Resolving selenium exposure risk: Spatial, temporal, and tissue-specific variability of an endemic fish in a large, dynamic estuary

A. Robin Stewart[a], Frederick Feyrer[b] and Rachel Johnson[c,d]

[a] U.S. Geological Survey, Water Mission Area, 345 Middlefield Rd. MS496, Menlo Park, CA USA, arstewar@usgs.gov

[b] U.S. Geological Survey, California Water Science Center, 6000 J Street, Placer Hall, Sacramento, CA USA, ffeyrer@usgs.gov

[c] NOAA Fisheries, Southwest Fisheries Science Center, Fisheries Ecology Division, 110 McAllister Way, Santa Cruz, CA USA, Rachel.Johnson@noaa.gov

[d] University of California Davis, Center for Watershed Sciences, 1 Shields Avenue, Davis, CA USA.

Corresponding author: A. Robin Stewart

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Abstract

Estuaries provide critical habitat for a vast array of fish and wildlife but are also a nexus for core economic activities that mobilize and concentrate contaminants that can threaten aquatic species. Selenium (Se), an essential element and potent reproductive toxin, is enriched in parts of the San Francisco Estuary (SFE) to levels known to cause toxicity, yet the risk of Se to species that inhabit the SFE is not well understood. We quantified Se concentrations in muscle, liver and ovary of the demersal cyprinid Sacramento Splittail from six regions in the SFE at three time points to evaluate Se exposure risk. Selenium levels exceeded proposed EPA criteria in ovary and thresholds of concern for liver in 15% and 20%, respectively, of fish collected in the fall of 2010, preceding the discovery of juvenile Splittail displaying a high incidence (>40%) of spinal deformities characteristic of Se toxicity, and again in 2011. No exceedances were detected in muscle tissue. Selenium concentrations varied significantly among regions for muscle ($F_{5,15}$ = 21.4, $p < 0.0001$), liver ($F_{5,115}$ = 30.0, $p < 0.0001$) and ovary ($F_{5,114}$ = 20.5, $p < 0.0001$) but did not vary between the wet and dry years, nor were they influenced by foraging trophic level or prey selection. Foraging location along the salinity gradient, defined by $\delta^{34}S$ values, explained regional Se exposures in Splittail. Relationships between tissues varied among regions for muscle and liver and muscle and ovary, but a single global relationship could be defined for ovary and liver Se concentrations. Our results suggest that the proposed EPA Se criteria for muscle tissue in Splittail may be under-protective as it would not have predicted exceedances in liver or ovary tissue and that the relationship between muscle tissue and ovary and liver may be Se concentration and seasonal dependent.

1. Introduction

Estuaries provide invaluable habitat for migratory birds, shellfish, juvenile fish and serve as migration corridors for anadromous fish (Nichols et al., 1986). Yet, estuaries are under threat from a range of stressors including climate change, eutrophication, freshwater diversions, invasive species and contaminants (Cloern and Jassby, 2012). Estuaries are arguably among the most challenging environments to evaluate and manage contaminant risk due to complexities of physical transport in a tidal environment, movement of species between interconnected habitats, biogeochemical gradients, and competing resource needs of fish and wildlife and economic development. Selenium is both an essential element and potent teratogen (Lemly, 2004) and its environmental presence is strongly tied to core economic activities (e.g. agriculture, mining, energy production)(Chapman et al., 2010). In the San Francisco Estuary (SFE), Se is introduced primarily through the import of agriculturally irrigated salinized soils containing high levels of geologically derived Se in the San Joaquin Valley (Presser and Luoma, 2006; Presser et al., 1994) and within estuary point-source loading from oil refining effluents leading to elevated Se levels in fish and wildlife (Cutter, 1989; Cutter and San Diego-McGlone, 1990; Ohlendorf et al., 1986). How movements of fish and wildlife across the SFE Se exposure landscape at different points during their life history affect exposure risk of Se has been difficult to resolve due to the hydrologic, biogeochemical and geomorphic complexity of the region and multiple sources.

Selenium is incorporated into proteins supporting certain enzyme systems, most notably glutathione peroxidase (Stadtman, 1974). It is physiologically dynamic (rapid rates of uptake and loss) and has a narrow range separating levels that are nutritionally limiting and those that are toxic (Lemly, 1982; Lemly, 1997; Lemly, 1999) making it a particularly challenging contaminant to manage in nature. When limiting in diets, Se can result in serious deficiency disorders including white muscle disease in sheep, mulberry heart disease in pigs (Muth et al., 1958) and cardiomyopathy "Keshan disease" in humans (Chinese Medical Association, 1979). Conversely, when in excess, Se has been shown to cause acute toxicity in fish and birds including teratogenesis, mortality, loss of mass in adults, reduced growth in juveniles and immune suppression (Skorupa et al., 1998). Effects are often realized in a single season resulting in the losses of entire age classes of fish and birds (Paulsson and Lundbergh, 1989).

Evaluating the risks posed by Se in the environment to food webs has been shown to depend on multiple physical/chemical and biological factors. Physical and chemical factors include loading, chemical speciation (Cutter and Cutter, 2004), particle composition (Doblin et al., 2006), and associated partitioning coefficients (Presser and Luoma, 2010). Biological factors include taxa specific accumulation (Baines and Fisher, 2001; Baines et al., 2001; Schlekat et al., 2002; Schlekat et al., 2004), ontogenetic shifts in predator diets (Feyrer et al., 2003), foraging behavior of predators (Stewart et al., 2004), developmental stage (Heinz and Hoffman, 1998), and physiological stress (Heinz and Fitzgerald, 1993; Lemly, 1993). Ecosystem models for Se have been developed that incorporate many of these elements to evaluate the potential risks of Se in different environments, including the SFE (Presser and Luoma, 2010). However, refinement of

87 the factors and processes applied in these models, and in particular, how to account for spatial
88 and temporal variation in Se concentrations in tissues of migratory species, is still needed to
89 effectively estimate individual and population-level impacts of Se on fish and wildlife.
90
91 Quantifying and understanding spatial and temporal variation is critical for characterizing the
92 extent of contamination risk not only to individuals, but also to populations during sensitive
93 time periods that are most critical to biological processes (e.g. juvenile growth, reproduction).
94 Understanding of spatiotemporal variation is also needed to identify and resolve sources of
95 contamination and exposures. Regulatory criteria are usually static and applied uniformly
96 across ecosystems, which can result in either under- or over-protection given how contaminant
97 effects manifest in nature. This approach is particularly problematic for estuaries, which
98 encompass a series of interconnected habitats with large chemical gradients (e.g. salinity, point
99 source loadings) that vary across hourly, daily, seasonal and interannual timescales, within
100 which species may occupy different habitats for different periods depending upon life stage
101 requirements (Lopez et al., 2006).
102
103 Several waterways in the SFE have been listed as impaired under the Clean Water Act. Recent
104 regulatory efforts to address this impairment include a proposed revision to the US EPA
105 protective criteria for Se (Environmental Protection Agency, 2016) and the development of a
106 total maximum daily load (TMDL) for northern San Francisco Bay (Baginska, 2015). In each
107 case, the regulatory statutes apply to a large geographic area and are static through time. Long-
108 term monitoring of the bivalve, *Potamocorbula amurensis*, has identified large spatial and

109 temporal gradients in Se concentrations in the estuary that could potentially influence Se
110 exposures of benthivorous predator species such as White Sturgeon (*Acipenser transmontanus*)
111 and diving ducks (Stewart et al., 2013; Stewart et al., 2004). Both regulatory actions in the SFE
112 have focused predominantly on the native White Sturgeon as it is a species of great societal
113 interest due to sport fishing and human consumption. Ecotoxicological studies of White
114 Sturgeon are challenging due to the fact they are large-bodied fishes with expansive foraging
115 ranges exposing them to a range of diets and Se exposures from a multitude of sources both
116 within the estuary and the freshwater Delta. While some laboratory studies have linked Se
117 bioaccumulation in White Sturgeon with reproductive toxicity in the laboratory (Linville, 2006),
118 similar linkages have not been possible for individuals in nature due to the difficulty of
119 collecting fertilized embryos or even locating juveniles. Integrating Se exposures and effects
120 based on field data is needed to fully evaluate ecosystem risks of Se to migratory predators in
121 estuaries such as the SFE.
122
123 Sacramento Splittail (*Pogonichthys macrolepidotus*) is an endemic species to the SFE that has been
124 relatively well studied in terms of its developmental biology (Deng et al., 2012) and Se
125 toxicology (Teh et al., 2004) and is an opportune species to evaluate the risk of Se exposure over
126 its lifetime. Splittail are found throughout the region from the freshwater tributaries and delta
127 through the upper estuary and because they have dietary overlap with White Sturgeon there is
128 commonality of exposure as Se is predominantly accumulated via the diet (Schlekat et al., 2001;
129 Stewart et al., 2010; Stewart et al., 2004). Splittail are a relatively large (400 mm), moderately
130 long-lived (8 years) demersal cyprinid that exhibit a semi-anadromous life cycle spending the

majority of its adult life in low to moderate salinity (0–12 psu) habitats (Feyrer et al., 2015). Upon reaching sexual maturity at approximately 2 years they migrate upstream into freshwater rivers and seasonally inundated floodplains for spawning during late winter and spring, although some remain on spawning grounds year-round (Daniels and Moyle, 1983). In the spring of 2011, young-of-year Splittail displaying a high incidence (>40%) of spinal deformities characteristic of Se toxicity were discovered at the site of a water diversion station in the San Joaquin Valley of the Delta (U.S. Department of the Interior, Bureau of Reclamation Tracy Fish Collection Facility). The chronology of Se exposure in the affected juvenile fish was documented based on Se concentrations in their otoliths (ear bones) and identified significant post-hatch Se exposures originating from exogenous feeding (Johnson et al., submitted). However, the affected cohort also showed elevated, but highly variable, Se concentrations originating from their parents known to have been foraging in the estuary based on isotopic markers in their otoliths that may have contributed to the observed deformities.

In this study, we quantified adult Splittail Se concentrations and how they vary spatially and temporally in the SFE. Selenium concentrations were determined in muscle, ovary and liver tissue in fish collected from five regions of the upper portion of the SFE in the fall of 2010, preceding the discovery of deformed juveniles and again in the fall of 2011, and from their spawning grounds in the freshwater Delta in the spring of 2017. The main goals of the study were to determine if Se concentrations: 1) exceeded regulatory criteria or thresholds of concern, 2) varied between years and among regions, 3) were influenced by foraging behavior, based on bulk isotopic tracers ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$), and 4) shared similar relationships between tissues

across regions and years allowing Se concentrations in one tissue to serve as a surrogate for another. The intention of this work was to illustrate the dynamic nature of tissue specific Se concentrations in relation to fish movements and foraging behavior that will assist managers in more effectively parameterizing ecosystem Se models and estimating the risk of Se to fish and wildlife inhabiting estuarine environments.

2. Materials and Methods

2.1 Study design and site descriptions

The study was conducted in the northern reach of the SFE and adjacent freshwater Delta, located on the Pacific Coast of the United States in the state of California (Figure 1). There are several sources of Se in the region including agricultural, industrial, and municipal discharges that mobilize and concentrate geologic sources of Se (Baginska, 2015; Presser and Barnes, 1984; Presser and Ohlendorf, 1987; Presser et al., 1994) (Figure 1). The Sacramento River to the north provides the largest contribution of freshwater to the estuary (Kimmerer, 2002) and lower Se concentrations compared to the San Joaquin River to the south, which has proportionally lower freshwater inflows and higher aqueous Se concentrations (Cutter and Cutter, 2004). There are no known significant point sources of Se on the Petaluma or Napa Rivers. Historically, Se concentrations tend to be highest in the food web in Suisun Bay (Linville et al., 2002; Stewart et al., 2013; Stewart et al., 2004), which receives Se loads from three local oil refineries as well as other municipal discharges, including one directly into Pacheco Creek (Figure 1). Ambient Se concentrations monitored over a period of 22 years in the bivalve *P. amurensis* indicate that Se

exposures are strongly influenced by seasonal and interannual variation in freshwater inflows whereby they tend to be elevated in late fall and winter and are reduced by seasonal increases in freshwater inflow in the spring or during high flow water years (Stewart et al., 2013).

Fish were collected by gill net in the late fall (November to December) of 2010 and 2011 from sites aggregated into five regions representing the full range of Splittail distributions across tidal freshwater rivers to estuarine habitats including Petaluma and Napa Rivers, Pacheco Creek bordering Suisun Bay, Suisun Cutoff and the Confluence of the Sacramento and San Joaquin Rivers (Figure 1). These regions are hereafter abbreviated to Petaluma, Napa, Pacheco, Suisun and Confluence. The 2010/11 fish were previously collected as a part of another study investigating Splittail population characteristics (Feyrer et al., 2015). The selected regions for fish collections are important foraging and in some cases nursery habitat for Splittail, but also serve other anadromous and resident fishes, as well as migratory birds, year-round species of diving ducks, shorebirds and terns (Bennett et al., 1996; Conomos, 1979; Moyle et al., 2004; Nichols et al., 1990; Ohlendorf et al., 1989; Sommer et al., 2001). Climatological conditions varied between sample years with freshwater inflows to the estuary being lower in 2010 (dry water year) and higher in 2011 (wet water year) based on long term historical data accessed from California's Department of Water Resources (DWR) website at http://www.water.ca.gov/dayflow/output/Output.cfm on 19 April 2018.

A second set of fish were collected in the spring of 2017 (wet water year) from the freshwater Delta, in the Yolo bypass; a seasonally inundated floodplain to the north that is commonly used

by Splittail for spawning and rearing of juveniles (including Chinook Salmon) and has historically lower Se concentrations in water and prey compared to the upper estuary (Cutter and Cutter, 2004; Lucas and Stewart, 2005; Sommer et al., 2001). The Delta fish provided an opportunity to quantify Se concentrations at the time of spawning, when maternal transfer to eggs occurs (Conley et al., 2014), and how those concentrations differ from adults foraging in the upper estuary in the fall.

2.2 Fish processing and tissue preparation

We used a randomized-stratified design to select 90 mature females from the total of 178 fish collected from the 5 regions of the SFE in 2010/11 by Feyrer et al. (2015) resulting in 8–10 individuals per region and year ranging in age from 2-8 years and fork length (FL) ranging from 182-376 mm. Splittail become sexually mature at approximately 180 mm FL as they near age 2 and undergo an ontogenetic shift in their diet to include the bivalve *P. amurensis* (Feyrer et al., 2003). Methods for fish collection and processing are described in Feyrer et al. (2015). Briefly, fish were transported live to the laboratory and processed within approximately 12 hours. Fish FL (mm) and whole fish wet weight (g) were recorded, skinless muscle fillets, liver, and gonad tissues and otoliths were extracted, liver and gonad wet weights recorded, and tissues were frozen in polyethylene bags at −20°C, until they could be freeze-dried and ground into a fine powder. Age was determined by counting annual rings using thin sections of otolith microstructures as described in Feyrer et al. (2015). Thirty-two fish, spanning a similar size and age range as those collected in 2010/11, were collected from the Delta during February–March of 2017. All but one fish analyzed were visually identified as female with ovaries in various stages

of development, but 14 also had undeveloped testes. Synchronous hermaphrodism has not officially been reported for Splittail, but has been documented for other species (Teh et al., 2000). Upon collection, Delta fish were immediately frozen individually in polyethylene bags and stored at -20°C until one year later when fish were thawed, their FL, whole fish wet weight recorded, and tissues were extracted and processed as described for the 2010/11 fish. In addition to skinless muscle fillets, liver, and gonad (ovary and teste), the remaining gastrointestinal tract and carcasses were retained and processed. Carcasses were chopped into smaller pieces and run through a stainless-steel clamp-on hand grinder with 4.8 mm grinding plates (LEM part #1384). A sub-sample of carcass homogenate (~ 40 mL) was then transferred to a pre-weighed polypropylene Falcon tube, a wet weight taken, the sub-sample frozen at -80°C, freeze dried and ground using a small electric grinder with stainless steel blades. This method of sub-sampling a larger quantity of grossly homogenized tissue for further freeze drying and grinding has been shown to be representative of whole mass measurements in the case of caloric content determinations (Glover et al., 2010). Morphological data including length, age (for 2010/11 fish from Feyrer et al. 2015), and whole fish and tissue wet weights are provided in Table 1. Ages were not directly determined for Delta fish but were estimated using length-age relationships developed for 2010/11 fish.

2.3 Selenium and stable isotope analyses

Selenium concentrations were determined by isotope dilution-hydride generation-inductively coupled plasma-mass spectrometry (ID-HG-ICP-MS) as in Kleckner et al. (2017). In brief, a small tissue mass (10-20 mg dry weight) was added to a 6 mL PFA vial (Savillex, 10321 West

241 70th Street Eden Prairie, MN 55344-3446 USA part number 200-006-20) along with 100 μL
242 enriched ^{82}Se isotope spike and 600 μL of 16N HNO_3 and heated in a benchtop autoclave at
243 126°C/20 psi for 3 hours. This step was followed by a second oxidative step where 200 μL of
244 30% H_2O_2 was added to the cooled samples, allowed to react and then heated to near dryness on
245 a hotplate. In the final pre-analysis step, reconstituted samples (by addition of 2% HCl) were
246 reduced (to convert all selenate to selenite) by adding equivalent volumes of sample and 12N
247 HCl for 50% v/v solution that was heated in a water bath and boiled for 30 minutes. The ICP-
248 MS used for analysis of Se was an Elan DRC II (Perkin-Elmer SCIEX, Shelton, Connecticut,
249 USA). The ICP-MS was combined with a Flow Injection System FIAS 400 (Perkin-Elmer SCIEX,
250 Shelton, Connecticut, USA) for hydride generation (HG).
251
252 Quality assurance/quality control (QA/QC) data are provided in (Stewart, 2019) and include, for
253 each analytical run, procedural blanks (n=3), duplicates (10% of each sample run), and certified
254 reference materials (CRMs) (two SRM matrices, n=3), including National Institute of Science and
255 Technology (NIST2976) mussel tissue, National Research Council Canada (NRCC) dogfish
256 muscle (DORM2), dogfish liver (DOLT3), and lobster hepatopancreas (TORT3). Background
257 concentrations for analytical calibration blanks quantifying instrument noise and procedural
258 blanks were subtracted from sample concentrations. Procedural blanks were always less than
259 10% of sample concentrations, precision for samples run in duplicate was on average <4.5%
260 (standard deviation/mean) and CRMs averaged 101% recoveries (n=73) with individual values
261 falling within ± 20% of the certified value. QA/QC data are provided along with raw Se
262 concentration data in Stewart (2019).

263

264 Isotope ratios of C, N and S were determined in muscle tissues at the Stable Isotope Facility
265 (SIF), University of California, Davis. Analyses were performed on a single tissue sub-sample
266 for C and N (~1-2 mg dry weight) using a Europa Scientific Hydra 20/20 continuous flow
267 isotope ratio mass spectrometer in conjunction with a Europa ANCA-SL elemental analyzer to
268 convert organic C and N into CO_2 and N_2 gas. Nitrogen isotope samples were standardized
269 against N_2 in air as follows:

270

271 $$\delta^{15}N\ (\%) = [(R_{sample} / R_{standard}) - 1] \times 1000$$

272

273 where $R = {}^{15}N/{}^{14}N$. A similar relation for $\delta^{13}C$ ($R = {}^{13}C/{}^{12}C$) was used to standardize carbon
274 isotope samples against Pee Dee Belemnite. Long-term instrument precision reported by the SIF
275 was 0.1% for carbon and 0.3% for nitrogen based on replicate analyses of standard reference
276 materials. Sulfur $\delta^{34}S$ isotope analysis of organic solid materials was done on a tissue sub-
277 sample (~3 mg dry weight) using an elemental analyzer and pre-concentration unit interfaced
278 with a continuous-flow isotope-ratio mass spectrometer. Final $\delta^{34}S$ values were obtained after
279 adjusting the provisional measurements such that correct $\delta^{34}S$ values for laboratory QA
280 materials are obtained (as above for ^{15}N). Laboratory reference materials were calibrated
281 directly against IAEA S-1, S-2, and S-3, as well as NBS-127, SO-5, and SO-6. The SIF reports
282 long-term reproducibility of this method for ^{34}S to be ± 0.4 ‰.

283

284 **2.4 Data analysis**

285 Selenium concentrations are reported on a dry weight (dw) basis in micrograms per gram
286 (μg/g). Whole body Se concentrations were estimated by dividing the summed mass of Se
287 measured in each tissue (muscle, liver, ovary, teste (if present), gastrointestinal tract and
288 carcass) by the summed mass of each tissue for each fish. Selenium concentrations were ln-
289 transformed (when required) to meet assumptions of heteroscedasticity and normality of
290 residuals. We used the statistical packages in JMP®14 (SAS Institute Inc., 2018). We applied a
291 tiered statistical approach to evaluate regional Se exposures in Splittail as outlined by our study
292 goals.
293
294 2.4.1 *Exceedance of regulatory criteria and thresholds of concern*: For each region, year, and tissue, we
295 determined the geometric means and standard error of Se concentrations. Geometric means are
296 preferable as the values were positively skewed. To do this, the means for natural log
297 transformed values were back transformed to linear values and standard errors were estimated
298 with the Delta method (Seber, 1982). We then compared values to proposed EPA regulatory
299 criteria (ERC) for Se in fish skin-less muscle (11.3 μg/g dw) and ovary (15.1 μg/g dw)
300 (Environmental Protection Agency, 2016). There are no ERC for liver tissue so we compared
301 values to a threshold level of concern (TLC) for liver (12 μg/g dw) derived by Presser and
302 Luoma (2006; 2010) based on literature values.
303
304 2.4.2 *Variation in Se concentrations between years and regions:* Differences in freshwater inflow
305 among years have been shown to influence Se concentrations in bivalves (Stewart et al., 2013)
306 and so we first tested for an effect of year on upper estuary Se concentrations for paired 2010

307 and 2011 fish, representing a dry and wet year, respectively. We used a fixed effects model that
308 included region + year as fixed effects and region x year as interactions, and ran the model
309 separately for each muscle, liver and ovary Se concentration. There was a significant interaction
310 between year and region for ovary tissue ($p = 0.02$) and so we used pairwise *t*-tests to determine
311 which regions differed between 2010 and 2011. Only one region (Confluence) showed a
312 statistical difference between years ($p = 0.007$), so we combined years within upper estuary
313 regions to test for differences among regions (including Delta 2017 fish) in a fixed effects model
314 with region as the main factor. Post-hoc differences in Se concentrations among regions were
315 evaluated by Tukey's HSD.
316
317 2.4.3 *Influence of spatiotemporal variation in foraging behavior on Se concentrations:* Selenium is
318 predominantly accumulated via the diet (Luoma et al., 1992) making understanding of foraging
319 behaviors (i.e., prey selection and foraging range) and how they vary spatially an important
320 step in resolving exposure patterns in fish. Ratios of bulk stable isotopes of carbon, nitrogen and
321 sulfur ($^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{34}S/^{32}S$) have been shown to be useful tools in understanding feeding
322 behaviors in fish (Peterson and Fry, 1987; Vander Zanden et al., 1998; Vander Zanden and
323 Rasmussen, 1999). Carbon isotopes can be used to identify contributions of carbon sources that
324 are shared between predator and prey (France, 1995) and when combined with nitrogen
325 isotopes, which estimate trophic level (Vander Zanden et al., 1997), provide an integrated
326 measure of prey selection (Peterson and Fry, 1987). In estuaries, isotopic gradients in $\delta^{13}C$ and
327 $\delta^{34}S$ can be used to estimate a predator's foraging range by matching the $\delta^{13}C$ and $\delta^{34}S$ values of
328 predator tissues to the isotopic signature of the source water (e.g. riverine vs. bay water) at the

329 location where the prey was consumed. For example, the $\delta^{13}C$ signature of dissolved inorganic
330 carbon (DIC) is enriched along the salinity gradient, moving from land (freshwater) to sea
331 (marine) (Spiker & Schemel 1979). As phytoplankton cells grow, they incorporate the salinity-
332 specific DIC $\delta^{13}C$ signature (Canuel et al. 1995, Cloern et al. 2002), which is transferred to
333 consumers providing a means to geographically bound their foraging ranges. The
334 freshwater/marine isotopic gradient is even sharper for $\delta^{34}S$, whereby the depleted $\delta^{34}S$
335 signature of sulfur found in reducing sediments in freshwater habitats is rapidly enriched upon
336 exposure to marine sulfate, which strongly influences $\delta^{34}S$ values in food webs even at low
337 salinities (Peterson et al., 2017).
338
339 To evaluate the influence of foraging behavior on Se concentrations in fish, we first created
340 biplots ($\delta^{15}N$ by $\delta^{13}C$ and $\delta^{34}S$, mean±95% CI) by region and year. We then used a fixed effects
341 model that included region + year as fixed effects and region x year as interactions and ran the
342 model separately for each of the isotopes for the upper estuary. There was a significant
343 interaction between year and region for all isotopes (see results) and so we tested the effect of
344 region by year for the upper estuary and Delta, using pairwise *t*-tests to determine which
345 regions and years differed from the global average for all regions and years. We then tested for
346 the influence of isotopic signatures directly on Se concentrations using a fixed effects model
347 with region + $\delta^{13}C$ + $\ln\delta^{15}N$ + $\delta^{34}S$ as factors. Only sulfur was found to have a significant effect on
348 Se concentrations in all three tissues and so we re-ran the initial fixed effects model with both
349 region and $\delta^{34}S$ as fixed effects to see if the addition of sulfur isotopic signatures improved the
350 original model performance to estimate tissue Se concentrations based on AIC scores.

2.4.4 *Relationships between tissue Se concentrations:* Tissues selected for monitoring purposes are often different from those tissues used to set regulatory criteria. As a result, Se concentrations in one tissue are often used to estimate Se concentrations in another. However, relationships between Se concentrations in tissues have been shown to vary among tissues, species and developmental stages (Janz et al., 2010) and it is not well known how relationships vary among regions or seasons, particularly in environments with sharp gradients in concentration. We evaluated relationships between muscle, liver and ovary for all regions of the upper estuary and between whole body and muscle, liver and ovary for Delta 2017 fish. The years 2010 and 2011 were combined within regions for the analyses because there was no year by region interaction for any tissue combinations, except for ovary by muscle at Pacheco. We first determined regression relationships and report individual slopes and intercepts for each region. Regions and tissue combinations without significant relationships were dropped from further analyses (i.e. Petaluma). We then tested the relationships for differences in slope among regions using an ANCOVA model whereby lnSe tissue 1 = region + lnSe tissue 2 + region x lnSe tissue 2. We then removed the interaction term (where appropriate) and tested for differences in intercepts among regions to evaluate if a single global model was appropriate for all regions. Significant differences in intercept from the average model response were assessed for each region by *t*-test, which tests the null hypothesis that the difference in the response is zero. For tissue combinations where no regional differences in intercept were found (i.e. ovary by liver) region was removed from the model and a single global relationship was determined.

3. Results

3.1 Splittail Se concentrations and exceedance of regulatory criteria and thresholds of concern

Splittail Se concentrations spanned a broad range exceeding ERC and TLCs in some regions of the upper estuary in the fall of 2010 and 2011, but not on their spawning grounds in the North Delta in the spring of 2017. Selenium concentrations in fish were highest in ovary (3-20 μg/g dw) and liver (4-18 μg/g dw) and substantially lower in muscle tissue (0.8-2.1 μg/g dw) (Table 2). The proposed ERC for Se was never exceeded in muscle tissue (11.3 μg/g dw) but was exceeded in ovary (15.1 μg/g dw) in 10 of 15 fish at Pacheco, in 3 of 17 fish at Suisun and in 3 of 28 fish at Confluence. Liver TLCs (12 μg/g dw) were exceeded in 11 of 15 fish at Pacheco, in 3 of 17 fish at Suisun and in 3 of 28 fish at Confluence. Despite the consistently low muscle Se concentrations across all regions and years and no exceedances, the frequency of exceedance in liver and ovary were high for Pacheco ranging from 60-80% (range for both tissues and years), followed by Suisun in 2011 (33%) and the Confluence in 2010 (17%). Exceedances were not recorded for any fish from the Petaluma or the Delta.

3.2 Interannual and Regional differences in Se concentrations

Selenium concentrations in fish from the upper estuary were similar between the dry year of 2010 and wet year of 2011 for muscle ($F_{4,79} = 0.2$, $p = 0.7$), liver ($F_{4,80} = 0.01$, $p = 0.9$), and ovary ($F_{4,78} = 0.40$, $p = 0.5$) (Table 3). There was a significant interaction between region and year for ovary, but only for one region ($F_{4,78} = 3.0$, $p = 0.02$) (Table 3). Ovary Se concentrations at the Confluence in 2011 were almost 2-fold lower (t-Ratio = -2.76, $p = 0.007$) compared to 2010, but were not different between years for Petaluma, Napa, Pacheco or Suisun regions.

395

396 Selenium concentrations were found to be highly significantly different among regions
397 (including Delta fish) for muscle ($F_{5,15} = 21.4$, $p < 0.0001$), liver ($F_{5,115} = 30.0$, $p < 0.0001$) and ovary
398 ($F_{5,114} = 20.5$, $p < 0.0001$) (Table 3, Figure 2). Muscle Se concentrations (µg/g dw) were well below
399 ERCs with slightly higher geometric mean (roughly equivalent to median value of a log-normal
400 distributed data) concentrations (±SE) (pooled years by region) in Pacheco (1.95±0.14) and the
401 Delta (1.56±0.08), followed by Suisun (1.26±0.05) and the Confluence (1.11±0.06), Napa
402 (0.98±0.05) and the Petaluma (0.94±0.04) (Figure 2A). Considerably sharper regional gradients
403 were observed for liver and ovary with geometric mean Se concentrations being markedly
404 higher at Pacheco (liver: 17.51±2.10, ovary: 20.21±3.10) relative to other regions (Figure 2B and
405 C). The remaining regions had similar geometric mean concentrations decreasing in order from
406 Suisun (liver: 7.15±0.70, ovary: 7.43±1.25) and the Confluence (liver: 6.49±0.62 ovary: 6.76±0.97),
407 followed by the Napa (liver: 5.84±0.53, ovary: 5.03±0.46), Petaluma (liver: 5.02±0.25, ovary:
408 6.94±0.98) and with the lowest being found in the Delta (liver: 3.55±0.27, ovary: 3.16±0.31)
409 (Figure 2B and C).

410

411 **3.3 Influence of foraging behavior on Se concentrations**

412 Overall foraging behavior, as reflected in carbon, nitrogen and sulfur stable isotope signatures,
413 was more variable within than among regions and between years although some differences
414 were evident, particularly for sulfur (Figure 3). For the upper estuary regions, there was a
415 significant interaction between region and year for $\delta^{13}C$ ($F_{4,80} = 3.06$, $p = 0.02$), $\delta^{15}N$ ($F_{4,80} = 4.00$, $p =$
416 0.005), and $\delta^{34}S$ ($F_{4,80} = 5.64$, $p = 0.0005$). With the exception of the Petaluma ($F_{10,111} = 8.89$, $p <$

0.0001), which differed between years and were significantly more enriched in $\delta^{15}N$ values, Splittail appeared to feed at a similar trophic level across regions and between years, as inferred by a less than 3.4‰ difference in regional mean $\delta^{15}N$ values (Napa, Pacheco, Suisun, Confluence, and Delta regions range = 13.99 to 15.27; Petaluma range = 16.28 to 19.2) (Table 1, Figure 3). Petaluma fish showed a large drop in $\delta^{15}N$ values between 2010 and 2011 (*t*-Ratio = -3.55, $p = 0.007$). There were no sharp gradients in carbon isotope values among regions but fish within each region tended to reflect the general position of the region along the salinity gradient ranging from -25.6 ‰ to -22.2, with those from the less saline regions of the Delta and the Confluence being the most depleted (Table 1, Figure 3A). Carbon isotopic signatures were not different between the dry and wet years in the upper estuary, except at Suisun where $\delta^{13}C$ values were more depleted in the wet year of 2011 (*t*-Ratio = -2.78, $p = 0.006$). There were small statistical differences in $\delta^{13}C$ values among regions in the upper estuary, but only in 2011 with the Confluence being more depleted than the Petaluma (*t*-Ratio = -3.61, $p = 0.0005$) and Suisun being more depleted than Petaluma (*t*-Ratio = -4.15, $p = 0.0001$) and Pacheco (*t*-Ratio = -3.63, $p = 0.0004$). Delta fish $\delta^{13}C$ were not different from regions sampled in 2010/11 with the exception of being more depleted than the Petaluma (*t*-Ratio = -4.48, $p < 0.0001$) and Pacheco (*t*-Ratio = -3.81, $p = 0.0002$) in 2011. Sulfur isotope values were the most variable within (CV range 23 to 63%) and among (CV 28%) regions with $\delta^{34}S$ values becoming progressively more enriched in fish moving seaward from the freshwater regions of the Delta ~Petaluma ~ Confluence < Napa ~ Pacheco (Figure 3B) ($F_{10,111} = 15.6$, $p < 0.0001$). There were no significant or consistent differences between years within regions, although the Confluence $\delta^{34}S$ values were lower in 2011 relative to 2010 (Figure 3B, *t*-Ratio = -3.58, $p = 0.0005$).

After statistically accounting for the effects of region by year (i.e., upper estuary regions are tested separately for each year), sulfur isotopic signatures were found to have significant effect on muscle, liver and ovary tissue Se concentrations, while nitrogen had a marginal effect on Se concentrations in muscle ($F_{10,107} = 5.22$, $p = 0.02$). Addition of $\delta^{34}S$ values as a main effect in a fixed effects model with region (there were no significant interactions for $\delta^{34}S$ x region) resulted in a slight improvement of the model performance compared to the model with region alone, as indicated by lower AIC scores, in explaining variation in Se concentrations in muscle, liver and ovary throughout the upper estuary and Delta (Table 3).

3.4 Relationships between tissue Se concentrations

Tissue Se concentration combinations – muscle by liver, muscle by ovary, and liver by ovary – were significantly related to each other within each region in the SFE and the Delta with one tissue explaining between 18 to 74% (adjusted R^2) of the variation in the other tissue's Se concentrations (Table 4, Figure 4). The only exception was the Petaluma where no significant relationships were found between any of the tissues and so the Petaluma was not considered in further analyses. The relationship slopes were not statistically different among regions for liver by muscle (ANCOVA $F_{4,98} = 1.07$, $p = 0.4$) or ovary by muscle (ANCOVA $F_{4,98} = 1.91$, $p = 0.1$) and was marginally significant for ovary by liver (ANCOVA $F_{4,98} = 2.52$, $p = 0.05$). We dropped the interaction term (lnSe tissue x region) from the ANCOVA model for liver and ovary by muscle to assess differences in intercepts among the regions. Relationships between muscle and liver or muscle and ovary differed across regions as indicated by a significant region effect in the

ANCOVA model for liver by muscle ($F_{4,102}$ = 55.8, p < 0.0001) and ovary by muscle ($F_{5,112}$ = 33, p < 0.0001) (Table 5). For example, for a given muscle Se concentration liver Se concentrations were significantly higher at Pacheco (t Ratio = 5.58, p <0.0001) and Confluence (t Ratio = 2.05, p = 0.04) and significantly lower at the Delta (t Ratio = -13.96, p = 0.0001) compared to the mean intercept for all regions (Table 5). Similarly, ovary Se concentrations were higher at Pacheco (t Ratio = 3.92, p = 0.0002) and Confluence (t Ratio = 2.63, p = 0.01) and lower at the Delta (t Ratio = -12.00, p < 0.0001) relative to the mean intercept for all regions for a given muscle Se concentration. For liver and ovary, region did not have a significant effect on the relationships between liver and ovary Se concentrations either when tested with separate slopes ($F_{4,98}$ = 1.10, p = 0.36) or with the interaction term removed ($F_{4,102}$ = 0.77, p = 0.5) (Table 5). Removing the effect of region from the model we estimated a global linear relationship between liver and ovary for Splittail in the SFE of lnSe Ovary = -0.26 + 1.12 x lnSe Liver (R^2 = 0.77, p < 0.0001).

Whole body Se concentrations measured in Delta fish were significantly related to muscle, liver and ovary tissue Se concentrations explaining ~60% of the variation in the tissues (Table 4). The relationship slopes between whole body Se and other tissues were lowest for muscle (0.55) followed by liver (0.76) and ovary (1.0) and fell within the range reported for other tissue combinations.

4. Discussion

4.1 Selenium exposure risk in the San Francisco Estuary

483 Selenium concentrations exceeded thresholds of concern in liver in 20% (8 of 41 fish) and EPA
484 regulatory criteria in ovary in 15% (6 of 39 fish) of adult Sacramento Splittail collected in the
485 upper estuary of San Francisco Bay during the pre-spawning season (late fall) of 2010. The
486 frequency of exceedances in individual fish was highest in the Pacheco (liver – 80%, ovary –
487 60%) and Suisun (liver – 33%, ovary – 33%) (Table 2) regions located in the more saline portions
488 of the upper estuary near several industrial sources of Se. This is also a region where their prey
489 *P. amurensis* are abundant and have Se burdens that exceeded ERC for bivalves (Stewart et al.,
490 2013). The toxic Se levels measured in liver and ovary of adult Splittail were consistent with
491 elevated levels of Se detected in otoliths of juvenile Splittail collected from the freshwater Delta
492 in the following spring of 2011 that also exhibited deformities characteristic of Se toxicity
493 (Johnson et al. submitted). Johnson et al.(submitted) identified that Se in the inner rings or the
494 maternally-deposited regions of the otoliths (i.e., during yolk absorption and prior to exogenous
495 feeding 1-10 days post-hatch; Deng et al., 2012) originated from parents that spent time foraging
496 in the estuary rather than the North Delta based on unique strontium isotopic values. While it is
497 not possible to directly establish that elevated Se concentrations in adult SFE Splittail were
498 causing the spinal deformities in the juveniles, these results establish that Se levels were
499 sufficiently elevated to cause toxicity in offspring based on established regulatory criteria. The
500 frequency of exceedances of ERCs and TLCs in ovary and liver was similar in 2011, despite it
501 being a wet year. Conversely, Se concentrations were uniformly low in tissues in adult Splittail
502 collected in the spring of 2017 on their spawning grounds in the North Delta. While adult
503 Splittail might be expected to have recently migrated to the Delta from the estuarine portion of
504 SFE to spawn, it is unclear given the high rate constant of loss of Se from tissues (Baines et al.,

505 2002; Stewart et al., 2004) what their tissue concentrations may be after feeding on prey with
506 lower Se concentrations for a period of weeks and the impact of those concentrations on
507 developing eggs. Selenium concentrations in water in the North Delta compared to those in the
508 South Delta and the San Joaquin River have consistently been lower (Cutter and Cutter, 2004).
509 Without examining the strontium isotopic signature of the Delta fish, it is not possible to know
510 the recent foraging location of the Delta Splittail. Notably, none of the muscle Se concentrations
511 measured in the present study approached ERCs for muscle tissue (11.3 μg/g dw), indicating
512 that muscle tissue Se concentrations would not have predicted the exceedances in liver or ovary
513 tissues, potentially underestimating Se exposure risk in this species. EPA's regulatory criteria
514 for muscle tissue was developed based on relationships with Se concentrations in other tissues
515 in species where Se toxicity has been documented in the laboratory and field (Environmental
516 Protection Agency, 2016); however, these relationships appear to vary across Se concentrations
517 in nature (see section 4.6).
518
519 **4.2 Sharp regional estuarine gradients drive Se exposures**
520 Selenium concentrations in Splittail were strongly linked to the region where the fish were
521 collected and their foraging range. Although Splittail move throughout the northern reach of
522 the SFE, they appear to exhibit strong site foraging fidelity as evidenced by sharp regional
523 gradients in Se concentrations (Figure 2). Region had the largest effect on Se exposures in a
524 fixed effects model followed by muscle sulfur isotopic signatures (Table 3). Splittail from the
525 Pacheco region, near the mouth of Pacheco Creek and near several point source discharges from
526 oil refineries and wastewater treatment plants, had distinctly higher Se concentrations in all

527 tissues compared to other regions. Adjacent regions, Suisun Cutoff, and the Confluence of the
528 Sacramento and San Joaquin Rivers, had the next highest Se concentrations but also were highly
529 variable.
530
531 The higher levels of Se in adult Splittail from the Suisun Bay region of the SFE correspond to
532 those observed throughout their food web starting with filtered water (Cutter and Cutter, 2004),
533 particulate material (Doblin et al., 2006) and the estuarine bivalve *P. amurensis* (Linville et al.,
534 2002; Stewart et al., 2013). The pathway of accumulation whereby dissolved Se is actively taken
535 up at the base of the food web by algae and bacteria (Baines and Fisher, 2001; Baines et al., 2004)
536 and particulate associated Se is efficiently assimilated by deposit and filter-feeding bivalves
537 (Lee et al., 2006; Schlekat et al., 2000) has been well described. Biogeochemical models
538 describing behavior and distributions of Se in the SFE (Chen et al., 2012; Meseck and Cutter,
539 2006) also indicate higher Se concentrations in water and particulate material in the Carquinez
540 Strait/Suisun Bay portions of the SFE compared to less saline regions. Long-term (17+ years)
541 measurements in *P. amurensis* have found, despite fluctuations with freshwater inflow, clam Se
542 concentrations are persistently and significantly higher in the region of Carquinez Straight, near
543 Pacheco Creek (Stewart et al., 2013). White sturgeon collected in Suisun Bay and the upper
544 estuary have higher Se concentrations compared to those apparently foraging in the freshwater
545 habits of the SFE or seaward towards South Bay (Sun et al. unpublished data; Linares-Casenave
546 et al., 2015).
547
548 **4.3 Effect of freshwater inflow on Se exposures**

549 Selenium concentrations did not differ in Splittail collected in the dry year of 2010 and wet year
550 of 2011, with the exception of ovary tissue at the Confluence which was lower in 2011 compared
551 to 2010. This was surprising as Se concentrations in Splittail prey *P. amurensis* have been shown
552 to vary both seasonally and interannually in response to changes in freshwater inflow (Stewart
553 et al., 2013). It is thought that large contributions of Sacramento River water that is comparably
554 lower in Se serves to dilute within estuary sources of Se (Cutter and Cutter, 2004). Selenium
555 concentrations in *P. amurensis* in the fall months (September – November) declined by 30% and
556 10% at U.S. Geological Survey long-term water quality monitoring stations 4.1, near our
557 Confluence region, and 8.1, near our Pacheco region, respectively, between 2010 and 2011
558 (Stewart et al., 2013). The interannual difference between Se concentrations in *P. amurensis* is
559 small, particularly at the station closest to Pacheco, compared to the >60-80% regional difference
560 between Se concentrations in liver of fish from the Confluence and Pacheco in either year.
561 Indeed, the larger interannual difference in Se concentrations in *P. amurensis* at station 4.1 may
562 explain why a year effect was only detected at the Confluence. Small differences in fish Se
563 concentrations between years may also be further confounded by fish movements between
564 regions indicating that larger samples sizes would be needed to detect more subtle differences
565 between years due to changes in freshwater inflow.
566
567 **4.4 Effect of trophic level and prey selection on Se**
568 Selenium concentrations in Splittail in the upper estuary and delta did not appear to be
569 influenced by differences in trophic level or carbon pathway. Trophic enrichment is less
570 pronounced for Se than for mercury (Hg) (Stewart et al., 2010) and is highly dependent on the

physiological uptake of Se by its prey (Schlekat et al., 2002). Bivalve-based food sources were found to result in significantly higher Se levels in their consumers than crustacean-based food sources due to overall lower concentrations of Se in crustaceans stemming from faster loss rates from their tissues (Schlekat et al., 2004; Stewart et al., 2004). Thus, adult Splittail foraging on *P. amurensis* might be expected to have higher Se concentrations than those that were feeding on a different invertebrate species or even at a higher trophic level (i.e., fish) within a non-bivalve based food web. With few exceptions, Splittail did not appear to differ in trophic level based on the fact that $\delta^{15}N$ values in muscle tissue were consistent across regions, except for the Petaluma (see below). While it is possible that baseline $\delta^{15}N$ could have varied across regions (Cabana and Rasmussen, 1996), measured $\delta^{15}N$ values for *P. amurensis* collected from a monitoring station near the Confluence (station 4.1) and near Pacheco Creek (station 8.1) in the late fall of 2010 and 2011 (Kleckner et al., 2010) suggest that there was only a limited difference in $\delta^{15}N$ (2010 Δ = 1.41 ‰; 2011 Δ = 0.53 ‰) between these stations that might confound the interpretation of trophic level in Splittail. The average $\delta^{15}N$ value of Splittail across all regions and years was 14.70 ± 1.27, which is approximately 1 trophic level or ~ 3.4 ‰ above *P. amurensis*, based on an average $\delta^{15}N$ values for the preceding 3 months (September through November) of 10.21 ± 1.09 and a Δ of 4.49 ‰ (Cabana and Rasmussen, 1994; Vander Zanden et al., 1997). While Splittail forage on a range of food including organic matter, insects and bivalves, since *P. amurensis* invaded the SFE this clam has been an important component of Splittail diets in habitats where these clams are found (Feyrer et al., 2003). The enriched $\delta^{15}N$ values in Splittail from the Petaluma River in both years indicate either an enriched baseline, often associated with water treatment or urbanization, or foraging at a higher trophic level (e.g. fish). Age-0 Splittail collected from the

Petaluma in 2002 and 2003 were found to eat predominantly invertebrates, based on gut analysis, and had similar $\delta^{15}N$ values (~18‰) as adult Splittail in this study, suggesting that an elevated baseline in $\delta^{15}N$ was likely the cause of the higher values at the Petaluma (Feyrer et al., 2007).

4.5 Effect of foraging location on selenium exposures

The positive relationship between sulfur isotopes and Se concentrations in fish tissues suggests that as fish spend more time foraging in the more saline regions of the SFE, in this case Suisun Bay, they may be exposed to higher Se (Table 3). Unlike the discrete assignment of fish to a specific region, sulfur isotopes provided an opportunity to examine the continuous relationship between Se concentrations and foraging location in the SFE as has been shown for other species in South San Francisco Bay (Peterson et al., 2018) and estuaries and watersheds elsewhere (Hesslein et al., 1991). The foraging gradient represented by continuous $\delta^{34}S$ values of individual fish did identify a point in the estuary (or salinity field) that was associated with a rapid and significant increase in Se concentrations in Splittail tissues. Beyond a $\delta^{34}S$ value of ~14‰, concentrations of Se increased in liver and ovary from a median of 4.78 and 4.62 to 7.57 and 9.50, which constitutes an increase of 58% and 106%, respectively. Although region had the largest effect on Se concentrations in Splittail, $\delta^{34}S$ was also a significant explanatory variable. This suggests that $\delta^{34}S$ may be a particularly useful biomarker for other species (e.g., White Sturgeon, birds) that show less site foraging fidelity making the discrete variable "Site" a less effective predictor of Se site exposures in environments where exposures vary along the salinity

614 gradient. Further, combining $\delta^{34}S$ with isotope markers in fish otoliths may provide further
615 resolution of exposure history (Johnson et al., submitted).
616
617 **4.6 Variability in tissue Se relationships across concentration gradients**
618 Selenium concentrations in tissues were significantly related to each other for all tissues and
619 regions with the exception of the Petaluma. Excluding Petaluma, relationships were slightly
620 better in regions where Se concentrations were higher such as Pacheco (range $R^2 = 0.55\text{-}0.74$) and
621 between liver and ovary (mean for 5 regions $R^2 = 0.54$) tissues rather than muscle and liver
622 (mean for 5 regions $R^2 = 0.39$) or ovary (mean for 5 regions $R^2 = 0.39$). While a single global
623 relationship could be used to relate liver Se concentrations to ovary Se concentrations
624 independent of region, year or season (i.e., fall pre-spawning fish in upper estuary verses
625 spawning fish in the Delta), the same was not possible for liver or ovary by muscle. Those
626 relationship shared similar slopes, but different intercepts depending on the region. For a given
627 muscle Se concentration the predicted liver and ovary concentration would be significantly
628 higher at the Pacheco and Confluence and significantly lower at the Delta compared to a global
629 model. The lower predicted concentrations for Delta fish may not be unexpected as the fish
630 were spawning or getting ready to spawn which could affect how Se was being partitioned into
631 liver and ovary. Indeed, lower liver Se might be expected if Se is being transferred from the
632 liver to eggs in the form of yolk vitellogenin and then the Se enriched eggs are removed from
633 the ovary during spawning, a process well described in White Sturgeon (Kroll and Doroshov,
634 1991; Linares-Casenave et al., 2003; Linville, 2006). The higher Se concentrations in liver and
635 ovary relative to muscle tissue at Pacheco and the Confluence is more difficult to explain, but

perhaps related to a similar mechanism. As the collections took place in late fall (November/December), it is possible that Splittail were undergoing egg development resulting in a rapid translocation of recently accumulated Se to liver and ovary tissue for vitellogenesis and ovary maturation. This indeed was observed between pre-vitellogenic and vitellogenic White Sturgeon females who had significantly different Se concentrations, but also relative differences between Se concentrations in muscle, liver and gonad tissue (Linares-Casenave et al., 2015). Selenium concentrations in gonad and liver relative to muscle tissue were considerably higher in vitellogenic relative to pre-vitellogenic White Sturgeon females. These results suggest that muscle to liver or ovary relationships may vary both in response to Se exposure concentrations, but also season and spawning status. The exceedances of toxicity thresholds and criteria in liver and ovary, but not muscle, are further suggestive that inter-species differences may be important in developing relationships between tissues for regulatory criteria. The proposed EPA criteria were developed based on White Sturgeon tissues and translation factors between tissues that may not be appropriate for fish species with different physiologies and life histories. For example, offloading of mercury from muscle to liver and eggs/embryos has been shown to increase with mercury concentrations in some fish species depending on their reproductive strategy (Drevnick et al., 2006; van Hees and Ebert, 2017). Further studies of relationships among Se concentrations in muscle and liver and ovary across a range of species are warranted.

4.7 Evaluating risk across sharp exposure gradients and sensitive time periods

Our study provided a unique opportunity to evaluate Se sources and factors influencing Se exposures in the endemic minnow Sacramento Splittail, *Pogonichthys macrolepidotus,* in relation to proposed protective criteria and thresholds of concern for Se developed to protect fish and wildlife inhabiting the SFE. Selenium concentrations in 6 ovary and 7 liver of the 41 individual Splittail collected from the upper estuary in 2010 were found to exceed proposed EPA criteria for ovary and thresholds of concern for liver. Despite the exceedances in liver and ovary tissue, no fish muscle Se concentrations exceeded EPA criteria. These elevated Se liver and ovary concentrations preceded the discovery of juvenile Splittail in the freshwater Delta in 2011 displaying spinal deformities characteristic of Se exposures and a chronology of Se exposure associated with both maternal foraging in the upper estuary and post-hatch exogenous foraging in the San Joaquin River region (Johnson et al., submitted). Despite the wide distribution of Se sources in the SFE and movements of Splittail among regions and migration to the freshwater Delta for spawning, Se exposures were found to be spatially distinct and well described by collection sites grouped by region. Differences in tissue Se levels among regions were much larger than interannual differences due to freshwater inflows resulting in similar exceedances in liver and ovary tissue in 2010 and 2011 and matched geographical distributions of Se concentrations in Splittail prey. While the highest levels of Se appear to be constrained to a relatively narrow geographical region of the SFE, it is a region highly utilized by fish whose populations are at risk from multiple stressors (Sommer et al., 2007). Bulk isotopes of nitrogen and carbon confirmed that Splittail did not vary across regions in terms of prey selection or trophic level, while sulfur isotopes helped resolve individual variation in foraging behavior, not considered in the discrete assignment of region. Our results suggest that the proposed EPA

679 criteria for muscle tissue in Splittail may be under-protective as they would not have predicted
680 Se exceedances in liver or ovary tissue and that the relationship between muscle tissue and
681 ovary and liver may be concentration and seasonally dependent.
682
683 Selenium muscle concentrations observed for Splittail in the SFE (0.83 to 2.09 μg/g dry weight)
684 appeared to be in the range observed for fish species in other marine environments including
685 off the coast of Long Island NY (0.27 to 0.44 μg/g wet weight ~1.37 to 2.2 μg/g dry weight)
686 (Karimi et al., 2013) or Portugal (0.42 to 0.92 μg/g wet weight ~2.1 to 4.6 μg/g dry weight)
687 (Cabañero et al., 2005) but lower than those reported for fish from a seagrass food web in
688 Australia (4 to 9.3 μg/g dry weight). Indeed, Se concentrations in the Australian study exceeded
689 levels shown to elicit sub-lethal effects in freshwater fish, although no Se concentrations were
690 provided for other tissues (Barwick and Maher, 2003). A study of fish Se and Hg levels in the
691 Guanabara Bay estuary on the southern Brazilian coast showed a similar range in median Se
692 concentrations in muscle tissue for four fish species (0.27 to 2.01 μg/g dry weight) but a smaller
693 range in median liver Se concentrations (2.61 to 8.1 μg/g dry weight) compared to SFE Splittail
694 (liver range 4.97 to 17.85 μg/g dry weight). This lack of coherence between Se concentrations in
695 muscle verses other tissues both across fish species and ecosystems appears to be consistent
696 with earlier conclusions that extrapolation of tissue-tissue relationships should be site, species,
697 and life stage specific (Janz et al., 2010).
698
699 These results highlight some of the challenges faced by managers tasked with developing
700 regulatory approaches and protective criteria that are appropriate across aquatic species in

estuaries with distributed contaminant source loading. Physiology (i.e., uptake and depuration rates, translocation among tissues) combined with life histories that include movements across large geophysical and chemical gradients that vary by season, influence a species' individual and population exposure risk. Despite the extensive spatial variation, these results also offer some considerations for identifying factors to include in large scale ecosystem models based on the regulatory goals and processes (e.g. reproduction) they are meant to address and protect.

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6. References

Baginska B., 2015. Total Maximum Daily Load Selenium in North San Francisco Bay. Appendix C. Staff report for the proposed basin plan amendment San Francisco Bay Regional Water Quality Control Board, Oakland, CA. http://www.waterboards.ca.gov/sanfranciscobay/water_issues/programs/TMDLs/Setmdl.shtml.

Baines S.B., Fisher N.S., 2001. Interspecific differences in the bioconcentration of selenite by phtyoplankton and their ecological implications. Marine Ecology Progress Series. 213, 1-12. https://doi.org/10.3354/meps213001.

Baines S.B., Fisher N.S., Doblin M.A., Cutter G.A., 2001. Uptake of dissolved organic selenides by marine phytoplankton. Limnology and Oceanography. 46, 1936-1944. https://doi.org/10.4319/lo.2001.46.8.1936.

Baines S.B., Fisher N.S., Doblin M.A., Cutter G.A., Cutter L.S., Cole B., 2004. Light dependence of selenium uptake by phytoplankton and implications for predicting selenium incorporation into food webs. Limnology and Oceanography. 49, 566-578.

Baines S.B., Fisher N.S., Stewart R., 2002. Assimilation and retention of Se and other trace elements from crustacean food by juvenile striped bass (*Morone saxatilis*). Limnology and Oceanography. 47, 646-655. https://doi.org/10.4319/lo.2002.47.3.0646.

Barwick M., Maher W., 2003. Biotransference and biomagnification of selenium copper, cadmium, zinc, arsenic and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW, Australia. Marine Environmental Research. 56, 471-502. https://doi.org/https://doi.org/10.1016/S0141-1136(03)00028-X.

748 Bennett W.A., Moyle P.B., Hollibaugh J.T., 1996. Where have all the fishes gone? Interactive
749 actors producing fish declines in the Sacramento-San Joaquin Estuary. San Francisco
750 Bay: The Ecosystem. Pacific Divison, American Association for the Advancement of
751 Science, San Francisco, CA, 1996, pp. 519-542.

752 Cabana G., Rasmussen J.B., 1994. Modelling food chain structure and contaminant
753 bioaccumulation using stable nitrogen isotopes. Nature. 372, 255-257.
754 https://doi.org/10.1038/372255a0.

755 Cabana G., Rasmussen J.B., 1996. Comparison of aquatic food chains using nitrogen isotopes.
756 Proceedings of the National Academy of Sciences, USA. 93, 10844-10847.
757 https://doi.org/10.1073/pnas.93.20.10844.

758 Cabañero A.I., Carvalho C., Madrid Y., Batoreu C., Cámara C., 2005. Quantification and
759 speciation of mercury and selenium in fish samples of high consumption in Spain and
760 Portugal. Biological Trace Element Research. 103, 17-35.
761 https://doi.org/10.1385/BTER:103:1:017.

762 Chapman P.M., Adams W.J., Brooks M., SETAC (Society), 2010. Ecological assessment of
763 selenium in the aquatic environment. Taylor & Francis, Boca Raton.
764 https://doi.org/10.1201/EBK1439826775.

765 Chen L., Meseck S.L., Roy S.B., Grieb T.M., Baginska B., 2012. Modeling Fate, Transport, and
766 Biological Uptake of Selenium in North San Francisco Bay. Estuaries and Coasts. 35,
767 1551-1570. https://doi.org/10.1007/s12237-012-9530-y.

768 Chinese Medical Association, 1979. Observations on the effects of sodium selenite in prevention
769 of Keshan disease. Chinese Medical Journal. 92, 471-476.

770 Cloern J.E., Jassby A.D., 2012. Drivers of change in estuarine-coastal ecosystems: Discoveries
771 from four decades of study in San Francisco Bay. Reviews of Geophysics. 50, RG4001.
772 https://doi.org/10.1029/2012RG000397.

773 Conley J.M., Watson A.T., Xie L., Buchwalter D.B., 2014. Dynamic Selenium Assimilation,
774 Distribution, Efflux, and Maternal Transfer in Japanese Medaka Fed a Diet of Se-
775 enriched Mayflies. Environmental Science & Technology. 48, 2971-8.
776 https://doi.org/10.1021/es404933t.

777 Conomos T.J., 1979. San Francisco Bay - the urbanized estuary, investigations into the natural
778 history of San Francisco Bay and Delta with reference to the influence of man. American
779 Association for the Advancement of Science, San Francisco, California.

780 Cutter G.A., 1989. The Estuarine Behavior Of Selenium In San-Francisco Bay. Estuarine, Coastal
781 and Shelf Science. 28, 13-34. https://doi.org/10.1016/0272-7714(89)90038-3.

782 Cutter G.A., Cutter L.S., 2004. Selenium biogeochemistry in the San Francisco Bay estuary:
783 changes in water column behavior. Estuarine, Coastal and Shelf Science. 61, 463-476.
784 https://doi.org/10.1016/j.ecss.2004.06.011.

785 Cutter G.A., San Diego-McGlone M.L.C., 1990. Temporal variability of selenium fluxes in San
786 Francisco Bay. Science Of the Total Environment. 97/98, 235-250.
787 https://doi.org/10.1016/0048-9697(90)90243-N.

788 Daniels R.A., Moyle P., 1983. Life history of the splittail (Cyprinidae: Pogonichthys
789 macrolepidotus) in the Sacramento–San Joaquin Estuary. Fishery Bulletin. 84, 105-117.

790 Deng X., Teh S.J., Doroshov S.I., Hung S.S.O., 2012. Embryonic and Larval Development of
791 Sacramento Splittail Pogonichthys macrolepidotus. San Francisco Estuary and
792 Watershed Science. 10. https://doi.org/10.15447/sfews.2012v10iss1art1.

793 Doblin M.A., Baines S.B., Cutter L.S., Cutter G.A., 2006. Sources and biogeochemical cycling of
794 particulate selenium in the San Francisco Bay estuary. Estuarine, Coastal and Shelf
795 Science. 67, 681-694. https://doi.org/10.1016/j.ecss.2006.01.007.

796 Drevnick P.E., Sandheinrich M.B., Oris J.T., 2006. Increased ovarian follicular apoptosis in
797 fathead minnows (Pimephales promelas) exposed to dietary methylmercury. Aquatic
798 Toxicology. 79, 49-54. https://doi.org/10.1016/j.aquatox.2006.05.007.

799 Environmental Protection Agency. Water Quality Standards; Establishment of Revised Numeric
800 Criteria for Selenium for the San Francisco Bay and Delta, State of California. 81, 2016.

801 Feyrer F., Herbold B., Matern S.A., Moyle P.B., 2003. Dietary shifts in a stressed fish assemblage:
802 Consequences of a bivalve invasion in the San Francisco Estuary. Environmental Biology
803 of Fishes. 67, 277-288. https://doi.org/10.1023/A:1025839132274.

804 Feyrer F., Hobbs J., Acuna S., Mahardja B., Grimaldo L., Baerwald M., et al., 2015.
805 Metapopulation structure of a semi-anadromous fish in a dynamic environment.
806 Canadian Journal of Fisheries and Aquatic Sciences. 72, 709-721.
807 https://doi.org/10.1139/cjfas-2014-0433.

808 Feyrer F., Sommer T., Hobbs J., 2007. Living in a dynamic environment: Variability in life
809 history traits of age-0 splittail in tributaries of San Francisco Bay. Transactions of the
810 American Fisheries Society. 136, 1393-1405. https://doi.org/10.1577/T06-253.1.

811 France R.L., 1995. Differentiation between littoral and pelagic food webs in lakes using stable
812 carbon isotopes. Limnology and Oceanography. 40, 1310-1313.
813 https://doi.org/10.4319/lo.1995.40.7.1310.

814 Glover D.C., DeVries D.R., Wright R.A., Davis D.A., 2010. Sample Preparation Techniques for
815 Determination of Fish Energy Density via Bomb Calorimetry: An Evaluation Using
816 Largemouth Bass. Transactions of the American Fisheries Society. 139, 671-675.
817 https://doi.org/doi:10.1577/T09-110.1.

818 Heinz G.H., Fitzgerald M.A., 1993. Overwinter survival of mallards fed selenium. Archives of
819 Environmental Contamination and Toxicology. 25, 90-94.
820 https://doi.org/10.1007/BF00230717.

821 Heinz G.H., Hoffman D.J., 1998. Methylmercury chloride and selenomethionine interactions on
822 health and reproduction in mallards. Environmental Toxicology and Chemistry. 17, 139-
823 145. https://doi.org/10.1002/etc.5620170202.

824 Hesslein R.H., Capel M.J., Fox D.E., Hallard K.A., 1991. Stable isotopes of sulfur, carbon, and
825 nitrogen as indicators of trophic level and fish migration in the lower Mackenzie River
826 basin, Canada. Canadian Journal of Fisheries and Aquatic Sciences. 48, 2258-2265.
827 https://doi.org/10.1139/f91-265.

828 Janz D.M., DeForest D.K., Brooks M.L., Chapman P., Gilron G., Hoff D., et al., 2010. Selenium
829 toxicity to aquatic organisms. in: Chapman P, Adams WJ, Brooks ML, Delos CG, Luoma
830 SN, Maher WA, et al., editors. Ecological Assessment of Selenium in the Aquatic
831 Environment: Summary of a SETAC Pellston Workshop. Society of Environmental
832 Toxicology and Chemistry (SETAC), Pensacola, FL (USA), 2010, pp. 141-231.
833 https://doi.org/10.1201/EBK1439826775.

834 Johnson R.C., Stewart A.R., Limburg K.E., Huang R., Cocherell D., Feyrer F., submitted.
835 Lifetime chronicles of selenium exposure linked to deformities in an imperiled
836 migratory fish. Environmental Science & Technology.

837 Karimi R., Frisk M.G., Fisher N.S., Swadling K., 2013. Contrasting food web factor and body size
838 relationships with Hg and Se concentrations in marine biota. PloS one. 8, e74695.

839 Kimmerer W.J., 2002. Effects of freshwater flow on abundance of estuarine organisms: physical
840 effects or trophic linkages. Marine Ecology Progress Series. 243, 39-55.
841 https://doi.org/10.3354/meps243039.

842 Kleckner A.E., Kakouros E., Stewart A.R., 2017. A practical method for the determination of
843 total selenium in environmental samples using Isotope Dilution-Hydride Generation-
844 Inductively Coupled Plasma-Mass Spectrometry. Limnology and Oceanography:
845 Methods. 15, 363-371. https://doi.org/10.1002/lom3.10164.

Kleckner A.E., Stewart A.R., Elrick K.A., Luoma S.N., 2010. Selenium concentrations and stable isotopic compositions of carbon and nitrogen in the benthic clam *Corbula amurensis* from Northern San Francisco Bay, California: May 1995–February 2010. U.S. Geological Survey Open-File Report 2010-1252.

Kroll K.J., Doroshov S.I. Vitellogenin: Potential vehicle for selenium bioaccumulation in oocytes of white sturgeon. In: Williot P, editor. *Ancipenser*. CEMAGREF, Bordeau, France, 1991, pp. 99-106.

Lee B.G., Lee J.S., Luoma S.N., 2006. Comparison of selenium bioaccumulation in the clams *Corbicula fluminea* and *Potamocorbula amurensis* : A bioenergetic modeling approach. Environmental Toxicology and Chemistry. 25, 1933-1940.

Lemly A.D., 1982. Response of juvenile centrarchids to sublethal concentrations of waterborne selenium. I. Uptake, tissue distribution, and retention. Aquatic Toxicology. 2, 235-252. https://doi.org/10.1016/0166-445X(82)90027-3.

Lemly A.D., 1993. Metabolic stress during winter increases the toxicity of selenium to fish. Aquatic Toxicology. 27, 133-158. https://doi.org/10.1016/0166-445X(93)90051-2.

Lemly A.D., 1997. A teratogenic deformity index for evaluating impacts of selenium on fish populations. Ecotoxicology and Environmental Safety. 37, 259-266. https://doi.org/10.1006/eesa.1997.1554.

Lemly A.D., 1999. Selenium impacts on fish: An insidious time bomb. Human and Ecological Risk Assessment (HERA). 5, 1139-1151. https://doi.org/10.1080/10807039.1999.10518883.

Lemly A.D., 2004. Aquatic selenium pollution is a global environmental safety issue. Ecotoxicology and Environmental Safety. 59, 44-56. https://doi.org/10.1016/S0147-6513(03)00095-2.

869 Linares-Casenave J., Kroll K.J., Van Eenennaam J.P., Doroshov S.I., 2003. Effect of ovarian stage
870 on plasma vitellogenin and calcium in cultured white sturgeon. Aquaculture. 221, 645-
871 656. https://doi.org/10.1016/S0044-8486(03)00134-0.

872 Linares‐Casenave J., Linville R., Van Eenennaam J., Muguet J., Doroshov S., 2015. Selenium
873 tissue burden compartmentalization in resident white sturgeon (Acipenser
874 transmontanus) of the San Francisco Bay Delta estuary. Environmental Toxicology and
875 Chemistry. 34, 152-160. https://doi.org/10.1002/etc.2775.

876 Linville R. Effects of excess selenium on the health and reproduction of White sturgeon
877 (*Acipenser transmontanus*): Implications for San Francisco Bay-Delta. Ecology. University
878 of California, Davis, California, 2006, pp. 232.

879 Linville R.G., Luoma S.N., Cutter L., Cutter G.A., 2002. Increased selenium threat as a result of
880 invasion of the exotic bivalve Potamocorbula amurensis into the San Francisco Bay-
881 Delta. Aquatic Toxicology. 57, 51-64. https://doi.org/10.1016/S0166-445X(01)00265-X.

882 Lopez C.B., Cloern J.E., Schraga T.S., Little A.J., Lucas L.V., Thompson J.K., et al., 2006.
883 Ecological values of shallow-water habitats: Implications for restoration of disturbed
884 ecosystems. Ecosystems. 9, 422-440. https://doi.org/10.1007/s10021-005-0113-7.

885 Lucas L.V., Stewart A.R., 2005. Tranport, transformation, and effects of selenium and carbon in
886 the delta of the Sacramento-San Joaquin Rivers: Implications for ecosystem restoration.
887 Ecosystem Restoration Program Project No. ERP-01-C07 Calfed, Sacramento, CA.

888 Luoma S.N., Johns C., Fisher N.S., Steinberg N.A., Oremland R.S., Reinfelder J.R., 1992.
889 Determination of selenium bioavailability to a benthic bivalve from particulate and
890 solute pathways. Environmental Science & Technology. 26, 485-491.

891 Meseck S.L., Cutter G.A., 2006. Evaluating the biogeochemistry of selenium in San Francisco
892 Bay through modeling. Limnology and Oceanography. 51, 2018-2032.
893 https://doi.org/10.4319/lo.2006.51.5.2018.

894 Moyle P.B., Baxter R.D., Sommer T., Foin T.C., Matern S.A., 2004. Biology and Population
895 Dynamics of Sacramento Splittail (*Pogonichthys macrolepidotus*) in the San Francisco
896 Estuary: A Review. San Francisco Estuary and Watershed Science. 2.
897 https://doi.org/10.15447/sfews.2004v2iss2art3.

898 Muth O.H., Oldfield J.E., Remmert L.F., Schubert J.R., 1958. Effects of Selenium and Vitamin E
899 on White Muscle Disease. Science. 128, 1090-1090.
900 https://doi.org/10.1126/science.128.3331.1090.

901 Nichols F.H., Cloern J.E., Luoma S.N., Peterson D.H., 1986. The Modification of an Estuary.
902 Science. 231, 567-573. https://doi.org/10.1126/Science.231.4738.567.

903 Nichols F.H., Thompson J.K., Schemel L., 1990. Remarkable invasion of San Francisco Bay
904 (California, USA) by the Asian clam Potamocorbula amurensis . II. Displacement of a
905 former community. Marine Ecology Progress Series. 66, 95-101.

906 Ohlendorf H.M., Lowe R.W., Harvey T.E., Kelly P.R., 1989. Selenium and heavy metals in San
907 Francisco Bay diving ducks. J.Wild.Manag. 50, 64-71.

908 Ohlendorf H.M., Lowe R.W., Kelly P.R., Harvey T.E., 1986. Selenium and Heavy Metals in San
909 Francisco Bay Diving Ducks. The Journal of Wildlife Management. 50, 64-70.
910 https://doi.org/10.2307/3801489.

911 Paulsson K., Lundbergh K., 1989. The selenium method for treatment of lakes for elevated levels
912 of mercury in fish. Science of the Total Environment. 87-88, 495-507.
913 https://doi.org/10.1016/0048-9697(89)90256-8.

914 Peterson B.J., Fry B., 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and
915 Systematics. 18, 293-320.

916 Peterson S.H., Ackerman J.T., Eagles-Smith C.A., 2017. Mercury contamination and stable
917 isotopes reveal variability in foraging ecology of generalist California gulls. Ecological
918 Indicators. 74, 205-215. https://doi.org/10.1016/j.ecolind.2016.11.025.

919 Peterson S.H., Ackerman J.T., Eagles-Smith C.A., Herzog M.P., Hartman C.A., 2018. Prey fish
920 returned to Forster's tern colonies suggest spatial and temporal differences in fish
921 composition and availability. Plos One. 13. https://doi.org/10.1371/journal.pone.0193430.

922 Presser T., Luoma S.N., 2006. Forecasting selenium discharges to the San Francisco Bay-Delta
923 Estuary: ecological effects of a proposed San Luis Drain extension. U.S. Geological
924 Survey Professional Paper 1646.

925 Presser T., Luoma S.N., 2010. A methodology for ecosystem-scale modeling of selenium.
926 Integrated Environmental Assessment and Management. 6, 685-710.
927 https://doi.org/10.1002/ieam.101.

928 Presser T.S., Barnes I., 1984. Selenium concentrations in waters tributary to and in the vicinity of
929 the Kesterson National Wildlife Refuge, Fresno and Merced counties, California. Water-
930 Resources Investigations Report 84-4122. U.S. Geological Survey,
931 http://pubs.er.usgs.gov/publication/wri844122.

932 Presser T.S., Ohlendorf H.M., 1987. Biogeochemical cycling of selenium in the San Joaquin
933 Valley, California, USA. Environ.Manag. 11, 805-821. https://doi.org/10.1007/BF01867247.

934 Presser T.S., Sylvester M.A., Low W.H., 1994. Bioaccumulation of selenium from natural
935 geologic sources in Western States and its potential consequences. Environ.Manag. 18,
936 423-436. https://doi.org/10.1007/BF02393871.

937 SAS Institute Inc. JMP® 14 Fitting Linear Models. SAS Institute Inc, Cary, NC, 2018.

938 Schlekat C.E., Dowdle P.R., Lee B.G., Luoma S.N., Oremland R.S., 2000. Bioavailability of
939 particle-associated Se to the bivalve Potamocorbula amurensis Environmental Science &
940 Technology. 34, 4504-4510.

941 Schlekat C.E., Lee B.G., Luoma S.N., 2002. Assimilation of selenium from phytoplankton by
942 three benthic invertebrates: effect of phytoplankton species. Marine Ecology Progress
943 Series. 237, 79-85. https://doi.org/10.3354/meps237079.

944 Schlekat C.E., Lee B.G., Luoma S.N., Newman M.C., Roberts M.H., Hale R.C., 2001. Dietary
945 metals exposure and toxicity to aquatic organisms: Implications for ecological risk
946 assessment. Coastal and estuarine risk assessment. CRC Press, Boca Raton, 2001, pp.
947 151-188. https://doi.org/10.1201/9781420032451.

948 Schlekat C.E., Purkerson D.G., Luoma S.N., 2004. Modeling selenium bioaccumulation through
949 arthropod food webs in San Francisco Bay. Environmental Toxicology and Chemistry.
950 23, 3003-3010. https://doi.org/10.1897/03-4.1.

951 Seber G.A.F. The estimation of animal abundance, and related parameters. Macmillan Pub. Co.,
952 New York :, 1982.

953 Skorupa J.P., Frankenberger W.T., Jr., Engberg R.A., 1998. Selenium poisoning of fish and
954 wildlife in nature: Lessons from twelve real-world experiences. Environmental
955 chemistry of selenium. Marcel Dekker, New York, 1998, pp. 315-354.

956 Sommer T., Armor C., Baxter R., Breuer R., Brown L., Chotkowski M., et al., 2007. The collapse
957 of pelagic fishes in the Upper San Francisco Estuary. Fisheries. 32, 270-277.
958 https://doi.org/10.1577/1548-8446(2007)32[270:Tcopfi]2.0.Co;2.

959 Sommer T., Harrell B., Nobriga M., Brown R., Moyle P.B., Kimmerer W., et al., 2001. California's
960 Yolo Bypass: Evidence that flood control can be compatible with fisheries, wetlands,
961 wildlife and agriculture. Fisheries. 26, 6-16. https://doi.org/10.1577/1548-
962 8446(2001)026<0006:CYB>2.0.CO;2.

963 Stadtman T.C., 1974. Selenium Biochemistry. Proteins containing selenium are essential
964 components of certain bacterial and mammalian enzyme systems. 183, 915-922.
965 https://doi.org/10.1126/science.183.4128.915.

966 Stewart A.R., 2019. Selenium concentrations in tissues of Sacramento Splittail in the San
967 Francisco Estuary, California 2010-11. U.S. Geological Survey data release.
968 https://doi.org/10.5066/P9GI73P9

969 Stewart A.R., Grosell M., Buchwalter D.B., Fisher N., Luoma S.N., Mathews T., et al., 2010.
970 Bioaccumulation and trophic transfer of selenium. in: Chapman PM, Adams WJ, Brooks
971 ML, Delos CG, Luoma SN, Maher WA, et al., editors. Ecological Assessment of Selenium
972 in the Aquatic Environment: Summary of a SETAC Pellston Workshop. Society of
973 Environmental Toxicology and Chemistry (SETAC), Pensacola, FL (USA), 2010, pp. 93-
974 139. https://doi.org/10.1201/EBK1439826775.

975 Stewart A.R., Luoma S.N., Elrick K.A., Carter J.L., van der Wegen M., 2013. Influence of
976 Estuarine Processes on Spatiotemporal Variation in Bioavailable Selenium. Marine
977 Ecology Progress Series. 492, 41-56. https://doi.org/10.3354/meps10503.

978 Stewart A.R., Luoma S.N., Schlekat C.E., Doblin M.A., Hieb K.A., 2004. Food Web Pathway
979 Determines How Selenium Affects Aquatic Ecosystems: A San Francisco Bay Case
980 Study. Environmental Science & Technology. 38, 4519-4526.
981 https://doi.org/10.1021/es0499647.

982 Teh S.J., Deng X., Deng D.F., Teh F.C., Hung S.S.O., Fan T.W.M., et al., 2004. Chronic effects of
983 dietary selenium on juvenile Sacramento splittail (Pogonichthys macrolepidotus).
984 Environmental Science & Technology. 38, 6085-6093. https://doi.org/10.1021/es049545+.

985 Teh S.J., Miller C.E., Hinton D.E., 2000. Hermaphroditism in Laboratory-Cultured Albino
986 Western Mosquitofish. Journal of Aquatic Animal Health. 12, 78-80.
987 https://doi.org/10.1577/1548-8667(2000)012<0078:HILCAW>2.0.CO;2.

988 van Hees K.E., Ebert D.A., 2017. An evaluation of mercury offloading in two Central California
989 elasmobranchs. Science of The Total Environment. 590-591, 154-162.
990 https://doi.org/https://doi.org/10.1016/j.scitotenv.2017.02.191.

991 Vander Zanden M.J., Cabana G., Rasmussen J.B., 1997. Comparing trophic position of
992 freshwater fish calculated using stable nitrogen isotope ratios (Î 15 N) and literature
993 dietary data. Canadian Journal of Fisheries and Aquatic Sciences. 54, 1142-1158.
994 https://doi.org/10.1139/cjfas-2017-0381.

995 Vander Zanden M.J., Hulshof M., Ridgway M.S., Rasmussen J.B., 1998. Application of stable
996 istope techniques to trophic studies of age-0 smallmouth bass. Trans.Amer.Fish.Soc. 127,
997 729-739. https://doi.org/10.1577/1548-8659(1998)127<0729:AOSITT>2.0.CO;2.

998 Vander Zanden M.J., Rasmussen J.B., 1999. Primary consumer δ13C and δ15 N and the trophic
999 position of aquatic consumers. Ecology. 80, 1395-1404. https://doi.org/10.1890/0012-
1000 9658(1999)080[1395:PCCANA]2.0.CO;2.
1001

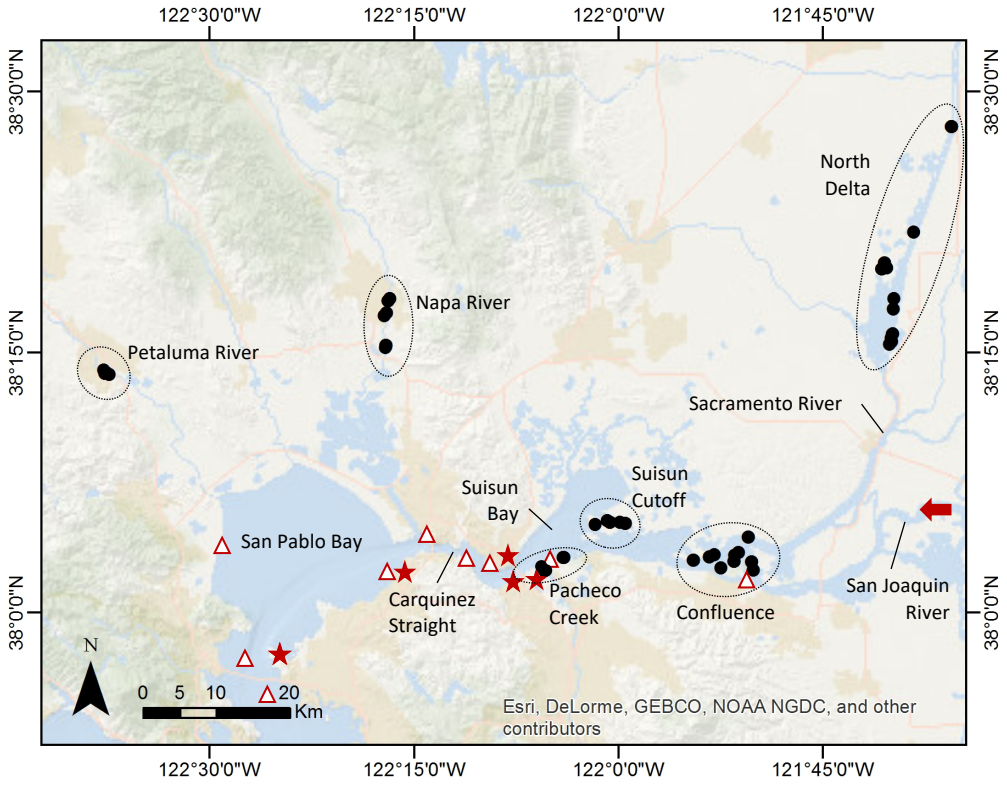
Figure captions

Figure 1. Map of the northern reach of the San Francisco Estuary showing regions where Sacramento Splittail were collected in the fall of 2010 and 2011 (Petaluma River, Napa River, Pacheco Creek, Suisun Cutoff, Confluence) and in the North Delta in the spring of 2017. Black filled circles – sites of fish collections. Dashed black shapes: Regions. Red arrow – agricultural irrigation sources from the San Joaquin Valley. Red stars – oil refineries. Red triangles – wastewater treatment plants.

Figure 2. Selenium (Se) concentrations in Sacramento Splittail tissues by region in the San Francisco Estuary. A. Muscle. B. Liver. C. Ovary. Box plot values: median (horizontal line), box (1st and 3rd quartile), whiskers (minimum and maximum quarter of values), circles (outliers - 1.5 times interquartile range). Dashed lines represent proposed EPA criterion level (muscle – 11.3 (not shown), ovary – 15.1) or threshold level of concern (liver - 12). Regions (years combined) with different lowercase letters are different ($p < 0.05$). Petaluma – dark blue. Napa – orange. Pacheco – yellow. Suisun – grey. Confluence – light blue. Delta – green.

Figure 3. Biplots of stable isotope values in Sacramento Splittail muscle from the San Francisco Estuary by region and year. A. $\delta^{15}N$ versus $\delta^{13}C$. B. $\delta^{15}N$ versus and $\delta^{34}S$. Values are means ± 95% CI. Petaluma – dark blue. Napa – orange. Pacheco – yellow. Suisun – grey. Confluence – light blue. Delta – green. Filled circles – 2010. Open circles – 2011. Filled square – 2017.

Figure 4. Relationships between selenium concentrations (Se) in Sacramento Splittail tissues for regions of the San Francisco Estuary. A. Liver verses muscle. B. Ovary verses muscle. C. Ovary verses liver. Petaluma – dark blue. Napa – orange. Pacheco – yellow. Suisun – grey. Confluence – light blue. Delta – green. Dashed black line represents equivalent concentrations between tissues (shown only for ovary verses liver).


122°30'0"W
122°15'0"W
122°0'0"W
121°45'0"W
38°30'0"N
38°15'0"N
38°0'0"N
North
Delta
Napa River
Petaluma River
Sacramento River
Suisun
Bay
Suisun
Cutoff
San Pablo Bay
Carquinez
Straight
Pacheco
Creek
Confluence
San Joaquin
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N
0 5 10 20
Km
Esri, DeLorme, GEBCO, NOAA NGDC, and other
contributors

Figure 1

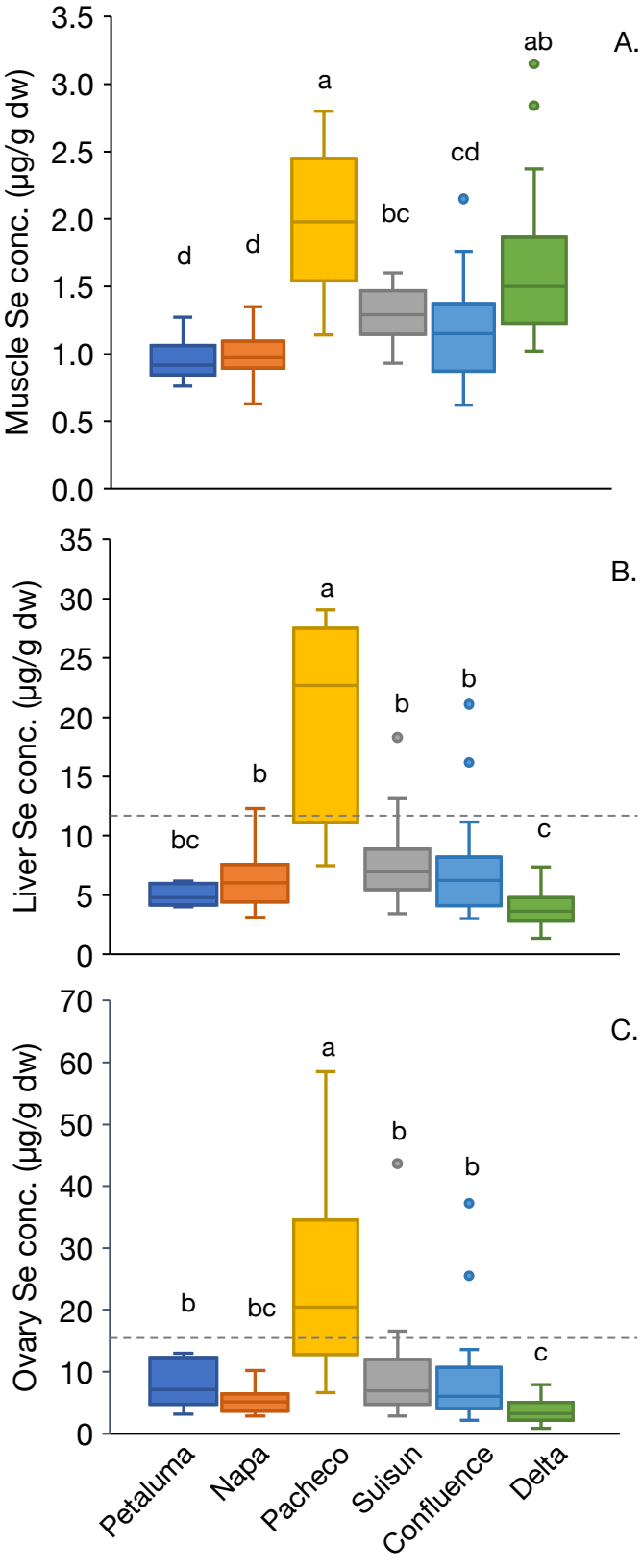
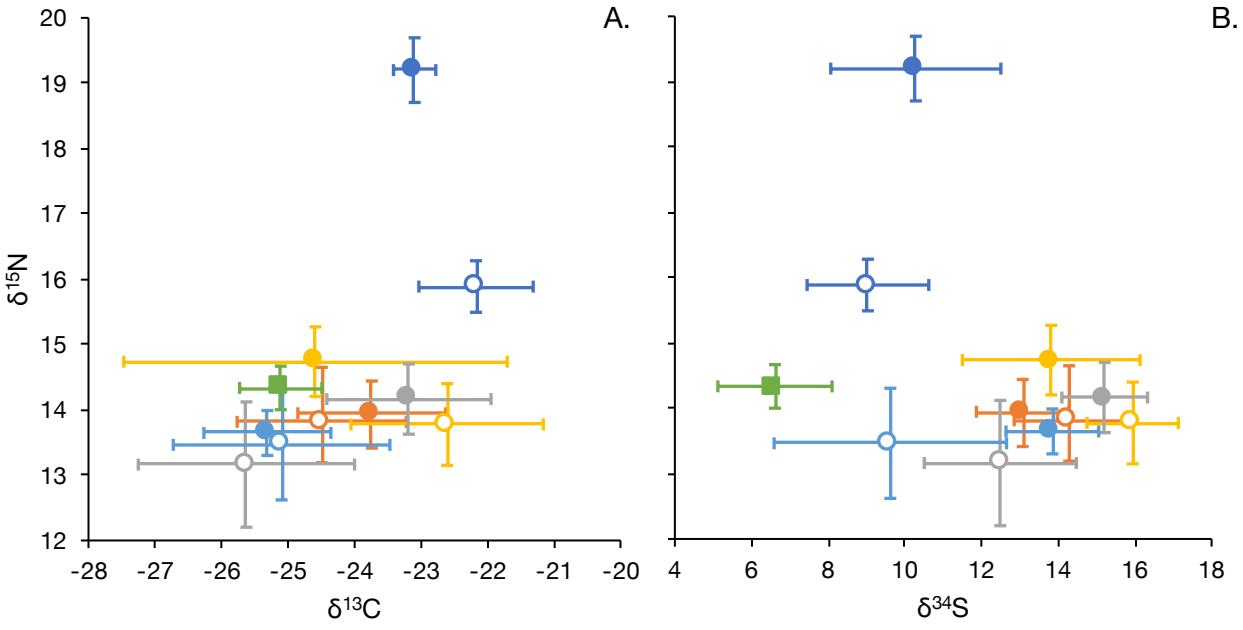

A.
Muscle Se conc. (µg/g dw)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
d
d
a
bc
cd
ab
B.
Liver Se conc. (µg/g dw)
35
30
25
20
15
10
5
0
bc
b
a
b
b
c
C.
Ovary Se conc. (µg/g dw)
70
60
50
40
30
20
10
0
b
bc
a
b
b
c
Petaluma
Napa
Pacheco
Suisun
Confluence
Delta

Figure 2


A.
B.
δ15N
δ13C
δ34S
20
19
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13
12
-28
-27
-26
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Figure 3

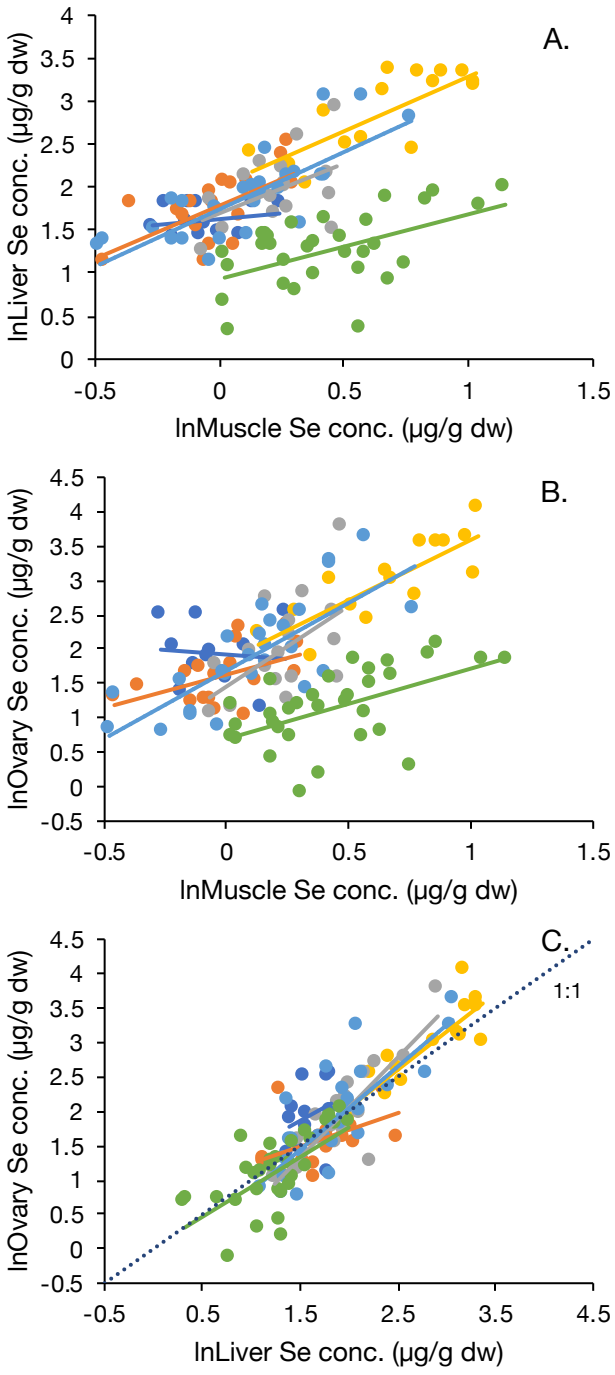

A.
lnLiver Se conc. (µg/g dw)
lnMuscle Se conc. (µg/g dw)
B.
lnOvary Se conc. (µg/g dw)
lnMuscle Se conc. (µg/g dw)
C.
1:1
lnOvary Se conc. (µg/g dw)
lnLiver Se conc. (µg/g dw)

Figure 4

Table 1. Fish length, whole body weight, age, liver and gonad tissue weights and muscle $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ isotope signatures for Sacramento Splittail from regions of the San Francisco Estuary. Values are regional and yearly means ± SD, n represents the number of fish sampled.

Region	Year	n	Fork length (mm)	Weight[a] (g ww)	Age[b] (yr)	Gonad (g ww)	Liver (g ww)	Muscle $\delta^{13}C$	Muscle $\delta^{15}N$	Muscle $\delta^{34}S$
Petaluma	2010	2	193 ± 15	99 ± 17	2.0 ± 0.0	2.1 ± 0.1	2.3 ± 0.8	-23.10 ± 0.32	19.2 ± 0.49	10.29 ± 1.24
	2011	10	238 ± 22	172 ± 48	3.5 ± 1.0	7.1 ± 4.4	2.9 ± 1.0	-22.17 ± 1.20	16.28 ± 0.55	9.04 ± 2.22
Napa	2010	8	289 ± 28	319 ± 85	5.3 ± 1.0	22.5 ± 13.4	4.1 ± 1.3	-23.74 ± 1.33	14.44 ± 0.59	13.07 ± 1.44
	2011	10	297 ± 38	372 ± 147	5.5 ± 1.1	21.2 ± 16.5	4.5 ± 2.2	-24.49 ± 1.78	14.65 ± 1.17	14.29 ± 2.00
Pacheco	2010	5	275 ± 43	307 ± 141	4.4 ± 1.5	15.8 ± 17.5	4.3 ± 1.5	-24.59 ± 2.32	15.27 ± 0.43	13.82 ± 1.87
	2011	10	272 ± 32	277 ± 99	4.5 ± 1.1	8.3 ± 5.8	2.9 ± 1.2	-22.61 ± 2.02	14.4 ± 0.87	15.94 ± 1.67
Suisun	2010	8	308 ± 25	412 ± 87	5.8 ± 0.9	27.8 ± 14.6	5.2 ± 1.0	-23.18 ± 1.48	14.7 ± 0.64	15.21 ± 1.34
	2011	9	297 ± 36	369 ± 131	5.4 ± 0.9	13.5 ± 11.6	3.8 ± 1.4	-25.62 ± 2.12	14.12 ± 1.25	12.49 ± 2.58
Confluence	2010	18	297 ± 38	379 ± 150	5.6 ± 1.0	23.2 ± 18.9	4.6 ± 1.9	-25.31 ± 1.92	13.99 ± 0.69	13.85 ± 2.43
	2011	10	331 ± 41	562 ± 204	6.5 ± 1.2	41.9 ± 31.2	6.5 ± 3.2	-25.10 ± 2.28	14.31 ± 1.18	9.62 ± 4.24
Delta	2017	32	310 ± 36	430 ± 133	5.9 ± 1.2	30.5 ± 40.0	5.1 ± 3.2	-25.11 ± 1.71	14.67 ± 0.93	6.61 ± 4.13

[a] wet weight (ww)— recorded upon collection, except for Delta fish (and tissues) which were recorded after freezing/thawing.

[b] ages were determined from counting rings on fish otoliths as reported in Feyrer et al. 2015, except for Delta fish which were estimated based on length by age relationship derived for fish from other regions (Fork length = 28.7 x Age + 136.9; $R^2 = 0.91$, $p < 0.0001$).

Table 2. Geometric mean fish tissue selenium (Se) concentrations in Sacramento Splittail tissues for regions of the San Francisco Estuary for each region and year (± SE), n represents the number of fish sampled. Geometric mean values that exceeded regulatory criteria or thresholds of concern are underlined and the number and percentage of individual fish exceedances are reported for a given tissue, region and year.

Region	Year	n	Muscle Se (µg/g dw[a])	Number of individual exceedances	Liver Se (µg/g dw)	Number of individual exceedances	Ovary Se (µg/g dw)	Number of individual exceedances
Petaluma	2010	2	0.83 ± 0.07	0	5.25 ± 0.68	0	12.26[b]	0
	2011	10	0.97 ± 0.05	0	4.97 ± 0.29	0	6.56 ± 0.94	0
Napa	2010	8	0.88 ± 0.07	0	5.80 ± 0.80	1 (13%)	4.38 ± 0.28	0
	2011	10	1.07 ± 0.04	0	6.66 ± 0.75	0	5.62 ± 0.86	0
Pacheco	2010	5	2.09 ± 0.24	0	<u>17.85 ± 4.64</u>	4 (80%)	<u>20.29 ± 7.22</u>	3 (60%)
	2011	10	1.88 ± 0.18	0	<u>16.56 ± 2.37</u>	7 (70%)	<u>20.19 ± 3.31</u>	7 (70%)
Suisun	2010	8	1.23 ± 0.07	0	5.69 ± 0.54	0	5.71 ± 1.00	0
	2011	9	1.30 ± 0.08	0	9.73 ± 1.19	3 (33%)	9.38 ± 2.47	3 (33%)
Confluence	2010	18	1.19 ± 0.09	0	7.26 ± 0.97	3 (17%)	8.62 ± 1.54	3 (17%)
	2011	10	1.00 ± 0.08	0	5.30 ± 0.46	0	4.47 ± 0.84	0

Delta	2017	32	1.56 ± 0.08	0	3.55 ± 0.27	0	3.16 ± 0.31	0

[a] dw – dry weight

[b] single value was determined

Table 3. Fixed effects models explaining factors influencing ln-transformed selenium (Se) concentrations in Sacramento Splittail tissues for regions of the San Francisco Estuary. Significant model effects are noted with an asterisk.

Tissue Model	Effect	DF	Sum of Squares	F Ratio	*p*-value	AIC score
lnSe Muscle = Region[a] +	Model	9	5.58	11.46	<0.0001*	
Year + Region x Year	Region	4	4.99	23.1	<0.0001*	
	Year	1	0.01	0.20	0.65	
	Region x Year	4	0.43	1.99	0.10	
lnSe Liver = Region[a] +	Model	9	15.9	10.2	<0.0001*	
Year + Region x Year	Region	4	12.8	18.4	<0.0001*	
	Year	1	0.001	0.008	0.93	
	Region x Year	4	1.43	2.05	0.09	
lnSe Ovary = Region[a] +	Model	9	22.2	6.95	<0.0001*	
Year + Region x Year	Region	4	17.2	12.1	<0.0001*	
	Year	1	0.14	0.40	0.53	
	Region x Year	4	4.26	3.01	0.02*	
lnSe Muscle = Region[b]	Model	5	6.82	21.4	<0.0001*	19
lnSe Liver = Region[b]	Model	5	26.8	30.0	<0.0001*	143
lnSe Ovary = Region[b]	Model	5	37.2	20.5	<0.0001*	228
lnSe Muscle = Region[b] +	Model	11	8.11	13.3	<0.0001*	9.9
$\delta^{34}S$	Region	10	7.94	14.4	<0.0001*	
	$\delta^{34}S$	1	0.87	15.7	0.0001*	
lnSe Liver = Region[b] +	Model	11	29.8	16.7	<0.0001*	140
$\delta^{34}S$	Region	10	13.8	8.53	<0.0001*	
	$\delta^{34}S$	1	1.49	9.22	0.003*	

lnSe Ovary = Region[b] +	Model	11	44.1	12.6	<0.0001*	220
$\delta^{34}S$	Region	10	21.2	6.65	<0.0001*	
	$\delta^{34}S$	1	2.53	7.96	0.0057*	

[a]Model does not include Delta fish

[b]Model includes Delta fish, upper estuary 2010 and 2011 data combined

Table 4. Relationships between selenium (Se) concentrations in Sacramento Splittail tissues for regions of the San Francisco Estuary and Delta. Slopes are reported with ±95% confidence intervals. Significant relationships are noted with an asterisk.

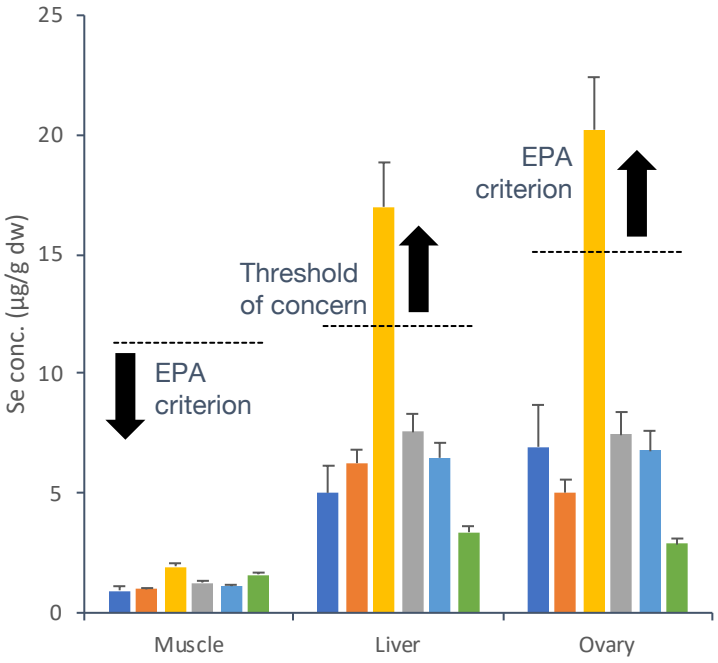
Region	Regression Model	n	Intercept *a*	Slope *b*	R^2	*p*-value
Petaluma	lnSe Muscle = *a* + *b*lnSe Liver	12	1.63	0.27±0.80	-0.04	0.5
Napa		18	1.79	1.24±0.80	0.38	0.005*
Pacheco		15	2.02	1.27±0.64	0.55	0.0009*
Suisun		17	1.69	1.16±1.16	0.18	0.05*
Confluence		27	1.74	1.34±0.44	0.59	<0.0001*
Delta		31	0.93	0.75±0.45	0.26	0.002*
Petaluma	lnSe Muscle = *a* + *b*lnSe Ovary	11	1.92	-0.27±2.2	-0.1	0.79
Napa		18	1.63	0.97±0.92	0.19	0.04*
Pacheco		15	1.84	1.75±0.72	0.65	0.0002*
Suisun		17	1.46	2.34±1.89	0.27	0.02*
Confluence		26	1.69	1.98±0.70	0.57	<0.0001*
Delta		32	0.70	1.02±0.59	0.27	0.001*
Petaluma	lnSe Liver = *a* + *b*lnSe Ovary	11	0.72	0.76±1.9	-0.02	0.40
Napa		18	0.75	0.49±0.47	0.18	0.04*
Pacheco		15	-0.17	1.11±0.38	0.74	<0.0001*
Suisun		17	-0.86	1.46±0.50	0.70	<0.0001*
Confluence		27	-0.37	1.21±0.36	0.64	<0.0001*
Delta		31	0.03	0.87±0.37	0.43	<0.0001*
Delta[a]	lnSe Wholebody = *a* + *b*lnSe Muscle	32	0.10	0.55±0.16	0.62	<0.0001*
	lnSe Wholebody = *a* + *b*lnSe Liver	31	0.79	0.76±0.24	0.58	<0.0001*
	lnSe Wholebody = *a* + *b*lnSe Ovary	32	0.51	1.0±0.31	0.59	<0.0001*

[a]Whole body Se measurements were only done on Delta fish.

Table 5. ANCOVA model relationships between selenium (Se) concentrations in Sacramento Splittail tissues for regions of the San Francisco Estuary. Terms are model coefficients and *t*-tests test if estimates are different from zero. Significant estimates are noted with an asterisk.

Tissue Model[a]	Term	Estimate	SD Error	*t* Ratio	Prob>|t|
Liver by Muscle	Intercept *a*	1.64	0.05	33.03	<0.0001*
	lnSe Muscle *b*	1.09	0.13	8.59	<0.0001*
	Region[Napa]	0.15	0.08	1.83	0.07
	Region[Pacheco]	0.50	0.09	5.58	<0.0001*
	Region[Suisun]	0.07	0.07	1.03	0.3
	Region[Confluence]	0.13	0.06	2.05	0.04*
	Region[Delta]	-0.85	0.06	-13.96	<0.0001*
Ovary by Muscle	Intercept *a*	1.50	0.07	21.32	<0.0001*
	lnSe Muscle *b*	1.51	0.18	8.41	<0.0001*
	Region[Napa]	0.14	0.11	1.24	0.2
	Region[Pacheco]	0.49	0.13	3.92	0.0002*
	Region[Suisun]	0.15	0.10	1.46	0.1
	Region[Confluence]	0.24	0.09	2.63	0.01*
	Region[Delta]	-1.02	0.09	-12	<0.0001*
Ovary by Liver	Intercept *a*	-0.11	0.18	-0.58	0.6
	lnSe Liver *b*	1.05	0.09	11.49	<0.0001*
	Napa	-0.12	0.09	-1.43	0.2
	Pacheco	0.12	0.12	0.95	0.3
	Suisun	0.05	0.09	0.63	0.5
	Confluence	0.04	0.07	0.56	0.6
	Delta	-0.09	0.09	-0.96	0.3
Ovary by Liver	Intercept *a*	-0.26	0.11	-2.28	0.02*
(global model)	lnSe Liver *b*	1.13	0.06	18.94	<0.0001*

[a]Tissue models are linear regressions where lnSe Tissue 1 = *a* + regional estimate adjustment + *b*lnSe Tissue 2. E.g., Suisun lnSe Liver = 1.71 + 1.09 x lnSe Muscle.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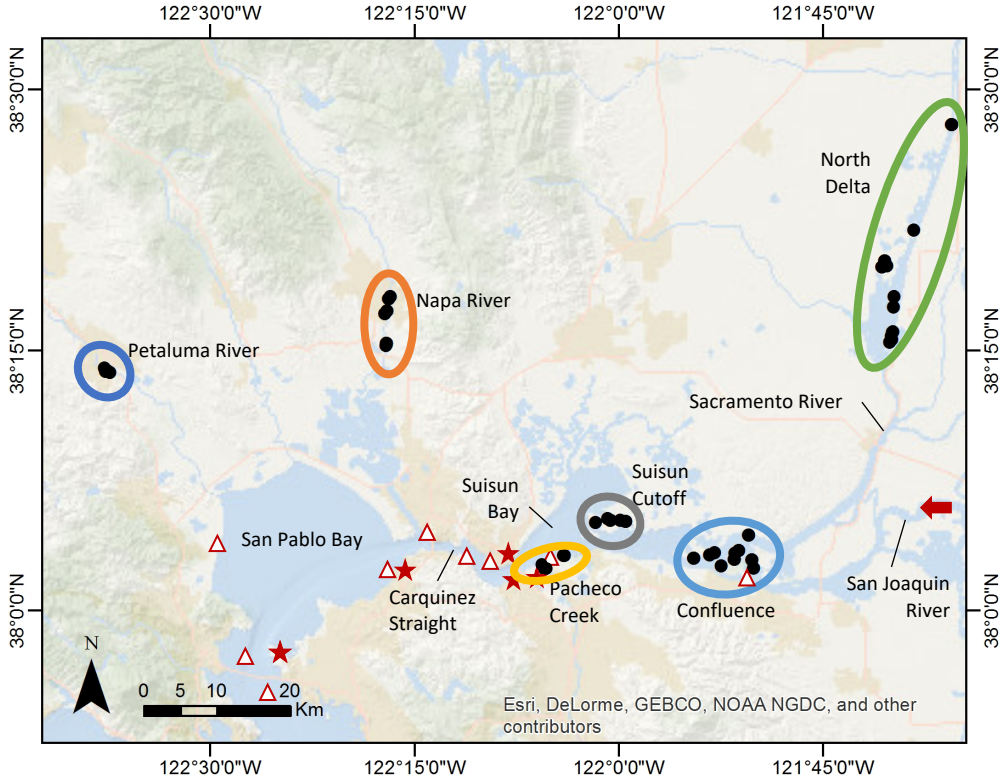
15
10
5
0
EPA criterion
Threshold of concern
EPA criterion
Muscle
Liver
Ovary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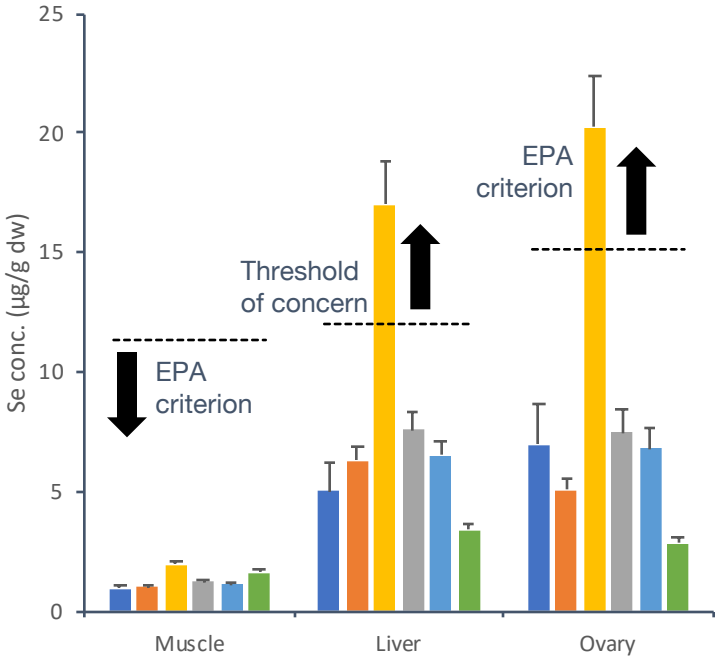
122°15'0"W
122°0'0"W
121°45'0"W
38°30'0"N
38°15'0"N
38°0'0"N
North Delta
Napa River
Petaluma River
Sacramento River
Suisun Bay
Suisun Cutoff
San Pablo Bay
Carquinez Straight
Pacheco Creek
Confluence
San Joaquin River
N
0 5 10 20
Km
Esri, DeLorme, GEBCO, NOAA NGDC, and other contributors


Se conc. (µg/g dw)
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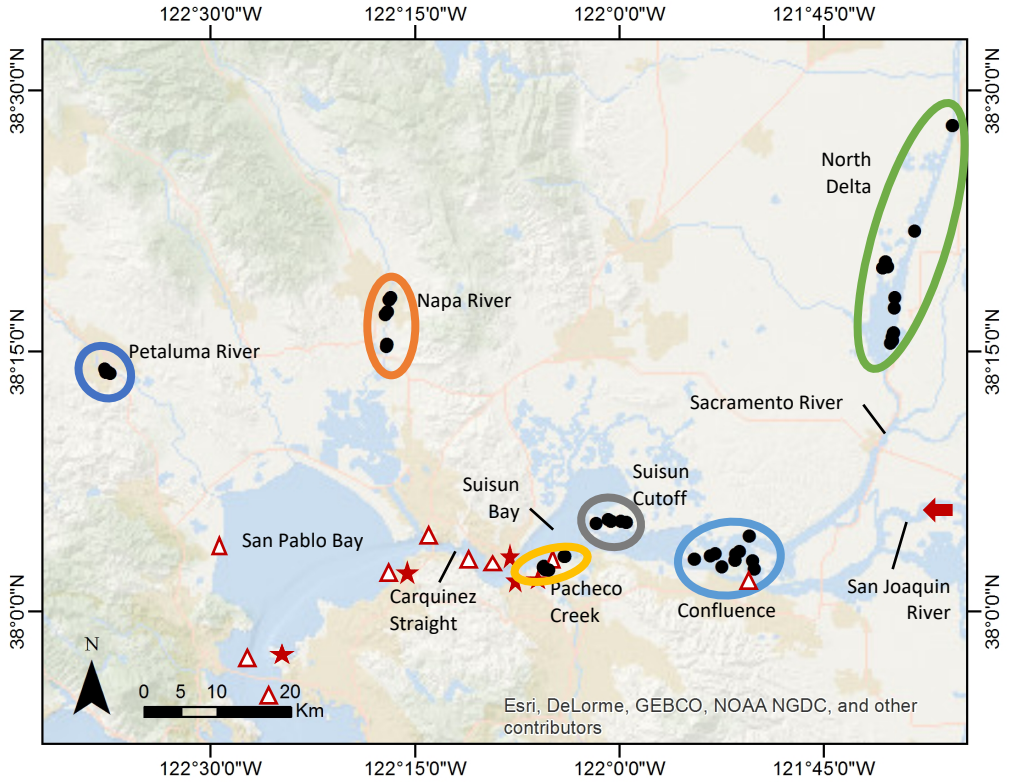
20
15
10
5
0
EPA criterion
Threshold of concern
EPA criterion
Muscle
Liver
Ovary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122°15'0"W
122°0'0"W
121°45'0"W
38°30'0"N
38°15'0"N
38°0'0"N
North Delta
Napa River
Petaluma River
Sacramento River
Suisun Bay
Suisun Cutoff
San Pablo Bay
Carquinez Straight
Pacheco Creek
Confluence
San Joaquin River
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