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Selenium in Muscle Plugs of White Sturgeon from North San Francisco Bay, 2015-2017

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Executive Summary

This report presents the findings from a study evaluating selenium concentrations in white sturgeon (*Acipenser transmontanus*) muscle tissues collected from live sturgeon during the California Department of Fish and Wildlife's fall sturgeon tagging studies in North San Francisco Bay. The goal of this study was to non-lethally collect a large number of sturgeon muscle plugs and analyze them for selenium to (1) establish an understanding of current status, trends, and causes of variability in sturgeon muscle selenium concentrations; and (2) evaluate the potential for long-term sturgeon selenium monitoring with muscle plugs, through development of field and laboratory methods and informing the monitoring design. Monitoring of selenium in white sturgeon is needed to evaluate the effectiveness of the North Bay Selenium Total Maximum Daily Load (TMDL) in protecting sturgeon from selenium toxicity. This technical report provides documentation of the study and presents its major findings in the context of all historically available data on sturgeon muscle selenium concentrations in San Francisco Bay.

Sample collection was conducted through a collaboration with the California Department of Fish and Wildlife (CDFW), which conducts an annual sturgeon tagging survey in August-October in Suisun and San Pablo Bays to track trends in the population. The CDFW effort presents a unique, long-term opportunity to non-lethally collect many sturgeon muscle tissue samples for selenium analysis. Through this collaboration, 30 samples in 2015, 38 in 2016, and 58 in 2017 were collected and analyzed for selenium. The present study established monitoring in collaboration with CDFW as a feasible, cost-effective, and minimally invasive method for collecting sturgeon muscle tissue samples for monitoring sturgeon selenium concentrations.

The Muscle Plug Study presented an opportunity not only to establish a 3-year baseline of current adult (>105 cm total length) sturgeon selenium concentrations, but also to evaluate the effect of annual hydrology on sturgeon selenium concentrations. Compared to previous measurements in the Bay, selenium concentrations were elevated in adult sturgeon muscle in 2015 (mean = 11.8 µg/g dw, standard error ± 1.3 µg/g dw), a critically dry water year, and slightly lower but still elevated in 2016, a below normal water year (mean = 10.6 µg/g dw, standard error ± 0.9 µg/g dw). In contrast, concentrations in 2017, a wet water year, were statistically significantly lower (mean = 7.3 µg/g dw, standard error ± 0.4 µg/g dw) than in either 2015 or 2016. This suggests that the elevated sturgeon selenium concentrations observed between fall 2015 and spring 2017 in the present study and a companion study (the Sturgeon Derby Study – Sun *et al.* 2018) were driven largely by hydrology rather than changes in selenium sources or water column concentrations.

Hydrology, fish length, and several additional potential causes of sturgeon muscle selenium variability were further analyzed using all available historical data for San Francisco Bay. These included biological factors (fish length or age, sex, and reproductive stage) and environmental factors that affect dietary selenium, including annual and seasonal hydrology (freshwater inflow from the Delta, assessed using water year type and month of sampling) and

estimated foraging location (approximated using fish capture location and isotope data). Analyses of these individual factors were then used to inform both statistical analyses of long-term trends and the long-term monitoring design for sturgeon muscle selenium.

The results of the larger analysis indicate that annual hydrology, fish length, and foraging location are significant contributors to variability in sturgeon muscle selenium concentrations. Water year type could be evaluated only with the results from this study – the only one with a consistent sampling design spanning multiple water year types. Juveniles (< 105 cm total length) were found to have significantly lower selenium concentrations than adults, but no significant relationship between length and selenium concentrations was found among adults. In contrast, sex was not found to be a significant driver of selenium concentrations. Additionally, available data do not suggest a strong effect of either reproductive stage or season on sturgeon selenium concentrations, but data were too limited and sparsely distributed to enable a robust statistical evaluation of the effect. Elevated selenium concentrations were found in North Bay compared to the Delta or other regions of the Bay.

The results of these analyses suggest that future monitoring should focus only on adults, target an equal distribution of lengths across the target size range (115-181 cm total length), and does not need to examine sex or reproductive stage through blood plasma sex steroid analyses. Several factors cannot be controlled (annual hydrology) in the future monitoring design, or are fixed based on the CDFW monitoring design (fall monitoring; capture location in the North Bay). However, the significant influence of annual hydrology on sturgeon muscle selenium concentrations indicates that water year type should be included as a covariate in future long-term trend analyses.

Mixed effects models were used to control for individual or interacting effects of these drivers on sturgeon muscle selenium concentrations while analyzing for long-term selenium trends in sturgeon muscle selenium. The most parsimonious model indicated a significant declining trend in selenium between 1986 and 2017. However, the long-term trend was weak and the robustness of this analysis is limited by the sparse data available to run these models. As a result, additional data may change model results and the conclusions of this analysis.

Overall, this study successfully established an approach for long-term sturgeon muscle plug monitoring and a current baseline of selenium concentrations against which long-term trends can be evaluated. Data show that selenium concentrations have occasionally been elevated in recent years compared to previous measurements in the Bay, including one annual mean concentration above the TMDL numeric target; however, trend analyses do not suggest long-term increases in concentrations. Continued long-term monitoring of sturgeon muscle plugs, using a consistent monitoring design informed by the results of this pilot study (Grieb *et al.* 2018), will ultimately provide valuable information on long-term trends in selenium concentrations in white sturgeon in the North Bay, as well as on the effectiveness of the North Bay Selenium TMDL.

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*), particularly in North San Francisco Bay. 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 occurs naturally 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 runoff 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 (SFRWQCB) 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 dry weight [dw], respectively), which were subsequently adopted as numeric targets for the North Bay in the Basin Plan. 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 US Environmental Protection Agency (USEPA) also released draft revised Clean Water Act criteria for fish tissue in the entire San Francisco Bay-Delta (USEPA 2016c). 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 monitoring implementation guidance for USEPA's freshwater selenium criteria recommends using a t-test to compare the mean of all fish tissue data for a single species to the fish criteria (USEPA 2016b). The draft implementation guidelines 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 criteria 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 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 indicate that this invasive clam species is up to ten 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 the North Bay, which hosts a large population of overbite clam. Attainment of the TMDL target in white sturgeon is expected to be protective of other species in the Bay as well, include green sturgeon (*Acipenser medirostris*), which are currently listed as a threatened species.

To support implementation of the TMDL, the Selenium Workgroup of the Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) has been developing a monitoring method that will allow for the routine collection of large numbers of white sturgeon muscle tissue samples. During RMP Status and Trends sport fish sampling in 2009 and 2014, and the 2016 and 2017 RMP Sturgeon Derby special study, paired muscle plug and fillet samples were analyzed for selenium as part of an effort to establish a non-lethal and efficient method of collecting sturgeon muscle tissue. Results from these studies show that muscle plug and muscle fillet selenium are strongly correlated, indicating that muscle plugs can be used as proxies for muscle fillets to monitor selenium in sturgeon muscle tissue (Sun *et al.* 2017a,b; Sun *et al.* 2018).

The RMP piloted this muscle plug monitoring method on live white sturgeon in 2014 through collaboration with the California Department of Fish and Wildlife (CDFW), which collected samples for the RMP *pro-bono* during its annual sturgeon population tagging survey in North Bay (Sun *et al.* 2016). This pilot study identified several challenges in field sample collection (i.e., staff capacity and sample storage) and laboratory sample processing (i.e., sample storage, skin removal). The purpose of the present study was to non-lethally collect and analyze a large number of sturgeon muscle plugs for selenium, address the logistical challenges in sampling and analysis, establish a baseline of current sturgeon muscle selenium concentrations, and further assess this opportunity to conduct long-term sturgeon selenium monitoring. This study addressed several objectives:

1. evaluate the current status and long-term trends in sturgeon muscle selenium concentrations;
2. assess factors influencing variability in sturgeon muscle selenium concentrations to constrain variability in future monitoring designs and statistical analyses;
3. pilot the muscle plug monitoring and selenium analysis methods; and
4. inform the long-term monitoring design.

This report presents results from the 2015-2017 Muscle Plug Study and evaluates the current status and trends in sturgeon muscle selenium in the context of all available sturgeon muscle selenium data collected in San Francisco Bay. These analyses then provide the basis for recommendations for the development of a long-term monitoring plan and statistical analyses for tracking long-term trends in sturgeon selenium.

Methods

Field Sample Collection

Sturgeon tissue samples were collected through a *pro bono* collaboration with the CDFW, which conducts an annual sturgeon tagging survey in North Bay each August-October. While the CDFW survey includes both San Pablo and Suisun Bay, most sampling in recent years has focused in Suisun Bay (Figure 1). The survey does not include a spatially distributed screen of North Bay, but rather focuses on areas where catch is expected to be the greatest to allow the most fish to be tagged.

The RMP's target study design aimed to collect tissue samples from 60 adult sturgeon, equally distributed across each 10-cm size increment between 100 and 160 cm fork length (equivalent to a range of 115-181 cm total length). Sturgeon smaller than 100 cm in fork length were smaller than the sturgeon slot limit (40-60 in) and avoided when possible. In 2015 and 2016, US Fish and Wildlife Service (USFWS) staff collected muscle plug (selenium analyses) and blood plasma (sex steroid analyses) *pro bono* for the RMP, along with fin ray samples collected for a concurrent USFWS study for age estimation and microchemistry analyses. In 2016, RMP funds were not allocated for sample collection, but the CDFW and USFWS offered to collect samples later in the season. As a result, a smaller sample number and size range was sampled: 40% of the 38 sturgeon sampled were smaller than 100 cm fork length. In 2017, following the conclusion of the USFWS study, CDFW staff began collecting muscle plug samples for the RMP; due to staffing limitations, however, blood plasma and fin ray samples were no longer collected.

Two or three muscle plug samples were collected from each fish using a disposable 5-mm biopsy punch. Samples were collected through the skin from the epaxial muscle, just behind the dorsal fin and just offset from the midline, and stored chilled in a 2-mL cryovial with the skin on until the end of each sampling day when samples were frozen until processing. Blood plasma samples were drawn using a syringe and sealed vacutainer from a blood vessel just behind the anal fin. Whole blood samples were stored on wet ice and centrifuged at the end of each sampling day, at which time the blood plasma was drawn off into microcentrifuge tubes and stored frozen until analyzed. Lastly, small fin ray clips were collected using hand shears. Fin rays were dried under a fume hood and stored dried at room temperature.

The target study design and tissue collection methods are described further in the 2015 Sturgeon Muscle Plug Study Sampling and Analysis Plan (Appendix C). The full CDFW field sampling effort – including description of sampling methods, summary of concurrent non-RMP studies, summaries of the sturgeon surveyed, and maps of sampling locations – is further described in the CDFW 2015-2017 Field Season Summary reports for the Sturgeon Population Study (DuBois and Harris, 2015; DuBois and Harris, 2016; DuBois and Danos 2017).

Muscle Plug Sample Processing

Muscle plug samples with the skin on were stored chilled in the field and frozen in a commercial freezer at the end of each sampling day. In 2015, samples were stored frozen at a USFWS facility until the end of the field season, when they were transported to the analytical laboratory at USGS-Menlo Park and stored at -80 ºC until sample processing and analysis. In 2016, samples were transported to a commercial freezer at SFEI at the end of the field season, where they were stored until the end of the 2017 field season, and then transferred to USGS-Menlo Park. All samples from 2015 and 2016 were processed by USGS before digestion for selenium analyses. Skin was removed while the muscle plugs were still frozen, using a sharp, clean scalpel, which was used to remove the black skin disc along with about 2 mm of additional tissue below the skin. The sample vial weight was measured; the remaining muscle tissue was then returned to its sample vial and reweighed to obtain a wet tissue weight. Samples were subsequently freeze dried in preparation for analysis. This method was also used for the RMP 2014 Muscle Plug Study samples.

In 2017, samples were stored in a commercial freezer at the end of each field sampling day. On the last sampling day each week, frozen samples were brought back onto the sampling boat and transferred to SFEI at the end of the day with newly collected samples. Because all samples were thawed by the end of the day, thawed samples were then immediately processed that same day at SFEI. The skin disc, and in some cases a lipid layer immediately underneath the skin, were removed with dissection scissors by visual inspection of the plugs. Muscle tissue, skin, and lipid were differentiated by color (skin is black) and texture (lipid is more opaque than muscle tissue) when possible. When no lipid layer was apparent, the skin and muscle tissue were separated about 1-2 mm below the skin tissue; when lipid appeared to be present not in a layer but mixed with muscle tissue, it was not removed to preserve muscle tissue for analysis. A wet tissue weight was recorded. The skin-off tissue samples were then frozen in a commercial freezer until the end of the sampling season, when all samples were transported to USGS-Menlo Park and stored at -80ºC before the samples were re-weighed and freeze dried. Similar methods were used for the RMP 2015-2017 Sturgeon Derby Study muscle plug samples, which were processed in the field by RMP staff before freeze-drying and further processing in the laboratory.

Laboratory Analysis and QA/QC

Selenium

After muscle plugs (skin-off) were freeze-dried, a subsample was 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 by Kleckner *et al.* (2017). Due to the small sample masses, samples were not homogenized before digestion; instead, whole freeze-dried plugs were directly subsampled using a clean scalpel blade. The relationships between Se concentrations determined as muscle fillets (large masses

(grams) of homogenized muscle tissue) and muscle plugs (small masses (milligrams) of unhomogenized muscle tissue) for the same individual fish show excellent agreement for the fillet and muscle plug approaches with R^2 ranging from 0.96-0.99 for different sample years.

The 2015 samples were run in a single lab batch, and the 2016-2017 samples were run together across three lab batches. At least three method blanks and three replicates each of two different certified reference materials (CRMs) were run with each lab batch. Laboratory replicates were run at a minimum frequency of one for every ten field samples, except for the first 2016-2017 sample lab batch, during which no replicates were run. Matrix spike and matrix spike duplicates of either a CRM or field sample were run with each 2016-2017 lab batch. Accuracy was evaluated using CRMs with certified values for Se; precision was analyzed using duplicate samples, and selenium recovery was evaluated using the matrix spike samples. Selenium results are reported as blank-corrected dry-weight concentrations.

All samples analyzed met RMP QA/QC standards (Yee *et al.* 2017) in terms of procedural blanks, recovery of CRMs and duplicate measurements. For duplicates and rerun samples, the first rerun sample result was reported for the 2015 samples.

The 2016 and 2017 samples were analyzed together in three lab batches. No laboratory replicates were run in the first batch, so the second lab batch included both between-batch replicates (run in both the first and second batch) and within-batch replicates (run twice within the second lab batch). For two samples, results across the first two lab batches failed QA/QC precision standards (although duplicates of these samples run within the second batch did meet QA/QC precision standards), prompting a third analytical run with further between- and within-batch replicates.

In the final dataset, after three sample runs, eight samples (one collected in 2016; seven collected in 2017) were flagged by the analytical laboratory for failing QA/QC precision standards. It is not clear what caused variability among duplicates, but one possible cause may have been heterogeneous distribution of lipid and muscle tissue within these plugs; sample processing notes also indicate that several of these samples appeared to include substantial lipid that could not be separated from the muscle tissue. However, because confirmation of the lipid content in the samples analyzed was not possible, none of the flagged results were rejected.

For three other samples collected in 2017, results for several replicates were rejected based on sample processing notes indicating that the tissue analyzed appeared to have high lipid content and was not representative of muscle tissue. These rejects resulted in no reported values for two samples and a single result reported from the first lab batch for the third sample (17MP-WST-ST-21). For all 2016-2017 samples, selenium concentrations presented in this report are averages of all results reported by the laboratory, except for rejected results. Results were first averaged between duplicates in the same lab batch, and subsequently among averages for all lab batches. Further discussion of the QA/QC results is presented in Appendix B.

Isotopes

Carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) 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 (http://stableisotopefacility.ucdavis.edu/ 13cand15n.html; http://stableisotopefacility.ucdavis.edu/34s.html). $\delta^{13}C$ and $\delta^{15}N$ isotopes were run concurrently, while $\delta^{34}S$ isotopes were run separately. At least one lab replicate was run for each isotope in each lab batch, except for $\delta^{34}S$ isotopes in 2015, when not enough sample mass was available for a $\delta^{34}S$ 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.

The RMP QAPP has no measurement quality objectives for stable isotopes, but ideally results should vary less than the minimum difference observed between trophic levels; therefore, about 1‰ variation within replicates of a sample or of a reference material are typically adequate. Results in all lab batches met this standard, with the exception of $\delta^{34}S$ measured in 2017, which were flagged for variable precision. Detailed QA/QC results are presented in Appendix B.

Sex Steroids

Testosterone (T) and estradiol (E2) were extracted from blood plasma and measured by the USFWS Bozeman Fish Technology Center, following Fitzpatrick *et al.* (1986, 1987) and Feist *et al.* (1990). All samples were run in duplicate and reported as an average of the two results. Duplicate results with greater than 10% difference were rejected and samples were rerun. The lower limit of detection was 0.10 ng/mL for T and 0.16 ng/mL for E2.

The sex and reproductive stage of each fish were predicted based on T and E2 cutoff values established by the Webb Lab (Table 1). Most fish were categorized as non-reproductive females or non-reproductive males. However, the error rate for detecting the difference between non-reproductive males and females can be high, and the laboratory often does not differentiate between sexes for non-reproductive fish (Webb *et al.* 2002; Molly Webb, personal communication; USFWS, unpublished data from 2016 Sturgeon Derby). The error rate in assigning sex and reproductive stage to reproductively mature males and females is much lower, < 5% (Webb *et al*. 2002; Molly Webb, personal communication).

Method Development

The 2015-2017 monitoring and continued collaboration with CDFW further demonstrated the viability of the non-lethal muscle plug monitoring method piloted in 2014. In 2017, CDFW staff could collect samples directly for the RMP without USFWS staff assistance,

further establishing the CDFW sturgeon surveys as a potential continuing opportunity for long-term monitoring of selenium in sturgeon tissue. Muscle plug samples were successfully collected from many sturgeon with sufficient mass for selenium analyses. In 2015, all samples had sufficient mass for $\delta^{13}C$ and $\delta^{15}N$ analyses, and all but one had sufficient mass for $\delta^{34}S$ as well; in 2016-2017, 79 of 96 samples had sufficient mass for $\delta^{13}C$ and $\delta^{15}N$ analyses, and 78 had sufficient mass for $\delta^{34}S$ analyses. Additional work conducted as part of the 2015-2017 Sturgeon Derby Study and prior RMP Status and Trends monitoring efforts have also established muscle plugs as good proxies for muscle fillet selenium concentrations (Sun *et al.* 2018).

However, substantial variability observed in the 2016-2017 selenium results indicated that further sample processing method development is needed. Notably, the same selenium analysis methods have been used for all muscle plugs analyzed by USGS-Menlo Park since 2014. In prior sample sets, duplicates subsampled from non-homogenized muscle plugs were consistent and met measurement quality objectives (RPD < 35%). Therefore, the variability observed in the 2016 and 2017 samples was unexpected, particularly given that the 2015 and 2016 samples were processed using identical methods at USGS-Menlo Park.

Further development is needed to ensure a consistent methodology for muscle plug skin and lipid removal during sample processing. Improved methods could be also be explored to remove lipid from muscle plugs and/or homogenize plug tissue before analysis. Laboratory or method inter-comparisons conducted on true laboratory replicates will not be possible given the small sample masses of plug samples; however, comparisons could be conducted using field replicates sampled from sturgeon fillets collected during the 2019 RMP Status and Trends study or from tissue archived in previous years.

Data Analyses

Statistical Analyses

The combined dataset indicated that selenium concentrations in sturgeon muscle tissue are log-normally distributed. Statistics were conducted using parametric methods on data that were log-transformed to meet assumptions of normality and heterogeneity of variances. In some cases, unequal sample group sizes and unequal variances required the use of alternative statistical tests as described in the text.

Data Classification

Water Year Type – Water years were classified as Wet, Above Normal, Below Normal, Dry, or Critical based on the classification index used by the California Department of Water Resources to characterize conditions in the Sacramento and San Joaquin Rivers. In this report, a 6-month time lag is used to classify sturgeon muscle tissue selenium concentrations with water year types, based on an estimated 6-month time lag between selenium in the water column and

sturgeon muscle tissues (Beckon 2016). For further discussion, see "Hydrology" section, "Water Year Type" subsection in the Results and Discussion.

Fish Length Size Classes – Estimates of the approximate age and length of maturation in white sturgeon are variable; for the Sacramento-San Joaquin population, females are estimated to mature around 12-16 years or 95-135 cm fork length, and males at around 10-12 years or 75-105 cm fork length (Moyle 2002). Linares-Casenave *et al.* (2015a,b) delineated three size classes in their 2015 study, with 105 cm total length as the cutoff between the first and second size classes (roughly, juveniles and small adults). In this report, 105 cm total length is used to distinguish between juveniles and adults. When total length data were not available, the following regression was used to estimate total length, calculated from all available studies with both total and fork length data reported (RMP S&T 2014, RMP Muscle Plug Study [2015-2016 only], Linares-Casenave *et al.* 2015a,b):

Total Length = (1.10 x Fork Length) + 4.50 ($R^2 = 0.99$, p = <0.0001)

The target design for the current study, as previously described, aimed to collect tissue samples from 60 adult sturgeon, equally distributed across each 10-cm increment between 100 and 160 cm fork length. This design was developed to target sturgeon primarily within the sport fish regulation slot limit (40-60 inches fork length, or approximately 102-152 cm fork length). In the current study, both fork length and total length were measured in 2015 and 2016; in these years, all sturgeon within the target fish length range (100-160 cm fork length) would also be considered adults based on the 105 cm total length cutoff. For consistency and simplification of the sampling protocol, it is recommended that future monitoring continue targeting sturgeon within this slot limit.

Foraging Location – In this report, foraging location was primarily approximated using the location the sturgeon were captured, as these data were easy to collect and available for most samples collected in previous studies. Additionally, in the current study, available $\delta^{13}C$ isotope data could be compared to carbon isotopes in *Potamocorbula amurensis* collected at multiple fixed locations in North Bay (Robin Stewart, USGS, unpublished data) to further assess whether the sturgeon were likely to have been foraging in the regions where they were captured. A comparison of $\delta^{15}N$ in sturgeon muscle tissue and *Potamocorbula* over the same period suggests that (Robin Stewart, USGS, unpublished data), as expected, bivalves were a major component of the diet of the sturgeon sampled in this study, and therefore a comparison of carbon and sulfur isotopes in sturgeon and *Potamocorbula* can provide a useful estimate of foraging location.

The majority of fish in the current study were sampled within North Bay, and $\delta^{13}C$ and $\delta^{34}S$ measured in muscle plugs suggest that most sturgeon collected during this study were likely foraging predominantly in North Bay (see the subsection "Foraging Area"). In 2017 eight fish were caught upstream of Honker Bay; however the $\delta^{13}C$ and $\delta^{34}S$ do not provide strong evidence that they were predominantly foraging outside of North Bay. Thus, all sturgeon collected in the

current study were considered to have been foraging in North Bay, including those that were captured outside of North Bay.

Results and Discussion

Muscle selenium concentrations were measured in 30 sturgeon in 2015, 38 in 2016, and 58 in 2017. Selenium concentration ranges, means, medians, variances, and percentages of all adult sturgeon samples exceeding the TMDL numeric target for each year are presented in Table 2. Thirteen samples from 2016 and five from 2014 were from juveniles (≤ 105 cm TL) and are not included in the table. Samples from an additional 28 sturgeon collected in 2015 are archived at -80 ºC at USGS-Menlo Park. Selenium concentrations and $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ isotope ratios for individual samples are presented in Appendix A and are available through the Contaminant Data Display and Download tool (CD3, www.sfei.org/cd3) as well as the California Environmental Data Exchange Network (CEDEN, http://ceden.waterboards.ca.gov/AdvancedQueryTool).

Dataset Summary and Comparison to TMDL Target

Selenium concentrations in adult sturgeon were variable and log-normally distributed, with coefficients of variation ranging from 43% to 62% across years from 2015-2017 (Table 2; Figures 2-4). Mean and median selenium concentrations in adult sturgeon decreased each year between 2015 and 2017. The mean concentration in 2015 (11.8 µg/g dw, standard error ± 1.3 µg/g dw) was above the TMDL numeric target (11.3 µg/g dw) while the median concentration was just below it (10.9 µg/g dw), and 47% of individual samples exceeded the target. Concentrations in 2016 were slightly lower, with the median slightly higher than the mean selenium concentration (median=11.0 µg/g dw, mean=10.6 µg/g dw, standard error ± 0.9 µg/g dw, 44% exceedances). In contrast, concentrations measured in 2017 were significantly lower (median = 6.8 µg/g dw, mean = 7.3 µg/g dw, standard error ± 0.4 µg/g dw, 12% exceedances; Welch's one-way ANOVA, $F(2,52.7) = 7.6$, $p = 1.3 \times 10^{-3}$; Games-Howell post-hoc test, 2015 vs 2017: $p = 0.04$; 2016 vs 2017: $p = 2.9 \times 10^{-3}$).

The sturgeon sampled in 2016 included a substantial number of juveniles (smaller than 105 cm total length). When results from juveniles were included, the mean and median concentrations for 2016 were substantially lower (median = 7.6 µg/g dw; mean = 8.8 µg/g dw; 29% above the target).

Sources of Variability

Several factors contributed to inter- and intra-annual variability in observed selenium concentrations (Figures 4-5). In this section, six factors that have been measured in previous sturgeon selenium studies are evaluated, including biological factors (fish length or age, sex, and reproductive stage) and environmental factors that affect dietary selenium (annual and seasonal

hydrology [freshwater inflow from the Delta, assessed using water year type and month of sampling] and estimated foraging location [approximated using fish capture location and isotope data]). Understanding these factors influencing muscle tissue selenium will inform the following two objectives:

1. evaluating long-term trends – determining what contributors to variability should be controlled for in regression analyses, or used to exclude data that are not comparable to most of the historical dataset; and
2. developing a long-term monitoring design – assessing how to constrain or eliminate sources of variability in the monitoring design that may confound the detection of long-term trends (e.g., focusing on a specific fish length or season).

These factors are analyzed in the context of the 2015-2017 Muscle Plug Study data, as well as all historically available data on selenium in sturgeon muscle from the Bay. A summary of the key findings and their implications for the two objectives is presented in Table 3. Linear regression analyses were also used to conduct a more robust analysis of the interactive effects of key causes of variability in sturgeon selenium concentrations ("Long-Term Trend Analysis" section).

Fish Length or Age and Maturity

While age is relatively difficult to measure and frequently not reported with fish contaminant data, length data are easily collected and reported, and sturgeon age and length are correlated (Linares-Casenave *et al.* 2015a,b; Brennan and Cailliet 1989). In this report, length is used as a proxy for fish age and maturity (i.e., juvenile or adult). Selenium generally is not known to reach higher concentrations with increasing age. However, higher muscle tissue selenium concentrations in larger sturgeon size classes were reported by Linares-Casenave *et al.* (2015a,b), who postulated this could be due to differences in foraging behavior between juvenile and adult sturgeon.

In 2016, significantly lower selenium concentrations were found in juveniles (≤ 105 cm total length) compared to adults (Welch's t-test, $p = 3.5 \times 10^{-4}$), consistent with the findings reported by Linares-Casenave *et al.* (2015a, b). Although the present study targeted adult fish, 35% of samples (13 of 38 samples) collected in 2016 were from juveniles because sampling was not coordinated by the RMP that year. In 2015 and 2017, no fish smaller than the slot limit were sampled.

Among adults, no consistent correlation was found between fish length and selenium concentration. No significant relationship between fork length and selenium concentration was found for adults in 2016 and 2017 (linear regression, 2016: $p = 0.93$; 2017: $p = 0.87$; fork length was used here because total length was not directly measured in 2017). These results are consistent with data from Linares-Casenave *et al.* (2015a,b) in which no significant relationship was found among adult size classes ($p = 0.50$). In 2015, there was a negative relationship between selenium concentration and fish size, with the highest concentrations in the smaller

adults (linear regression, $p = 2.5 \times 10^{-3}$, $R^2 = 0.26$). Reanalyzing the data in the same manner as Linares-Casenave *et al.* (2015a,b), who compared two adult size classes (106-150 cm TL and >150 cm TL), showed similar relationships between selenium concentration and fish size by year (Welch's t-test, 2015: $p = 2.7 \times 10^{-3}$, 2016: 0.63, 2017: 0.87, Linares-Casenave *et al.* 2015a,b: $p = 0.13$). A similar negative relationship between fish length and muscle selenium concentrations was found in adults measured in the Selenium Verification Study (SVS; Figure 6; CSWRCB 1987, 1988, 1989, 1991). That study included some of the highest selenium concentrations in the entire Bay dataset, and was conducted during critically dry and dry water years, suggesting a potential interaction between water year type or flow and fish length (Figure 7).

These results indicate that fish length and sturgeon muscle tissue selenium are related. Lower concentrations were found in juveniles compared to adults; among adults, no clear linear relationship was observed, although there may be an interaction between annual hydrology and fish length. This suggests that long-term selenium trend monitoring should focus on adults, and may not need to control for size among adults. Interactions between the effect of length and other environmental factors on selenium concentrations in adults can be further explored using regression models ("Long-Term Trend Analysis" section). Future long-term monitoring should continue to focus on adults, distributed across the target size range (100-160 cm fork length, or 115-181 cm total length) when possible, to obtain further information and support efforts to better control for any effects of sturgeon age or length on selenium concentrations.

Sex and Reproductive Stage

Differences in tissue selenium levels among sturgeon of different sexes and reproductive stages are important to consider, given that the primary mechanism of selenium impairment is maternal transfer to vitellogenin and egg yolk proteins. Vitellogenic, or pre-spawning, females (called "reproductive" in this report) are therefore a particularly sensitive population. White sturgeon are iteroparous, spawning every two to four years (Chapman *et al.* 1996), so only a subset of the mature females are reproductive in any one year. The spawning season predominantly occurs in March and April, though spawning females have been found between February and May (Doroshov *et al.* 1994, Kohlhorst 1976. While selenium concentrations in ovary and liver tissues can be expected to be higher in vitellogenic females, given the incorporation of selenium into vitellogenin proteins, the linkage between vitellogenesis and selenium in muscle tissue is less clearly established. However, Linares-Casenave *et al.* (2015a,b) found significantly higher selenium concentrations in the muscle tissue as well as liver and ovaries in vitellogenic compared to pre-vitellogenic sturgeon. No significant overall differences between males and females were observed by Linares-Casenave *et al.* To further examine these relationships, sex and reproductive stage were assessed in the present study in 2015 and 2016 using testosterone and estradiol levels measured in blood plasma (Methods section, above; Sex section, below).

Sex

In this study, males and females were relatively evenly represented, including 15 females and 15 males in 2015, and 17 females and 21 males in 2016. However, the blood hormones indicated that the vast majority of sturgeon measured in this study were non-reproductive (25 of 30 sampled in 2015 and all 38 sampled in 2016), and the prediction error rate between non-reproductive males and females is considered high (Webb *et al.* personal communication; Methods section). Therefore, evaluating the effect of sex on sturgeon muscle selenium in this study may not be reliable. Lethal sampling and direct sex identification based on gonads can reliably evaluate this effect, but is inconsistent with the goals of the non-lethal recommended sampling program.

No statistically significant differences were found between sexes in either the current study or previous studies conducted in the Bay (Welch's t-test, Table 4, Figure 8). The sample sizes in most prior studies involving lethal sample collection (i.e., more reliable sex identification) are small (Linares-Casenave *et al.* 2015a,b: n = 47; RMP Status and Trends 2003-2014, n = 7 to n = 12 per year), but thus far the data provide no evidence that muscle selenium differs between males and females. Therefore, any selenium trends observed in the population overall should reflect trends in female sturgeon as well.

Reproductive stage

Limited data from reproductive sturgeon are available from either the current study or previous studies to further evaluate the higher concentrations in reproductive females observed by Linares-Casenave *et al.* (2015a,b). The error rate for correctly identifying reproductive sturgeon is considered low (Molly Webb *personal communication*; Methods section); however, few reproductive sturgeon, particularly reproductive females, were identified in this study. Only two of 15 females (and 3 of 15 males) sampled in 2015 and none of the 17 females (and 3 of 21 males) sampled in 2016 were predicted to be at a mature reproductive stage. Sex steroids were not analyzed and reproductive stage was not predicted in 2017.

The two female sturgeon predicted to be vitellogenic in 2015 had low selenium concentrations (2.3 and 5.9 µg/g dw), contrary to expectations based on the Linares-Casenave *et al.* (2015a,b) study. Historically, sturgeon with the highest selenium concentrations have been collected in North Bay, suggesting higher dietary selenium levels in this region ("Foraging Area" subsection). Isotope results for these two sturgeon are inconclusive as to whether they were feeding predominantly on clams within North Bay. $\delta^{15}N$ for both sturgeon (15.18‰ and 16.09‰) are consistent with those expected for sturgeon feeding predominantly on *Potamocorbula* in Suisun Bay (Robin Stewart, *unpublished data*). However, low $\delta^{13}C$ in the first sturgeon (-27.12‰) suggests that it was feeding in the Delta. A particularly low $\delta^{34}S$ in the second sturgeon (7.8‰) also suggests it had been feeding predominantly outside of Suisun Bay, although the $\delta^{13}C$ in this sturgeon (-26.35‰) does not strongly support that hypothesis. It is possible that lower dietary selenium levels due to different foraging locations caused lower concentrations in these sturgeon than we would expect for vitellogenic females foraging in North

Bay. However, based on data from only two fish it is not possible to conclusively identify the potential cause of the low selenium concentrations observed in these two vitellogenic females, and whether these low fish are representative of the larger population of vitellogenic females foraging in North Bay in the fall.

Although results from RMP studies, including the current study, are inconsistent with the Linares-Casenave *et al.* (2015a,b) finding of higher muscle selenium in vitellogenic females, they are based on too few samples to clearly contradict it. Our analysis suggests, however, that dietary selenium levels have a greater impact on sturgeon muscle selenium concentrations than reproductive stage. Dietary selenium levels can be affected by a variety of factors, including feeding location or annual and seasonal hydrologic patterns affecting dilution of selenium inputs and hydrologic residence time (Presser and Luoma 2013, Stewart *et al.* 2013). It is possible that the effect observed by Linares-Casenave *et al.* (2015a,b) would be more easily observable in December-May when sturgeon tissues would be expected to have higher concentrations based on long-term monitoring of seasonal patterns in clam selenium concentrations (Stewart *et al.* 2013). Assuming an approximate 2-3 month lag between clam and sturgeon tissue selenium (Sun *et al.* 2018, Beckon 2016, Stewart unpublished data), sturgeon tissue in January and February would reflect clam selenium concentrations in October-December. Sturgeon tissues during the period of the current study (September-October), in contrast, would reflect clam concentrations in June-August, which are generally lower (Stewart *et al.* 2013). Notably, all of the vitellogenic females sampled by Linares-Casenave *et al.* were sampled between March and May, when sturgeon tissue selenium reflects a period (December-March) when clam selenium would be expected to be elevated, and this could have influenced the concentrations observed in those sturgeon.

It is notable that even in the 2016 RMP Sturgeon Derby Study, which was conducted in late January-early February, few reproductive females were found (2 of 9 females sampled), and they did not show elevated concentrations in muscle tissue (or ovary and liver tissue) compared to the non-reproductive females (Figure 6). However, the sample size for this study was small, and reproductive stage was estimated using sex steroids, rather than being confirmed with histology analyses.

From a toxicological perspective, it would be valuable to continue evaluating selenium concentrations in vitellogenic sturgeon specifically, to understand risks to this particularly sensitive population. Our current results are still inconclusive about the relative risks to the vitellogenic individuals compared to the overall sturgeon population, particularly during different seasons or water years. Further measurement of vitellogenic sturgeon leading up to and during the spawning season would allow for a better evaluation of this relationship. Additional monitoring of female sturgeon across seasons within similar water years - particularly wet years, during which we have the least data on female sturgeon - would also be necessary to better understand the relationship between reproductive stage and seasonal hydrology on muscle selenium concentrations.

From a regulatory perspective, however, it is not essential to focus additional study or future monitoring specifically on the sensitive vitellogenic female population, according to the draft USEPA monitoring guidance for the implementation of the 2016 USEPA ambient water quality criteria for selenium in freshwaters (USEPA 2016a,b). The current study also showed that few vitellogenic females are likely to be sampled during the fall, when long-term monitoring is proposed to occur. This matches expectations, given that this sampling period is about half a year before the spawning season. Therefore, any effect of reproductive stage on muscle selenium in vitellogenic sturgeon, if present, is unlikely to substantially contribute to variability observed during fall sturgeon monitoring. Furthermore, if future muscle plug monitoring continues through the current collaboration with CDFW, collecting blood plasma samples may also be logistically challenging due to limited staff capacity.

Therefore, based on the current information available, it does not seem necessary to continue monitoring sex steroids during long-term September-October muscle plug sampling. Additionally, given the limited data on reproductive stage available in previous studies, and the expectation that few vitellogenic females will be represented in future sampling, it appears acceptable to not account for reproductive stage in long-term trend analyses.

Hydrology – Annual (Water Year Type) and Seasonal (Month)

Annual and seasonal hydrologic patterns in freshwater inflow from the Delta have been shown to have a significant effect on selenium concentrations in *Potamocorbula* (Stewart *et al.* 2013), the dominant prey item of white sturgeon in North Bay. While selenium inputs from point-source dischargers in North Bay remain relatively constant year-round, freshwater inflows from the Delta vary significantly. High volumes of freshwater flow from the Delta during wet years and winter months can dilute other sources of selenium inputs to North Bay, reducing selenium concentrations in both the water column and prey such as *Potamocorbula*. Longer residence times in Suisun Bay are also observed during periods of low freshwater inflow; longer residence times in turn increase the likelihood of selenium uptake into the food web (Presser and Luoma 2006). Higher selenium concentrations in *Potamocorbula amurensis* in Suisun Bay have been observed during dry years and seasons (Stewart *et al.* 2013) and suggest that higher selenium concentrations could be observed in sturgeon during dry periods as well.

Sturgeon muscle tissue will respond more slowly than clams to hydrology-driven changes in selenium concentrations, but will also provide a more spatially and temporally integrated index of selenium concentrations. The consistency of the response to hydrologic patterns in clams in the North Bay suggests that a similar pattern would be observed in sturgeon.

Previously-measured sturgeon muscle selenium concentrations were unevenly distributed across water year types and seasons, and co-occurred with variation in other potential factors influencing fish tissue selenium (i.e., fork length, foraging location). Therefore, a robust statistical analysis of the effect of annual and seasonal hydrological variation on sturgeon muscle

selenium is not currently possible. However, qualitative data analysis and statistical evaluation of data from the current study are presented below.

Water Year Type

To evaluate the effect of interannual hydrologic variation, sturgeon muscle selenium data were compared with estimates of freshwater inflow from the Delta to the North Bay. The California Department of Water Resources uses a classification index to categorize water year types for the two dominant tributaries to the Bay, the Sacramento and San Joaquin Rivers, based on flows measured at various locations along each river. Water years are classified as wet, above normal, below normal, dry, or critical based on a weighted calculation that takes into account flow volume measured during the given water year, as well as the previous year's classification (http://cdec.water.ca.gov/cgi-progs/iodir/WSIHIST). Water year 2015 was considered critically dry on both rivers; flow in 2016 was considered below normal on the Sacramento River and dry on the San Joaquin River; and 2017 was considered a wet year on both rivers.

Correlating environmental selenium levels with sturgeon muscle selenium concentrations requires consideration of a lag time between ambient water selenium exposure at the bottom of the food web and uptake into sturgeon tissues. Stewart *et al.* (2013) estimated a 60-day lag time between selenium in the water column and selenium in clams based on a biodynamic bioaccumulation model following Lee *et al.* (2006). Through an empirical analysis of USGS clam data and RMP Sturgeon Derby data from spring 2015-2017, Stewart *et al.* (unpublished) estimated at least a 3-month time lag between selenium in clams and selenium in sturgeon muscle tissue. In another analysis, Beckon (2016) estimated a 50-120-day lag time between selenium in water and clams, and an approximately 6-month lag (178 days) between selenium in water and selenium in sturgeon muscle tissue, based on whole sturgeon tissue collected from the Grassland Bypass Project (Beckon 2016). For the purposes of this report, a 6-month time lag between water and sturgeon is used when describing comparisons of sturgeon selenium to water year types.

The present study, which spanned critically dry, below normal, and wet years on the Sacramento River as well as critically dry, dry, and wet years on the San Joaquin River, employing a consistent study design, presented an excellent opportunity to evaluate the effect of interannual variation in hydrology. Samples were collected during the same months each year (September and October) and from the same locations (Suisun Bay, focused in Grizzly Bay) throughout the study, minimizing the potential impact of seasonal variation and location on differences in observed selenium concentrations across years. Adult sturgeon muscle tissue selenium was found to be significantly higher in 2015 and 2016 (critically dry and dry years) than in 2017 (wet year); no significant difference was found between 2015 and 2016 ("Dataset Summary"). These results were consistent with expectations based on observed patterns in clam selenium concentrations in response to Delta flow (Stewart *et al.* 2013).

A qualitative analysis of data from prior studies indicates a similar pattern, with the highest selenium concentrations observed in sturgeon sampled in or immediately following critically dry or dry water years (Figures 9-10). Concentrations over 30 µg/g dw, for example, were observed only during critically dry, dry, or below normal water years, with the exception of a single sample collected in January of a wet water year (Stewart *et al.* 2004).

A similar effect has also been observed in largemouth bass in the Sacramento-San Joaquin Delta, which showed higher selenium concentrations during the dry year of 2007 than the wet years of 2000 or 2005 (Foe *et al.* 2010).

These data indicate that annual hydrology, as reflected in water year type designation, is a significant factor influencing sturgeon muscle tissue selenium concentration, with higher selenium concentrations observed during dry years. This suggests that the elevated selenium concentrations observed during the 2015-2017 Sturgeon Derby studies, for example, may have been driven largely by low flow conditions during these critically dry and below normal water years. To account for this effect, long-term trend analyses must consider the effect of water year type on selenium variation, either by including hydrology as a factor in statistical analyses or limiting trend analyses to similar water year types.

Season

Limited data are available to assess the effect of seasonal variation in hydrology on sturgeon muscle selenium. The current study was conducted during the fall season each year, and therefore cannot be used on its own to assess this effect. Two previous studies were conducted across multiple seasons, but variation in sampling month generally co-occurred with other drivers such as fish length, sampling location, or water year, making it difficult to isolate the seasonal effect. Furthermore, the effect of reproductive stage, which follows a seasonal pattern, on sturgeon muscle selenium is not entirely clear, further confounding the analysis of seasonal hydrologic patterns.

Assuming a two- to three-month lag between clam and sturgeon muscle selenium, and parallel seasonal patterns to those observed in clams, higher sturgeon muscle selenium concentrations would be expected during December-May, based on generally above-average clam concentrations in October-March (Stewart *et al.* 2013). Existing data are insufficient to assess this hypothesis. During the SVS, the highest sturgeon muscle concentrations were found in February and March, when concentrations regularly exceeded 20 µg/g dw. However, sturgeon sampled during most other months with lower mean concentrations were either juveniles (October and December) or their size was not recorded (April and May). Linares-Casenave *et al.* (2015a,b) found higher concentrations in April, May, and December than in March and June. However, only three fish were sampled in March and October; in July, most fish were juveniles, which are expected to show lower concentrations. Furthermore, the Linares-Casenave *et al.* study was conducted across wet and dry years.

Given that both annual and seasonal effects are driven largely by hydrology, an interaction between these two factors would be expected. Not enough data are available to separate the individual effects of these factors here, but long-term trend analyses may need to include a season-water year interaction term.

It should be noted that data from the current study and future fall monitoring should not be considered representative of year-round tissue selenium concentrations and associated risks. Assuming a 2-3 month lag between clam and sturgeon tissue selenium concentrations, sturgeon sampled in September and October would reflect clams consumed from approximately June-August. The highest clam concentrations are typically observed in October-March, suggesting that sturgeon tissue selenium would be higher in December-May. However, an advantage of monitoring consistently during the same time of year would be controlling for seasonal effects when evaluating long-term trends. Furthermore, consistent fall monitoring avoids the potential added seasonal variability observed in the spring due to possible reproductive stage effects. Most importantly, fall sampling in conjunction with the CDFW is also the most feasible sampling approach.

Foraging area

Selenium sources and food web processes differ significantly among regions of the Bay-Delta, making foraging location an important potential driver of selenium concentrations in sturgeon. North Bay receives nearly 90% of freshwater and sediment inflows to the Bay, including selenium loads from Central Valley agricultural drainage that move through the Delta, as well as oil refinery effluent, wastewater effluent, and other tributary inflows (SFBRWQCB 2015).

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 on foraging areas in Suisun Bay - particularly clam beds in the Grizzly Bay shallows - based on reported capture locations from both the present study and the 2015-2017 RMP Sturgeon Derby Study. 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).

Previous studies indicate that selenium concentrations are higher in sturgeon caught in North Bay, compared to Central Bay, South Bay, or the Delta. In RMP Status and Trends sport fish monitoring between 1997 and 2014, significantly higher concentrations were observed in sturgeon collected in North Bay compared to other Bay areas. Prior monitoring studies that included sturgeon from Suisun and San Pablo Bays (SVS, Stewart *et al.* 2004, 2017 Sturgeon

Derby) similarly showed higher mean concentrations in fish collected from Suisun Bay compared to San Pablo Bay or the Delta. In the present study, $\delta^{13}C$ measured in *Potamocorbula amurensis* in Carquinez Strait (Station 8.1) and at the landward end of Suisun Bay (Station 4.1), when compared with $\delta^{13}C$ measured in sturgeon muscle plugs, suggest that most sturgeon sampled in the current study were indeed foraging within North Bay, including those that were captured upstream of Station 4.1 (Figure 11).

In contrast, Linares-Casenave *et al.* (2015a,b) found significantly higher concentrations in both male and female sturgeon caught in San Pablo Bay compared to Suisun Bay (males: San Pablo Bay n = 15, Suisun Bay n = 6; females: San Pablo Bay n = 11, Suisun Bay n = 15). Unlike the previously studies, however, different sampling locations in this study also largely co-occurred with different sampling seasons (San Pablo Bay sturgeon were predominantly caught during the spawning season [March-May], including all vitellogenic females; Suisun Bay sturgeon were predominantly caught during the post-spawning season [July; one caught in March and one in October], and included no vitellogenic females), which may have confounded the observed pattern. Differences in selenium concentrations in sturgeon foraging in Suisun or San Pablo Bay could not be resolved using available isotope data.

The North Bay Selenium TMDL applies to the region between Broad Slough (at the confluence of the San Joaquin and Sacramento Rivers) and the Bay Bridge in Central Bay. The present study focused almost entirely on North Bay, with a few samples collected farther upstream in the rivers. Carbon, nitrogen, and sulfur isotopes measured in sturgeon muscle tissue suggests that the sturgeon collected upstream of Honker Bay were predominantly foraging within North Bay. Fishing for the CDFW survey generally takes place in both San Pablo and Suisun Bays, but the vast majority of sampling has occurred in Suisun Bay, and within Grizzly Bay in particular (Figure 1).

Most sturgeon sampled in previous studies were likely foraging in the North Bay region, based on capture location (sometimes estimated or anecdotal) and isotope data (see “Data Categorization” in the “Methods” section). The focus of sampling in this region contributes to the observed frequency of occurrence of selenium concentrations above the TMDL target. However, the North Bay is prime habitat for San Francisco Bay sturgeon, so the data from the Muscle Plug Study and other North Bay studies are appropriate indicators of sturgeon exposure.

Future sturgeon monitoring should continue to focus on North Bay, both to provide data for assessment of the North Bay TMDL and due to the logistical difficulty of regularly collecting large numbers of sturgeon tissue samples outside of North Bay. CDFW sampling may also largely continue to focus on Suisun Bay, and Grizzly Bay in particular, where sturgeon are abundant and by-catch is lower compared to San Pablo Bay. Monitoring this region to detect selenium trends in sturgeon is also valuable because Suisun Bay is the receiving water for selenium loads from the Delta.

Long-Term Trend Analysis

Muscle selenium concentrations measured in 2015-2017 fell within the range of observations from previous studies. The mean and maximum selenium concentrations measured during the critically dry water year of 2015 fell within the upper range of historical concentrations (Figure 5). Aside from samples collected in the present study in 2015-2016, the only studies that found measured selenium concentrations above 20 µg/g dw were the SVS and prior Sturgeon Derby studies (Stewart *et al.* 2004, Sun *et al.* in prep); aside from the present study in 2015, the only studies with annual mean concentrations above the TMDL numeric target were also the SVS and a Sturgeon Derby Study (2017).

As previously noted, significantly higher concentrations were found in adults in the dry years of 2015 (critically dry on both the Sacramento and San Joaquin Rivers) and 2016 (below normal on the Sacramento River, critically dry on the San Joaquin River) compared to 2017 (wet years on both rivers) (Table 2, Figure 2). The significantly lower concentrations in 2017 suggest that the relatively high concentrations observed across the three RMP studies between the summer of 2014 (2014 Status and Trends) and winter 2017 (2017 Sturgeon Derby) were driven by dry hydrologic conditions (Figure 3).

Considering the entire San Francisco Bay sturgeon muscle selenium dataset, there is very weak evidence for a long-term declining trend in concentrations (Figure 5; linear regression on log-transformed selenium concentration data, water years 1987-2017: $p = 0.05$, $R^2 = 6.5 \times 10^{-3}$; with the anomalously high years 1989-1990 removed, $p = 0.24$, $R^2 = 9.6 \times 10^{-4}$). However, multiple interacting drivers of selenium variability likely co-occurred during the period of the sparsely distributed historical sturgeon muscle tissue selenium dataset, which was compiled from a variety of studies that were not strictly designed to evaluate these factors, and could have obscured a stronger trend.

Regression Analyses

Mixed effect model and linear regression analyses were used to control for individual or interacting effects of factors influencing sturgeon muscle selenium concentrations while analyzing for long-term trends. Table 3 summarizes the key biological and environmental factors contributing to selenium variability evaluated in the previous section, and describes the evaluation and use of these factors in an initial set of mixed effect models.

The full mixed effect model included water year (continuous), water year type (categorical), and total length (continuous) as fixed effects, and log-transformed sturgeon muscle selenium as the dependent variable. Season was included as a random effect to evaluate the variance associated with this factor. The model was run on a dataset limited to adults (> 105 cm total length) collected within North Bay (n = 270, samples from 66% of sturgeon). Sex, reproductive stage, and interaction effects were excluded from this initial model due to limited

sex and reproductive stage information, as well as the sparse data available to assess these multiple factors.

An initial comparison between full models with and without the random effect structure indicated that including season as a random effect in the model did not improve model performance. Variance explained by the random effect of season was shown to be negligible in the full model, indicating that season is not a strong driver of variability in sturgeon selenium concentrations. Further analyses of the fixed effects were conducted using standard multiple regression models.

A set of models with varying combinations of predictor variables was also evaluated. Models including water year as a measure of time were also compared against a null model and a model without water year, to assess the relative significance of changes in selenium concentrations over time compared to other causes of selenium variability (Table 5). The best model was identified using second-order Akaike Information Criterion coefficients (AIC_c, where the model with the smallest AIC_c is considered most parsimonious), and p-values were used to determine which predictors had a significant influence on sturgeon muscle selenium concentrations.

Table 5 summarizes the results of the model set with varying predictor variables. Among the models that did not include interacting variables, the best model included water year and water year type, but not sturgeon total length. A second model within $\Delta AIC \leq 2.0$ included all three variables, but showed no significant trend across fish total length ($p = 0.18$). The results of the best model indicate a significant ($p = 8.16 \times 10^{-4}$) but very weakly negative temporal trend ($R^2 = 0.09$). Significantly lower selenium concentrations were found during wet years compared to critically dry years ($p = 0.001$) and wet years and dry years ($p = 0.03$).

Several additional models were run to test interaction effects. Among all model possibilities, the most parsimonious model included a significant negative temporal trend ($p = 5.4 \times 10^{-4}$), as well as significant interaction effects between water year type and total length ($p = 1.6 \times 10^{-3}$), but not water year type and water year ($p = 0.09$). Significantly higher selenium concentrations were found during critically dry and dry years compared to below normal years ($p = 0.03$ in both cases). Additionally, the relationship between total length and sturgeon muscle selenium was found to be significantly different during dry and critically dry years compared to wet years. However, the adjusted R^2 for the overall model was low ($R^2 = 0.15$). Figures 9 and 10 demonstrate the paucity of data available to evaluate the effect of each factor included in this model, which limited the robustness of this analysis. The significant trends identified by this model were therefore weak and should be re-examined after additional data collection is conducted.

Conclusions

This muscle plug selenium monitoring study has established fall muscle plug monitoring as a cost-effective and feasible means of non-invasively collecting large numbers of samples of sturgeon muscle for selenium analyses. Additional laboratory sample processing method development is needed, but analysis of selenium in the small muscle plug samples is feasible. Results from this study also successfully established an understanding of current baseline sturgeon muscle selenium concentrations and enabled an analysis of key drivers of selenium variability, which helped to inform the design of a long-term sturgeon selenium monitoring plan, as well as long-term trend analyses.

Selenium concentrations were relatively high in 2015 and 2016 during critically dry and below normal water years, and were significantly lower in 2017, a wet water year. The mean concentration measured in 2015 (11. 8 µg/g dw) was slightly above the TMDL numeric target (11.3 µg/g dw), with 47% of individual samples exceeding the target. The mean concentration among adults in 2016 was slightly lower but still close to the target (10.6 µg/g dw, 44% of individual samples above the target). In contrast, the mean concentration measured in 2017 dropped to 7.3 µg/g dw, significantly lower than the 2015-2016 concentrations, with only 12% of individual samples above the TMDL target. These results suggest that hydrology is a significant contributor to observed variability in sturgeon selenium concentrations, with higher concentrations found under dry conditions. This in turn suggests the elevated sturgeon selenium concentrations observed in North Bay between fall 2014 and spring 2017 were driven by dry hydrologic conditions.

Further analysis of fish length in the context of all available historical data on sturgeon muscle selenium supports the finding that significantly higher selenium concentrations occur in adults compared to juveniles. Among adults, no significant relationship between fish length and muscle selenium was found. However, a significant interaction between fish length and water year type was found during the mixed effects model analysis. This suggests that future monitoring should focus on adults, and target an even size distribution of sturgeon across the target size range (100-160 cm fork length, or approximately 115-181 cm total length) to reduce variability and control for fish length in future statistical trend analyses.

Analysis of historical estimated foraging location data, approximated by sturgeon capture location, indicated that elevated concentrations occur in sturgeon collected in North Bay where future monitoring is planned to occur. Monitoring should continue to focus on this region, which receives selenium loads from the Delta and other pathways and supports a large population of sturgeon.

Several other factors evaluated were not found to be significant contributors to sturgeon muscle selenium variability. Historical data provided no evidence that sex was a significant factor, with no significant differences observed between males and females in multiple studies.

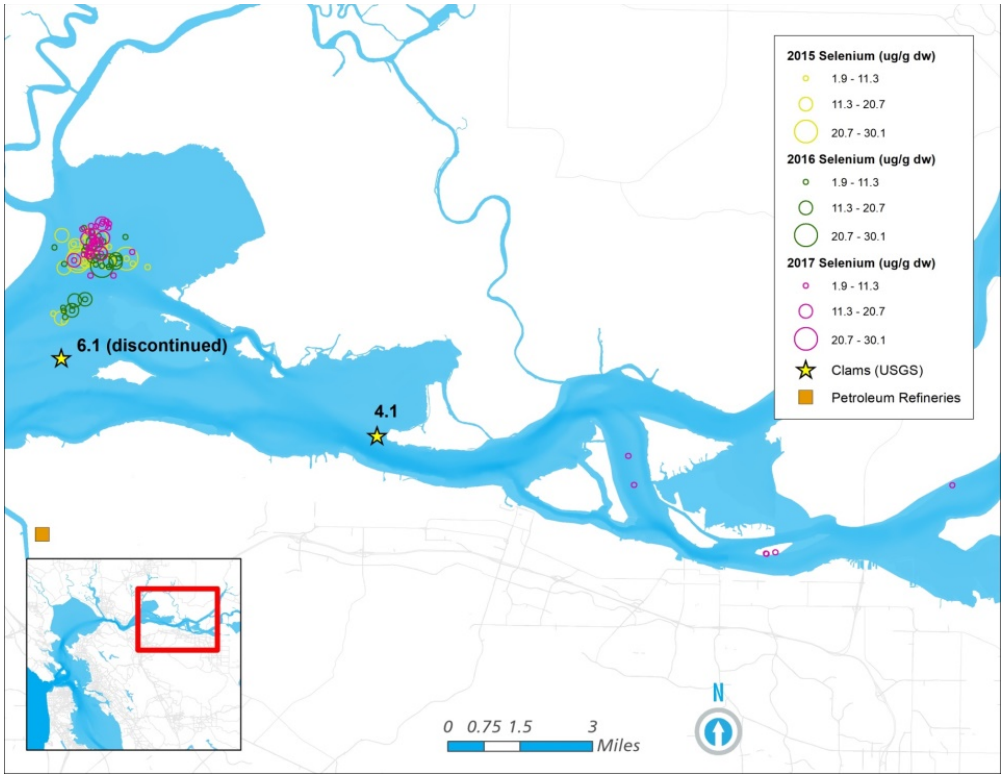
Data were too limited and sparsely distributed to statistically evaluate the effect of reproductive stage or season, although available data do not suggest a significant relationship between reproductive stage and sturgeon muscle selenium. Statistical analyses indicated that season explains almost no variability when included as a random effect in mixed effects models.

Limited data were available to conduct long-term trend analyses in the context of these many sources of variability. Between 1986 and 2017, a weak but significantly declining trend was found in both ordinary and multiple linear regression analyses that account for variability due to fish length and water year type. The sparse data used to conduct this regression analysis and large proportion of remaining unexplained variability indicate that the results of this model should be re-evaluated when more data are available. Long-term data collection through continued muscle plug monitoring that utilizes a consistent monitoring design will enable more robust analyses of long-term trends in the future (Grieb *et al.* 2018).

Figures

Figure 1. White sturgeon sampling locations, 2015-2017.

(A) Full extent of the sampling area, including samples collected upstream of Station 4.1 in 2017. Based on carbon, nitrogen, and sulfur isotopes, all sturgeon, including those collected upstream of Station 4.1, were likely all foraging in North Bay.



(B) Zoomed-in map of Grizzly Bay within Suisun Bay, where most sampling occurred.

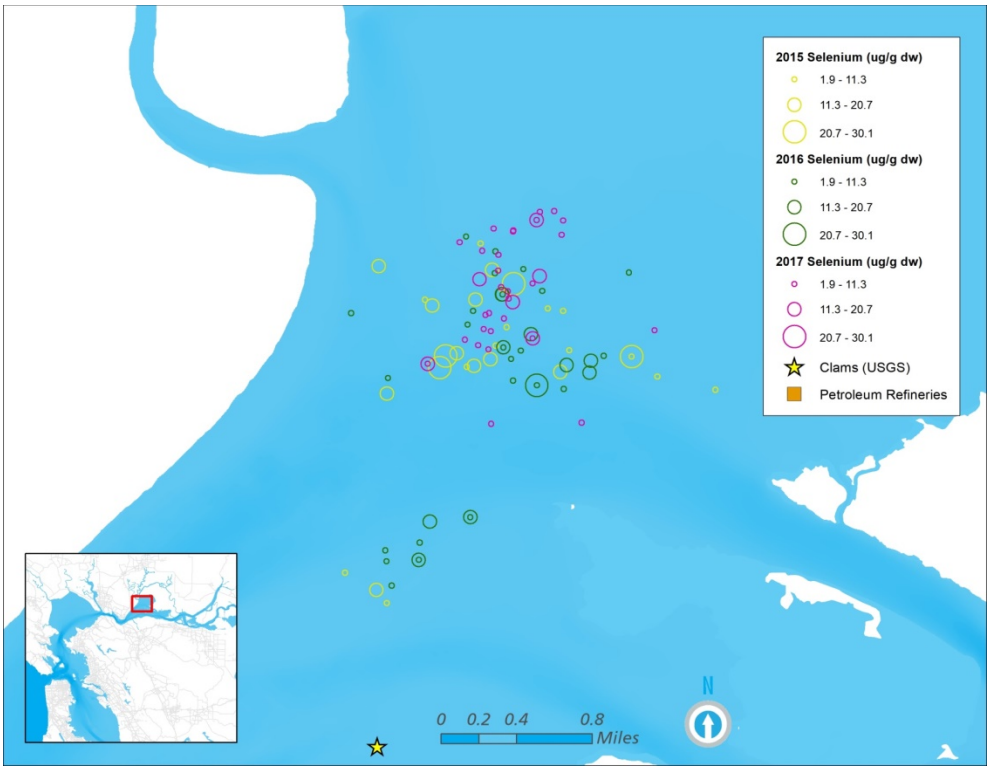


Figure 2. Selenium versus total fish length for all RMP Muscle Plug Study samples (2014-2017). Each point represents an individual sturgeon. Points shown with black outlines were flagged by the analytical lab for poor analytical precision across laboratory replicates. For 2016 and 2017, all points are shown as averages of all replicates measured for a given sturgeon, including those flagged by the lab. The grey bars represent the number of sturgeon sampled within each 11 cm total length range. The horizontal blue line indicates the North Bay TMDL target for selenium in sturgeon muscle tissue (11.3 µg/g dw). The vertical black line indicates the sturgeon total length (105 cm) used to distinguish between juvenile and adult sturgeon, based on size classes established by Linares-Casenave *et al.* (2015a,b).

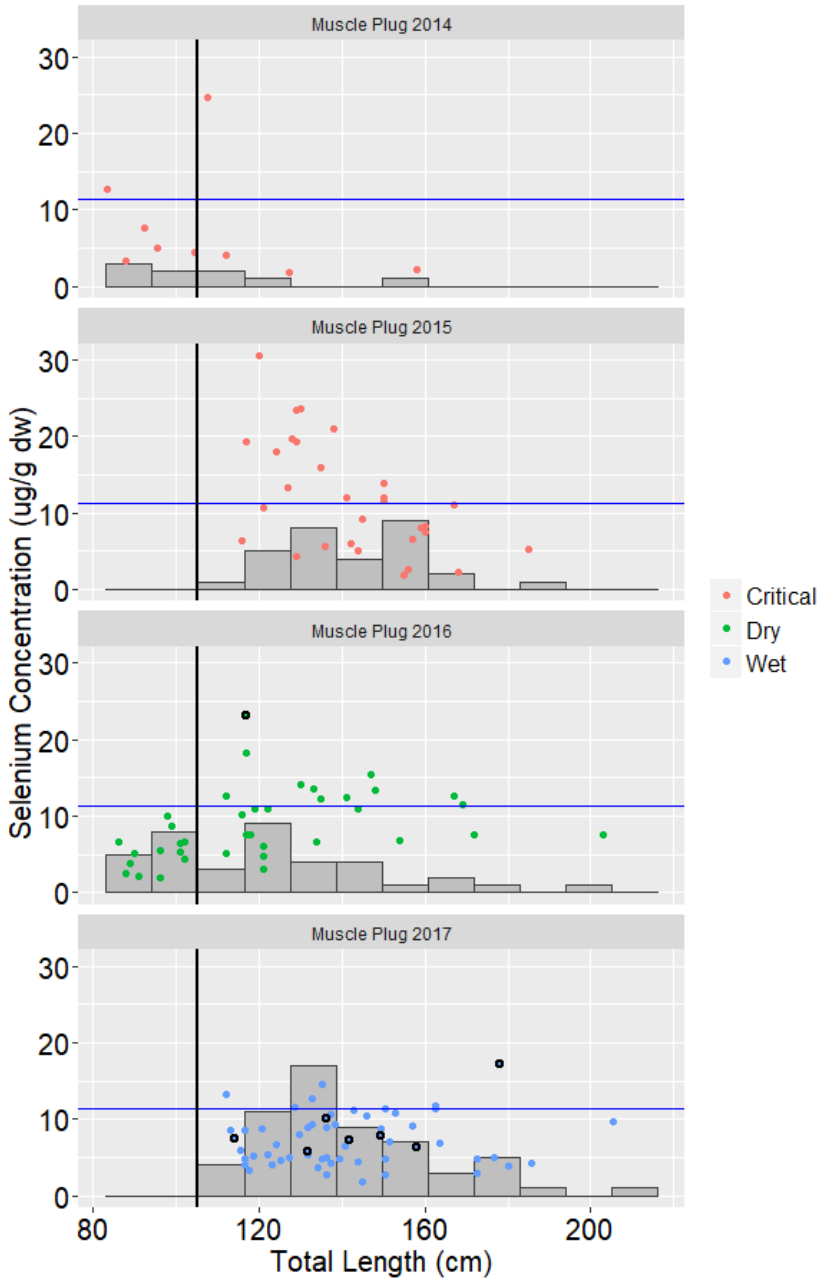


Figure 3. Selenium in white sturgeon muscle plugs from the 2015-2017 Muscle Plug Study for all adults (total length > 105 cm) presumed to have been foraging in North Bay. Each point represents an individual sturgeon. Points shown with black outlines were flagged by the analytical lab for poor analytical precision across laboratory replicates. For 2016 and 2017, all points are shown as averages of all replicates measured for a given sturgeon, including those flagged by the lab. Median concentrations are shown as white bars and mean concentrations as black diamonds. The horizontal blue line indicates the North Bay TMDL target for selenium in sturgeon muscle tissue (11.3 µg/g dw).

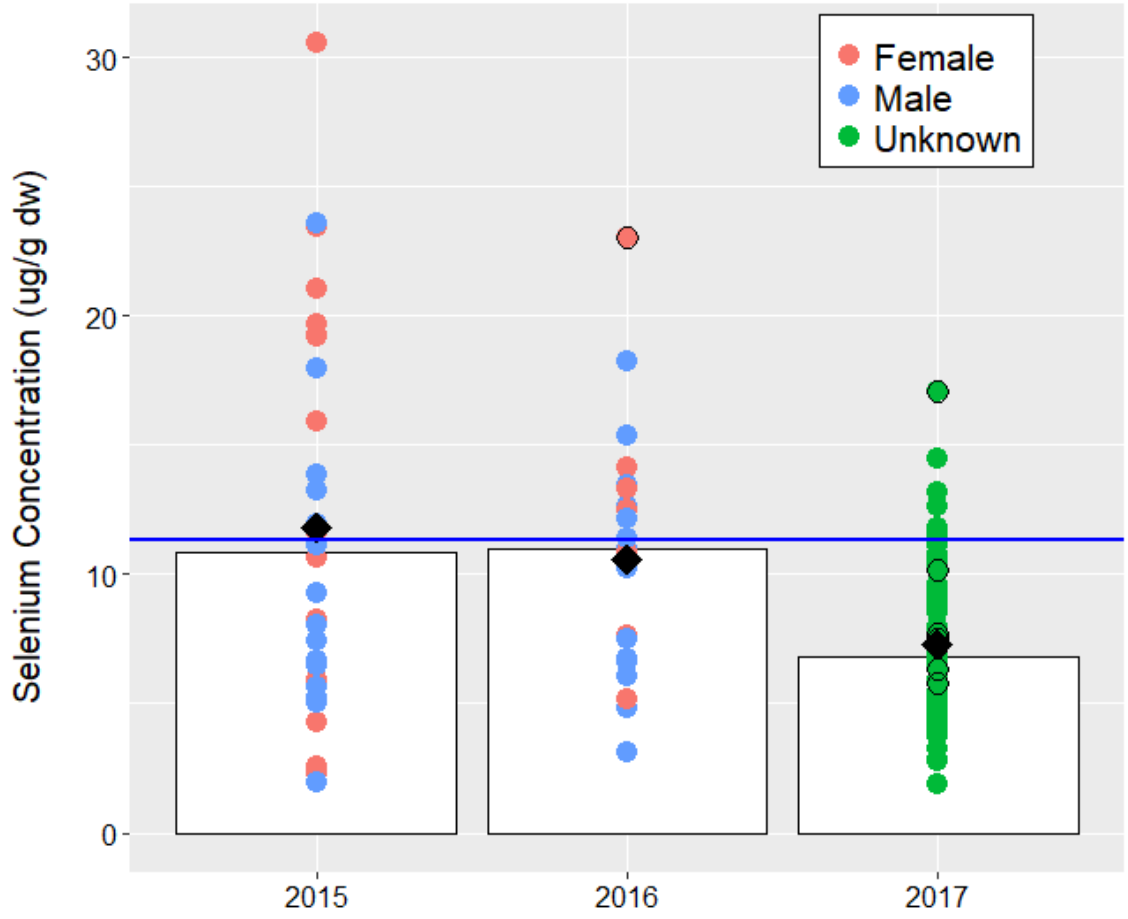


Figure 4. Selenium in white sturgeon muscle plugs for all adults (total length > 105 cm) presumed to have been foraging in North Bay. Each point represents an individual sturgeon. Points shown with black outlines were flagged by the analytical lab for poor analytical precision across laboratory replicates. For 2016 and 2017, all points are shown as averages of all replicates measured for a given sturgeon, including those flagged by the lab. Median concentrations are shown as white bars, and mean concentrations as black diamonds. The horizontal blue line indicates the North Bay TMDL target for selenium in sturgeon muscle tissue (11.3 µg/g dw).

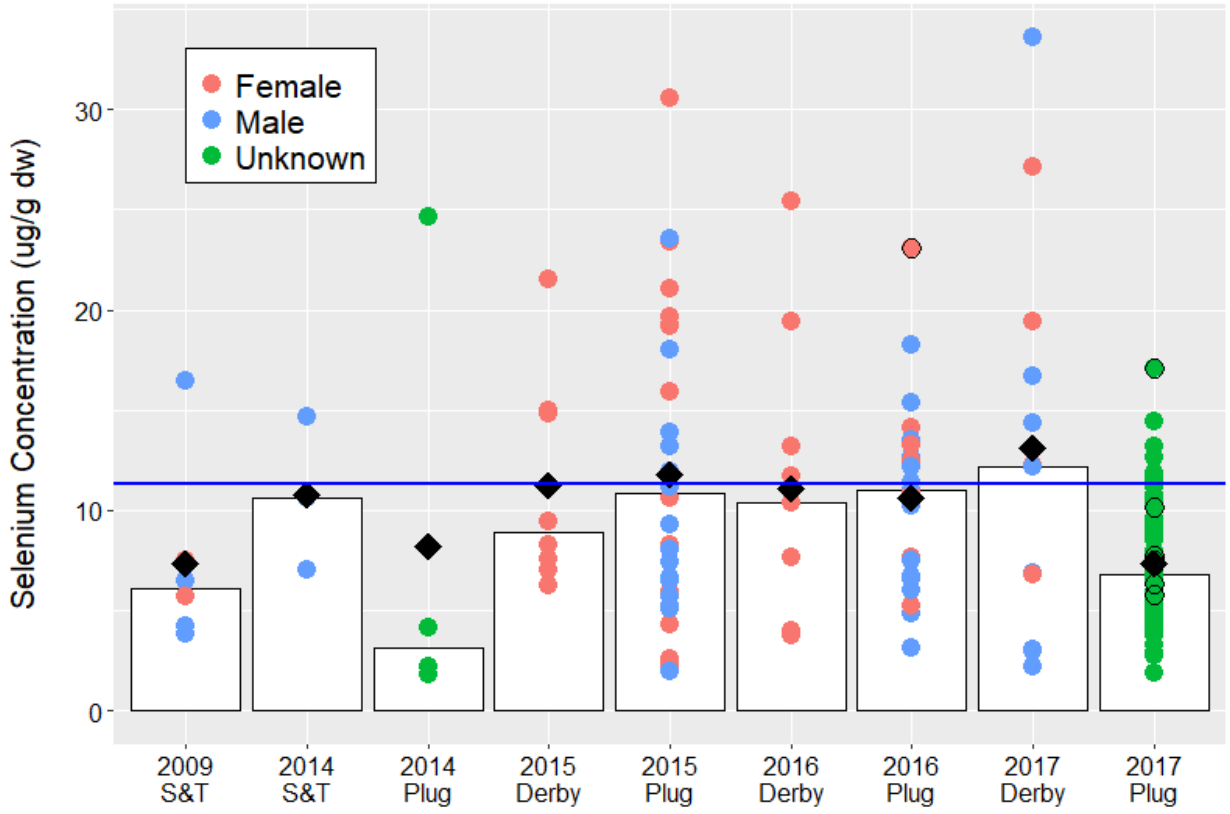


Figure 5. Selenium in white sturgeon muscle tissue (plugs and fillets), including sturgeon previously sampled in the Bay-Delta. For RMP studies in which both muscle plugs and fillets were collected, fillet results were used for the Status and Trends studies and plug results were used for the Sturgeon Derby studies. The horizontal blue line indicates the North Bay TMDL target for selenium in sturgeon muscle tissue (11.3 µg/g dw). Mean concentrations for each study, or each year of multi-year studies, are shown in black diamonds.

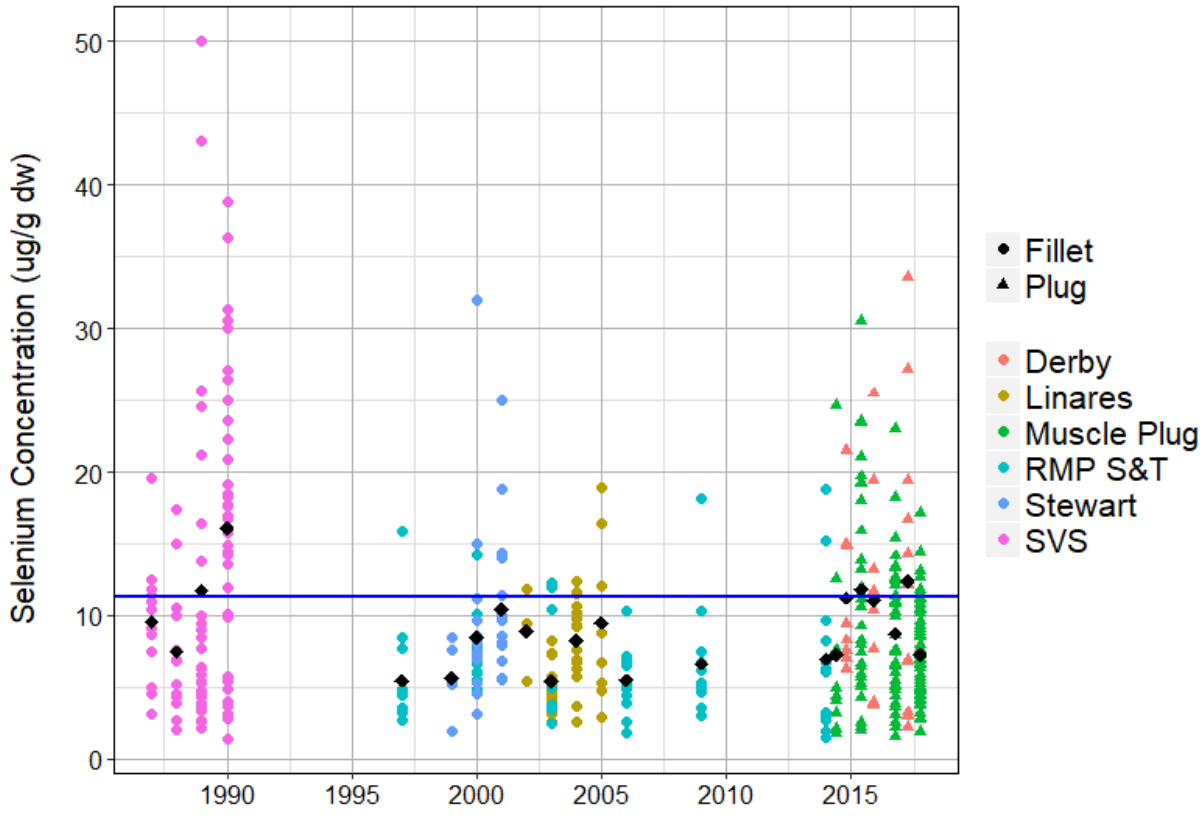


Figure 6. Selenium versus total length for all Bay studies. The horizontal blue line indicates the North Bay TMDL target for selenium in sturgeon muscle tissue (11.3 µg/g dw). The vertical black line indicates the sturgeon total length (105 cm) used to distinguish between juvenile and adult sturgeon, based on size classes established by Linares-Casenave *et al.* (2015a,b).

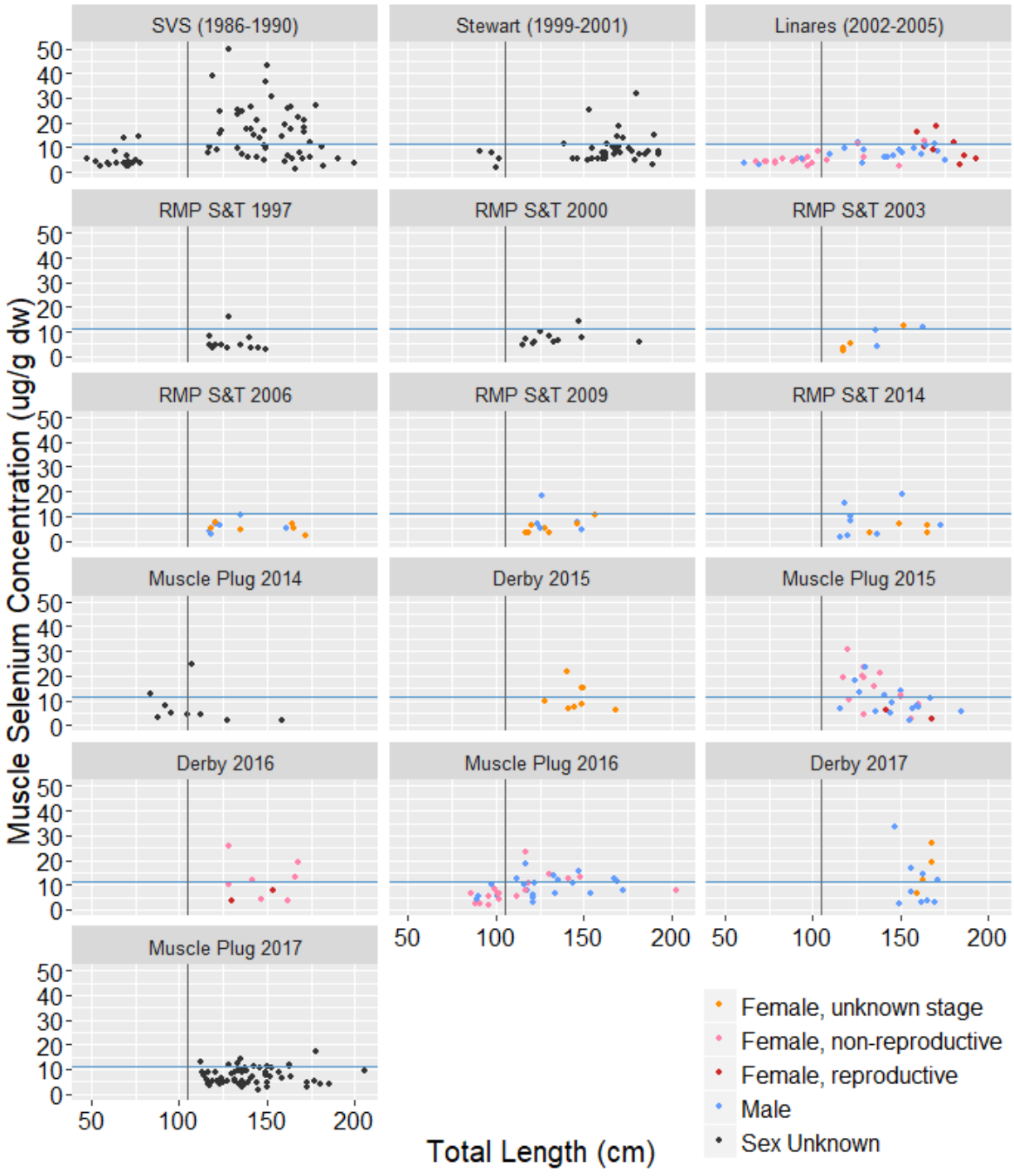


Figure 7. Selenium concentration relative to total length and water year type. Water year types are based on the California Department of Water Resources water year designations for the Sacramento River, assuming a 6-month lag between selenium in the water column and sturgeon muscle tissue. Data include all previous selenium results for white sturgeon sampled in North Bay. The grey bars represent the number of sturgeon sampled within each 11 cm total length range. The horizontal blue line indicates the North Bay TMDL target for selenium in sturgeon muscle tissue (11.3 µg/g dw). The vertical black line indicates the sturgeon total length (105 cm) used to distinguish between juvenile and adult sturgeon, based on size classes established by Linares-Casenave *et al.* (2015a,b).

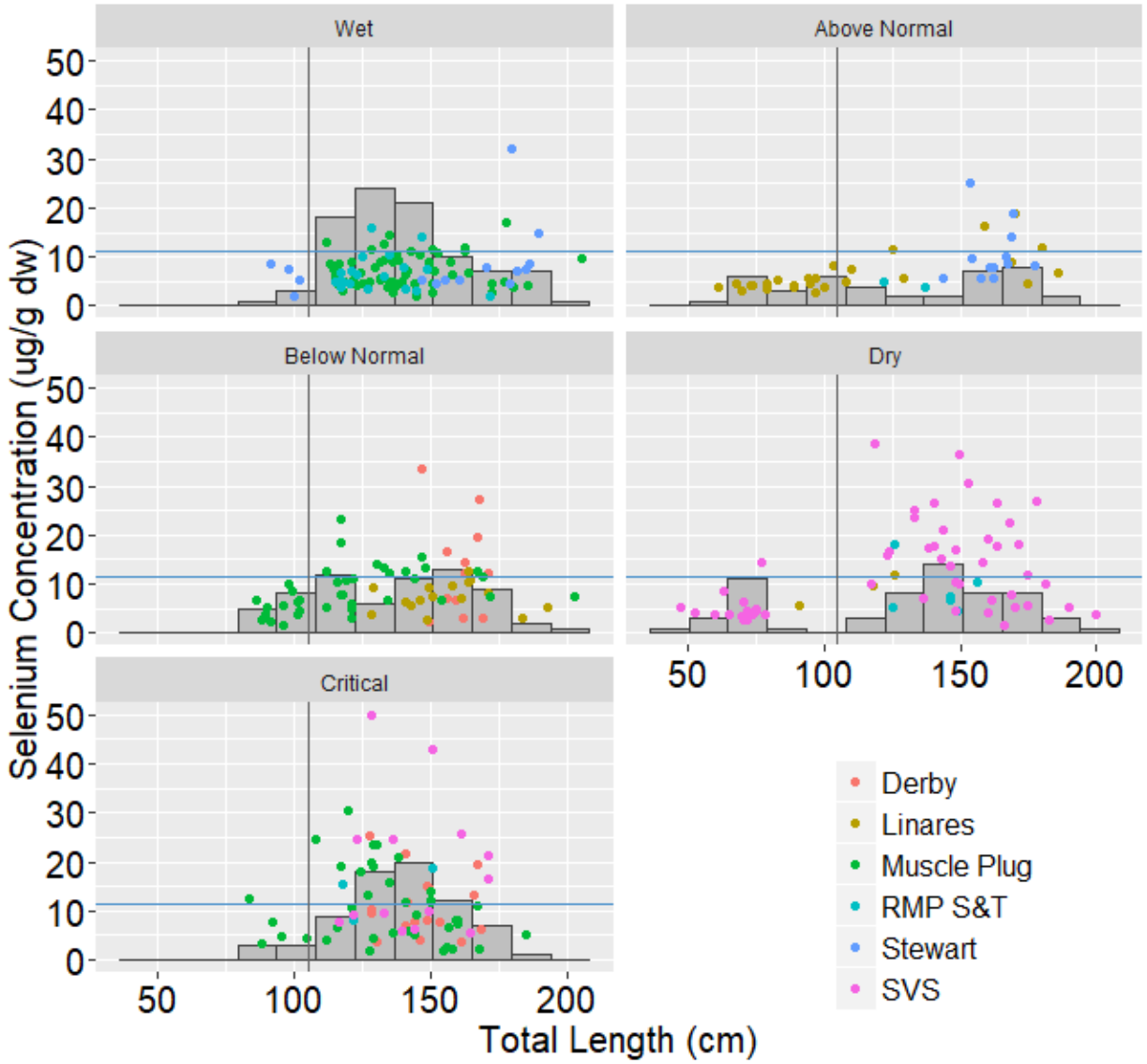


Figure 8. Selenium in female versus male white sturgeon, including all previous selenium results for white sturgeon sampled in the Bay-Delta. The horizontal blue line indicates the North Bay TMDL numeric target for selenium in sturgeon muscle tissue (11.3 µg/g dw). Boxes show the median and the quartiles.

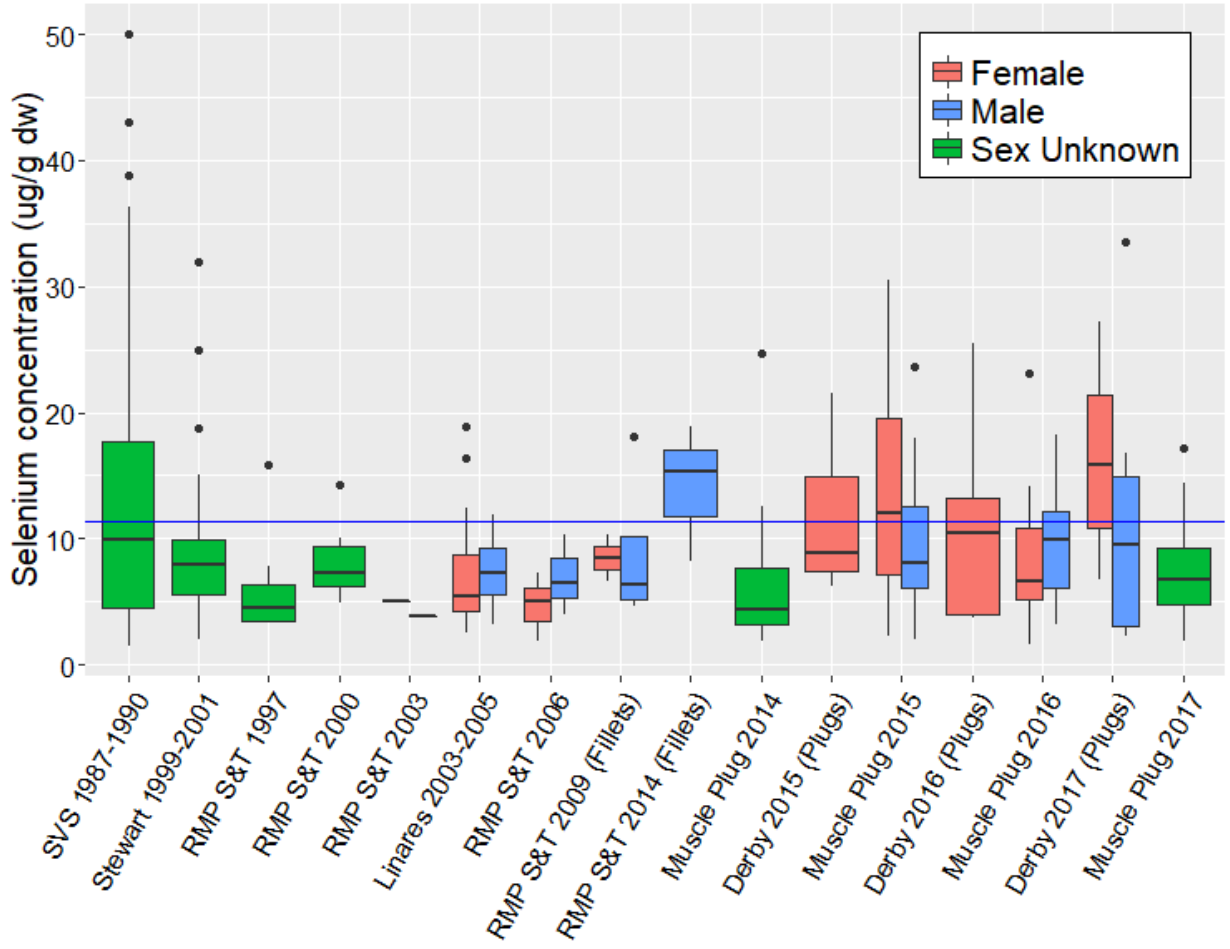


Figure 9. Selenium in white sturgeon muscle tissue across water year types and months. Water year types are based on the California Department of Water Resources water year designations for the Sacramento River, assuming a 6-month lag between selenium in the water column and sturgeon muscle tissue. Data include all historical selenium results for adult white sturgeon presumed to have been foraging in North Bay. The horizontal blue line indicates the North Bay TMDL numeric target for selenium in sturgeon muscle tissue (11.3 µg/g dw).

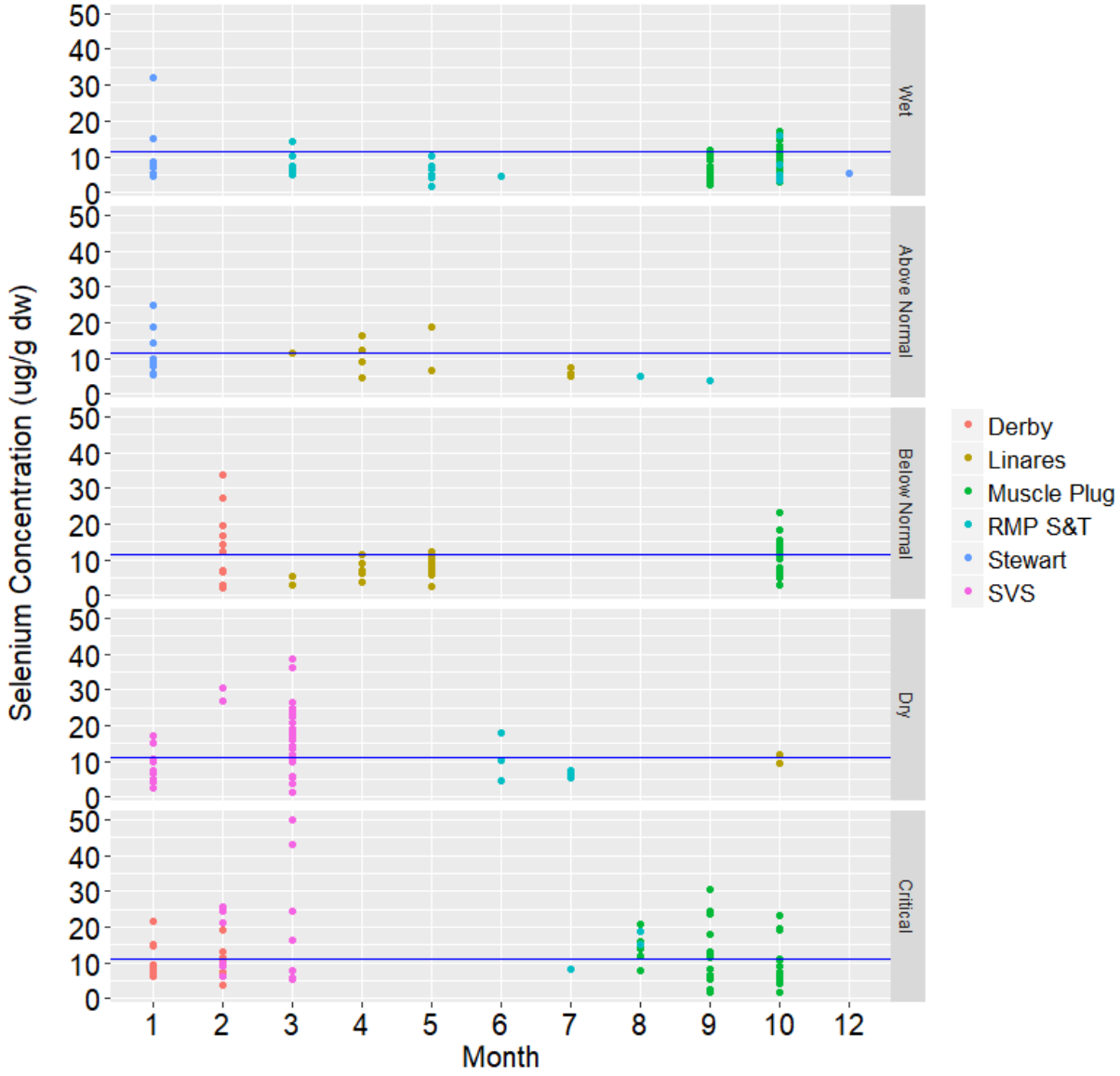


Figure 10. White sturgeon muscle tissue selenium trend across water year types. Water year types are based on the California Department of Water Resources water year designations for the Sacramento River, assuming a 6-month lag between selenium in the water column and sturgeon muscle tissue. Data include all historical selenium results for adult white sturgeon sampled in North Bay. Linear regression analyses were conducted on this dataset. The horizontal blue line indicates the North Bay TMDL numeric target for selenium in sturgeon muscle tissue (11.3 µg/g dw). Diamonds indicate annual averages.

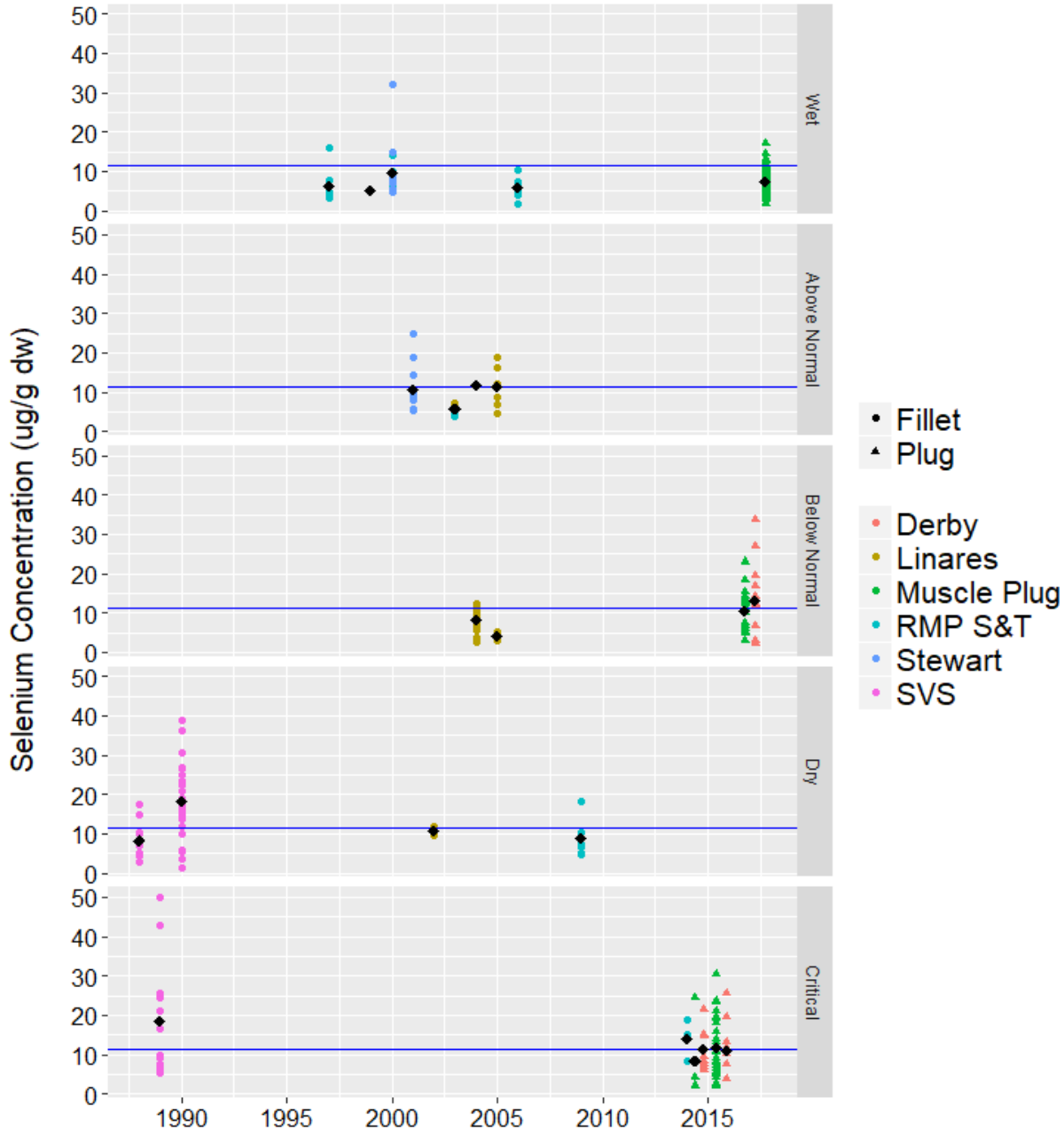
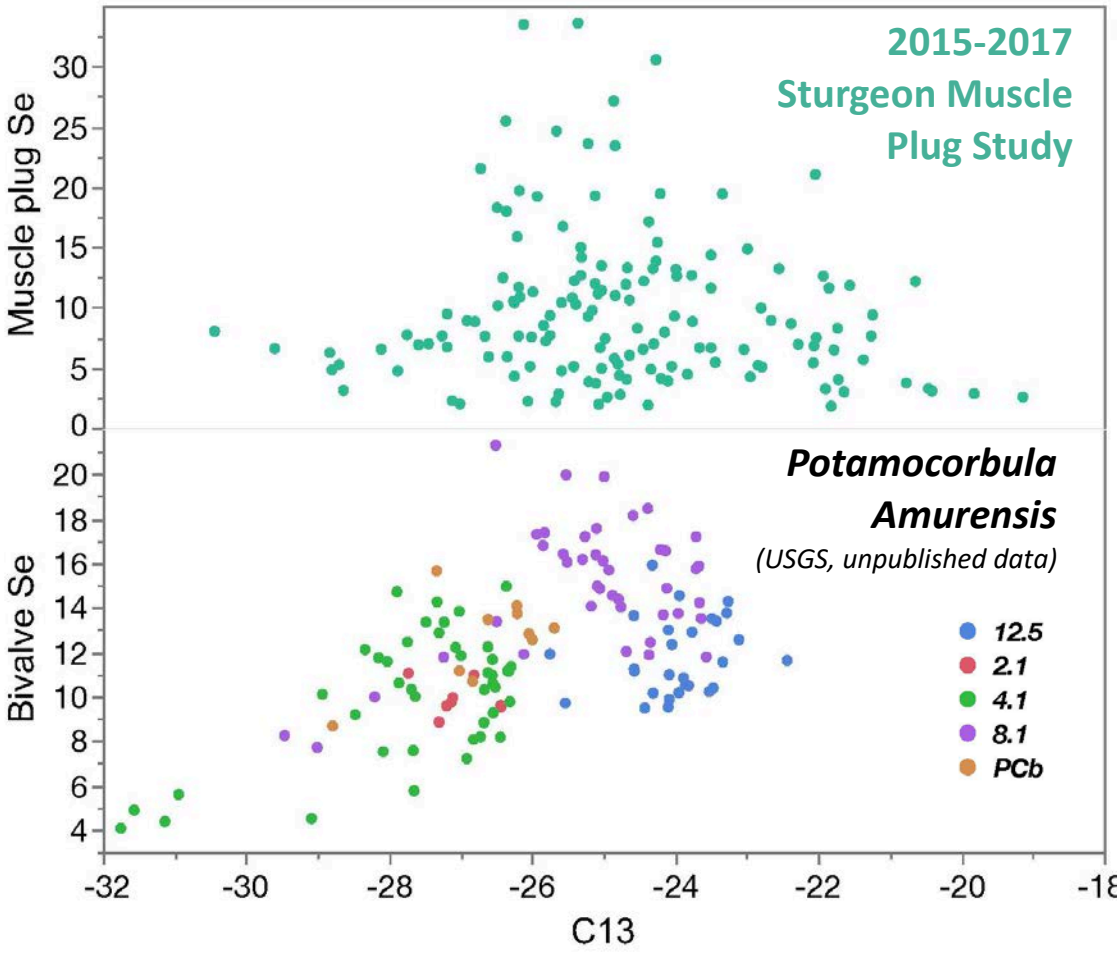


Figure 11. Selenium concentrations versus $\delta^{13}C$ in sturgeon muscle plugs and *Potamocorbula amurensis* (2015-2017). Carbon isotope enrichment between trophic levels is estimated to be approximately 1‰ (Stewart *et al.* 2004; Robin Stewart, personal communication). Each point in the muscle plug plot (upper panel) represents an individual sturgeon. Each point in the bivalve plot (lower panel) represents a monthly mean value, calculated as the average of five composite samples; colors represent the bivalve sampling station (Robin Stewart, USGS, unpublished data). Station 12.5 is located in San Pablo Bay, 8.1 in Carquinez Strait, and 4.1 in at the landward end of Suisun Bay (Stewart *et al.* 2013). This figure was provided by Robin Stewart at USGS-Menlo Park.



Tables

Table 1. Testosterone (T) and estradiol (E2) cutoff values used to estimate the sex and reproductive stage of each fish.

T	**E2**	**Predicted Sex / Reproductive Stage**	**Associated Developmental Stage**
T < 4	E2 < 1.5	Non-reproductive Female	Undergoing differentiation or pre-vitellogenic
T < 4	E2 ≥ 1.5	Female Undergoing Follicular Atresia (post-ovulatory or atretic)	Post-ovulatory or atretic
T ≥ 4	E2 ≥ 1.5	Reproductive Female	Early vitellogenic, vitellogenic, or undergoing oocyte maturation
40 > T ≥ 4	E2 < 1.0	Non-reproductive Male	Undergoing differentiation or pre-meiotic
T ≥ 40	E2 < 1.0	Reproductive Male	Undergoing onset of meiosis through spermiation
nd	nd	Non-reproductive; Unknown Sex	Post-spermiation

Table 2. Summary of selenium concentrations in adult white sturgeon muscle plugs, 2014-2017.

Year	Sample Number	Fork Lengths, cm (min-max; median)	Min (µg/g dw)	Max (µg/g dw)	Median (µg/g dw)	Mean (µg/g dw)	Standard Error (µg/g dw)	Coefficient of Variation	% of samples above North Bay TMDL threshold (11.3 µg/g dw)
2014	4	94-140; 105	1.8	25	3.1	8.2	5.5	135%	25%
2015	30	101-162; 124	2.0	31	10.9	11.8	1.3	62%	47%
2016	25	99-178; 114	3.1	23	11.0	10.6	0.9	43%	44%
2017	58	98-183; 121	1.9	17	6.8	7.3	0.4	45%	12%

Table 3. Summary of factors contributing to variation in sturgeon muscle selenium.

Factor	Model Use	Description
Water Year	Fixed effect (continuous)	Include as continuous variable for long-term trend analysis
Age/Length	Data constrained to include only adults	Historical data suggest that juveniles have lower muscle selenium concentrations than adults. Future monitoring will focus only on adults. Constraining the analysis to adults will reduce variability while focusing on the main population of interest for future monitoring.
	Fixed effect (continuous)	No clear trend in selenium concentrations among adults has been found. Further evaluation of the significance of this factor and any potential interaction effects can be evaluated by including this factor in model comparisons.
Sex	Not assessed in the model	Historical data suggest that there is no significant effect of sex on selenium concentrations, and there are not enough historical data to include this factor in the model.
Reproductive Stage	Not assessed in the model	There are not enough historical data to evaluate this effect, or include this factor in the model. EPA monitoring guidance indicates that monitoring should not be designed to target a segment of the population based on reproductive stage.
Water Year Type	Fixed effect (categorical)	Data from the current study suggest that water year type has a significant effect on sturgeon selenium, matching expectations based on clam selenium patterns. Similar effects have been observed in largemouth bass in the Sacramento-San Joaquin Delta (Foe *et al.* 2010), consistent with modeling predictions that indicate longer hydraulic residence times during dry years (Pressure and Lumoa 2006). Include as a categorical variable to evaluate effect. Use water year type designation for the Sacramento River, which is typically the dominant source of inflow to North Bay.
Season	Random effect (categorical)	Historical data are not extensive enough to statistically evaluate this factor. Qualitative data analysis and understanding of environmental and physiological processes suggest that higher concentrations should be expected in spring than summer or fall. Long-term monitoring in collaboration with the CDFW sturgeon tagging survey will occur during only one season (fall). The effect of season on sturgeon selenium will not affect the detection of long-term trends based on fall sampling with the CDFW. Therefore, additional data to further assess this factor will not be collected in the future, and assessing the effect of

		season on sturgeon selenium is a lower priority. Include as a random effect to control for any effect that may be present.
Foraging location (estimated)	Data constrained to include only fish collected in North Bay	Historical data suggest that higher concentrations are found in North Bay, which is the area of regulatory interest. 85% of historical samples were collected in North Bay and 100% of future samples will be collected in North Bay.
	Not included in the model	This constraint will reduce variability without substantially reducing the data evaluated by this analysis.

Table 4. Summary statistics and comparisons of muscle selenium concentrations in female versus male white sturgeon. All mean concentrations were below the North Bay TMDL target, with the exception of the mean for the four female sturgeon sampled during the 2017 RMP Sturgeon Derby.

Study	Geometric mean +/- SD, µg/g dw (n)		Welch's t-test p-value
	Male	Female	
RMP Status & Trends 2003	7.8 +/- 1.9 (3)	4.8 +/- 2.0 (4)	0.38
Linares-Casenave *et al.* 2015a,b (2002-2005)	6.8 +/- 1.5 (21)	5.9 +/- 1.7 (26)	0.32
RMP Status & Trends 2006	5.3 +/- 1.6 (6)	4.7 +/- 1.7 (6)	0.67
RMP Status & Trends 2009 (fillets)	7.4 +/- 1.7 (5)	4.9 +/- 1.6 (7)	0.21
RMP Status & Trends 2014 (fillets)	5.7 +/- 2.6 (8)	4.5 +/- 1.5 (4)	0.55
RMP Muscle Plug 2015	8.4 +/- 1.8 (15)	10.8 +/- 2.2 (15)	0.34
RMP Muscle Plug 2016	8.4 +/- 1.6 (21)	6.7 +/- 2.0 (17)	0.26
RMP Sturgeon Derby 2017 (plugs)	7.1 +/- 2.6 (9)	14.5 +/- 1.8 (4)	0.14

Table 5. Linear regression model selection results. The most parsimonious model is highlighted in yellow.

Predictor Variables	AICc	Factor p-values				
		Water Year	Water Year Type	Total Length	Total Length x Water Year Type	Water Year x Water Year Type
NULL	556.85					
Water Year + Water Year Type + Total Length	535.55	7.99E-04	4.41E-04	0.16		
Water Year + Total Length	548.33	1.13E-03		0.18		
Water Year + Water Year Type	535.60	8.16E-04	4.55E-04			
Water Year	548.17	1.14E-03				
Water Year + Total Length * Water Year Type	530.30	6.85E-04	3.26E-04	0.16	0.01	
Water Year * Water Year Type + Total Length	535.55	7.37E-04	3.91E-04	0.16		0.10
Water Year * Water Year Type + Water Year Type * Total Length	525.16	5.41E-04	2.46E-04	0.14	1.56E-03	0.09
Water Year Type*Total Length	530.11		3.43E-06	0.47	9.04E-03	

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APPENDIX A

Table A. Selenium and carbon, nitrogen, and sulfur isotopes for all sturgeon collected for the RMP Muscle Plug study (2014-2017). Results from samples collected in 2014, during which this study was piloted, are generally not discussed together with 2015-2017 samples in this report, but are shown below.

Sample ID	Sample Date	Sex (estimated)	Reproductive Status (estimated)	Fork Length (cm)	Total Length (cm)	Se (ug/g dw)	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
2014 Muscle Plugs									
ST-0924-01	9/24/2014	--	--	112	--	1.8	-21.8	14.8	
ST-0924-02	9/24/2014	--	--	91	--	4.4			
ST-0924-03	9/24/2014	--	--	140	--	2.2			
ST-0924-04	9/24/2014	--	--	94	--	25	-25.7	15.2	13.0
ST-0924-05	9/24/2014	--	--	83	--	4.9	-25.0	17.0	
ST-1022-01	10/22/2014	--	--	80	--	7.6	-21.3	18.1	16.2
ST-1022-02	10/22/2014	--	--	76	--	3.2	-21.9	18.5	
ST-1022-03	10/22/2014	--	--	72	--	13	-24.0	16.1	16.2
ST-1022-04	10/22/2014	--	--	98	--	4.1	-24.2	16.8	14.6
2015 Muscle Plugs									
NA-01	9/9/2015	Male	Non-reproductive	108	124	17.98	-26.4	15.3	12.5
NA-02	9/9/2015	Female	Non-reproductive	138	160	8.3	-24.5	16.3	
NA-04	9/10/2015	Female	Reproductive	123	142	5.9	-26.4	15.2	7.8
NA-05	9/10/2015	Male	Non-reproductive	113	127	13	-22.6	17.7	13.3
NA-09	10/5/2015	Female	Non-reproductive	113	129	23	-24.8	15.4	12.9
NA-11	10/8/2015	Male	Non-reproductive	129	145	9.3	-24.0	16.7	15.0
NA-13	10/8/2015	Female	Non-reproductive	105	121	11	-24.6	16.5	14.7
NA-16	10/13/2015	Female	Non-reproductive	105	117	19	-25.1	16.5	14.0
ST-02	8/26/2015	Male	Non-reproductive	133	150	14	-24.3	16.2	15.6
ST-03	8/26/2015	Male	Non-reproductive	143	159	8.0	-30.4	15.2	13.6
ST-06	8/27/2015	Female	Non-reproductive	121	138	21	-22.0	16.5	15.4

Sample ID	Sample Date	Sex (estimated)	Reproductive Status (estimated)	Fork Length (cm)	Total Length (cm)	Se (ug/g dw)	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
ST-08	8/31/2015	Female	Non-reproductive	138	150	12	-25.1	15.1	15.5
ST-09	8/31/2015	Female	Non-reproductive	118	135	16	-26.2	14.6	10.5
ST-10	9/1/2015	Male	Non-reproductive	137	157	6.7	-25.1	15.7	13.0
ST-11	9/1/2015	Male	Non-reproductive	117	130	24	-25.2	15.7	15.0
ST-15	9/9/2015	Female	Non-reproductive	132	150	12	-23.5	15.2	
ST-17	9/17/2015	Male	Non-reproductive	162	185	5.3	-28.7	15.9	13.9
ST-18	9/17/2015	Female	Non-reproductive	107	120	31	-24.3	15.7	13.7
ST-21	9/17/2015	Female	Reproductive	149	168	2.3	-27.1	16.1	14.0
ST-22	9/17/2015	Female	Non-reproductive	140	156	2.6	-19.1	18.0	15.8
ST-23	9/17/2015	Male	Reproductive	120	136	5.7	-21.4	17.4	16.0
ST-25	9/22/2015	Male	Non-reproductive	125	141	12	-24.7	17.5	16.8
ST-30	10/1/2015	Male	Reproductive	135	155	2.0	-25.1	16.6	16.5
ST-31	10/1/2015	Male	Non-reproductive	143	167	11	-25.1	15.9	14.5
ST-33	10/8/2015	Male	Reproductive	101	116	6.5	-21.8	17.1	12.4
ST-34	10/12/2015	Female	Non-reproductive	109	128	20	-26.2	16.3	14.5
ST-35	10/13/2015	Female	Non-reproductive	110	129	4.3	-26.3	14.2	7.9
ST-36	10/13/2015	Male	Non-reproductive	111	129	19	-25.9	15.3	15.4
ST-37	10/14/2015	Male	Reproductive	127	144	5.1	-22.8	18.7	17.1
ST-39	10/15/2015	Male	Reproductive	141	160	7.4	-25.0	16.2	16.8
2016 Muscle Plugs									
16MP-WST-ST-01	10/12/2016	Male	Non-reproductive	99	112	13	-21.9	18.7	15.2
16MP-WST-ST-02	10/13/2016	Female	Non-reproductive	178	203	7.6	-26.7	14.7	14.0
16MP-WST-ST-03	10/17/2016	Male	Reproductive	106	121	4.8	-28.8	14.6	-0.1
16MP-WST-ST-04	10/17/2016	Female	Non-reproductive	114	130	14	-25.3	15.9	12.4
16MP-WST-ST-05	10/17/2016	Male	Non-reproductive	115	133	13	-25.0	15.5	12.7
16MP-WST-ST-06	10/17/2016	Male	Non-reproductive	106	121	3.1	-28.6	15.7	12.1
16MP-WST-ST-07	10/17/2016	Male	Non-reproductive	87	101	3.7	-22.1	17.7	12.0
16MP-WST-ST-08	10/17/2016	Female	Non-reproductive	100	117	23	-26.1	15.9	13.6

Sample ID	Sample Date	Sex (estimated)	Reproductive Status (estimated)	Fork Length (cm)	Total Length (cm)	Se (ug/g dw)	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
16MP-WST-ST-09	10/18/2016	Female	Non-reproductive	77	91	2.2	-25.7	16.1	6.6
16MP-WST-ST-10	10/19/2016	Male	Non-reproductive	149	167	13	-23.8	15.9	13.7
16MP-WST-ST-11	10/19/2016	Male	Non-reproductive	85	98	10.0	-22.8	17.3	12.1
16MP-WST-ST-12	10/20/2016	Female	Non-reproductive	129	148	13	-24.7	16.1	14.0
16MP-WST-ST-13	10/25/2016	Female	Non-reproductive	77	88	2.6	-25.0	14.7	3.8
16MP-WST-ST-14	10/25/2016	Male	Non-reproductive	78	90	5.1	-24.1	16.0	11.3
16MP-WST-ST-15	10/26/2016	Male	Non-reproductive	108	122	11	-24.8	16.1	13.3
16MP-WST-ST-16	10/26/2016	Male	Non-reproductive	127	144	11			
16MP-WST-ST-17	10/26/2016	Male	Non-reproductive	136	154	6.7	-27.2	14.1	9.3
16MP-WST-ST-18	10/27/2016	Female	Non-reproductive	83	96	5.5	-23.4	17.0	11.9
16MP-WST-ST-19	10/27/2016	Female	Non-reproductive	89	102	6.7	-23.7	17.7	13.5
16MP-WST-ST-20	10/27/2016	Female	Non-reproductive	99	112	5.2	-22.8	17.4	12.1
16MP-WST-NA-01	10/13/2016	Male	Non-reproductive	107	118	7.6	-26.2	15.7	
16MP-WST-NA-02	10/13/2016	Male	Non-reproductive	78	89	3.9			11.9
16MP-WST-NA-03	10/17/2016	Female	Non-reproductive	87	99	8.7	-22.4	17.9	13.5
16MP-WST-NA-04	10/17/2016	Male	Non-reproductive	103	117	18	-26.5	15.7	13.5
16MP-WST-NA-05	10/17/2016	Male	Non-reproductive	107	121	6.0	-24.6	16.7	9.8
16MP-WST-NA-06	10/17/2016	Female	Non-reproductive	123	141	12	-26.4	15.0	11.9
16MP-WST-NA-07	10/17/2016	Female	Non-reproductive	81	96	1.6	-27.0	15.0	8.0
16MP-WST-NA-08	10/18/2016	Male	Non-reproductive	119	135	12	-24.4	16.2	11.6
16MP-WST-NA-09	10/19/2016	Female	Non-reproductive	106	119	11	-26.2	15.2	10.5
16MP-WST-NA-10	10/19/2016	Male	Reproductive	131	147	15	-24.3	16.3	14.4
16MP-WST-NA-11	10/20/2016	Female	Non-reproductive	75	86	6.5	-24.3	16.6	10.6
16MP-WST-NA-12	10/20/2016	Male	Non-reproductive	118	134	6.6	-29.6	15.1	7.7
16MP-WST-NA-13	10/20/2016	Male	Reproductive	146	169	11	-25.0	15.5	14.2
16MP-WST-NA-14	10/25/2016	Female	Non-reproductive	102	117	7.6	-27.3	15.3	13.3
16MP-WST-NA-15	10/25/2016	Female	Non-reproductive	90	102	4.4	-24.8	16.5	10.9
16MP-WST-NA-16	10/25/2016	Male	Non-reproductive	100	116	10	-25.4	16.2	12.4

Sample ID	Sample Date	Sex (estimated)	Reproductive Status (estimated)	Fork Length (cm)	Total Length (cm)	Se (ug/g dw)	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
16MP-WST-NA-17	10/27/2016	Male	Non-reproductive	153	172	7.5	-22.0	17.6	15.0
16MP-WST-NA-18	10/27/2016	Female	Non-reproductive	89	101	6.5	-23.0	16.5	11.2
2017 Muscle Plugs									
17MP-WST-ST-1	9/20/2017	--	--	116	--	5.3	-24.8	15.8	11.1
17MP-WST-ST-2	9/20/2017	--	--	183	--	9.6			
17MP-WST-ST-3	9/20/2017	--	--	107	--	5.5			
17MP-WST-ST-4	9/20/2017	--	--	139	--	9.1			
17MP-WST-ST-5	9/20/2017	--	--	133	--	4.7	-27.9	13.9	4.5
17MP-WST-ST-6	9/20/2017	--	--	102	--	4.9			
17MP-WST-ST-7	9/20/2017	--	--	110	--	4.5			
17MP-WST-ST-8	9/20/2017	--	--	103	--	3.3			
17MP-WST-ST-9	9/20/2017	--	--		--				
17MP-WST-ST-10	9/21/2017	--	--	118	--	3.7	-25.1	14.6	11.1
17MP-WST-ST-11	9/21/2017	--	--	135	--	11	-25.4	15.0	12.4
17MP-WST-ST-12	9/21/2017	--	--	144	--	11	-26.0	15.8	15.0
17MP-WST-ST-13	9/25/2017	--	--	128	--	1.9	-24.4	15.9	6.2
17MP-WST-ST-14	9/25/2017	--	--	144	--	12	-21.6	16.3	14.3
17MP-WST-ST-15	9/25/2017	--	--	129	--	10	-25.6	14.1	
17MP-WST-ST-16	9/26/2017	--	--	140	--	6.3	-25.2	15.1	13.5
17MP-WST-ST-17	9/26/2017	--	--	100	--	7.5	-21.2	18.1	13.6
17MP-WST-ST-18	9/26/2017	--	--	116	--	8.9	-26.9	13.2	5.5
17MP-WST-ST-19	9/26/2017	--	--	120	--				
17MP-WST-ST-20	9/27/2017	--	--	119	--	4.8			
17MP-WST-ST-21	9/27/2017	--	--	121	--	4.2			
17MP-WST-ST-22	9/27/2017	--	--	160	--	3.8			
17MP-WST-ST-23	9/27/2017	--	--		--				
17MP-WST-ST-24	9/27/2017	--	--	133	--	11			
17MP-WST-ST-25	10/2/2017	--	--	101	--	5.9	-26.6	13.2	11.5

Sample ID	Sample Date	Sex (estimated)	Reproductive Status (estimated)	Fork Length (cm)	Total Length (cm)	Se (ug/g dw)	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
17MP-WST-ST-26	10/2/2017	--	--	102	--	8.6			
17MP-WST-ST-27	10/2/2017	--	--	102	--				9.8
17MP-WST-ST-28	10/2/2017	--	--	117	--	13	-25.3	15.0	11.6
17MP-WST-ST-29	10/3/2017	--	--	109	--	6.7	-23.5	16.7	13.2
17MP-WST-ST-30	10/3/2017	--	--	121	--	11	-26.3	14.8	13.1
17MP-WST-ST-31	10/3/2017	--	--	145	--	6.9	-22.3	20.3	12.9
17MP-WST-ST-32	10/3/2017	--	--	120	--	5.1	-25.4	15.7	15.1
17MP-WST-ST-33	10/3/2017	--	--	153	--	2.9	-19.8	16.5	11.1
17MP-WST-ST-34	10/4/2017	--	--	132	--	8.8	-26.8	14.1	
17MP-WST-ST-35	10/4/2017	--	--	108	--	4.1			
17MP-WST-ST-36	10/4/2017	--	--	127	--	4.5	-23.8	16.5	15.2
17MP-WST-ST-37	10/4/2017	--	--	113	--	12	-21.8	17.2	9.6
17MP-WST-ST-38	10/4/2017	--	--	112	--	5.0			7.3
17MP-WST-ST-39	10/5/2017	--	--	126	--	11	-25.1	14.2	
17MP-WST-ST-40	10/5/2017	--	--	114	--				
17MP-WST-ST-41	10/5/2017	--	--	122	--	9.3	-25.8	15.0	3.3
17MP-WST-ST-42	10/5/2017	--	--	124	--	6.5	-28.1	13.1	
17MP-WST-ST-43	10/10/2017	--	--	157	--	5.0			12.7
17MP-WST-ST-44	10/10/2017	--	--	104	--	5.1	-26.0	16.7	6.7
17MP-WST-ST-45	10/10/2017	--	--	105	--	8.8	-23.8	14.8	11.8
17MP-WST-ST-46	10/11/2017	--	--	98	--	13	-24.0	15.6	11.7
17MP-WST-ST-47	10/11/2017	--	--	119	--	14			
17MP-WST-ST-48	10/11/2017	--	--	125	--	7.2	-25.8	14.5	11.0
17MP-WST-ST-49	10/11/2017	--	--	132	--	7.7	-27.8	15.1	7.4
17MP-WST-ST-50	10/11/2017	--	--	158	--	17	-24.4	15.5	14.6
17MP-WST-ST-51	10/11/2017	--	--	120	--	10	-26.4	16.0	10.0
17MP-WST-ST-52	10/11/2017	--	--	99	--	8.5	-25.8	14.2	11.0
17MP-WST-ST-53	10/16/2017	--	--	117	--	9.3	-25.2	15.1	13.5

Sample ID	Sample Date	Sex (estimated)	Reproductive Status (estimated)	Fork Length (cm)	Total Length (cm)	Se (ug/g dw)	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
17MP-WST-ST-54	10/16/2017	--	--	133	--	2.8	-24.8	15.1	6.3
17MP-WST-ST-55	10/17/2017	--	--	114	--	8.0	-24.2	15.8	14.6
17MP-WST-ST-56	10/17/2017	--	--	165	--	4.2	-23.0	17.2	14.5
17MP-WST-ST-57	10/17/2017	--	--	120	--	8.9	-22.7	16.5	9.0
17MP-WST-ST-58	10/18/2017	--	--	116	--	5.8	-24.9	15.7	8.1
17MP-WST-ST-59	10/18/2017	--	--	123	--	4.9	-24.3	16.6	7.4
17MP-WST-ST-60	10/18/2017	--	--	120	--	2.8	-25.6	15.6	7.4
17MP-WST-ST-61	10/18/2017	--	--	134	--	7.0	-24.4	16.5	4.7
17MP-WST-ST-62	10/25/2017	--	--	153	--	4.7	-25.6	16.3	6.2
17MP-WST-ST-63	10/25/2017	--	--	102	--	4.1	-24.7	16.0	

1

2 1 – $\delta^{34}S$ measured in 2017 were flagged for variable precision

APPENDIX B

Quality Assurance / Quality Control Summary

Selenium (USGS-Menlo Park)

2014

Results were reported for selenium and moisture in nine composite samples, four method blanks, and 10 certified reference materials. All samples in one batch were improperly frozen so were flagged with the non-censoring code "BY".

Overall the data were 100% reportable. Method detection limits (MDLs) were sufficient with detected selenium values found in all composite samples. Selenium was reported blank corrected, with the standard deviation of the method blank results below the average MDL, so no blank qualifiers were needed. Recoveries on certified reference materials (CRMs) were good with average error below 5%, well below the target of 35%. Precision for samples for other projects analyzed in the same batches averaged 5% relative standard deviation (RSD), well below the method quality objective (MQO) target of 35%.

2015 (analyzed together with samples from the 2016 Sturgeon Derby)

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 also 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.

2016-2017

Muscle plugs were analyzed for selenium and moisture in muscle plug samples collected from 39 fish in 2016 and 61 fish in 2017. A single moisture measurement was made in tissue for each unique fish. In contrast, a significant number of samples were analyzed for selenium in duplicate or triplicate across multiple lab batches, with a total of 60 samples run from plugs sampled in 2016, and 90 samples run from plugs sampled in 2017. Eight lab blanks, four matrix spikes (2 MS/MSD pairs) and 18 CRM results were reported for selenium. Nearly all the field data (~99%) were reportable, with two results flagged for processing issues (i.e., not representative of muscle tissue).

Overall the data set was acceptable, 100% detects, no uncorrected blank contamination, with variation in precision averaging ~33%, and errors in recovery averaging ~11% or better. The variations in field sample replicates (multiple plugs from one fish, sometimes very (up to 5x) different for individual pairs) were somewhat larger than typically seen in fish tissue composites, perhaps compounded by the very small sample sizes and inability to homogenize among replicates. Therefore, although aggregate (e.g.,

mean, median) results are likely representative for the population, differences among individual results (especially those not analyzed in replicate) should be regarded with caution.

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

2014 (analyzed together with samples from the 2015 Sturgeon Derby)

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 sulfur (S). Two field samples had no S isotopes reported. There were also four CRM analyses for C and N isotopes, along with 30 other lab control results for C and 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 reported as 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.

2015 (analyzed together with samples from the 2016 Sturgeon Derby)

The 2015 muscle plug and 2016 sturgeon derby isotope data were reported together. The data set included 39 field sample analyses and four lab replicates reported for C and N, as well as 37 field sample results and three lab replicates reported for S. There were also 41 analyses of control materials for C and N isotopes, and eight LCM results for C and 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‰ of the target for C and N isotopes in reference materials, and within 0.5‰ or better for S isotopes. Lab replicate precision was good, with standard deviations averaging <0.5‰ for field sample replicates on all the isotopes, although individual pairs sometimes differed by about 1‰.

2016-2017

C, N, and S masses and stable isotope distributions were reported for 40 muscle plug samples for C and N and 39 muscle plug samples for S in 2016, and 48 for C and N and 43 for S in 2017. Nearly all the data (> 99%) were reportable, with only one result rejected based on best professional judgement of possible subsampling issues (i.e., not representative of muscle tissue). 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.

Results for C and N were acceptable, with the standard deviation in replicates averaging < 1‰, and isotopes in reference materials deviating < 1‰ from expected values. For S isotopes, differences among

replicate analyses averaged > 1‰ (with a maximum standard deviation of ~5‰), so all S results were flagged for possible variable precision. More replicate analyses may be needed for samples in question if smaller differences needed to be distinguished among individual samples. Although averages and other central tendency statistics for S may be adequate, results for individual fish should be regarded with caution due to potential variability in results from these small samples.

APPENDIX C

2017 Sturgeon Muscle Plug Study Sampling and Analysis Plan

Introduction

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

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

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

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

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

Sampling Design

White sturgeon tissue samples will be collected by CDFW staff during their annual sturgeon tagging effort. Sturgeon will be collected aboard the *New Alosa* and *Striper II* in San Pablo and Suisun Bays between August and October 2017. The objective of the RMP study is to collect from 60 white sturgeon the following samples for analyses:

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

. Testosterone

a. Estradiol

Muscle plugs will be collected from a target of 60 fish within the 100-160 cm fork length size range. However, this target number may vary based on the number of fish caught, and any additional samples may be archived. On October 1, the catch relative to the target numbers in Table 1 will be evaluated. If it seems unlikely that the target of exactly 10 fish in each size range will be attained, the overall target number of 60 will be reached by obtaining more than 10 in the more abundant size ranges.

Table 1. Target samples collected.

Target Size Range (cm, fork length)	Target # of Fish	Target # of Muscle Plugs
101-110	10	2
111-120	10	2
121-130	10	2
131-140	10	2
141-150	10	2
151-160	10	2

Sampling Locations and Schedule

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

Table 2. Field Contacts

Name	Affiliation	Cell Phone #	Email
Jennifer Sun	RMP	(949) 202-6671	jennifers@sfei.org
Jay Davis	RMP	(530) 304-2308	jay@sfei.org
CDFW Staff 1	CDFW		
Jason DuBois	CDFW	(209) 639-2938	jason.dubois@wildlife.ca.gov
Marty Gingras	CDFW	(831) 372-2581	marty.gingras@wildlife.ca.gov

Sample Collection Method

Field sheets

The Organism ID, station code, date and time of fish collection, CDFW disc tag number, total length (cm), fork length (cm), and number of muscle plugs and blood samples collected will be recorded on field data sheets (Attachment 1). The date of collection, boat name, and disc tag

number will be used to cross-reference additional information about sampling location (latitude and longitude) and field sampling conditions recorded on CDFW field data sheets.

Each sturgeon from which samples are collected will be assigned a unique Organism ID 17MP-XX-## where:

17MP is the project ID (2017 Muscle Plug study)
XX is the Boat ID (NA for New Alosa, ST for Striper II)
is a unique number corresponding with the consecutive number of the sample collected on that boat (ie. 01, 02, 03….60)

Sample containers will be labeled with this unique Organism ID.

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

Muscle plugs

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

All plugs taken from the same fish can be stored in the same cryovial. Cryovials pre-labeled with the Organism ID will be provided by RMP staff. Cryovials will be frozen in a portable freezer in the field (-4 C) and transferred on wet ice to a commercial freezer (-20 C) at least once **every week** during the sampling season.

Figure 1. Location of muscle plug collection

<u>Sample Handling & Storage</u>

Muscle plug samples will be double-bagged in Ziploc freezer bags and stored at -4 C in a portable freezer in the field. Samples will be transported **at least once every week on dry ice** to SFEI, where they will be stored in a commercial freezer at -20 C until the end of the sampling season. Samples will then be transported on dry ice to USGS in Menlo Park, where they will be stored at -80 C until analysis.

Muscle plug samples should be stored at -80 C whenever possible, and should not be stored at -20 C for longer than 3 months. Muscle plug samples not analyzed for selenium or isotopes will be stored at -80 C at the USGS.

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

Sample Analysis

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

All samples must be maintained at -20C during transport to the respective laboratories. Any deviation should be noted and reported to RMP staff. Project samples will not be disposed of until all analyses are complete and analytical and QC results have been reviewed and approved by the RMP Project Manager and QA Officer.

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

Table 3. Shipping Contacts

Name	Affiliation	Function	Phone	Email	Shipping Address
Jennifer Sun	SFEI	RMP Project Manager	510-746-7393	jennifers@sfei.org	San Francisco Estuary Institute 4111 Central Ave. Richmond, CA 94804
Robin Stewart	USGS	Muscle plug processing, Se analysis, isotope sample preparation	650-329-4550	arstewar@sfei.org	U.S. Geological Survey Water Resources Division 345 Middlefield Rd. MS496 Menlo Park, CA 94025
Emily Schick	UC Davis	C, N, S isotope analyses	530-752-8100	sif@ucdavis.edu	UC Davis Stable Isotope Facility Dept of Plant Sciences – Rm 1210 PES One Shields Ave. Davis, CA 95616 USA

Table 4. Reportable Parameters

Tissue	Minimum Total Sample Required	Number of Samples[1]

		Se	**C & N isotopes**	**S isotope**
Muscle tissue plug	25 mg dw	60	60	60
Laboratory & Reporting Parameters				
Analytical Lab	--	USGS	UC Davis	UC Davis
Minimum Sample Mass Required	--	16 mg dw	1.5 mg dw (3 mg dw with dups)	3 mg dw (6 mg dw with dups)
Reporting Units	--	ug/g dw	delta units	delta units
Analysis Method	--	HG-ID-ICP-MS	EA-IRMS	EA-IRMS
Sample Container	--	2 mL cryovial		

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