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Executive Summary

The Original Sturgeon Derby (Derby) is an annual fishing derby held in Bay Point, CA that presents an opportunity to collect and analyze a variety of tissue samples from angler-harvested white sturgeon (*Acipenser transmontanus*) for selenium (Se). Collection and analysis of these tissues from the 2015 Derby helped to: 1) develop non-lethal sampling methods and 2) better understand the correlation between tissues that can be collected non-lethally (muscle plug, fin ray) with those that are of greater toxicological interest (ovary, liver). Otolith samples were also collected to inform the development of methods for fin ray Se microchemical analysis. On January 31 and February 1, 2015, tissue samples were obtained from eight female and 19 male white sturgeon collected at the Derby and analyzed for Se. The fin rays were also used to estimate age of the fish, and the muscle plugs were analyzed for carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) isotope ratios to provide information about recent habitat (e.g., primarily Delta, North Bay, or ocean) and foraging behavior (e.g., primarily clam- or fish-based diet).

Selenium concentrations were successfully measured in muscle plug samples collected from eight female sturgeon, and in fin rays collected from 26 male and female sturgeon. Laboratory methods for processing and analyzing selenium in low-sample mass muscle plug samples were further refined, and techniques for preparing and conducting microchemistry analyses on sturgeon fin rays were developed. Fin ray and otolith results are briefly discussed in this report, but will be presented in greater detail in a separate report (Palace *et al.*, in prep).

Se concentrations in muscle plug, liver, and ovary tissue samples were in the upper range of concentrations previously measured in San Francisco Bay white sturgeon. Three of the eight female muscle plug samples showed Se concentrations above the North Bay TMDL tissue target of 11.3 µg/g dw. Fish age and length were positively correlated (linear regression, $R^2=0.46$, $p=0.04$), but were not correlated with tissue Se, with the exception of a positive relationship between fork length and ovary Se (Spearman's rank correlation, rho=0.80, $p=0.02$). No clear correlation was observed among Se concentrations in muscle plug, liver, ovary, or fin ray samples (measured as either the maximum or average value in the outer annulus). Isotope data suggested most of the fish collected had been feeding on invertebrates in Suisun Bay, although one appeared to have been piscivorous and several may have been feeding in San Pablo Bay or the Delta.

Because of the small sample size and narrow size range of the fish collected at the Derby, the preliminary results from this first year of sampling do not definitively describe patterns of Se concentration among white sturgeon or correlations in selenium among various sturgeon tissues. Additional data collected at the Derby in 2016 and 2017 will help to better quantify these patterns and correlations.

Introduction

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

To address Se impairment, the San Francisco Bay Regional Water Quality Control Board initiated development of a Se total maximum daily load (TMDL) for North San Francisco Bay in 2007; the TMDL was formally approved in 2016. The TMDL established a target concentration of 11.3 µg/g dry weight (dw) in white sturgeon muscle tissue as the basis for evaluating impairment (Baginska 2015). White sturgeon is a bottom feeding species that is considered particularly vulnerable to Se exposure in the Bay because its diet consists primarily of the Se-rich overbite clam (*Potamocorbula amurensis*) (Stewart et al. 2004; Beckon and Maurer 2008). Although white sturgeon can be found from South San Francisco Bay to the upper reaches of the Sacramento and San Joaquin River systems, where they spawn, the San Francisco Bay white sturgeon population predominantly resides and feeds in North San Francisco Bay, which hosts a large population of overbite clam.

The Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) was established in 1993 to provide data to inform management decisions for the Bay. In 2014, the RMP formed a Selenium Workgroup with the goal of identifying low-cost, near-term Se monitoring elements to support decision-making, which now includes implementation of the North Bay TMDL. In 2014, the RMP funded a special study to collect a variety of tissue samples from white sturgeon at the 31st Annual Original Sturgeon Derby (Derby) for Se analysis to address the following objectives:

1. develop methods for non-lethal white sturgeon tissue sample collection and Se analysis, including muscle plugs and fin rays; and
2. evaluate correlations between tissues that can be monitored non-lethally (muscle plug or fin rays) and tissues that are more directly tied to adverse reproductive effects (ovary and liver).

Evaluation of non-lethal muscle plug monitoring began during the RMP 2009 and 2014 Status and Trends sampling events during which 12 paired muscle plug and fillet samples were collected each year at locations throughout the Bay (data can be accessed at cd3.sfei.org). The muscle plug method was further developed during the 2014 and 2015 RMP Sturgeon Muscle

Plug special studies, during which muscle plug samples were collected from live white sturgeon during annual California Department of Fish and Wildlife (CDFW) sturgeon tagging efforts each fall (DuBois and Harris 2015 - more information at http://www.dfg.ca.gov/delta/data/sturgeon/bibliography.asp; Sun *et al.* in prep.). Additional muscle plug samples collected during the Derby helped to increase the sample size of muscle plugs and to improve both the laboratory analysis and interpretation of this type of data.

The 2015 Sturgeon Derby also presented the opportunity to evaluate a second non-lethal monitoring method using fin ray samples. Dr. Vince Palace and Dr. Norman Halden at the University of Manitoba have developed a method for microchemical analysis of Se and other trace metals in sturgeon fin rays as an alternative to microchemical analysis in otoliths, which are known to be stable tissues that are good predictors of muscle fillet Se in several other species but cannot be collected non-lethally (Reash et al. 2014). Fin rays can be taken as a non-lethal clip, are easy to collect by non-specialists, and their removal has been shown to not be harmful to sturgeon (Collins and Smith 1996). Fin ray clips have already been collected by the U.S. Fish and Wildlife Service (USFWS) during the CDFW fall sturgeon tagging efforts in which muscle plug samples were collected in 2014 and 2015.

Because fin rays have a regular annual growth pattern similar to otoliths, concentrations of Se and other elements can be measured in each annual ring and assembled into a time series of Se concentrations. These data can potentially be used to better understand the temporal relationship between water, prey, and sturgeon tissue concentrations. The Derby also presented the opportunity to collect otoliths for comparative analysis to assess the chemical stability of fin ray samples.

In addition to developing non-lethal monitoring methods, the second objective of the Sturgeon Derby study was to examine correlations between tissues that can be monitored non-lethally (muscle plugs and fin rays) and tissues that are more directly tied to adverse reproductive effects (ovaries and liver). The primary pathway of Se exposure in white sturgeon is uptake as dietary seleno-methionine and maternal transfer to the egg yolk sac, where elevated Se concentrations can cause developmental deformities or mortality (Baginska 2015). In other species, correlations have been established between muscle plug and ovary concentrations (Osmundson and Skorupa 2011). A similar relationship might also be established using fin ray Se data.

The 2015 Derby study was implemented by the RMP, USFWS, the U.S. Geological Survey (USGS), and Vince Palace at the International Institute of Sustainable Development (formerly at Stantec, Inc.) (Table 1, Appendix A).

In addition to addressing the objectives previously described, the study was designed to contribute useful information relative to the following key questions identified by the RMP and the Selenium Strategy.

RMP Management Questions addressed by this study:

1. Are chemical concentrations in the Estuary at levels of potential concern and are associated impacts likely?
    b. What potential for impacts on humans and aquatic life exists due to contaminants in the Estuary ecosystem?

4. Have the concentrations, masses, and associated impacts of contaminants in the Estuary increased or decreased?
    b. What are the effects of management actions on the potential for adverse impacts on humans and aquatic life due to Bay contamination?

Selenium Strategy Questions addressed by this study:

1. What are appropriate thresholds?
2. Are the beneficial uses of San Francisco Bay impaired by selenium?
3. What is the spatial pattern of selenium impairment?
4. How do selenium concentrations and loadings change over time?

Methods

Field Sample Collection

The Sturgeon Derby is an annual angling competition held by the Foundation Sportsman's Club out of McAvoy Harbor in Bay Point, CA. The Derby provides a rare opportunity to collect tissue samples from white sturgeon harvested by anglers. USGS initiated sample collections from the Derby in 2000/2001 (Stewart et al. 2004), and USFWS has collected samples more recently over the past several years and helped facilitate the collection of additional samples for the RMP.

The Derby awards cash prizes to anglers who bring in fish closest to a chosen target length. The target size in 2015, chosen randomly from within the legal slot limit of 40-60 inches (101.6-152.4 cm fork length), was 51 inches (129.5 cm). Fish could be caught at any time between 7:00 am on January 31, 2015 and 1:00 pm on February 1, 2015, and anywhere in the San Francisco Estuary. Fish were brought alive to McAvoy Harbor to be measured, and in some cases, sacrificed and processed for their tissues.

Tissues were collected from all fish caught, except those that anglers wanted to release alive. A total of 27 fish were sampled, including 19 males and 8 females. Because the RMP was primarily interested in understanding Se impacts on white sturgeon reproduction, only samples collected from the eight female sturgeon were analyzed for the RMP. Tissue samples collected for analysis by the RMP included muscle plugs, ovary, fin rays, and otoliths. In addition, liver samples were collected and analyzed for this study pro-bono by the USGS in Menlo Park, and additional ovary samples were collected for pro-bono histology analysis by Stantec, Inc. Limited tissue samples were collected from males, and the only tissues analyzed were fin ray and otolith samples collected and analyzed pro bono by Vince Palace.

Additional tissue samples were collected for collaborating agencies. Blood samples were collected for Se analysis by USGS, and gut contents for diet analysis by Cramer Fish Sciences. USFWS collected a second set of fin ray and blood samples from each fish for aging and isotope analysis, as well as a variety of tissues for histopathology analyses. Liver and gut samples were not collected from any males, and in several cases, otolith, blood plasma, and gut contents could not be collected. A description of all samples collected at the Derby, including samples that were archived or analyzed by other agencies, is presented in the 2015 Sturgeon Derby Special Study Field Sampling Summary (Robinson and Sun 2015, included in this report as Appendix A). This report focuses on the muscle plug, liver, and ovary results. Fin ray and otolith analysis results will be discussed in a separate report (Palace et al. *in prep.*).

Once measured for length, fish were killed with a blow to the head by a 4 lb sledgehammer. The entire marginal fin ray was removed using a sharp knife, from the point of articulation down to the terminal end of the ray as described by Koch et al. (2008). Otoliths were accessed by cutting into the skull with a hacksaw. For liver and ovary samples, several small samples were collected over the length of the organ and composited for analysis. Muscle plug samples were taken in a manner similar to the method used to collect muscle plugs for the RMP 2014 Sturgeon Muscle Plug Special Study[1] (Sun et al. 2016). Two or three muscle plugs were collected from the region laterally adjacent to the dorsal fin with a 5 mm diameter biopsy punch, ensuring the collection of enough sample mass for Se and isotope analysis. Following sample collection, otoliths and fin rays were dried at room temperature. All other samples were frozen and sent to various labs for archiving or analysis. A more detailed description of the fish processing and sample collection methods is provided in the 2015 Sturgeon Derby Special Study Field Sampling Summary (Appendix A).

<u>*Laboratory Analysis and Data Quality Control / Quality Assurance*</u>

Muscle plug (skin off), liver, and ovary tissue were digested and analyzed for total Se and moisture by USGS (HG-ID-ICP-MS), following methods described in Kleckner et al. (2017). All samples were analyzed in one lab batch, including one lab duplicate for each of the three tissue types. Three method blanks and six certified reference material (CRM) analyses were also analyzed. Results were reported blank corrected. Accuracy was evaluated using CRMs with certified values for Se, and precision was evaluated using duplicate samples and samples collected from other studies.

Carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) isotope ratios were analyzed by UC Davis in one lab batch, together with the nine samples collected for the 2014 Selenium Muscle Plug Study (Sun et al. 2016). Samples were analyzed 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). One lab replicate was run for $\delta^{13}C$ and $\delta^{15}N$ isotopes, which were

[1] In contrast, muscle plugs collected during 2009 and 2014 RMP Status and Trends monitoring were collected using 8-mm biopsy punches. During that study, plugs were taken from an arbitrary location on a fillet collected from the posterior, dorsal side of each fish.

analyzed concurrently, but not enough sample mass was available to run a lab replicate for δ^{34}S isotopes. QA/QC analyses included four CRMs for δ^{13}C and δ^{15}N isotopes, seven laboratory control materials (LCMs) for δ^{13}C and δ^{15}N percent masses, over 30 LCM results for δ^{13}C and δ^{15}N isotopes, and 24 LCMs for δ^{34}S isotopes. No method blanks were analyzed.

Se microchemical analysis was conducted on 26 fin ray samples by IISD, including samples taken from both male and female fish. To prepare each sample for laboratory analysis, an approximately 2 mm-thick cross section of the fin ray tissue was cut near the conspicuous curve of the fin ray, as close as possible to the articulating process, using a small Dremel saw. Each sample was then mounted on a glass slide and analyzed using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). This method enables the analysis of Se down to the ng/g level from the core of the fin ray (representing early life) to the outer edge (representing late life) across each annular growth ring. Annuli were counted to estimate fish age. The fin rays were then analyzed using laser-ablation ICP-MS, resulting in continuous Se measurement across each annual growth zone. The resulting data were overlaid on the growth ring pattern and assembled into life history time series of Se concentrations

The average and maximum Se concentrations in the outer fin ray rings are presented in this report. Additional data analyses will be published by IISD**.** in a separate report (Palace et al., *in prep*). A similar method was used to conduct Se microchemical analyses in otoliths (Friedrich et al., 2011), which yield temporal signatures that are known to be chemically stable. The otolith results will then be used to evaluate the stability of the Se signature in fin rays.

Statistical Methods

Data analyses were performed using RStudio (v0.99.489). The relationship between age and length of Derby fish was evaluated using a linear regression model. Comparisons of data with previously published studies were conducted on log-transformed data using ANOVA with Tukey's Honestly Significant Difference posthoc test. Correlations among Se concentrations in different tissue types and between Se concentrations in tissue types and fork length or age were determined using the non-parametric Spearman's rank correlation test. All tests were evaluated using alpha=0.05.

Results and Discussion

Field Sample Collection

Tissues were obtained from a total of eight female white sturgeon collected at the Derby (Table 1). The target size was 51 inches (129.5 cm) fork length, and samples were collected from fish ranging from 112.7 to 149.2 cm (median = 129.4 cm; mean = 129.1 cm). Estimated ages ranged from 12 to 20 yr (median = 17 yr, mean = 16.6 yr). Exact sampling locations were not recorded, but conversations with anglers suggested that most fish were caught in Suisun Bay. Field measurements and the results of Se and isotope analyses in muscle plug, ovary, liver, and fin ray are presented in Table 1.

Quality Assurance Results

Se and $\delta^{13}C$ and $\delta^{15}N$ isotope ratios were analyzed in all eight female fish, but $\delta^{34}S$ was measured only in a subset of samples with enough sample mass for analysis. All selenium and isotope ratios met measurement quality objectives (Yee et al. 2015).

Selenium was detected in all samples and results were blank corrected, with the standard deviation of the blanks well below the method detection limit. CRM recoveries showed an average 7% error, well within the 35% measurement quality objective target. Relative standard deviations on lab replicates averaged 13%, also well within the 35% measurement quality objective target. Isotope ratios were reported in all samples above the limit of quantitation. The standard deviation among replicates was less than 1‰ for all isotopes, while the relative standard deviations for carbon, nitrogen, and sulfur mass in replicates were < 25%.

Summary Statistics for Selenium

Se concentrations in muscle plugs collected from the eight female fish ranged from 6.2 to 22 µg/g dw, with a median of 8.9 and mean of 11.2 µg/g dw (coefficient of variation of 17%), within the range of the TMDL target of 11.3 µg/g dw (Table 2; Figure 1). Three of the eight muscle plugs (38%) had Se concentrations above the TMDL target (15, 15, and 22 ug/g dw), while the remainder of the samples ranged between 6.2 and 9.5 µg/g dw.

Se concentrations in ovaries ranged from 3.5 to 47 µg/g dw, with a median of 17 and mean of 20 µg/g dw. The standard deviation of ovary Se concentrations was relatively high, at 5.1 µg/g dw (coefficient of variation of 25%). The distribution was driven by two particularly high values (47 and 32 µg/g dw; the next highest values were 23 and 17 µg/g dw).

Se concentrations in the liver were higher, ranging from 11 to 74 µg/g dw, with a median of 31 and mean of 36 µg/g dw. The standard deviation of liver Se concentrations was 6.8 µg/g dw (coefficient of variation of 22%). Again, the high mean Se concentration was driven in part by one particularly high Se value (74 µg/g dw) measured in the largest fish.

One fish (WHST-27M) accounted for an interesting combination of the extreme values in this small dataset. This individual had the lowest muscle plug Se value but the highest ovary and liver Se concentrations measured. It was the largest, although not the oldest, fish.

Comparison with Historical Data

Most tissue Se concentrations measured in the 2015 Derby Study were in the upper end of the range of those measured previously in white sturgeon in San Francisco Bay (Figure 1). The muscle plug Se concentrations measured during the Derby (median = 8.9 µg/g dw, range= 6.2-22 µg/g dw; n=8) were significantly greater than the plug or fillet concentrations measured in female sturgeon in RMP Status and Trends monitoring (plugs: median 5.3 µg/g dw, range 2.6-7.5 µg/g dw, n=11 measured in 2009 and 2014, p=0.02; fillets: median 4.7 µg/g dw, range 1.8-12 µg/g dw, n=22 measured in 1997, 2000, 2003, 2006, 2009, and 2014, p=0.01). Se concentrations in Derby plugs were also toward the upper end of the range of plug Se measured in muscle fillets of female fish collected in 2003-2005 reported by Linares-Casenave et al. (median 5.4 µg/g dw;

range 2.6 – 19 µg/g dw; p=0.1) and the RMP 2014 Muscle Plug Study (median 4.4 µg/g dw; range 1.8-25 µg/g dw; n=8; p=0.08) (Sun et al. 2016).

Ovary and liver tissue Se concentrations measured during the 2015 Derby were similarly relatively high compared to previous studies (Figure 1). Ovary Se concentrations measured in Derby study (median 17 µg/g dw; range 3.5-47 µg/g dw; n=8) were significantly greater than those reported by Linares-Casenave et al. (median 3.2 µg/g dw; range 1.1-47 µg/g dw; n=26; p=0.04), and higher but not significantly different from samples collected during the RMP 2014 Status and Trends monitoring event (median 7.3 µg/g dw; range 4.3-7.4 µg/g dw; n=3; p=0.49; note the small sample size of this dataset). Liver Se concentrations (median 31 µg/g dw; range 11-74 µg/g; n=8) were even more elevated compared to concentrations previously reported in female sturgeon by Linares-Casenave et al. (median 7.9 µg/g dw; range 4.7-34 µg/g dw; n=26; $p=6.1x10^{-5}$) and measured in female sturgeon by the RMP in 2009 (median 7.2 µg/g dw; range 4.5-16 µg/g dw; n=7; $p=8.9x10^{-5}$).

Three factors may be contributing to the high Se concentrations observed in this study relative to previous studies. First, the relatively high Se concentrations measured during this study were likely driven by elevated Se concentrations in *P. amurensis*, the primary prey item of white sturgeon in the North Bay, in recent years. These higher concentrations are hypothesized to be due to drought conditions leading to low river flows (Robin Stewart, USGS, personal communication). Both muscle plug and liver concentrations measured during this study were similar to Se concentrations measured in white sturgeon by Stewart et al. (2004) in fish collected in January, after another period of low river inflows.

Second, the seasonal timing of sample collection may have contributed to the elevated Se concentrations observed. The primary mode of Se reproductive toxicity in sturgeon occurs through the substitution of Se for sulfur in proteins of the egg yolk precursor vitellogenin. Se concentrations increase in the liver and ovaries prior to spawning due to increased production of vitellogenin (Doroshev et al. 1994; Linville 2006). Female white sturgeon in San Francisco Bay spawn every two or more years in the spring – typically between late February and early May, with peak activity occurring in March and April (Kohlhorst 1976). Increased production of vitellogenin and egg yolk proteins in the liver and ovary of vitellogenic (pre-spawning) white sturgeon could be contributing to elevated Se concentrations measured in these tissues (Doroshev et al. 1994). This effect appeared to occur in a study published by Linares-Casenave et al. (2015), in which significantly higher Se levels were measured in the ovaries, liver, and muscle of vitellogenic compared to previtellogenic female white sturgeon (muscle: p=0.02; ovary: $p=3.7x10^{-5}$; liver: p=0.008; n=18 previtellogenic, n = 8 vitellogenic females). The authors of that study also suggested that dietary changes before spawning could also be contributing to elevated Se in tissues during the pre-spawning season.

Sturgeon sampled between April and August during RMP Status and Trends monitoring, or between August and October as part of the 2014 Muscle Plug study, would not be expected to be producing high levels of vitellogenin. However, some of the females collected in late January and early February during this Derby may have been producing a higher level of vitellogenin during this period leading up to the spawning season, and therefore might have been expected to have elevated tissue Se concentrations. Although reproductive stage was not identified for the sturgeon collected during the 2015 Derby study, seven of the eight females sampled were within the age range at which female white sturgeon typically mature (15 to 32 years).

Additionally, a comparison between Se measured in sturgeon caught during the Sturgeon Derby and sturgeon measured during the Linares-Casenave et al. study (2015) shows that Se concentrations measured in Sturgeon Derby females were more similar to Se concentrations previously measured in vitellogenic sturgeon compared to previtellogenic sturgeon (Figure 1). Ovary Se measured in the Derby study was significantly greater than ovary Se in previtellogenic females but not significantly different from ovary Se in vitellogenic females measured during the Linares-Casenave et al. study (Derby females vs. previtellogenic Linares-Casenave et al. females: $p=1.4x10^{-4}$; Derby vs. vitellogenic Linares-Casenave et al. females: p=0.92). A similar pattern was observed in muscle tissue (Derby females vs. previtellogenic Linares-Casenave et al. females: $p=3.4x10^{-3}$; Derby vs. vitellogenic Linares-Casenave et al. females: p=0.79). Liver Se concentrations in Derby females were higher than both groups in the Linares-Casenave et al. study, but closer to those measured in the vitellogenic females (mean liver Se: Derby females = 290 ug/g dw ; previtellogenic Linares-Casenave et al. females = 140 ug/g dw; vitellogenic Linares-Casenave et al. females = 170 ug/g dw). This analysis suggests that Se concentrations measured during the Sturgeon Derby are consistent with Se concentrations that may be expected in vitellogenic fish.

Histological analysis of ovaries collected during the 2016 and 2017 Sturgeon Derby special studies will allow a direct evaluation of the relationship between reproductive status on muscle and ovary Se concentrations.

Third, differences in the sample collection and analytical methods could be contributing to historical variation in measured Se concentrations. Two variations of the muscle plug sampling technique were used during this study and the 2014 Muscle Plug study (described in Appendix A and Sun et al., 2016) and the 2009 and 2014 RMP Status and Trends study (see footnote on page 7), which could have created a bias in the results using either method. Additionally, different laboratory tissue preparation, digestion, and analytical methods have been used in various studies of Se in sturgeon tissues. Muscle plug Se analyses in this study and the RMP Muscle Plug study were conducted by USGS-Menlo Park, while muscle plug and muscle fillet Se analyses for the RMP Status and Trends monitoring program were conducted by the Marine Pollution Studies Lab at Moss Landing Marine Laboratories (MPSL-MLML). Furthermore, sturgeon samples in the Linares-Casenave et al., 2015 study were processed and analyzed by UC Davis. A laboratory intercomparison study, particularly of muscle plugs collected using one or both previously used methods, may be useful to identify the impact of these sampling and analysis artifacts on historical patterns of Se concentrations measured in sturgeon.

Age, Length, and Tissue Se

Similar to other studies, a significant positive correlation was observed between age and fork length in the eight female Derby sturgeon (p=0.04, R^2=0.46) (Figure 2). However, when comparing tissue Se concentrations to age and fork length (Figure 3), a significant positive relationship was observed only between ovary Se and fork length, based on a non-parametric rank correlation test (Spearman's rank correlation, rho=0.80, p=0.02). No significant relationship between muscle plug Se and age or fork length was detected within this size range. In contrast, Linares-Casenave et al. (2015) found a significant positive relationship between length and Se in muscle, gonads, and liver, based on a comparison of concentrations measured in 21 male and 26 female fish separated into three size classes ranging from 53 to 170 cm (age 4-28 yr). All fish collected during the Derby fell within a narrower size and age range (112.7-149.2 cm fork length; 12-20 yrs), making it more difficult to assess the correlation between these characteristics and measured Se concentrations (Figure 3). The small size of this dataset may also make the detection of correlations more difficult. The results from the 2015 Derby Study should be considered preliminary given the small sample size and limited length range available for this dataset. Se and histology data from a different size range of fish collected during the 2016 Derby will increase the sample size and allow for further evaluation of the relationships reported by Linares-Casenave et al. (2015).

Se Correlations Among Tissues

No significant correlations in selenium concentrations were observed among the tissues sampled during this study (Figure 4). Varying rates of selenium uptake, depuration, and turnover influencing selenium accumulation in different tissues, in combination with variable diets and foraging locations over these accumulation periods, are likely contributors to the variability in the relationships between tissues observed. The small sample size in this study also impeded the detection of relationships in selenium concentration between tissue types.

In contrast, a stronger relationship was observed among these tissues in female sturgeon measured by Linares-Casenave et al. (2015), which included 26 female sturgeon collected throughout the year rather than the months immediately prior to the spawning season (Figure 5). The relationship between muscle plug and ovary selenium concentrations was statistically significant when including the entire dataset ($p=7x10^{-4}$, rho=0.62, n=26), but not when considering previtellogenic and vitellogenic females separately (previtellogenic: p=0.06; rho=0.45, n=18; vitellogenic: p=0.35, rho=0.38, n=8).

Higher concentrations and greater variability were observed in the tissues of vitellogenic (i.e., pre-spawning) females, particularly in the liver and ovary, where the egg yolk precursor protein vitellogenin is synthesized and stored. This suggests that selenium is disproportionately partitioned into these tissues during vitellogenesis, in the months prior to spawning. The higher variability in vitellogenic females suggests that in the months immediately prior to spawning, tissue Se concentrations may be more strongly influenced by variable factors such as recent foraging location and temporal proximity to spawning.

Additional data collected from fish of a potentially different size and age range during the 2016 and 2017 Sturgeon Derby studies, together with data on reproductive status, may help to better characterize the correlations of Se concentrations in these tissues.

C, N, and S Isotopes

δ^{13}C, δ^{15}N, and δ^{34}S isotope ratios were measured in a subset of the muscle plug samples with enough sample mass for analysis (Table 2, Figures 6 and 7) to provide information about habitat use and foraging behavior. The measured isotope ratios generally fell within the range of values previously observed in white sturgeon collected in the North Bay (Stewart et al. 2004).

δ^{15}N values ranged from 14.6 to 17.9‰, suggesting mixed foraging patterns, similar to what was observed in the 2014 Muscle Plug study (Sun et al. 2016). Fish with lower δ^{15}N values were likely foraging more on invertebrates, while fish with higher δ^{15}N were likely more piscivorous. The δ^{15}N values in fish collected in the 2014 Muscle Plug study and the 2015 Derby study suggest that most fish are feeding on *P. amurensis*. No clear correlation of muscle Se with δ^{15}N was apparent in the preliminary dataset (Figures 6 and 7).

δ^{13}C values ranged from -21.7 to -28.8 ‰ (Table 1), which is also consistent with expected values for sturgeon caught in north San Francisco Bay and the Delta, based on recent data on δ^{13}C in *P. amurensis* (Robin Stewart, personal communication). Across both studies, the highest muscle plug Se concentrations were measured in fish collected with δ^{13}C between -23.0 and -26.7‰ (Figures 6 and 7). This corresponds with δ^{13}C values for clams in Suisun Bay, where Se concentrations in *P. amurensis* are highest (Stewart et al. 2013). In contrast with fish caught in Suisun Bay during the fall 2014 Sturgeon Muscle Plug study, the generally lower δ^{13}C values in the Derby fish may reflect a seasonal shift in *P. amurensis* δ^{13}C values or a shift to foraging closer to the Delta.

δ^{34}S values ranged from 12.4 to 17.5‰, consistent with foraging in the fresh and brackish waters of the Bay (Figure 6). No clear relationship between ovary or liver selenium and muscle plug isotope ratios was apparent in this preliminary dataset (Figures 8-9).

Se in Fin Rays

Preliminary data on fin ray analyses were presented by Dr. Palace at the SETAC North America Conference in November 2015 (Palace et al. 2015). Multiple Se measurements can be made across a single annular ring of the fin ray, resulting in multiple measurements representative of sturgeon tissue Se for any given year. The average and maximum Se concentrations measured in the outermost annular ring for each of the eight female fish analyzed are presented in Table 1. Results for the remaining 18 male fish, in which fin rays and otoliths were analyzed for Se but not muscle, ovary, or liver tissues, will be published in the full fin ray report (Palace et al. *in prep*.)

Additional analyses of the data are planned, using different summation and averaging methods, to further probe the relationship between fin ray and other tissues, and the potential use of fin rays to non-lethally monitor Se in sturgeon. The following are some initial observations.

- The maximum Se concentrations measured in female fin ray tissue deposited in the most recent year ranged from 0.37 to 0.60 µg/g. The average Se concentration within this most recent growth ring ranged from 0.26 to 0.40 ug/g.

- Initial analyses show widely varying temporal patterns in the fin ray Se concentrations of the eight female fish. Time series of the maximum annual Se concentrations show either increasing or decreasing trends in different fish in recent years. However, across the eight females sampled, the maximum annual fin ray Se concentrations corresponding with the previous six years (2009-2015) showed no significant change over this period (Palace et al., *in prep*). The average of the maximum annual Se concentrations measured across all fish remained approximately at values just below 0.5 ug/g over this six year period. Temporal trend analyses were not conducted using years prior to 2009 because different numbers of fish are included in the average concentration prior to this year (i.e., not all fish were alive prior to 2009).
- Initial analyses have not indicated a significant correlation between fin ray and Se concentrations in other tissues (Figure 10). The maximum and average Se concentrations measured in the outer fin ray annulus of each fish, composed of the most recently deposited tissue, was not significantly correlated with Se muscle plug, ovary, and liver concentrations.

Additional fin rays were collected at the 2016 Sturgeon Derby. The combined dataset from 2015 and 2016 should allow firmer conclusions and will be published in a separate report (Palace et al., *in prep.*).

<u>*Se in Otoliths*</u>

Microchemical analyses were conducted on the 2015 Derby otoliths, together with the otoliths that were collected at the 2016 Sturgeon Derby in February 2016. A comparison of otolith and fin ray results will help evaluate the stability of Se concentrations in fin ray annuli and inform the interpretation of the fin ray data. Analyses of the combined 2015 and 2016 dataset will be included with the fin ray results (Palace et al., *in prep*).

Conclusions

Selenium concentrations were successfully measured in muscle plug samples collected from eight female sturgeon, and in fin rays collected from 26 male and female sturgeon. Laboratory methods for processing and analyzing selenium in low-sample mass muscle plug samples were further refined, and techniques for preparing and conducting microchemistry analyses on sturgeon fin rays were developed. Further discussion of the fin ray methods and results will be published in a separate report (Palace et al., *in prep*).

The muscle plug, ovary, and liver Se concentrations measured in this study were at the upper end of the range of previously measured values. The relatively high Se concentrations observed during this study may be influenced by the characteristics of the Derby fish, which were only females collected shortly before spawning season, during a period of low freshwater inflow, and in a region known to have high prey Se concentrations. The isotope data suggest that although some fish collected during the Derby appeared to have been foraging in the Delta and San Pablo Bay, the fish with the highest Se concentrations were likely feeding largely on *P. amurensis* in Suisun Bay, where clam Se concentrations are highest.

The sample size for this study was too small to draw firm conclusions on correlations among tissues. Poor correlations among Se concentrations in different tissues were observed. Additional data from a different size range of fish and reproductive status data collected during the 2016 Sturgeon Derby will be valuable in further evaluating these relationships. Otolith results will contribute to the full evaluation of fin ray Se as an index of Se bioaccumulation and Se concentrations in other tissues. Collection of these additional samples occurred on February 6-7, 2016 and the data will be presented in a separate report.

Tables and Figures

Table 1. 2015 Sturgeon Derby Study: field measurements, selenium and isotope results in eight female fish.

Sample ID	Sample Date	Fork Length (cm)	Age (yr)	Muscle Plug			Ovary	Liver	Muscle Plug			Fin Ray	
				Sample Weight (mg dw)	% Moisture	Selenium (µg/g dw)	Selenium (µg/g dw)	Selenium (µg/g dw)	$\delta^{13}C$ Isotope (‰)	$\delta^{15}N$ Isotope (‰)	$\delta^{34}S$ Isotope (‰)	Max Selenium (µg/g)	Average Selenium (µg/g)
WHST-03M	1/31/2015	124.5	16	64.5	53.8	7.0	4.6	30	-27.9	16.8	17.5	0.53	0.32
WHST-06M	1/31/2015	124.1	14	35.1	71.4	22	17	29	-26.7	16.5	17.2	0.47	0.28
WHST-08M	1/31/2015	131.9	17	15.5	74.0	15	32	11	-23.0	16.6	14.7	0.60	0.28
WHST-09M	1/31/2015	131.4	17	36.3	75.8	15	16	48	-25.3	17.0	17.2	0.40	0.27
WHST-10M	1/31/2015	112.7	12	31.4	74.4	9.5	3.5	22	-27.2	15.0	12.4	0.54	0.40
WHST-15M	1/31/2015	127.3	20	16.7	80.1	7.5	17	31	-26.0	15.4	14.0	0.45	0.30
WHST-17M	2/1/2015	131.4	18	38.0	79.1	8.3	23	45	-21.7	17.9	16.9	0.37	0.26
WHST-27M	2/1/2015	149.2	19	35.0	57.6	6.2	47	74	-28.8	14.6	15.1	0.51	0.29
Mean		**129.1**	**16.6**			**11**	**20**	**36**	**-25.8**	**16.2**	**15.6**	**0.48**	**0.30**
Median		**129.4**	**17.0**			**8.9**	**17**	**31**	**-26.4**	**16.5**	**16.0**	**0.49**	**0.29**
Standard Error		**3.6**	**0.9**			**1.9**	**5.1**	**6.8**	**0.9**	**0.4**	**0.7**	**0.03**	**0.02**

Figure 1. Muscle, ovary, and liver selenium concentrations measured in San Francisco Bay white sturgeon during the 2015 Sturgeon Derby and previous studies. The previous studies from which data are included on these graphs are Linares-Casenave et al. (2015) (muscle fillets), 2014 Muscle Plug (RMP 2014 Sturgeon Muscle Plug Special Study; Sun et al. 2016), RMP S&T (muscle fillets or plugs collected during RMP Status and Trends sport fish monitoring, 1997, 2000, 2003, 2006, 2009, and 2014 or 2009 and 2014, respectively) (Davis et al. 2011). The white bars show the median concentration for each set of samples, and points represent measurements of individual fish. Points are colored by sex and reproductive status, where data are available (M = male, U = unknown sex, F = female of unknown reproductive stage, F preVTG = previtellogenic female, F VTG = vitellogenic female). The black line is the fish muscle tissue numeric target (11.3 µg/g dw) established in the North Bay Selenium TMDL. Groups labeled with the same letter did not have significantly different means (log-transformed data, TukeyHSD, alpha=0.05).

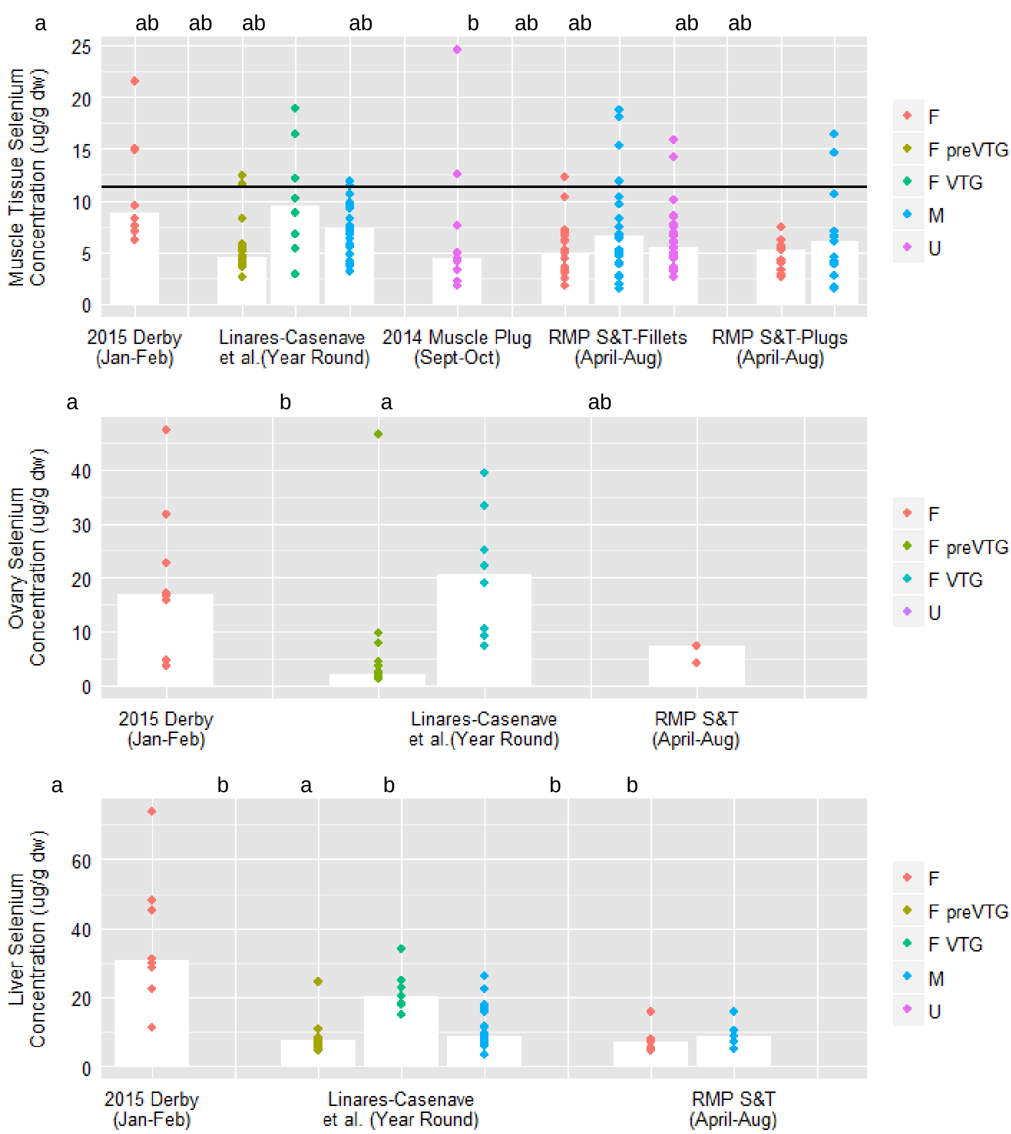


Figure 2. Relationship between fish age and fork length in eight female fish caught during the Derby.

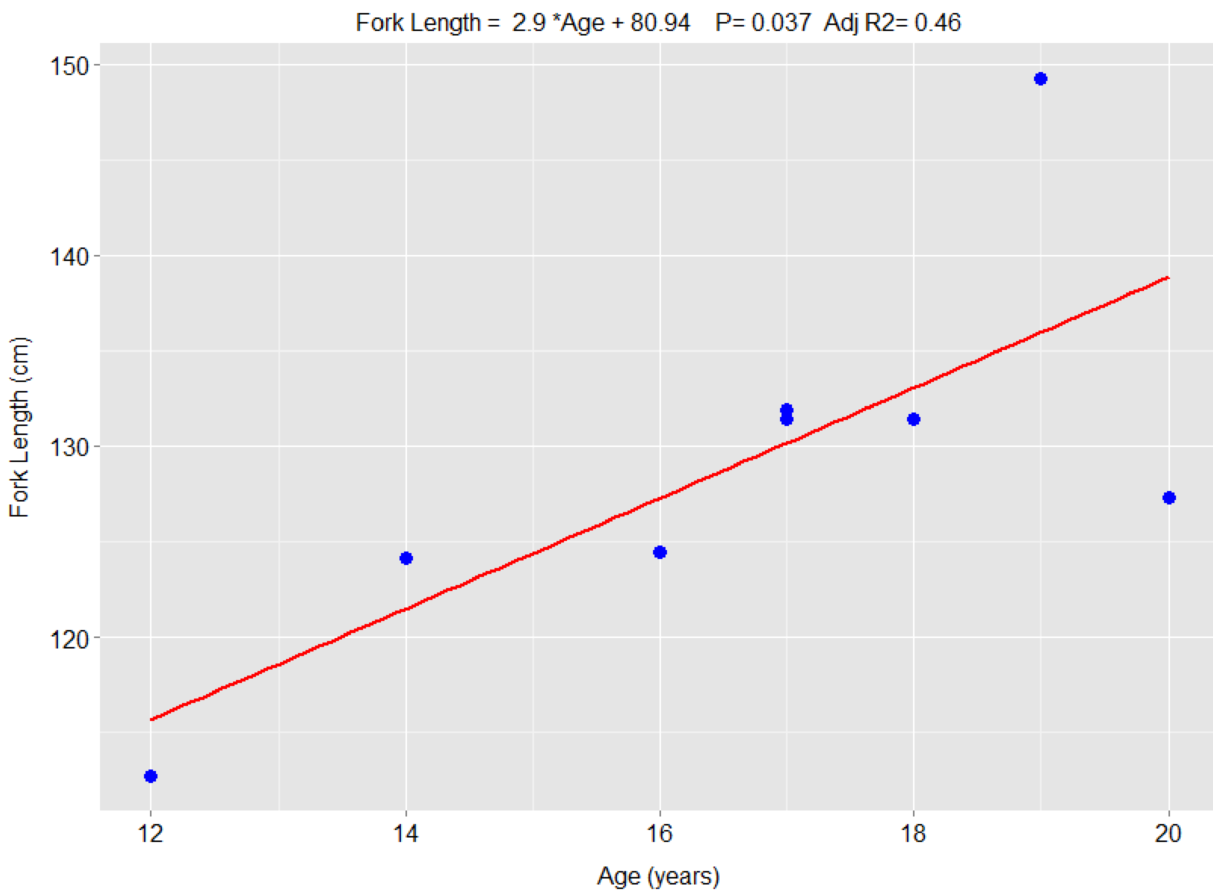


Figure 3. Relationship between selenium concentration in muscle plug, ovary, and liver tissue and fish age or length.

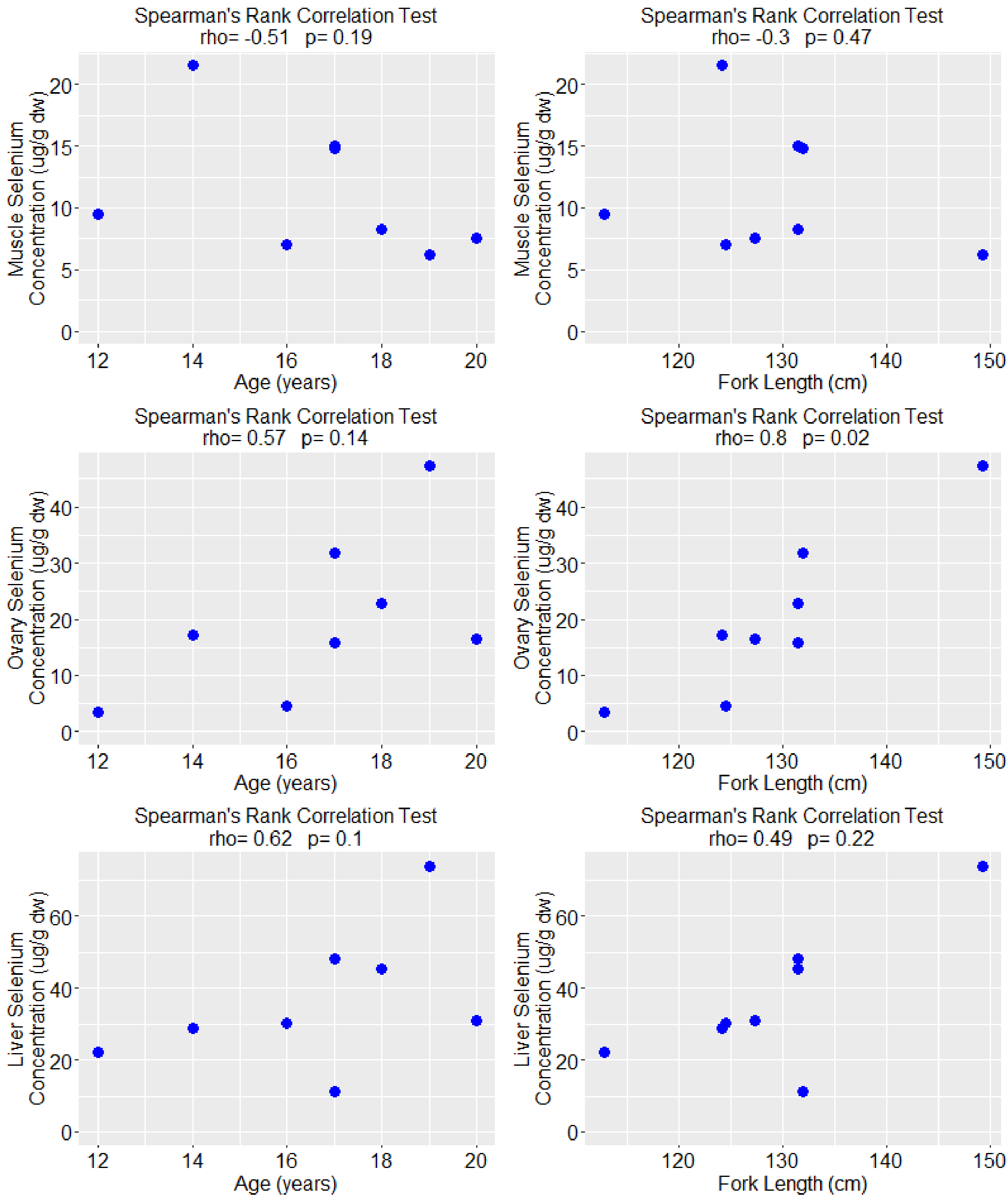


Figure 4. Relationships among selenium concentrations in muscle, ovary, and liver tissue collected from female sturgeon at the 2015 Sturgeon Derby. Points are labelled by SampleID. Correlations between tissue types are not statistically significant (Spearman's rank correlation test, alpha=0.05).

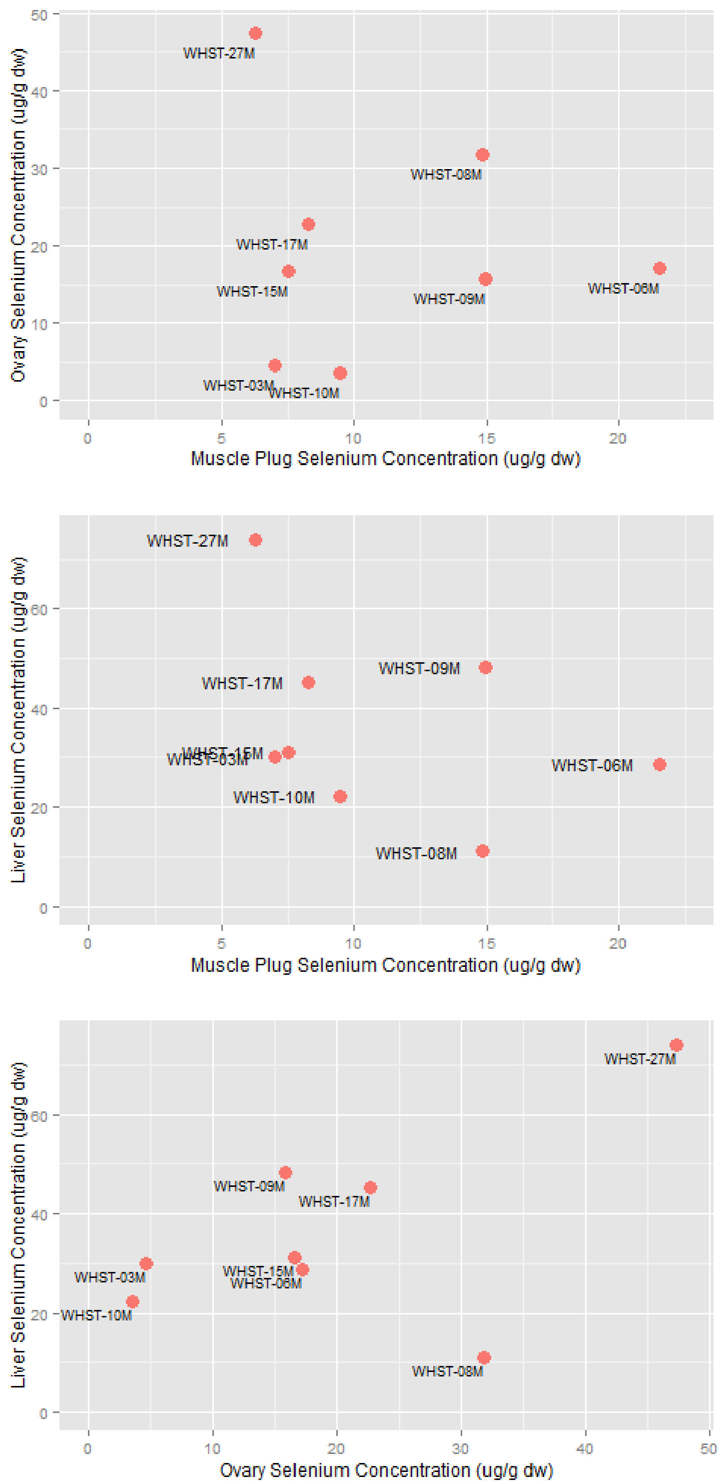


Figure 5. Relationships among selenium concentrations in muscle, ovary and liver tissue in female sturgeon, as reported in Linares-Casenave et al. (2015). Each point represents an individual fish, but some points may appear to be overlapping in the figure. 26 fish were sampled, including 18 previtellogenic and 8 vitellogenic fish. Positive correlations between selenium concentrations measured in all tissues were observed in the entire dataset (Spearman's rank correlation, alpha=0.05). However, no statistically significant correlations were observed in the previtellogenic or vitellogenic groups separately, with the exception of a significant relationship between ovary and liver, driven by high values in one previtellogenic fish caught in March (p=0.03, rho=0.50).

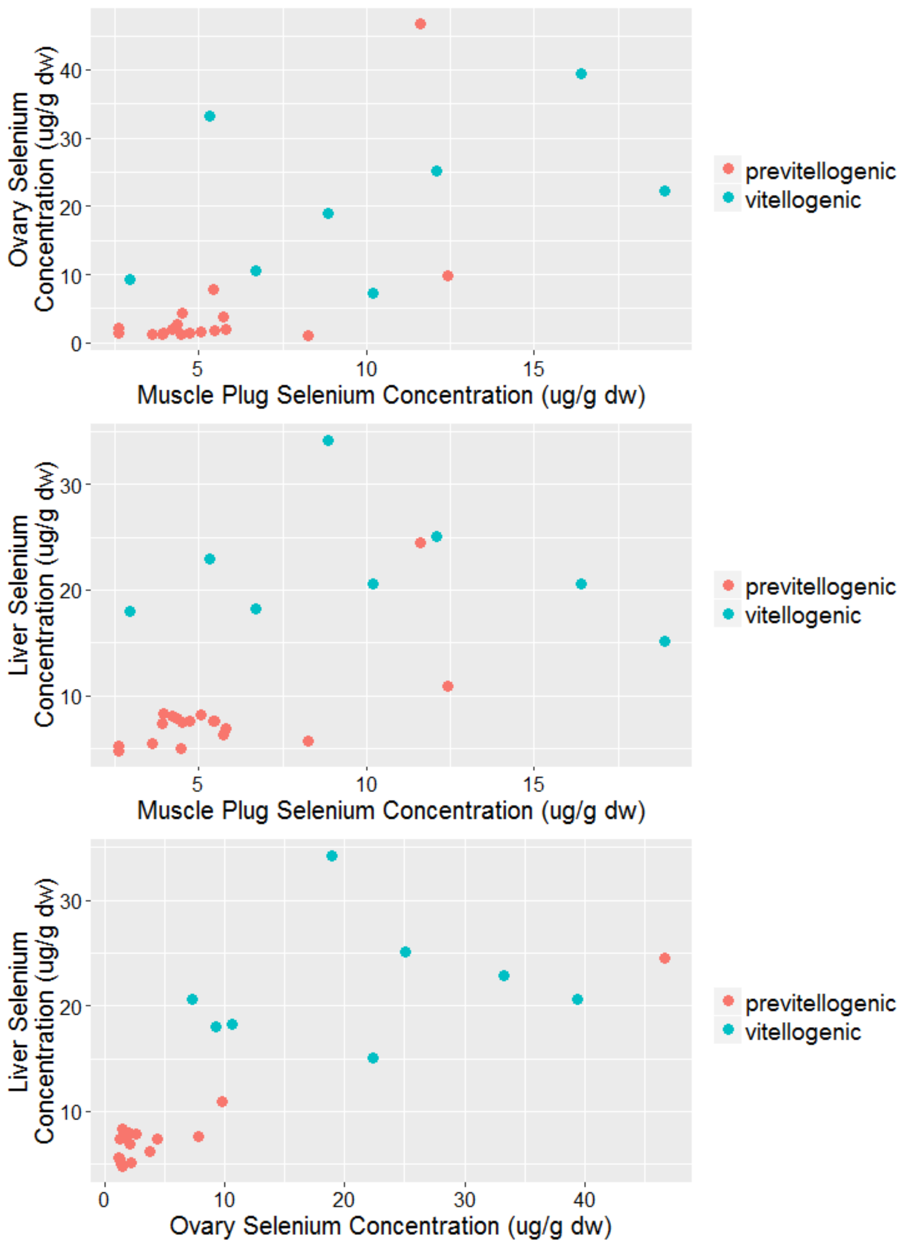
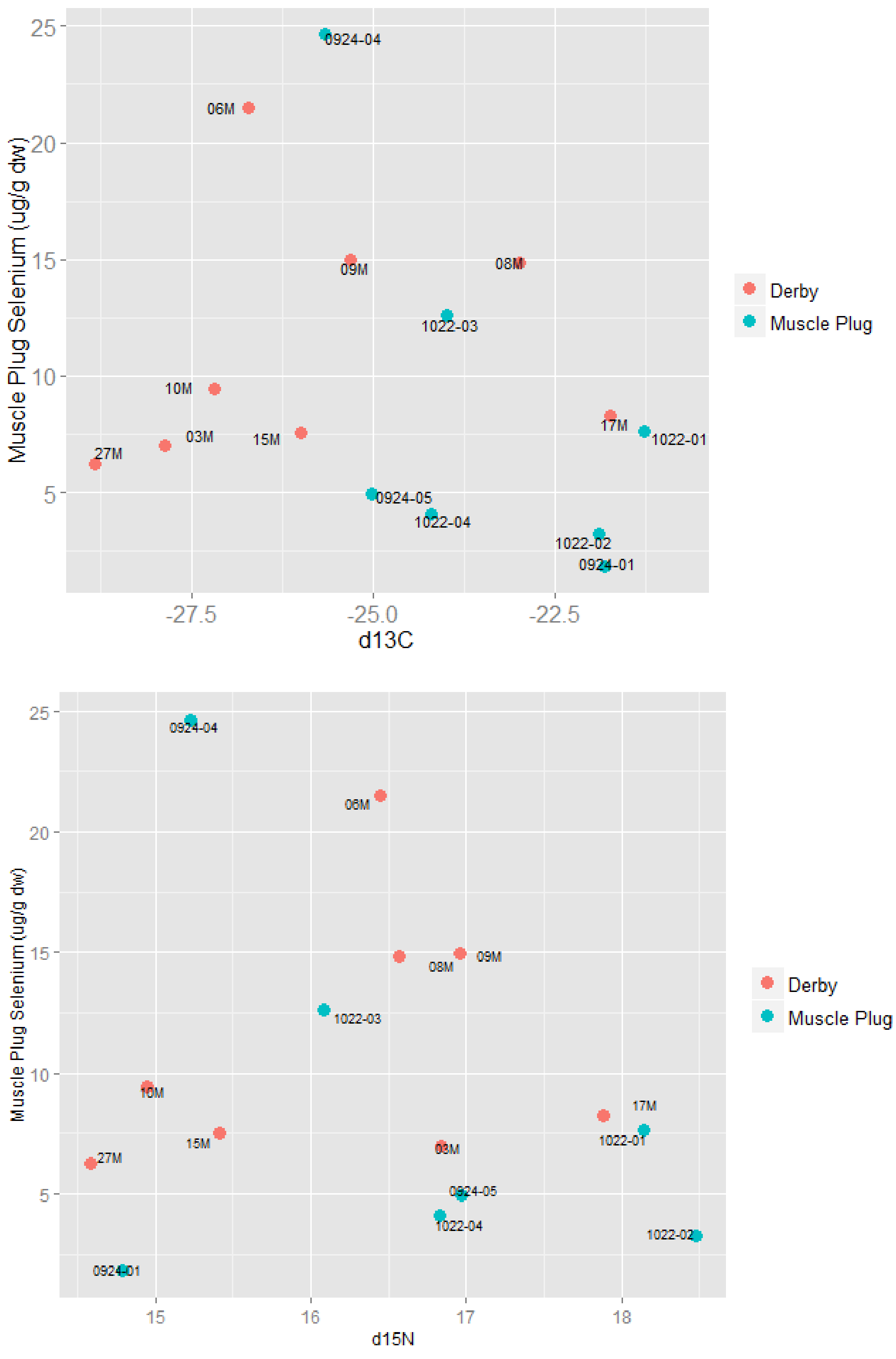
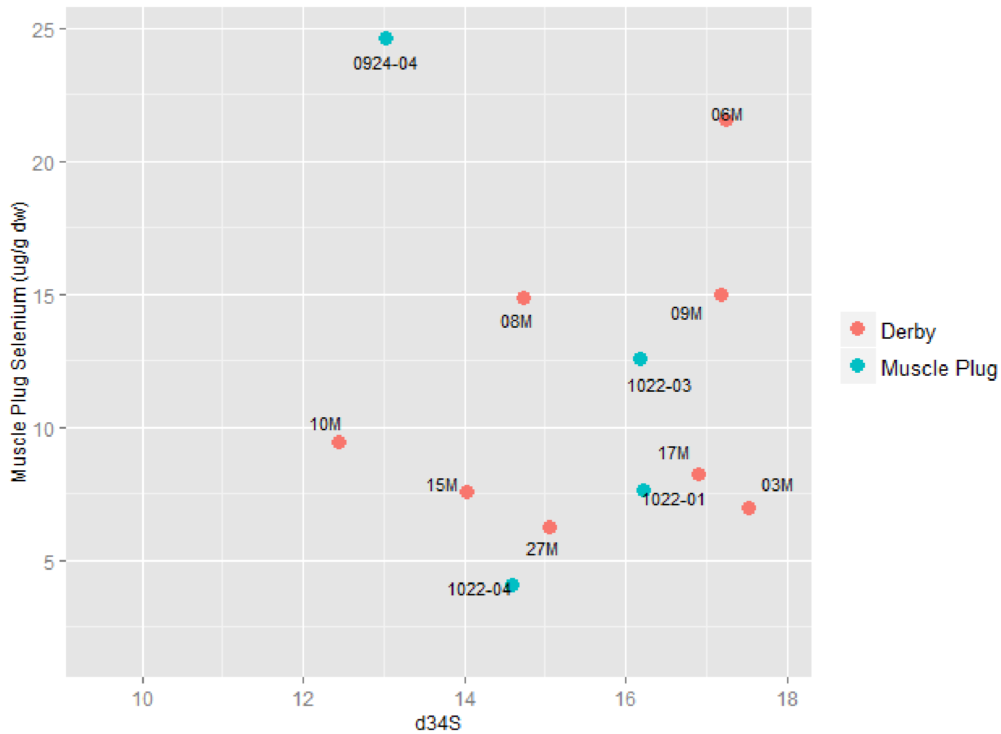


Figure 6. Relationship between muscle plug selenium and $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ isotope ratios in white sturgeon muscle plugs collected during the 2014 Muscle Plug study and 2015 Sturgeon Derby study. Each point represents an individual fish, and points are labeled by abbreviated SampleID (SampleIDs for Muscle Plug study samples are described in Sun et al., 2016). The highest muscle plug selenium concentrations correspond with $\delta^{13}C$ values between -23.0 and -26.7‰, corresponding with $\delta^{13}C$ values from clams in Suisun Bay. No clear relationship between selenium concentrations and $\delta^{15}N$ or $\delta^{34}S$ isotope ratios is apparent.





25
20
15
10
5
Muscle Plug Selenium (ug/g dw)
10
12
14
16
18
d34S
0924-04
06M
08M
09M
1022-03
10M
17M
15M
03M
1022-01
27M
1022-04
Derby
Muscle Plug

Figure 7. Muscle plug selenium concentration compared with $\delta^{13}C$ and $\delta^{15}N$ isotope ratios in white sturgeon collected during the 2014 Selenium in Muscle Plugs study and 2015 Sturgeon Derby study. Each point represents an individual fish, and points are labeled by muscle plug selenium concentration (µg/g dw). Fish with the highest selenium concentrations appear to have been foraging within Suisun Bay ($\delta^{13}C$ between -23.0 and -26.7‰) on invertebrates (i.e., *Potamocorbula amurensis*; $\delta^{15}N$ less than 17‰).

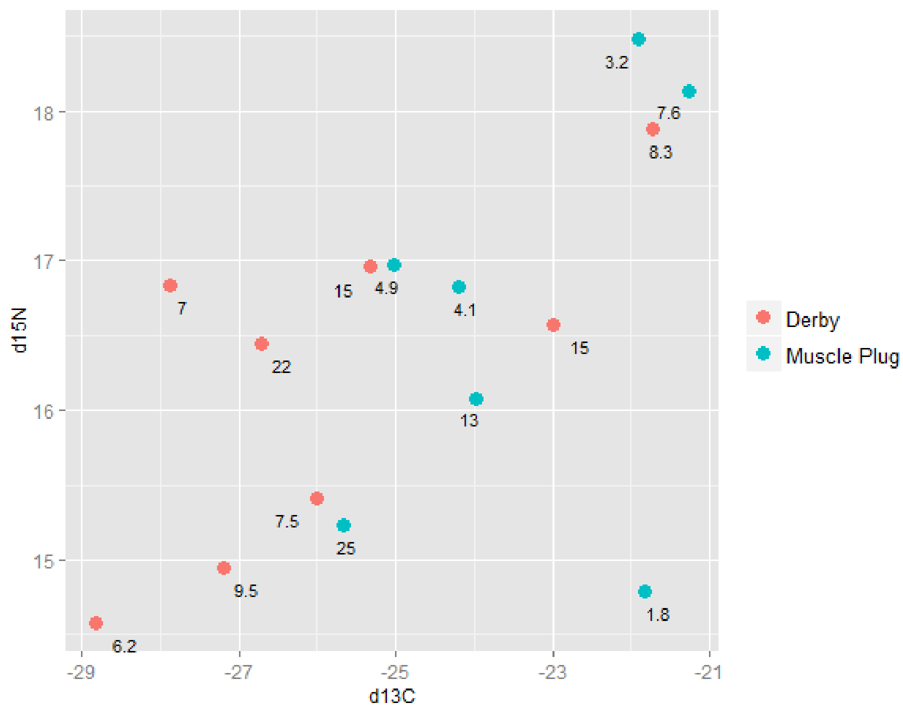


Figure 8. Relationship between ovary selenium and $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ isotope ratios in white sturgeon muscle plug samples collected during the 2015 Sturgeon Derby study. Each point represents an individual fish, and points are labeled by abbreviated SampleID. No clear relationship between ovary selenium concentrations and muscle plug $\delta^{13}C$, $\delta^{15}N$, or $\delta^{34}S$ isotope ratios is apparent.

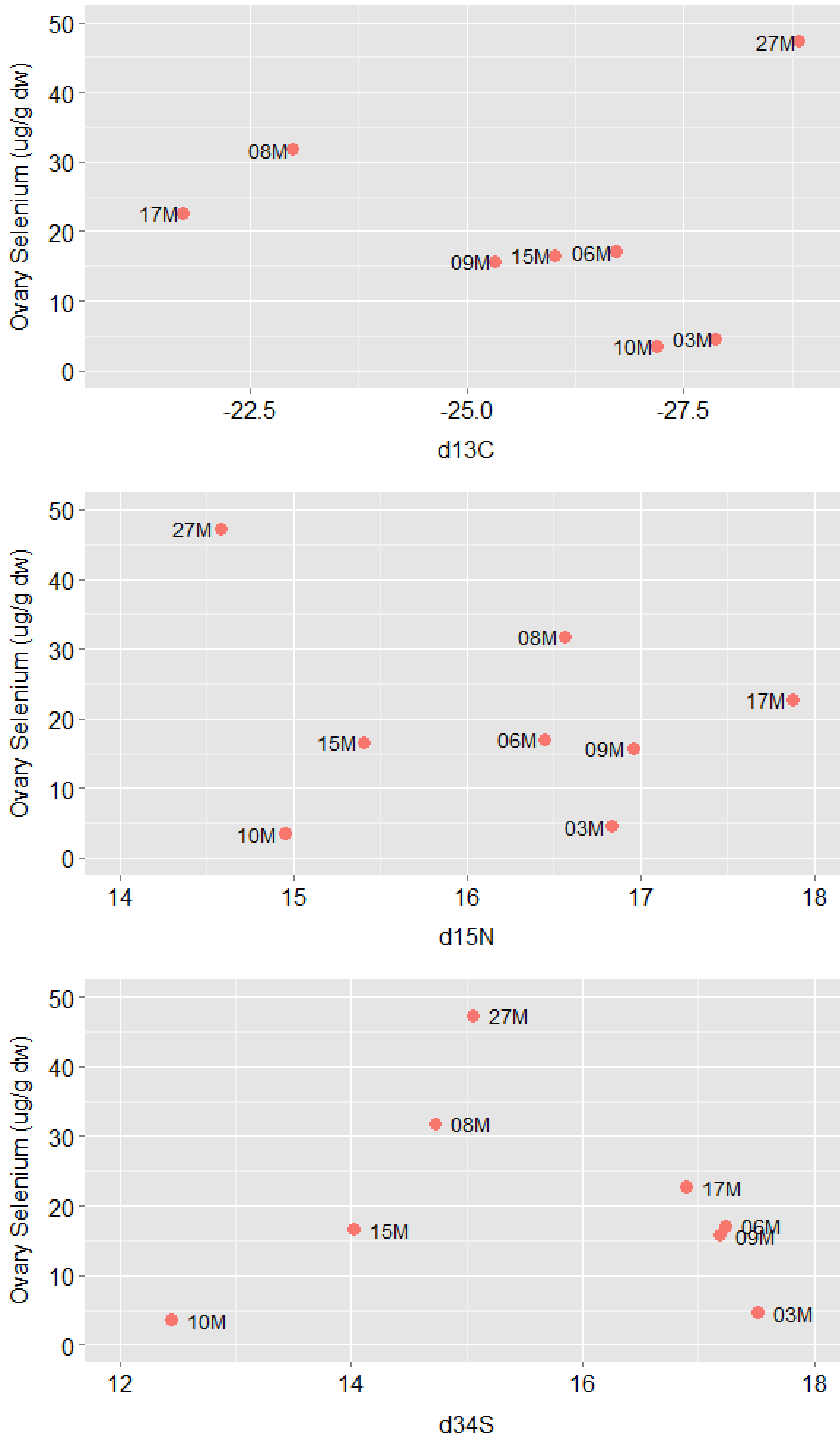


Figure 9. Relationship between liver selenium and δ^{13}C, δ^{15}N, and δ^{34}S isotope ratios in white sturgeon muscle plug samples collected during the 2015 Sturgeon Derby study. Each point represents an individual fish, and points are labeled by abbreviated SampleID. No clear relationship between liver selenium concentrations and muscle plug δ^{13}C, δ^{15}N, or δ^{34}S isotope ratios is apparent.

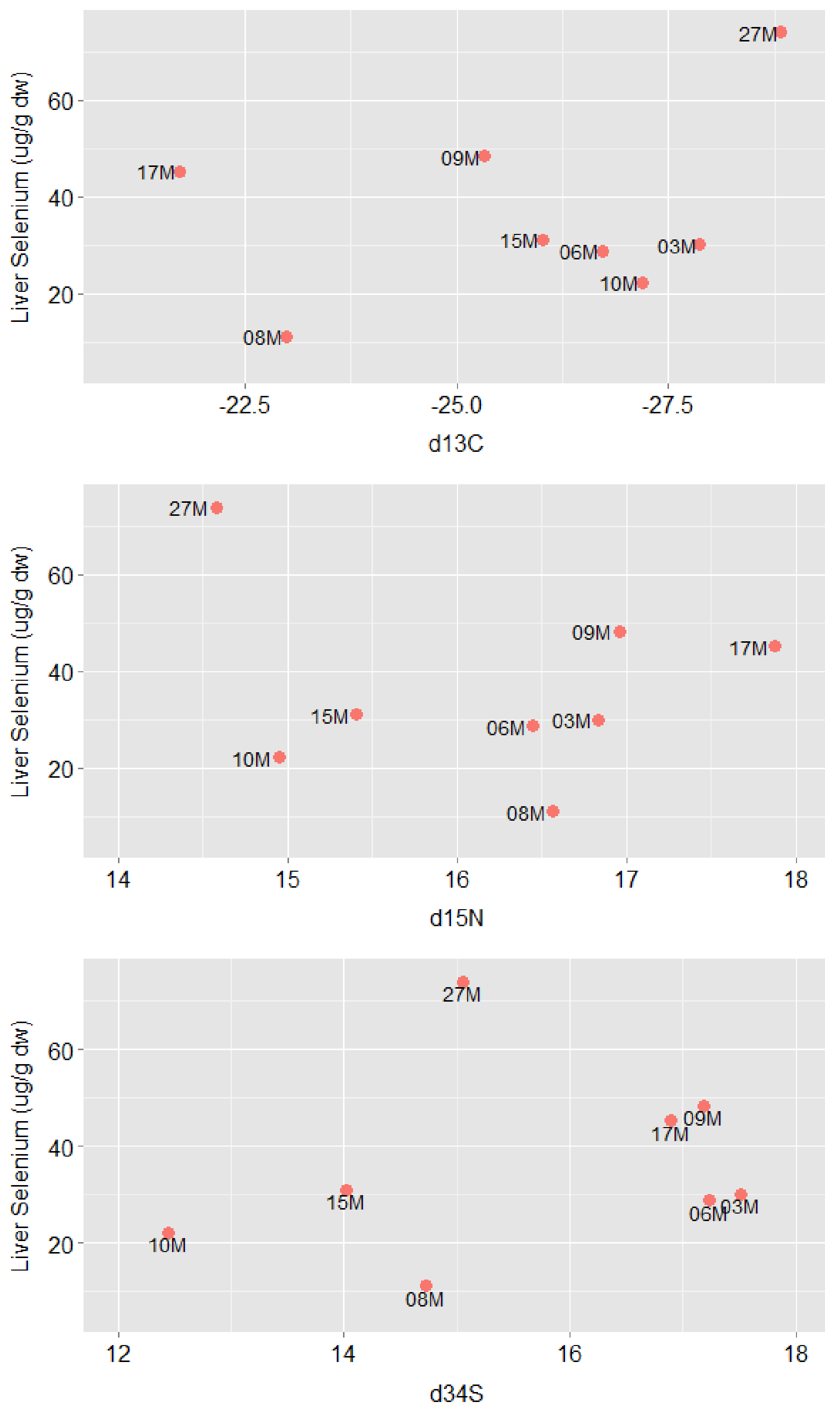
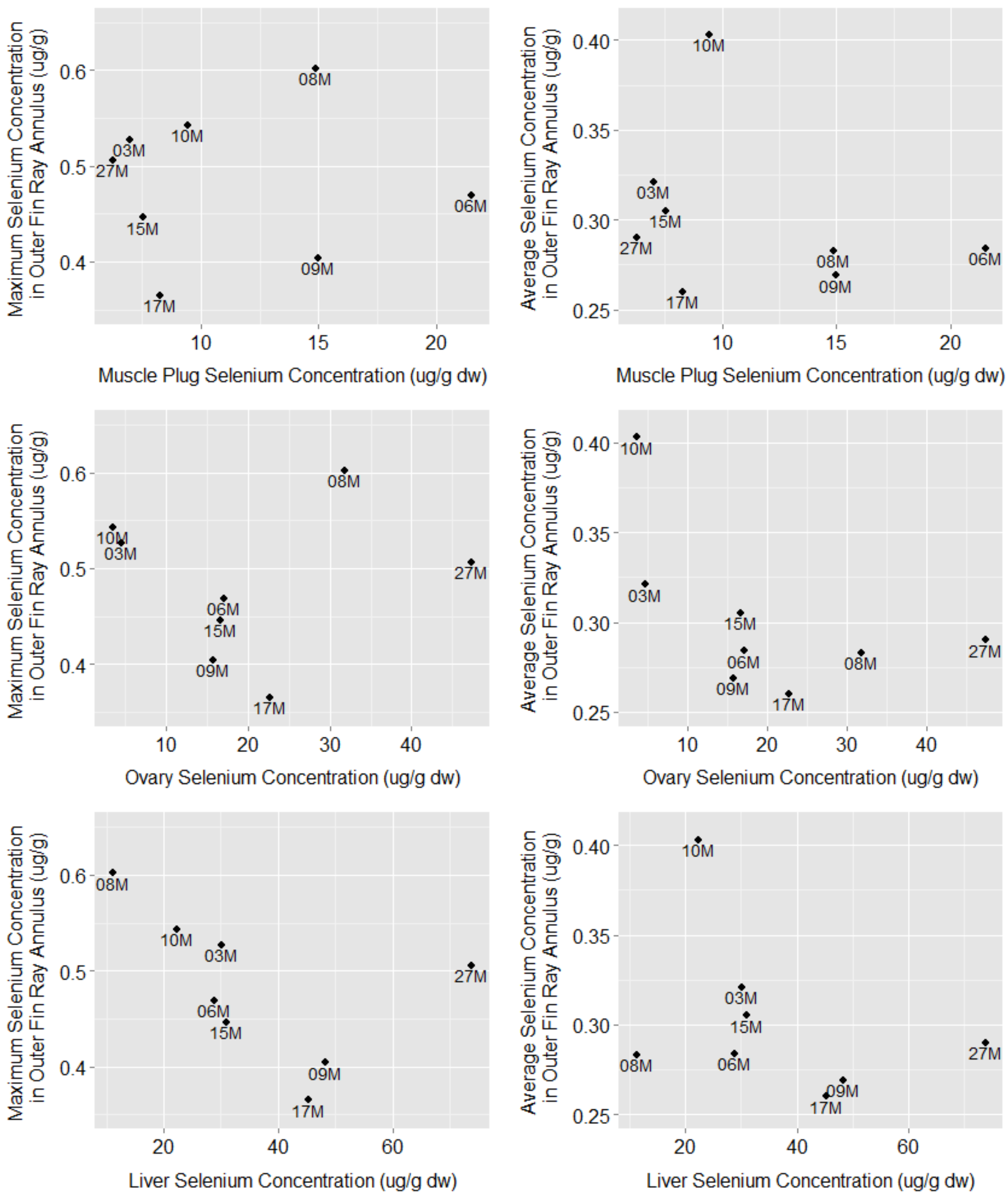


Figure 10. Relationship between the maximum and average selenium concentration measured in the outer fin ray annulus, and muscle plug, ovary, and liver concentrations. Different y-axis scales are used in plots of the maximum and average selenium concentrations in the outer fin ray annulus. Each point represents an individual fish, and points are labeled with abbreviated sample IDs. Correlations between tissue types are not statistically significant (Spearman's rank correlation test, alpha=0.05).



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Appendix A

2015 Sturgeon Derby Field Summary

The Sturgeon Derby, an annual angling competition held out of Martinez, CA, provides a rare opportunity to collect large numbers of tissue samples from white sturgeon. USFWS has developed a strong working relationship with the Derby organizers, the Foundation Sportsman's Club, over the past several years, and facilitated SFEI's collection of tissue samples for Se analysis from the Derby this year. These samples were collected to support the Se TMDL currently under development. Of particular interest for this study was collecting samples of both muscle and ovary tissue to examine the correlation between the two. In addition, fin ray and otolith samples were collected for microchemistry analysis to determine the fish age and changes in Se concentrations over time. Additional tissues were collected for analysis by USFWS, or to archive for potential future analysis by other organizations.

Methods

Sturgeon Collection

The Sturgeon Derby awards cash prizes to the anglers that bring in fish closest to a chosen target length. The target size this year, chosen randomly from within the legal slot limit of 40-60 inches (fork length), was 51 inches. The Derby took place from 7:00am on January 31, 2015 to 1:00pm on February 1, and fish could be brought in at any time within that window. Prizes were awarded to the 7 anglers whose fish were closest to the target for each day.

Sturgeon were collected by registered anglers in possession of a CDFW permit. Fish could be caught from anywhere in the Estuary but had to be brought alive to McAvoy Harbor to be measured. While no official record of where fish were collected was kept, anecdotally most fish this year were collected from Suisun Bay.

USFWS asked anglers for permission to take samples from their fish. The anglers were incredibly accommodating and supportive of the sampling. The only anglers who did not allow their fish to be sampled were those who wanted to release the fish alive. Anglers were interested in the results of the sampling, though far more interested in where the fish are from and the general health of the population, than the contaminant data. May be possible to leverage this as more of an outreach opportunity in the future.

Sample collection

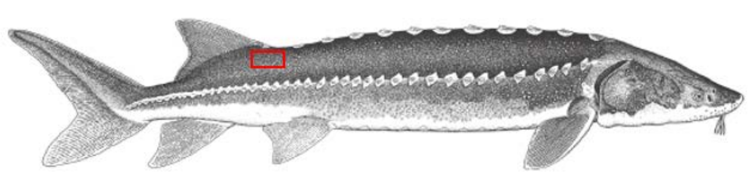
Samples collected for analysis for the RMP included muscle, ovary, fin rays and otoliths. In addition, SFEI collected liver, blood and gut contents to archive for future analysis by other groups (see Table 1). Two groups from USFWS were present, Zac Jackson's group which analyzed fin rays and blood for isotope analysis and the other Ken Nichols's group, which analyzed parasites (the samples for pathology).

Sturgeon were killed via a blow to the head with a mallet. Blood and fin ray samples were collected first, followed by the otolith and organ collection, and finally muscle plug collection. Blood samples were taken from the caudal artery via syringe. Blood was centrifuged in the field and separated into two plasma samples (one to be analyzed by USFWS, the other by USGS). Both marginal pectoral fin rays were removed using a sharp knife, from the point of articulation down to the terminal end of the ray

To access the liver, ovaries, and guts, we made an incision along the ventral length of the fish. For the ovaries and liver several small samples from across the length of the organ were collected. In addition a small piece of the ovary was collected separately to archive for future histology work. A large section of the intestines and stomach were collected for gut content analysis. For ease of sampling, sometimes additional organs were collected along with the guts, to be sorted out later by the lab.

To our knowledge otoliths have not been collected from white sturgeon prior to this study, and finding the otoliths proved challenging. Otoliths were accessed by cutting into the skull with a hacksaw. For the first 20 fish collected we were unable to find the otoliths while processing the fish, so we took a large section of skull to examine later. On the 21^{st} fish we discovered the otoliths were deeper in the skull than expected. We were able to recover otoliths from only a fraction of the previous fish that we had taken skull sections from. Vince Palace at Stantec is writing up a more detailed account of where in the skull the otoliths are located.

Muscle plug samples were taken using disposable 5mm biopsy punches, in a manner similar to muscle plugs collected for the RMP 2014 Selenium Study and Sportfish sampling. The skin was washed with DI water prior to sampling, and 2-3 plugs were taken from the epaxial muscle near or slightly in front of the dorsal fin, off-set from the midline:



Sample storage and shipping

Following sample collection, otoliths and fin rays were dried at room temperature. All other samples were kept frozen until they could be sent to the various labs for archiving or analysis. See Table 1 for details of sample fate, see Table 2 for details of how samples were stored.

Table 1. Number and fate of samples collected.

Sample	Males	Females	Fate
Plasma sample #1	19	6	Sent to USGS to archive for future Se analysis. Males will probably not be analyzed since there are no gonad or liver samples for comparison
Plasma sample #2	19	6	Sent to USFWS for microchemistry to identify movement patterns in white sturgeon during different life history stages.
Fin Ray Right	19	8	Send to IISD for aging and microchemistry.
Fin Ray Left	19	8	USFWS will analyze these to determine movement patterns, distinguishing SJ from Sac fish.
Otoliths	5	4	Send to IISD for aging.
Muscle Plug	0	8	Send to USGS for Se and isotope analysis.
Ovary	0	8	Send to USGS for Se analysis.
Ovary Histology	0	8	Archived at SFEI.
Liver	0	8	Sent to USGS for Se analysis.
Gut Contents	5	7	Archived at Cramer Fish Sciences for future diet analysis.

Table 2. Sample containers and storage.

Tissue	Container Type	Preservative	Storage
Muscle plug	Polystyrene vials	None	Wet ice
Ovary	Vials	None	Dry ice
Ovary - Histology	Vials	Buffered formalin	N/A
Fin rays	Envelopes	None	Dried
Otoliths	Envelopes	None	Dried
Blood	Centrifuge tubes	None	Wet ice
Liver	Ziploc/Whirl-Pak bags	None	Dry ice
Gut content	Ziploc / Whirl-Pak bags	Buffered formalin or ethanol	Wet ice

Measuring sturgeon fork length

Blood collection

Fin ray collection

Dried fin ray sample


Fin ray cross section showing growth rings



Otolith collection and dried otolith sample

Sturgeon dissection for organ collection

Fin ray close-up