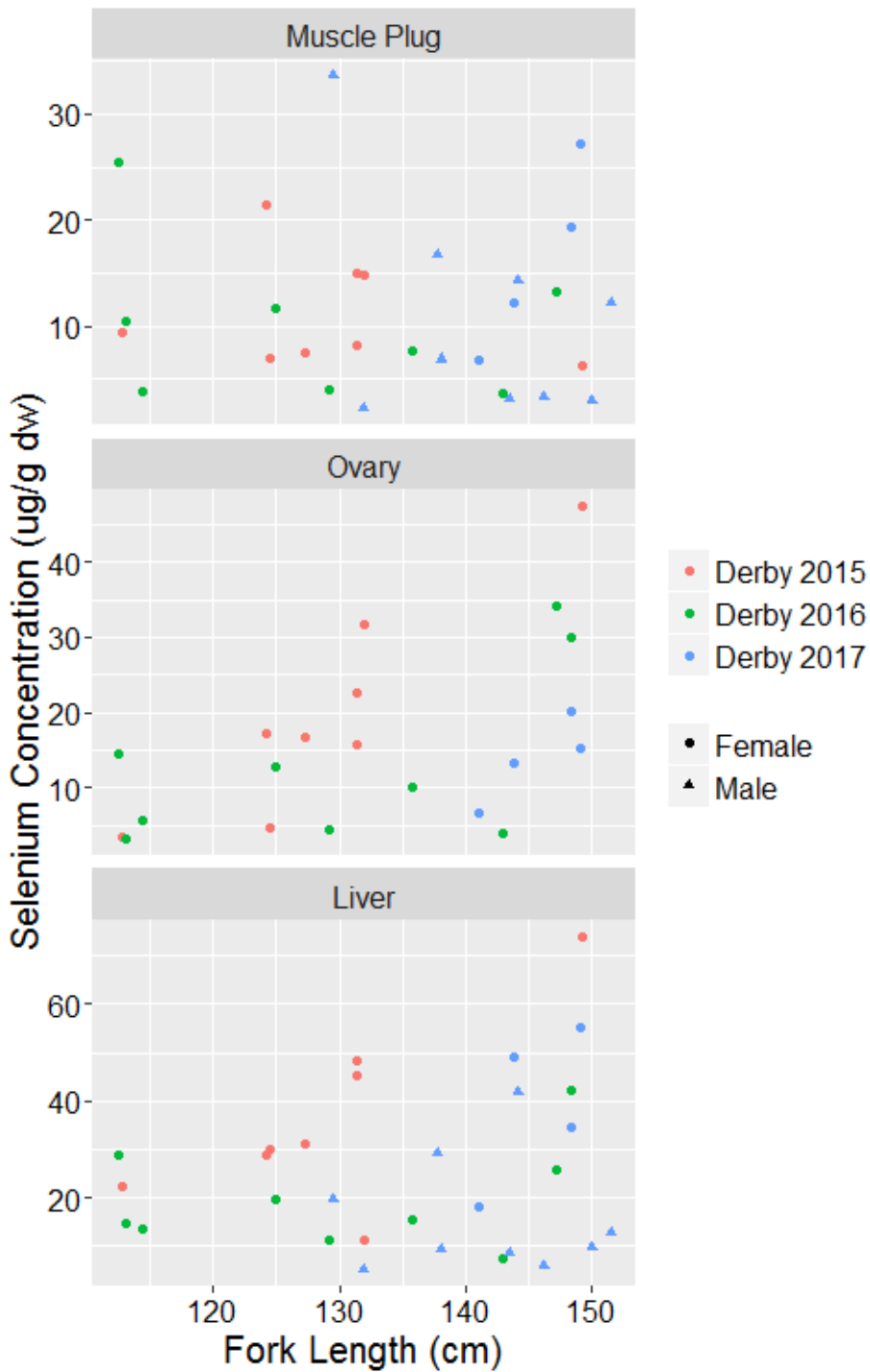
Figure 3A. Selenium concentrations (µg/g dw) in sturgeon muscle, ovary, and liver tissues, 1986-2017. Black dots represent average concentrations. Points represent individual fish. Symbology for muscle tissue indicates whether muscle tissue was collected as a plug or fillet; symbology for ovary and liver indicates whether the paired muscle tissue sampled from the same fish was collected as a plug or fillet. The blue line represents the 11.3 µg/g dw fish muscle tissue numeric target established by the North Bay Selenium TMDL. This figure includes data collected from other RMP and external data sources, described in the Methods section of the text, under "Additional Data Sources."
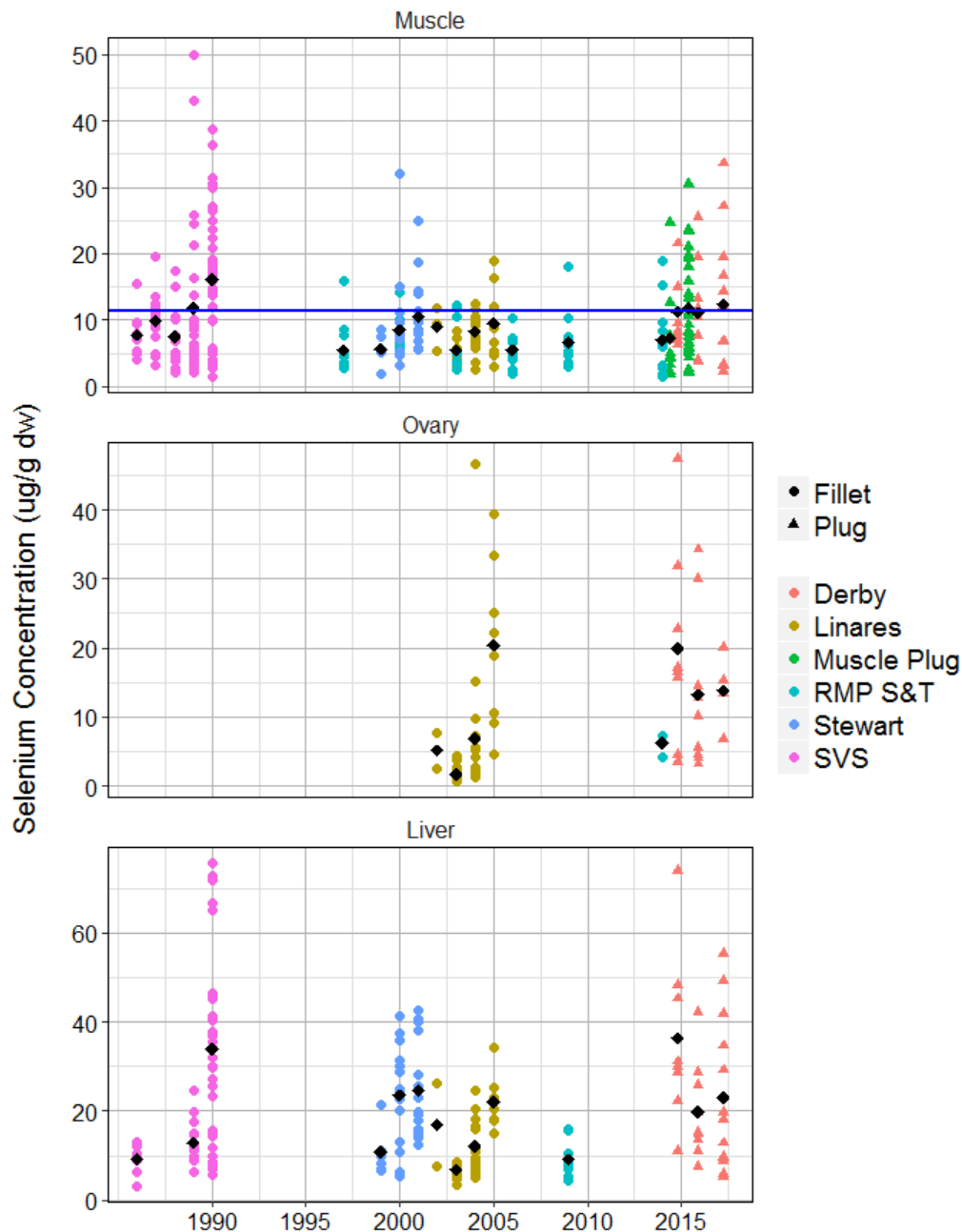
Figure 3B. Enlarged view of the muscle tissue plot presented in Figure 3A. Selenium concentrations (µg/g dw) in sturgeon muscle tissue, 1986-2017. Black dots represent average concentrations. Points represent individual fish. Symbology indicates whether muscle tissue was collected as a fillet or a plug. The blue line represents the 11.3 µg/g dw fish muscle tissue numeric target established by the North Bay Selenium TMDL. Figure includes data collected from other RMP and external data sources, described in the Methods section of the text, under "Additional Data Sources."
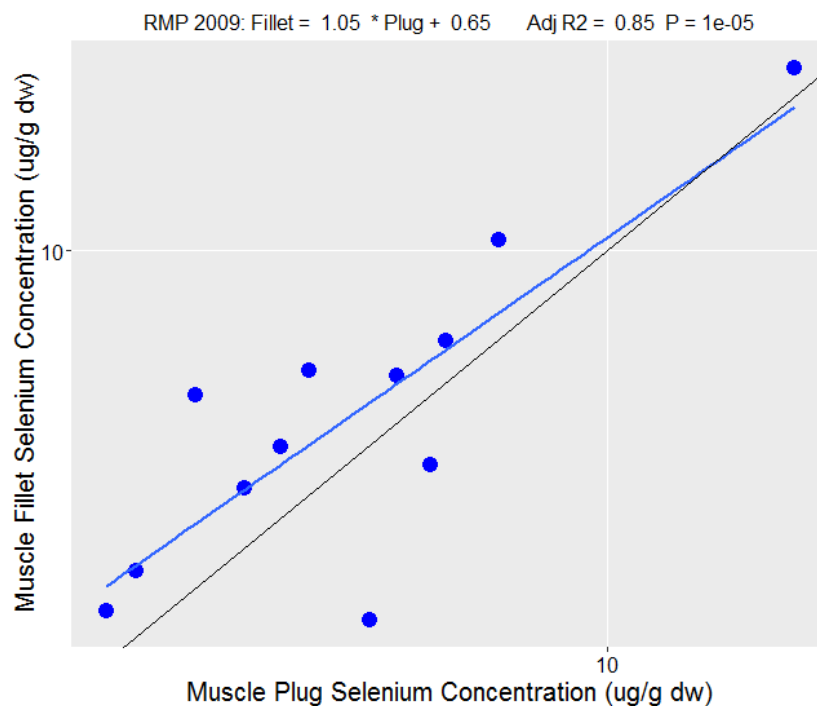
Data were obtained from the CDFW and SWRCB's SVS (CSWRCB 1987, 1988, 1989, 1991); Stewart et al. 2004; a USGS study of fish collected during the 1999-2001 Sturgeon Derbies; Linares-Casenave et al. 2015, including fish collected throughout North Bay and the Delta by UC Davis, CDFW, and Bureau of Reclamation in 2002-2005; the RMP's Status and Trends monitoring events from 1997-2014; the RMP's fall muscle plug sampling effort conducted in collaboration with CDFW and the USFWS (2014-2017; 2015-2017 data unpublished); and the 2015-2017 Sturgeon Derby events discussed in this report.
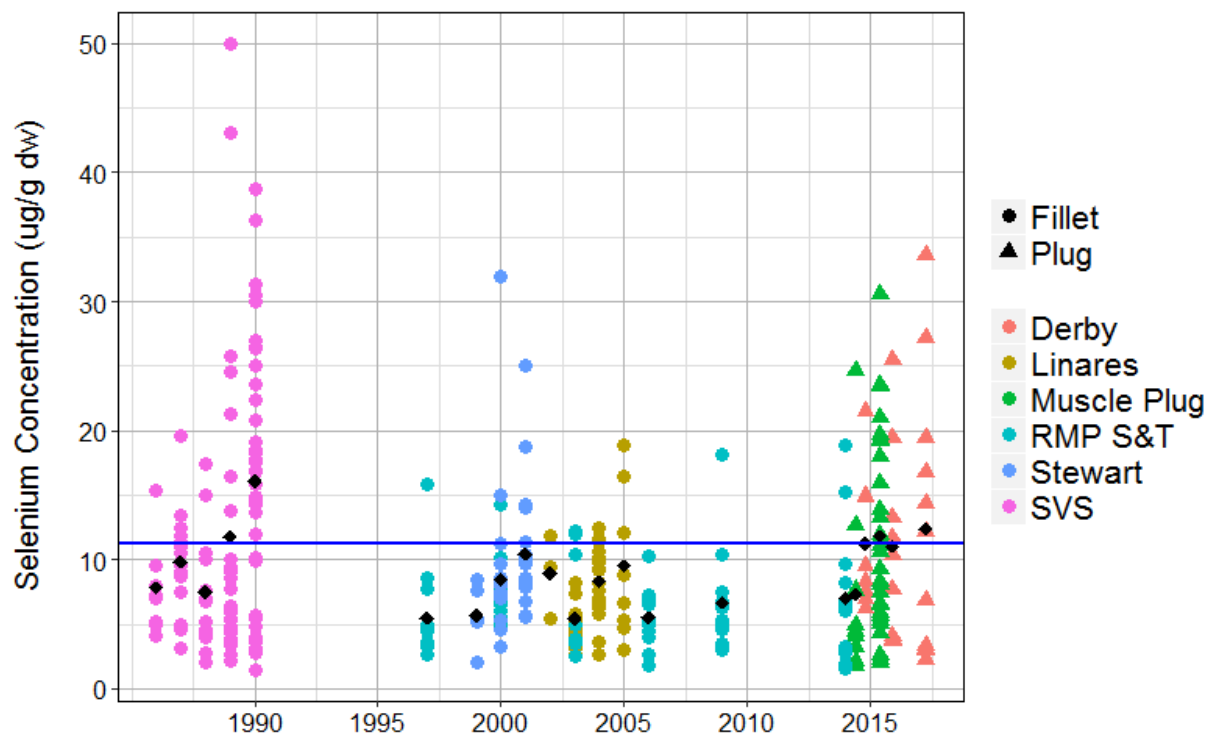
Figures 4A-D. Selenium concentrations (µg/g dw) in paired samples of muscle plugs and muscle fillets of white sturgeon from San Francisco Bay. Points represent individual fish. Data include samples collected during the RMP's Status and Trends monitoring events in 2009 and 2014 (A-B), as well as the 2016 and 2017 Sturgeon Derby events (C-D). Data are log-normally distributed, and linear regressions were conducted on log-transformed data. Linear regressions established for each year were highly significant, but the slope of the regression (represented by blue lines in figures) varied across studies. The plug-fillet relationship is close to 1:1 (represented by black lines in figures) in the Derby studies, with high R^2 (> 0.94), while the relationship was more variable across samples collected during the RMP's Status and Trends monitoring events. These differences are likely attributable to differences in tissue sampling methods, which are further described in the text.

A. 2009 RMP Status and Trends

RMP 2009: Fillet = 1.05 * Plug + 0.65   Adj R2 = 0.85 P = 1e-05

B. 2014 RMP Status and Trends

RMP 2014: log(Fillet) = 1.11 * log(Plug) + -0.04   Adj R2 = 0.904 P = 1.3e-06

Muscle Fillet Selenium Concentration (ug/g dw)

Muscle Plug Selenium Concentration (ug/g dw)

C. 2016 RMP Sturgeon Derby

Derby 2016: log(Fillet) = 0.99 * log(Plug) + -0.05   Adj R2 = 0.94 P = 1.3e-05

Muscle Fillet Selenium Concentration (ug/g dw)

Muscle Plug Selenium Concentration (ug/g dw)

D. 2017 RMP Sturgeon Derby

Derby 2017: log(Fillet) = 0.98 * log(Plug) + 0.03   Adj R2 = 0.99 P = 8.7e-13

Muscle Fillet Selenium Concentration (ug/g dw)

Muscle Plug Selenium Concentration (ug/g dw)

Figure 5. Comparison of sturgeon muscle tissue sampling methods. jj

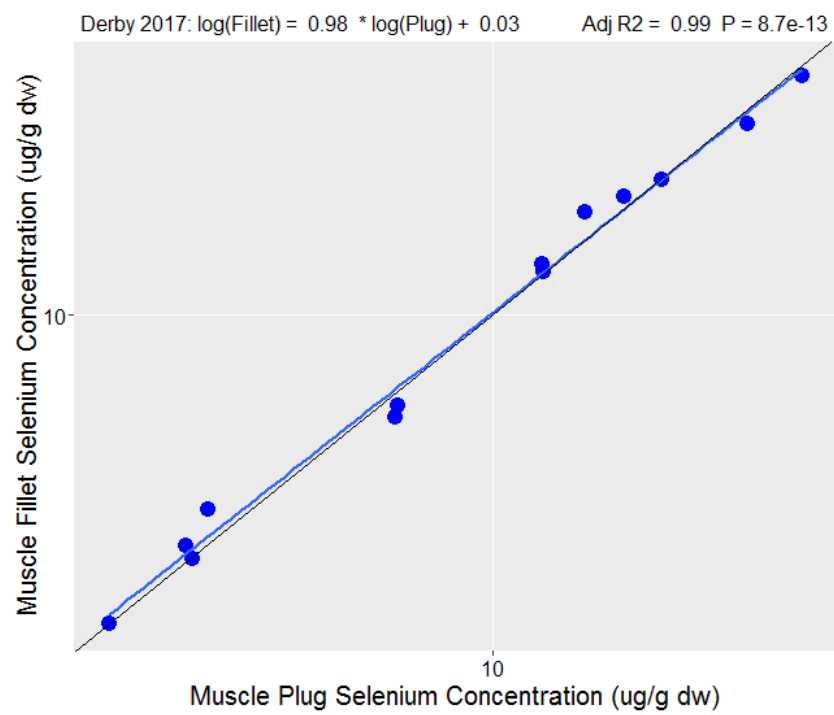| Sampling Event	Status and Trends	Sturgeon Derby and Muscle Plug Studies
Method	Moss Landing Marine Laboratories: EPA 200.8	USGS: ID-HG-ICP-MS
Field sample collection	Fish section collected from behind the anal vent to the caudal keel	Muscle plug collected from the epaxial muscle, just in front of the dorsal fin Fillet collected directly adjacent to and behind the muscle plug sample
Fillet vs. plug collection locations	Fillet and plug samples collected from opposite sites (left and right) of the fish section	Fillet and plug samples collected from adjacent locations
Tissue sample depth	Plug sampled through the skin, skin subsequently removed Fillet sampled from a section with skin removed first; depth of skin removal varied	Fillet and plug both sampled through the skin, with skin subsequently cut off. About 2 mm of tissue below the skin was removed for plugs; depth of skin removal for fillets varied
Sample Size	Plug: 2 or 3 plugs per fish, 8-mm diameter Fillet: approximately 6 x 6 cm square, varies by fish	Plug: 2 or 3 plugs per fish, 5-mm diameter Fillet: approximately 2 x 3 x 5 cm section
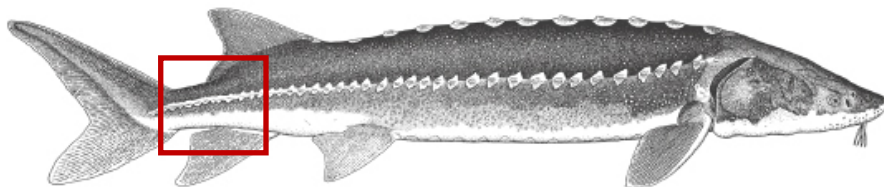
Location of tissue sample collection, Status & Trends.
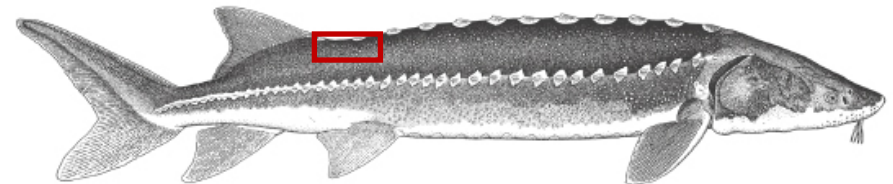
Location of tissue sample collection, Sturgeon Derby & Muscle Plug studies

Figure 6A. Relationship between selenium concentrations (µg/g dw) measured in paired muscle and ovary samples. Data are shown on a log scale. Muscle tissue samples include both muscle plugs (Derby studies, RMP S&T 2014) and muscle fillets (Linares-Casenave et al. 2015). Each point represents an individual fish. Data from the RMP Sturgeon Derby Study are shown as dots outlined in black; data from external data sources are shown as simple colored dots, and are described in the Methods section of the text, under "Additional Data Sources." There was a statistically significant relationship between ovary and muscle selenium concentrations (linear regression, all studies: R^2=0.44, p=1.1x10^{-7}).

Figure 6B. Relationship between selenium concentrations (µg/g dw) measured in paired muscle and liver samples. Data are shown on a log scale. Muscle tissue samples include both muscle plugs (Derby studies, RMP S&T 2009) and muscle fillets (SVS [CSWRCB 1987, 1988, 1989, 1991], Linares-Casenave et al. 2015, Stewart et al. 2004) Each point represents an individual fish. Data from the RMP Sturgeon Derby Study are shown as dots outlined in black; data from external data sources are shown as simple colored dots, and are described in the Methods section of the text, under "Additional Data Sources." There was a statistically significant relationship between liver and muscle selenium concentrations (linear regression, all studies: $R^2=0.6$, $p=2.2 \times 10^{-16}$).
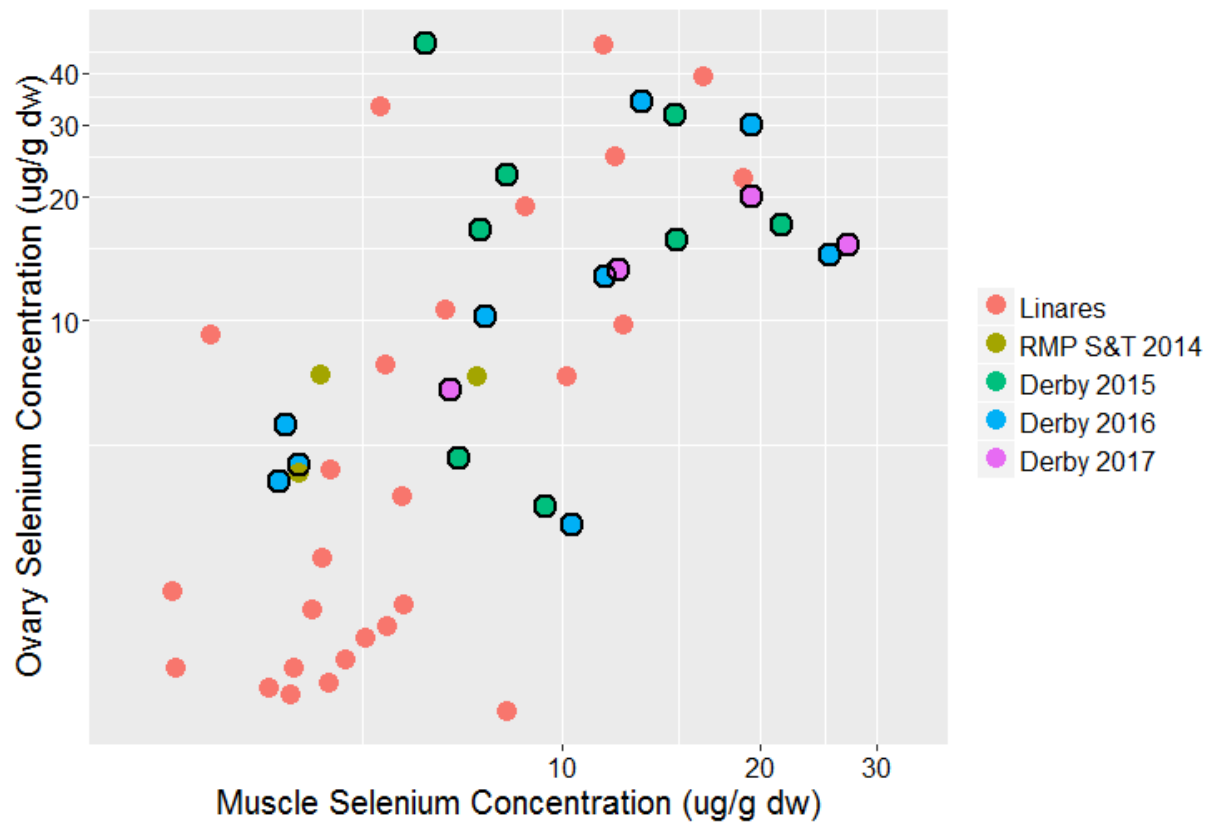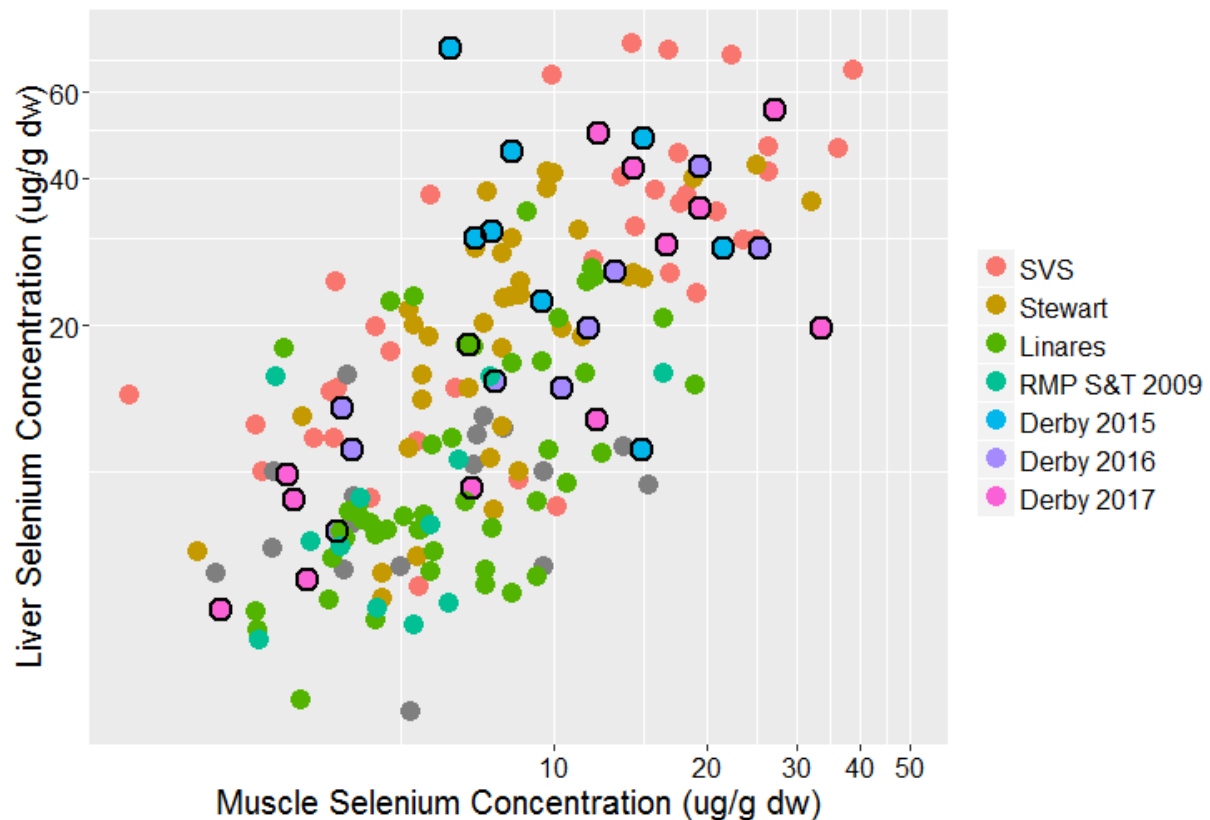
Figure 6C. Relationship between selenium concentrations (µg/g dw) measured in paired liver and ovary samples. Data are shown on a log scale. Each point represents an individual fish. Data from the RMP Sturgeon Derby Study are outlined in black; data from external data sources are shown as simple colored dots (Linares-Casenave et al. 2015), and are described in the Methods section of the text, under "Additional Data Sources." There was a statistically significant correlation between ovary and liver selenium concentrations (linear regression, all studies: R^2=0.64, p=2.9x10^{-16}).
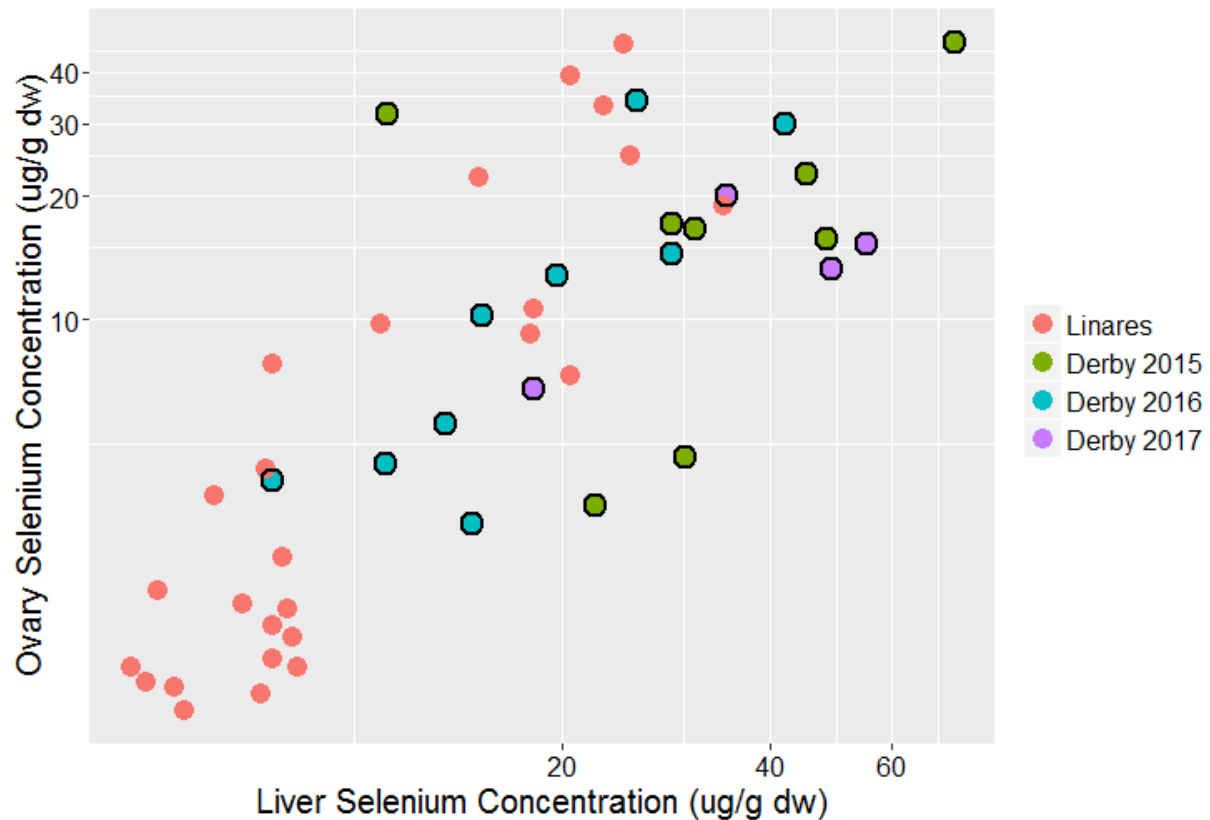
Figure 7. Muscle plug selenium concentrations at the approximate locations of capture in 2017. Locations were anecdotally provided by anglers. Colored points represent individual fish. Black diamonds represent average concentrations, and white bars represent median concentrations. One fish was caught in the Delta, eight in Suisun Bay, three in San Pablo Bay (two points are overlapping, at 3.0 and 3.1 µg/g dw), and one in South Bay.

Tables

Table 1. Summary of field samples collected

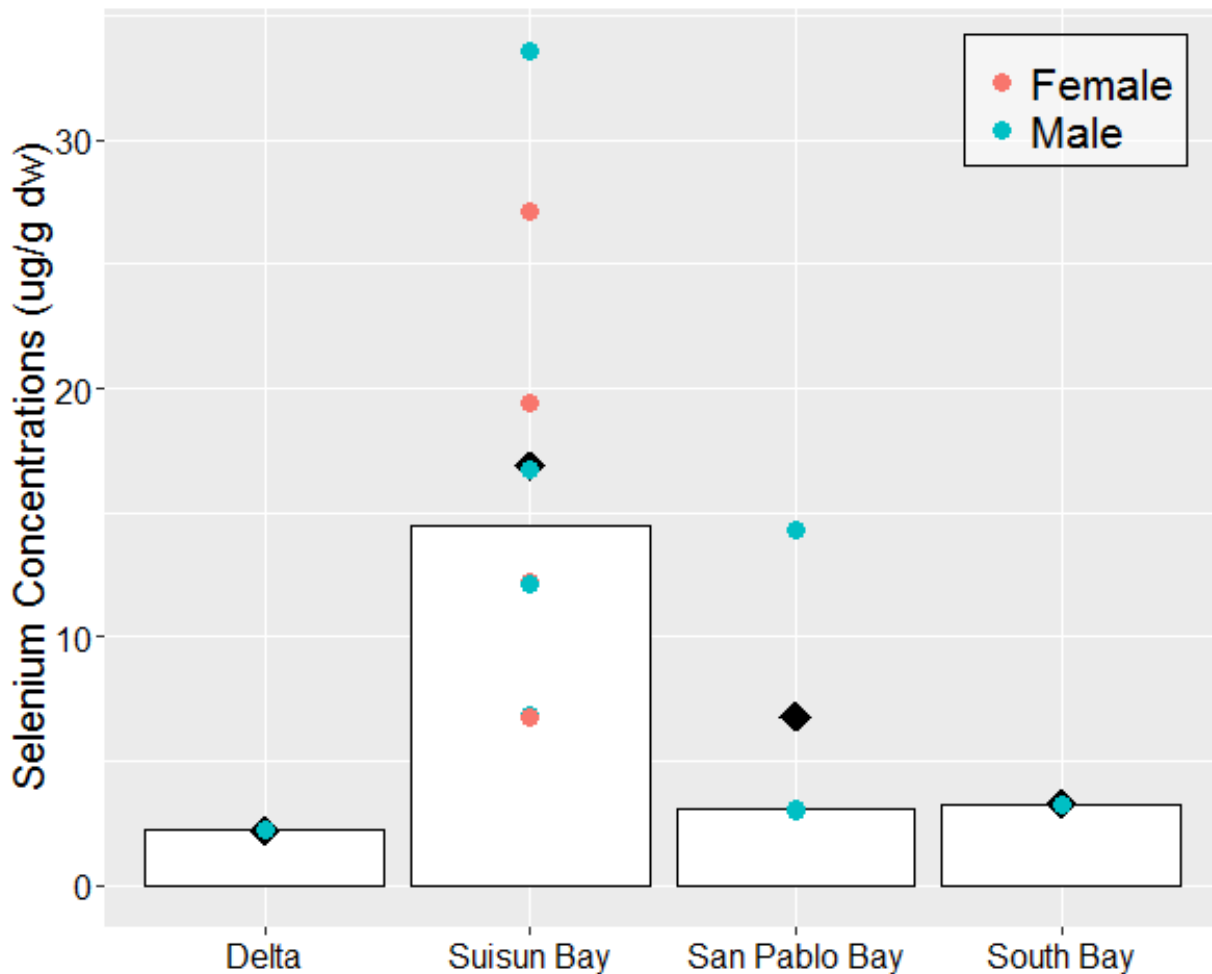| Field Sampling Information	2015		2016		2017			
Sampling Dates	1/31 - 2/1		2/6 - 2/7		2/4 - 2/5			
Fork Length range (cm)	113-149		104-151		130-152			
Samples Collected: RMP	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Analyses**	**Analytical Lab**
Muscle Plug	8	--	9	--	4	9	Selenium; C, N, S isotopes	USGS, Menlo Park; UC Davis
Muscle Fillet	--	--	9	--	4	9	Selenium	USGS, Menlo Park
Ovary	8	--	9	--	4	--	Selenium	USGS, Menlo Park
Liver	8	--	9	--	4	9	Selenium	USGS, Menlo Park
Ovary - Histology	8	--	9	--	4	--	Histology	Stantec
Fin Ray	8	19	9	13	4	9	Selenium; Age	IISD / University of Manitoba
Otolith	4	5	9	13	4	9	Selenium; Age	IISD / University of Manitoba
Endolymph	--	--	8	8	4	9	Selenium	IISD / University of Manitoba
Samples Collected: non-RMP							**Analyses**	**Sample Collection Agency or Archive Location**
Fin Ray	8	19	9	13	--	--	Microchemistry; Age	Agency - USFWS
Otolith	--	--	8	10	--	--	Age	Agency - USFWS
Blood Plasma	6	19	9	12	--	--	Sex Steroid	Agency - USFWS
Ovary - Histology (duplicate sample)	--	--	7	--	--	--	Histology	Agency - USFWS (2016)
Testes - Histology	--	--	--	13	--	9	Histology	Agency - USFWS (2016) OR Archived - SFEI (2017)
Testes	--	--	--	--	--	9	Selenium	Archived - USGS, Menlo Park
Blood Plasma	6	19	9	12	4	9	Selenium	Archived - USGS, Menlo Park (2015) Archived - SFEI (2016-2017)
Gut Contents	5	7	--	--	description only		Diet	Archived - Cramer Fish Sciences

Table 2. Summary of selenium (µg/g dw)

Year	Sample Number	Sex	Min	Max	Median	Mean	Standard Error	Coefficient of Variation	% of samples above threshold
Muscle Plug									**North Bay TMDL: 11.3 µg/g dw**
2015	8	F	6.2	22	8.9	11	1.9	0.48	38%
2016	9	F	3.7	25	10	11	2.5	0.68	44%
2017	13	F and M	2.2	34	12	12	2.7	0.79	54%
Ovary									**USEPA Freshwater Criteria: 15.1 µg/g dw**
2015	8	F	3.5	47	17	20	5.1	0.72	75%
2016	9	F	3.2	34	10	13	3.8	0.87	22%
2017	4	F	6.8	20	14	14	2.8	0.40	50%
Liver									**Stewart et al. 2004: 15 µg/g dw**
2015	8	F	11	74	31	36	6.8	0.53	88%
2016	9	F	7.6	42	15	20	3.6	0.54	56%
2017	13	F and M	5.2	55	18	23	4.8	0.75	54%

References

Beckon, W.N., and T.C. Maurer. 2008. Species at Risk from Selenium Exposure in San Francisco Estuary. Final report to the USEPA. United States Department of the Interior, Fish and Wildlife Service.http://www.waterboards.ca.gov/sanfranciscobay/water_issues/programs/TMDLs/northsfbayselenium/Species_at_risk_FINAL.pdf

California State Water Resources Control Board (CSWRCB). 1987. Selenium verification study, 1986. Sacramento (CA): California State Water Resources Control Board. 79 p + 9 appendices.

California State Water Resources Control Board (CSWRCB). 1988. Selenium verification study, 1986–1987. Sacramento (CA): California State Water Resources Control Board. 60 p + 8 appendices.

California State Water Resources Control Board (CSWRCB). 1989. Selenium verification study, 1987–88. Sacramento (CA): California State Water Resources Control Board. 81 p + 11 appendices

California State Water Resources Control Board (CSWRCB). 1991. Selenium verification study, 1988–1990. Sacramento (CA): California State Water Resources Control Board. 79 p + 9 appendices.

California State Water Resources Control Board (CSWRCB). 2016. Approving an amendment to the Water Quality Control Plan for the San Francisco Bay basin to establish a Total Maximum Daily Load and implementation plan for selenium in North San Francisco Bay. Resolution No. 2016-017.

Collins, M.R., and T.I.U. Smith. 1996. Sturgeon fin ray removal is nondeleterious. *North American Journal of Fisheries Management* 16:939-941

Deng, D., S.S.O. Hung, and S.J. Teh. 2007. Selenium depuration: Residual effects of dietary selenium on Sacramento splittail (*Pogonichthys macrolepidotus*). *Science of the Total Environment* 377: 224-232.

De Riu, N. J. Lee, S.S.Y. Huang, G. Moniello, and S.S.O. Hung. 2014. Effects of dietary selenomethionine on growth performance, tissue burden, and histopathology in green and white sturgeon. *Aquatic Toxicology* 148: 65-73.

Gunderson, D.T., S.C. Zeug, R.B. Bringolf, J. Merz, Z. Jackson, and M.A.H. Webb. 2017. Tissue contaminant burdens in San Francisco Estuary white sturgeon (*Acipenser transmontanus*): Implications for population recovery. *Archives of Environmental Contamination and Toxicology* 73: 334-347.

Kleckner, A.E., E. Kakouros, and A.R. Stewart. 2017. A practical method for the determination of total selenium in environmental samples using isotope dilution-hydride generation-inductively coupled plasma-mass spectrometry. *Limnology and Oceanography: Methods* 15: 363-371.

Lemly, A.D. 1997. A Teratogenic Deformity Index for Evaluating Impacts of Selenium on Fish Populations. *Ecotoxicology and Environmental Safety*. 37: 259-266.

Linares-Casenave, J., R. Linville, J.P. Van Eenennaam, J.B. Muguet, and S.I. Doroshov. 2015a. Selenium tissue burden compartmentalization in resident white sturgeon (*Acipenser transmontanus*) of the San Francisco Bay Delta Estuary. *Environmental Toxicology and Chemistry* 34: 152-160.

Linares-Casenave, J., R. Linville, J.P. Van Eenennaam, J.B. Muguet, and S.I. Doroshov. 2015b. Selenium tissue burden compartmentalization in resident white sturgeon (*Acipenser transmontanus*) of the San Francisco Bay Delta Estuary - Corrigendum. *Environmental Toxicology and Chemistry* 34: 943.

Linville, R. 2006. Effects of excess selenium on health and reproduction of white sturgeon *Acipenser transmontanus*: Implications for San Francisco Bay-Delta. PhD Dissertation. University of California, Davis, CA.

Moyle, P.B. 2002. Inland fishes of California. University of California Press. Berkeley, California

Osmundson, B., and J. Skorupa. 2011. CO-Selenium in Fish Tissue: Prediction Equations for Conversion between Whole Body, Muscle, and Eggs. United States Fish and Wildlife Service, Western Colorado Field Office, Grand Junction, CO. https://catalog.data.gov/dataset/co-selenium-in-fish-tissue-prediction-equations-for-conversion-between-whole-body-muscle-a

Palace, V. et al. *in prep*. xx

Peragón, J., F. Ortega-García, J. B. Barroso, M. de la Higuera and J. A. Lupiáñez. 1992. Alterations in the fractional protein turnover rates in rainbow-trout liver and white muscle caused by an Aminoacid-based diet and changes in the feeding frequency. *Toxicological & Environmental Chemistry* 36:3-4, 217-224. doi: 10.1080/02772249209357844

Peragón, J., J. B. Barroso, L. Garcia-Salguero, M. de la Higuera and J. A. Lupiáñez. 1994. Dietary protein effects on growth and fractional protein synthesis and degradation rates in liver and white muscle of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 124: 35-46.

Presser, T.S., and S.N. Luoma. 2013. Ecosystem-scale selenium model for the San Francisco Bay-Delta Regional Ecosystem: Restoration implementation plan. *San Francisco Estuary and Watershed Science* 11(1): 1-9.

Reash, R., L. Friedrich, and N. Halden. 2014. Selenium bioaccumulation patterns in tissue and otoliths for fish from wastewater exposure and reference sites. Poster at the Society of Environmental Toxicology and Chemistry North America 35th Annual Meeting. Vancouver, BC, Canada. November 9-13, 2014.

RStudio Team (2016). RStudio: Integrated Development for R. RStudio, Inc. Boston, MA. http://www.rstudio.com/.

San Francisco Bay Regional Water Quality Control Board (SFBRWQCB). 2015. Total Maximum Daily Load Selenium in North San Francisco Bay: Staff Report for Proposed Basin Plan Amendment. Report prepared for the California Regional Water Resources Control Board, San Francisco Bay Region, November 2015. San Francisco Bay Regional Water Quality Control Board,

Oakland, CA. http://www.waterboards.ca.gov/ sanfranciscobay/board_info/agendas/2015/November/6_appendix_c.pdf

Stewart, A.R., S.N. Luoma, C.E. Schlekat, M.A. Doblin, and K.A. Hieb. 2004. Food web pathway determines how selenium affects aquatic ecosystems: a San Francisco Bay case study. *Environmental Science and Technology.*. 38: 4519-4526. http://wwwrcamnl.wr.usgs.gov/Selenium/Library_articles/stewart04.pdf

Stewart, A.R., S.N. Luoma, K.A. Elrick, J.L. Carter, and M. van der Wegen. 2013. Influence of estuarine processes of spatiotemporal variation in bioavailable selenium. *Marine Ecology Progress Series* 492: 41-46.

Stewart, A.R., et al., *in prep.*

Sun, J., A. Robinson, and J. Davis. 2016. 2014 RMP Selenium in Sturgeon Muscle Plug Special Study report. SFEI Contribution #774. San Francisco Estuary Institute, Richmond, CA.

Sun, J., J.A. Davis, S.N. Bezalel, J.R.M. Ross, A. Wong, R. Fairey, A. Bonnema, D.B. Crane, R. Grace, R. Mayfield, and J. Hobbs. 2017a. Contaminant Concentrations in Sport Fish from San Francisco Bay, 2014. SFEI Contribution #806. San Francisco Estuary Institute-Aquatic Science Center, Richmond, CA.

Sun, J., A. Robinson, J.A. Davis, P.T. Trowbridge, A.R. Stewart, V. Palace, and Z. Jackson. 2017b. Selenium in White Sturgeon Tissues: 2015 Sturgeon Derby. SFEI Contribution #834. San Francisco Estuary Institute-Aquatic Science Center, Richmond, CA.

Sun, J. J.A. Davis, and A.R. Stewart. 2019. Selenium in Muscle Plugs of White Sturgeon from North San Francisco Bay, 2015-2017. SFEI Contribution #929. San Francisco Estuary Institute, Richmond, CA.

Tashjian D.H., T.S.J. Sogomonyan, and A. Hung. 2006. Bioaccumulation and chronic toxicity of dietary L-selenomethionine in juvenile white sturgeon (*Acipenser transmontanus*). *Aquatic Toxicology* 79: 401–409.

United States Environmental Protection Agency (USEPA). 2016a. Water Quality Standards; Establishment of Revised Numeric Criteria for Selenium in the San Francisco Bay and Delta, State of California. 81 FR 46030. https://www.gpo.gov/fdsys/pkg/FR-2016-07-15/pdf/2016-16266.pdf

United States Environmental Protection Agency (USEPA). 2016b. Aquatic Life Ambient Water Quality Criterion for Selenium - Freshwater 2016. EPA 822-R-16-006. Office of Water, Office of Science and Technology. Washington, D.C. June. https://www.epa.gov/sites/production/ files/2016-07/documents/aquatic_life_awqc_for_selenium_-_freshwater_2016.pdf

Yee, D., A. Franz, A. Wong, J. Ross, and P.T. Trowbridge. 2017. Quality Assurance Program Plan for the Regional Monitoring Program for Water Quality in San Francisco Bay. San Francisco Estuary Institute, Richmond, CA, USA. http://www.sfei.org/documents/2017-quality-assurance-program-plan-regional-monitoring-program-water-quality-san-francisco-bay

Zeug S.C., A. Brodsky, N. Kogut, A.R. Stewart, and J.E. Merz. 2014. Ancient fish and recent invaders: white sturgeon *Acipenser transmontanus* diet response to invasive-species-mediated changes in a benthic prey assemblage. *Marine Ecology Progress* Series 514: 163-174. https://doi.org/10.3354/meps11002

APPENDIX A

Sample ID	Sample Date	Fork Length (cm)	Sex	Selenium (µg/g dw)				Isotope (‰) - Muscle Plugs			Catch Location[1]
				Muscle Fillet	Muscle Plug	Ovary	Liver	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)	
2015											
WHST-03M	1/31/2015	124.5	F	--	7	4.6	30	-27.9	16.8	17.5	Unknown (assumed Suisun Bay)
WHST-06M	1/31/2015	124.1	F	--	22	17	29	-26.7	16.5	17.2	Unknown (assumed Suisun Bay)
WHST-08M	1/31/2015	131.9	F	--	15	32	11	-23	16.6	14.7	Unknown (assumed Suisun Bay)
WHST-09M	1/31/2015	131.4	F	--	15	16	48	-25.3	17	17.2	Unknown (assumed Suisun Bay)
WHST-10M	1/31/2015	112.7	F	--	9.5	3.5	22	-27.2	15	12.4	Unknown (assumed Suisun Bay)
WHST-15M	1/31/2015	127.3	F	--	7.5	17	31	-26	15.4	14	Unknown (assumed Suisun Bay)
WHST-17M	2/1/2015	131.4	F	--	8.3	23	45	-21.7	17.9	16.9	Unknown (assumed Suisun Bay)
WHST-27M	2/1/2015	149.2	F	--	6.2	47	74	-28.8	14.6	15.1	Unknown (assumed Suisun Bay)
2016											
16SD-04	2/6/2016	114.3	F	3.7	3.8	5.5	14	-25.2	15.9	9.9	Suisun Bay - near Montezuma Slough
16SD-06	2/6/2016	148.4	F	19	19	30	42	-24.2	15.6	14.9	Suisun Bay - near Port Chicago
16SD-07	2/6/2016	124.9	F	8.1	12	13	20	-26.2	16.2	14.9	Suisun Bay - near Mothball Fleet
16SD-11	2/6/2016	143.0	F	3.9	3.7	4.0	7.6	-20.8	17.0	14.4	Unknown (assumed Suisun Bay)
16SD-14	2/7/2016	129.2	F	2.9	4.0	4.5	11	-21.7	18.2	9.9	Suisun Bay - Seal Islands
16SD-16	2/7/2016	135.7	F	7.6	7.7	10	15	-25.8	15.3	11.1	Suisun Bay - Grizzly Bay
16SD-17	2/7/2016	147.2	F	12	13	34	26	-24.3	16.0	15.3	Suisun Bay - Montezuma Slough, about 0.5 miles from the mouth
16SD-19	2/7/2016	112.4	F	23	25	14	29	-26.4	16.2	15.1	Suisun Bay
16SD-22	2/7/2016	113.0	F	6.9	10	3.2	15	-26.3	14.0	6.1	Suisun Bay - Mothball Fleet

Sample ID	Sample Date	Fork Length (cm)	Sex	Selenium (µg/g dw)				Isotope (‰) - Muscle Plugs			Catch Location[1]
				Muscle Fillet	Muscle Plug	Ovary	Liver	δ13C (‰)	δ15N (‰)	δ34S (‰)	
2017											
17SD-01	2/4/2017	137.8	M	18	17		29	-25.6	15.8	13.0	Suisun Bay - Grizzly Bay
17SD-02	2/4/2017	146.2	M	3.9	3.3		6.0	-20.5	19.8	14.0	South Bay - Alviso Slough
17SD-03	2/4/2017	150.0	M	3.3	3.0		9.9	-21.6	18.6	15.8	San Pablo Bay - near Sonoma River mouth
17SD-04	2/4/2017	144.2	M	17	14		42	-23.5	16.8	15.0	San Pablo Bay
17SD-05	2/4/2017	131.9	M	2.2	2.2		5.2	-26.1	14.5	2.2	Delta - Prospect Slough
17SD-06	2/4/2017	138.1	M	6.4	6.9		9.3	-27.6	14.3	5.4	Suisun Bay - Grizzly Bay near Ryer Island
17SD-07	2/4/2017	143.5	M	3.0	3.1		8.8	-20.4	18.6	14.0	San Pablo Bay
17SD-08	2/4/2017	148.4	F	19	19	20	35	-23.3	15.9	14.2	Suisun Bay - Carquinez Strait
17SD-09	2/5/2017	141.1	F	6.1	6.8	6.7	18	-22.1	17.2	12.9	Suisun Bay
17SD-10	2/5/2017	143.8	F	12	12	13	49	-25.4	15.4	13.3	Suisun Bay - Grizzly Bay
17SD-11	2/5/2017	129.5	M	32	34		20	-25.4	15.6	13.7	Suisun Bay
17SD-12	2/5/2017	149.1	F	25	27	15	55	-24.9	16.0	15.1	Suisun Bay
17SD-13	2/5/2017	151.6	M	13	12		13	-20.6	16.5	14.6	Suisun Bay - Carquinez Straight, just upstream of Benicia Bridge

1 -- Approximate sturgeon catch location reported by anglers. Exact sampling coordinates were not provided. Locations may not represent long-term sturgeon foraging location.

APPENDIX B
Quality Assurance / Quality Control Summary

Selenium (USGS-Menlo Park)

<u>**2015**</u>

Selenium analyses included 27 field sample analyses (a mix of 2016 derby composite tissues and 2015 muscle plugs reported together), with 3 lab replicates, and 6 certified reference material (CRM) analyses, and 3 laboratory blanks. Overall the field data was 100% reportable.

The method was sufficient so none of the field samples were non-detects. There was no uncorrected blank contamination. Recoveries on the certified reference materials (CRMs) were good, averaging 7% error. Lab replicate precision averaged 13% relative standard deviation (RSD), well within the 35% target, so no added precision flags were needed.

<u>**2016**</u> (analyzed together with samples from the 2015 Muscle Plug Study)

Results were reported for moisture and selenium in fish tissues: 30 field samples for the Muscle Plug Study, 36 for the Derby Study. Lab replicates were reported for selenium in three samples for the Muscle Plug Study and two samples for the Sturgeon Derby Study. Three lab blanks and triplicates for three CRMs were also reported. All (100%) the data were reportable, and no results were rejected. MDLs were sufficient for all samples, with none reported as non-detects.

Results were reported all blank corrected, but the variation in the blank standard deviation was larger than the MDL, so results were all qualified. Results were all still much higher than this variation in blank signal, so results were flagged but not censored. Precision was good on lab replicates, averaging <15% RSD, and recovery on CRM samples was good, averaging within 8% of the target value, so no flags were added for precision or recovery issues.

<u>**2017**</u>

Selenium and moisture were reported for 50 (43 field samples and 7 lab replicates) composite samples, as well as 10 additional non-project composites for QC use. A total of 6 lab blanks, 4 MSs (2 MS/MSD pairs) and 15 CRM results (12 tissue and 3 sediment). All the field data (100%) were reportable.

Overall the data set was acceptable, with 100% detects, no uncorrected blank contamination, variation in precision averaging ~5%, and errors in recovery averaging ~13% or better. Precision was tighter than is typically seen in muscle plugs, likely because this sample set included muscle fillets, which unlike muscle plugs are large enough to be homogenized prior to analysis.

Carbon, Nitrogen, Sulfur Stable Isotopes (UC Davis Stable Isotope Facility)

<u>**2015**</u> (analyzed together with samples from the 2014 Muscle Plug Study)

The 2014 muscle plug and 2015 sturgeon derby isotope data were reported together. The data set included 16 field sample analyses reported for carbon (C) and nitrogen (N), including 1 lab replicate, but only 14 results for S. Two field samples had no S isotopes reported. There were also 4 CRM analyses for C&N isotopes,

along with 30 other lab control results for C&N isotopes interspersed with samples. Sulfur isotopes were reported for only 24 lab control samples. Field data were reportable for all samples.

The method was sufficient so none of the field samples were non-detects. Recoveries were good, averaging within <1 per mil of the target for C & N isotopes in CRMs and laboratory control materials (LCMs; <0.5 per mil difference generally), and slightly higher for sulfur (S) isotopes (maximum of ~1.3 per mil difference). Lab replicate precision was good, with a standard deviation of <1 per mil for field sample replicates on all the isotopes. The $\delta^{13}C$ averaged -25 vs PDB for the field samples, $\delta^{15}N$ about 16 vs air N_2, and around 14 for $\delta^{34}S$ vs CDT. These were similar to RMP 2014 sturgeon, with -21 for $\delta^{13}C$, 19 for $\delta^{15}N$, and 15 for $\delta^{34}S$.

2016 (analyzed together with samples from the 2015 Muscle Plug Study)

The 2015 muscle plug and 2016 sturgeon derby isotope data QC were reported together. The data set included 43 field sample analyses reported for C&N, including lab replicates (but only 40 results for S). There were also 41 analyses of control materials for C&N isotopes, and 8 LCM results for C&N percent masses. Sulfur isotopes were reported for 46 control material samples. Field data were reportable for all samples.

The method was sufficient so none of the field samples were reported as non-detects. Recoveries were good, averaging within 0.2 per mil of the target for C & N isotopes in reference materials, and within 0.5 per mil or better for S isotopes. Lab replicate precision was good, with standard deviations averaging <0.5 per mil for field sample replicates on all the isotopes, although individual pairs sometimes differed by about 1 per mil. The average isotope results appear reasonably similar to the previous years' sturgeon results, with the same isotopes within about 1 per mil between years.

2017

C, N, and S masses and stable isotope distributions were reported for 14 (13 field samples and 1 lab replicate) fish composite samples, with replicate analyses of 4 reference materials (37 analyses for C & N, 50 for S) . All the field data (100%) were reportable. The RMP QAPP has no MQOs for stable isotopes, but generally there is desire/need for variations less than the minimum difference in trophic levels; therefore, about 1 per mil variation within replicates of a sample, or of a reference material, are typically adequate.

Overall the data set was acceptable, with stdev in replicates averaging <1 per mil, and isotopes in reference materials deviating <1 per mil from expected values. For S isotopes, although averages were within 1 per mil of expected values in reference materials, differences for individual replicate analyses sometimes differed by up to 1.6 per mil, so more replicate analyses may be needed if smaller differences need to be distinguished among individual samples.