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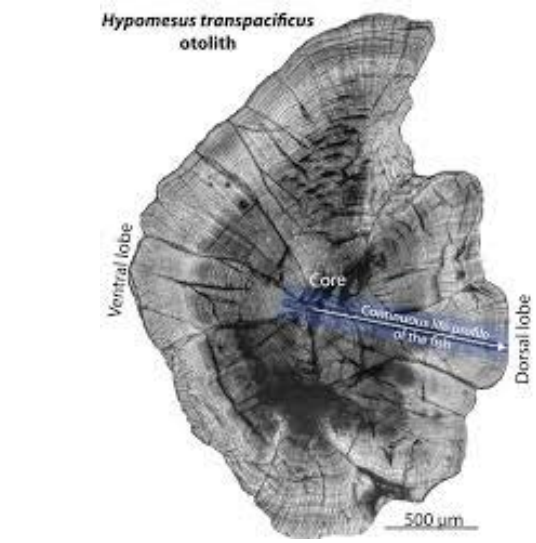
Managing Water in the West

Directed Outflow Project
Technical Report 1



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14. ABSTRACT The U.S. Bureau of Reclamation (USBR) and California Department of Water Resources (DWR), along with collaborators, are continuing efforts to evaluate the hypothesized benefits of outflow and outflow alteration for Delta Smelt. The collective aim of these efforts is to better inform management actions that will bolster and stabilize the Delta Smelt population. The planned five-year Directed Outflow Project (DOP) seeks to assist in evaluating the overarching hypothesis that habitat quality and quantity is improved in the summer/fall when X2 is below 81 km and the LSZ occurs in Suisun Bay and Marsh, and this improvement in habitat conditions will translate into a greater catch density, health, and growth for Delta Smelt using this area. The DOP hypotheses and predictions are largely based on the conceptual models within Baxter et al. (2015) and predictions in Brown et al. (2014). The DOP expands the biological and physical monitoring to test outflow-related hypotheses and associated predictions. In this technical report we present research findings related to the DOP. This work may also assist in evaluation of other actions such as the North Delta Flow Action (Yolo Bypass Toe Drain outflows) and Suisun Bay Marsh Salinity Control Gate Action.

15. SUBJECT TERMS
Directed Outflow Project, Fall X2, Salinity, Delta Smelt, Zooplankton, Contaminants, Growth, Condition, San Francisco Bay, Delta

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Background and Purpose

In spring 2016, U.S. Fish and Wildlife Service (USFWS) requested augmentation of summer outflow from the Sacramento River to benefit the Delta Smelt *Hypomesus transpacificus* population. The objective and rationale provided by the Service (agency communication) for augmentation of summer outflow was:

> *An X2 (location of 2 ppt salinity isohaline) of no more eastward than 81 km would provide favorable habitat conditions for the Delta Smelt population during the summer of 2016 by allowing the LSZ to overlap with higher quality habitat in Suisun Bay and Marsh and still allowing smelt to occupy habitats east of the Sacramento-San Joaquin river confluence like the lower Yolo Bypass and Sacramento Deep Water Shipping Channel. It was expressed that Delta Smelt had positive recruitment in only two years (2006 and 2011) since 2002. In both of those years, Delta Smelt experienced high quality habitat conditions in Suisun Bay and Marsh in the spring and summer. In the other 13 years, X2 was located east of the confluence for much or all of the summer, and salinities in Suisun Bay and Marsh limited habitat quality in these western portions of the designated critical habitat.*

However, the requested 2016 action did not occur as the amount of water available for outflow was deemed insufficient to provide the intended benefit. The concept of altering outflow to benefit rearing stages of Delta Smelt is not new. Action 4 (Fall X2 Action) of the USFWS Biological Opinion on the Long-Term Operational Criteria and Plan for Coordination of the CVP and SWP (Delta Smelt Bi-Op; USFWS 2008) requires implementation of Delta outflow augmentation during the fall to improve fall habitat for Delta Smelt when the preceding water year was above normal.

During spring and summer of 2016, the Delta Smelt Resiliency Strategy (DSRS) was circulated and a final draft released in July 2016 (CNRA 2016). This science-based document was intended to address both immediate and near-term needs of Delta Smelt and promote their resiliency to drought conditions as well as future variability in habitat conditions. The document articulated a suite of actions that could be implemented in the next few years to benefit Delta Smelt based on concepts detailed in Baxter et al. (2015). The DSRS described numerous actions including augmentation of Delta outflow to push the low salinity zone westward and routing of water through Yolo Bypass Toe Drain to promote food production as potential management actions that would benefit Delta Smelt production.

Since the DSRS's release in 2016, water management actions related to Delta Smelt resiliency have continued through the Collaborative Science and Adaptive Management Program (CSAMP). This group has served the functions of annual planning to ensure actions are pursued, coordination to enhance the monitoring and science being implemented, and stimulate communication and learning about these actions. CSAMP identified there was uncertainty and disagreement (presented and discussed at 5/8/17 and 7/12/17 CSAMP meetings) regarding the hypothesized mechanistic relationships between summer or fall Delta outflow variability and Delta Smelt vital rates and habitat needs.

Reduced outflow has been linked to reductions in habitat suitability in Suisun Bay and Marsh and movement of the LSZ to the Confluence of the Sacramento-San Joaquin River where little connection to shallow open water habitats exists. However, there is disagreement among scientists and stakeholders regarding the efficacy of short-term flow alteration actions in the summer or fall. Specifically, there is considerable uncertainty regarding the ability of such actions to translate to measurable responses in Delta Smelt habitat, condition, growth and survival.

The U.S. Bureau of Reclamation (USBR) and California Department of Water Resources (DWR), along with collaborators, are continuing efforts to evaluate the hypothesized benefits of outflow and outflow alteration for Delta Smelt. The collective aim of these efforts is to better inform management actions that will bolster and stabilize the Delta Smelt population. The planned five-year Directed Outflow Project (DOP) seeks to assist in evaluating the overarching hypothesis that habitat quality and quantity is improved in the summer/fall when X2 is below 81 km and the LSZ occurs in Suisun Bay and Marsh, and this improvement in habitat conditions will translate into a greater catch density, health, and growth for Delta Smelt using this area. The DOP hypotheses and predictions are largely based on the conceptual models within Baxter et al. (2015) (figures 48 and 49 in particular) and predictions in Brown et al. (2014). The DOP expands the biological and physical monitoring to test outflow-related hypotheses and associated predictions (Table 1). Predictions in Table 1 not related to chapters in the first DOP technical report may be addressed in subsequent DOP reports, however, the DOP is not intended to test all possible hypotheses and associated predictions related to outflow and outflow alterations. This work may also assist in evaluation of other actions such as the North Delta Flow Action (Yolo Bypass Toe Drain outflows) and Suisun Bay Marsh Salinity Control Gate Action.

Paired monitoring of fish communities and aquatic habitat for the DOP was implemented in July of 2017 in collaboration with agency partners. Additional biotic habitat measures began in September of 2017. The DOP is seeking to continue the additional paired fish-habitat monitoring in coordination and collaboration with the USFWS's Enhanced Delta Smelt Monitoring program (EDSM) through 2021. This work is coordinated with, and will augment, the California Department of Fish and Wildlife's (CDFW) ongoing fish and habitat surveys mandated by the Delta Smelt Bi-Op (USFWS 2008) and Revised Water Right Decision 1641 (SWRCB 2000).

In this technical report we present research findings related to the DOP. Each chapter within this report is intended for eventual submittal to a peer-review scientific journal, thus formatting varies among chapters. Comments at the top of the title page of each chapter will alert the reader of those chapters already published or submitted to a peer-reviewed journal.

The DOP is a direct contributor to the Interagency Ecological Program, Flow Alteration - Management, Analysis, and Synthesis Team (FLOAT-MAST) and its draft current technical report (FLOAT-MAST 2019). FLOAT-MAST technical report objectives include: summarizing data collected from flow-related special studies and long-term monitoring programs for the water year of 2017, with an emphasis on data relevant to the fall low-salinity habitat component of the RPA (USFWS 2008); provide synthesis of new and previous data to further assess the validity of the hypotheses underlying the Fall X2 Action and to provide a baseline for future evaluations;

update the current conceptual models for Delta Smelt; put results from the FLOAT-related studies into the context of the overall body of knowledge on Delta Smelt (FLOAT-MAST 2019).

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Table 1. Qualitative Predictions Regarding the Effect of X_2 (location of 2 ppt salinity isohaline) in or Near the Suisun Bay/Marsh Area During Summer and Fall Compared to Other Regions, and within this Area During Summer and Fall X_2 Action Periods

Dynamic Abiotic Habitat Components	**X2 in/near Susuin Region During Summer or Fall Compared to Other Regions and Within Suisun Region During Summer or Fall X2 Action Periods (in parentheses)**	**Chapters within DOP Technical Report 1 With Related Data**
Low-salinity Habitat Area	Higher (Increases)	
Habitat Complexity	Higher (Increases)	
Hydrodynamic Complexity	Higher (Increases)	
Water Temperature	Lower (Decreases)	Chapters 3, 4, 9
Turbidity	Higher (Increases)	Chapter 9
Contaminants*	Lower (Decreases)	Chapter 6
Dynamic Biotic Habitat Components		
Delta Smelt Prey Density and Biomass	Higher (Increases)	Chapter 9
Phytoplankton Density and Biomass	Higher (Increases)	
Harmful Algal Constituents / Cyanotoxins	Lower (Decreases)	
Impact of Non-native Competitors	Lower	
Impact of Non-native Predators	Lower	
Delta Smelt Responses		
Occupancy/Residence	Greater (Increases)	Chapter 9
Health	Greater (Increases)	Chapters 2, 6
Growth	Higher (Increases)	Chapters 3, 4, 5
Survival	Higher (Increases)	
Prey Quality, Foraging Success	Better (Increases)	Chapters 1 (general diet), 7
Fecundity	Higher	
Population Range/Distribution	Broader, less constricted	Chapters 3, 9
Life History Diversity	Greater, more even spread	Chapter 3

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Chapter 1: Patterns of Zooplankton Consumption by Juvenile and Adult Delta Smelt (*Hypomesus transpacificus*)

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Abstract

Delta Smelt is an imperiled fish species endemic to the upper San Francisco Estuary and associated Sacramento-San Joaquin Delta. Management actions to benefit Delta Smelt include freshwater outflow augmentation, however it is unclear how flow affects Delta Smelt foraging. Our study generated diet information from 1,962 Delta Smelt collected from 2011-2017 to evaluate hypotheses related to the feeding ecology of Delta Smelt among seasons and habitats (salinity) over several years of varying flow conditions in the upper estuary, including 2017, an extremely wet year.

Cyclopoid and calanoid copepods were the numerically dominant prey in the guts of Delta Smelt during most years and seasons and relatively dominant in terms of prey biomass in the guts of Delta Smelt for young juveniles during summer. As Delta Smelt matured, larger prey items such as mysids, amphipods, and larval fishes contributed more to stomach contents, the latter item being important to adults during the spring period only. The wet year of 2017 was dominated by copepods, cladocerans, and amphipods in terms of prey biomass. The importance of amphipods in diet contrasts with prior years, where for most years amphipods were not a large biomass component of Delta Smelt diet including 2011, another wet year. Gut fullness was also higher in 2017, particularly in the low salinity zone (0.5 to 6 ppt) relative to other salinity areas. We found no relationship between gut fullness and condition factor, likely due to these measures operating

on different time scales. Our results revealed that prey categories consumed varied seasonally and among habitats (salinity), yet were similar among recent years.

Introduction

The Delta Smelt *Hypomesus transpacificus* is a small pelagic fish endemic to fresh and brackish waters of the upper San Francisco Estuary (Estuary) and Sacramento-San Joaquin Delta (Delta), California, USA. Though once numerous, Delta Smelt has suffered a long-term decline in abundance associated with changes in habitat conditions in the Estuary (Moyle et al. 2016). The Estuary receives fresh water from the Sacramento and San Joaquin River Delta that flows toward the Pacific Ocean through a series of rivers, channels, and bays. The amount of fresh water flow shifts seasonally from the wet period of winter and spring to the dry period of summer and fall. The tidal mixing of fresh and ocean waters results in a gradient of brackish water, of which the low salinity zone (LSZ; 0.5-6.0 ppt) is important rearing habitat for many young fishes including Delta Smelt (Dege and Brown 2004, Kimmerer et al. 2013). The amount of Estuary fresh water flow is managed by a complex series of reservoir releases and freshwater pumping extraction, both of which influence the location and size of the LSZ (Feyrer et al. 2007, Kimmerer et al. 2013). Habitat features important to Delta Smelt include turbid waters, cool temperatures, and prey availability (Baxter et al. 2015).

Delta Smelt is largely a zooplanktivore that consumes an array of prey that increase in size as the fish matures (Moyle et al. 1992; Feyrer et al. 2003; Mager et al. 2004, Hammock et al. 2019). Delta Smelt larvae hatch at 5-6 mm fork length (FL) (Wang 1986) with feeding starting within about one week of hatching (i.e. ~6 mm FL, Mager et al. 2004). Nobriga (2002) found the smallest Delta Smelt larvae consumed mostly copepod nauplii and copepodites, with larger larvae (~20 mm) switching to mostly adult copepods. The calanoid copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi*, and cyclopoid copepods were the dominant prey consumed with Delta Smelt showing positive selection for both *E. affinis* and *P. forbesi* (Nobriga 2002). Slater and Baxter (2014) showed similar patterns with selection for *E. affinis* and *P. forbesi* extending well into the juvenile life stage during summer. During the summer the authors found *P. forbesi* adults became the major food item by number and weight with *Limnoithona* spp. of noted occurrence as well. During this period, the smaller *Limnoithona* spp. were selected against, but were consumed when at extremely high densities and other prey were limited. Types of prey consumed is also a function of regional differences in availability (Baxter et al. 2015, Hammock et al. 2017). Adult Delta Smelt consume larger zooplankton prey including mysids and larval fishes (Baxter et al. 2015; Hammock et al. 2017). Laboratory feeding experiments show similar patterns with Delta Smelt larvae transitioning to larger copepod prey as fish mature, with selection for larger calanoid copepods *E. affinis* and *P. forbesi* over smaller zooplankton life stages and species (e.g., *Limnoithona* spp.) (Sullivan et al. 2016).

The pelagic foodweb, on which Delta Smelt depends, has undergone radical changes over the last ~50 years. Slater and Baxter (2014) summarized the substantial changes in the prey of Delta Smelt from the 1970s through the 1990s as a result of numerous species introductions. Most notable changes in the upper Estuary and Delta occurred in the late 1980s with new zooplankton species, notably copepods, and the reduction in primary and secondary production following invasion of the bivalve *Potamocorbula amurensis.* The invasions of the Delta by the bivalves

Corbicula fluminea and *P. amurensis* are thought to strongly suppress phytoplankton via grazing and reduce zooplankton abundance through competition and predation. These impacts have had a negative effect on a suite of zooplankton (Winder and Jassby 2011), such as mysids, that are historically important to Delta Smelt (Moyle 2002, Feyrer et al. 2003, Baxter et al. 2015).

The decline of the Delta Smelt population has been attributed in part to changes in the food web (Bennett and Moyle 1996; Moyle 2002; Sommer et al. 2007; Mac Nally et al. 2010; Baxter et al. 2015, Moyle et al. 2016). More specifically, it is thought that Delta Smelt are food limited during the spring through fall periods (Bennett and Moyle 1996; Bennet 2005). Kimmerer (2008) found summer to fall survival was significantly related to calanoid zooplankton biomass in the low-salinity zone (0.5-2.1 psu). Slater and Baxter (2014) suggest low calanoid copepod abundance in August and September may have affected feeding and survival in 2005 and 2006. However, while prey availability is an undoubtedly vital component of Delta Smelt habitat and survival, some uncertainties in this relationship exist.

Outflow-related management actions to benefit Delta Smelt are currently in place or proposed (USFWS 2008; CNRA 2016; Frantzich et al. 2018; Schultz et al. 2018). However, how such actions affect food availability and prey use by Delta Smelt is uncertain. The prevailing hypotheses are that food production, food quality and feeding success for Delta Smelt increases as the salinity field moves seaward, as a function of increased Delta freshwater outflow (USBR 2012; Brown et al. 2014). In this study we examined Delta Smelt collected over a 7-year period to describe prey found in stomachs to address the following questions: (1) Did Delta Smelt have increased feeding success (gut fullness) in 2017 relative to previous years? (2) Was there a relationship between fullness and body condition? (3) How did prey consumption change among seasons (life stages) for Delta Smelt? and (4) Did prey consumption differ among years and habitat (salinity)?

Methods

Study Area. – The study area ranged from San Pablo Bay in the western part of the upper Estuary upstream into the connecting Delta to Stockton on the San Joaquin River, Hood on the Sacramento River, and the Sacramento River Deep Water Ship Channel (Figure 1-1). Daily net freshwater outflow (cfs) past Chipps Island estimates were obtained from the DWR DAYFLOW website (https://water.ca.gov/Programs/Environmental-Services/Compliance-Monitoring-And-Assessment/Dayflow-Data) and summarized as monthly trends among water years, along with the Sac Valley water year index (W = wet, AN = above normal, BN = below normal, D = dry, and C = critically dry). Note that water years in California are October 1-September 30 (e.g. water year 2011 is October 1, 2010-September 30, 2011).

Delta Smelt. – We used Delta Smelt captured during monitoring surveys conducted by the California Department of Fish and Wildlife (CDFW; 2011-2017) and U.S. Fish and Wildlife Service (USFWS; 2017) participating in the Interagency Ecological Program (IEP). CDFW IEP surveys included Summer Townet (STN), Fall Midwater Trawl (FMWT), Spring Kodiak Trawl (SKT), and also a special study in 2014 the Gear Efficiency Survey (GES) (for more details on survey design see Hammock et al. 2017). The USFWS survey Enhanced Delta Smelt Monitoring program (EDSM) begun in 2017 and used a Kodiak Trawl. Fish surveys for CDFW

employed a fixed-station design and USFWS surveys used a generalized random-tessellation stratified sampling design (Stevens and Olsen 2004; Starcevich et al. 2016). Temperature (°C), Secchi disk depth (cm), and specific conductivity (µS/cm) were measured from boats at each sampling location. Salinity (parts-per-thousand, ppt) was calculated from specific conductance (uS/cm) corrected to 25°C then using the equation ppt = ((0.36966/(((µS/cm *0.001)^-1.07)-0.00074)*1.28156). Diet data were organized into the following salinity categories <0.5 ppt, 0.5-6.0 ppt, and >6.0 ppt; the Low Salinity Zone (LSZ) recognized as ~0.5-6.0 ppt.

Delta Smelt were preserved in liquid nitrogen on the boats using methods described in Teh et al. (2016) and transferred to University of California at Davis (UCD). Thawed specimens were measured for fork length (mm) and total body weight (g) and then rapidly dissected (~5–10 min per fish). Delta Smelt length-weight data was summarized via a scatterplot and the relationship reported as a power function (Supplement Data: Figures, Figure B1). The gastro-intestinal tract, including esophagus, stomach, and intestine, was preserved in 95% ethanol and sent to CDFW's Diet Study Laboratory for analysis (Stockton, CA). Body weights of 13 fish were not recorded at the start of the study in 2011 as attempts to weigh fish in the field were found too variable, subsequent measures were recorded in the laboratory. We calculated Fulton's condition factor for each fish as follows:

$$K = (W / L^3) * 100{,}000,$$

where W is body weight (g) and L is fork length (mm) (Neumann et al. 2012).

Fullness and Prey Use. – Data related to stomach content identification and fullness largely followed methods in Slater and Baxter (2014) and Hammock et al. (2017). Gastro-intestinal tracts were taken out of vials and rinsed to remove ethanol. The intestine was removed and the stomach was opened to expose contents. Stomach contents were placed in water in a Petri dish and all items were identified to the lowest practical taxon and counted. Intestine contents were not examined as items were heavily digested. In addition to counting items, a length was recorded for mysids, amphipods, and larval fish, when intact. A body length (mm) estimate was assigned to mysids, amphipods and larval fish that were heavily digested or in pieces; assigned lengths were from the intact prey of the same type from the same stomach or same type from a stomach of a fish collected close in time and location (e.g. same station or nearby station). Lengths were recorded for a subset of other zooplankton types, when intact (cumaceans, terrestrial invertebrates, isopods, others). We categorized amphipods as either *Gammarus* spp. or *Corophium* spp. based on distinct body shapes of the genera but did not identify them to species. We determined wet weight of prey in guts by multiplying the count of each prey type by a wet weight estimate (Supplemental Data: Tables, Table A1) or from lengths using length-weight equations for mysids, amphipods, and larval fish (Supplemental Data: Tables, Table A2). Recorded lengths of prey were summarized as scatterplots (Supplemental Data: Figures, Figures B2 and B3). We summed calculated weights of the various prey types for each fish stomach. The calculated weight of prey in stomachs was divided by the total number of prey to generate average prey mass per fish. The various prey categories were grouped by species or genera for a total of 19 categories.

Gut fullness was calculated as stomach content weight as a percentage of body weight (%BW), with wet weight of the stomach contents (g) divided by fish body wet weight (g) multiplied by

100 (Bush 2003). Stomach contents were found in various stages of digestion, so at times only parts of an organism were found (e.g. telson from amphipod). Therefore, the sum of calculated prey weights could exceed actual mass if only parts of prey items are present, or the opposite could occur if materials not enumerated like unidentified animal and plant material were present. Calculated stomach weights and fullness values that exceeded 4% were removed from the analysis (N = 24), as they exceeded double the "full" percentage of 2% and so were believed to be outliers. We assessed the percent fullness and assigned a relative index of fullness rank using the scale 0 = empty, 1 = 1-25% full, 2 = 25-50% full, 3 = 50-75% full and 4 = 75-100% full, similar to Cohen and Bollens (2008). The fullness rank was an additional measure added during the study, so data does not exist for all samples.

We organized data to allow comparison among years for seasons (June-August, September-November, and December-May) that follow closely to gear types used to track the various life stages of Delta Smelt (juveniles, sub-adults, and adults, respectively). Results of diet analysis were reported as percent by number (%N), by weight (%W), and by frequency of occurrence (%FO). Numeric diet data allows examination of prey consumption relative to prey availability, but small numerically abundant prey can outweigh contribution of larger, less frequently consumed prey to the diet. Mass diet data allows examination of patterns relative to stomach fullness, but can overestimate importance of large, less frequently consumed prey. Unidentified animal and plant material were not included in diet by %N, %W, or %FO as these items could not be enumerated.

We used a non-parametric Kruskal-Wallis test to determine whether there were significant differences ($P < 0.05$) in gut fullness across years, salinities, and seasons. A boxplot was generated to show the distribution of calculated fullness (%) values relative to the observed stomach fullness by rank (SYSTAT 13). We used least squares linear regression to assess the relationship between gut fullness and condition factor. A Conover-Iman post-hoc test was applied to test for significance differences among the pairwise comparisons when the Kruskal-Wallis test was significant.

Multivariate analyses were conducted to examine patterns in zooplankton consumption by Delta Smelt from stomach content data among years, habitats (salinity) and seasons using PRIMER 7. Fish with empty stomachs (N = 66) were not included in the multivariate analyses of prey consumption. A square-root transformation was applied to mean diet by percent number, and mean diet by percent weight data, and Bray-Curtis similarity matrices (abundance) were produced. We used one-way Analysis of Similarity (ANOSIM) to test for statistical differences in diet between year, seasons, and salinity ranges. An ANOSIM R value close to zero indicates no difference between groups, an R value close to 1 indicates strong differences between groups, and the maximum value of 1 is the greatest level of dissimilarity possible (Clarke and Warwick 2001, Sampson et al. 2009). We used Non-metric Multidimensional Scaling (NMDS) on the Bray-Curtis matrices to illustrate diet overlap. Similarity Percentage (SIMPER procedure) was used to determine which prey categories contributed to the differences in diets, if any, revealed by ANOSIM. We did use ANOSIM to test for a difference in diet among fish collected by agency (CDFW vs USFWS) and found no significant difference in the global test in diet between agencies (R = 0.075, P = 0.286), so no further analyses for this variable were conducted.

Results

Freshwater outflow as calculated at Chipps Island in the upper Estuary was highly variable during this study. Mean monthly flow followed seasonal trends for the Estuary with wet winters and springs followed by dry summers and falls, with several years of extreme drought (i.e., dry and critically dry water years, 2013-2016), two below normal water years (2012 and 2016), and two wet water years (2011, 2017), one of which (2017) was one of the wettest years on record (Figure 1-2).

Delta Smelt. – There were 1,866 Delta Smelt collected by CDFW studies (2011-2017) and 96 collected by USFWS EDSM (2017) for a total of 1,962 fish that were examined for gut contents (Table 1-1). Feeding incidence was highly positive with some amount of prey present in n=1,896 (98%) stomachs. Delta Smelt in this study were collected at temperatures ranging from 8 to 26 °C, at Secchi depths ranging from 10 to 130 cm, at times between 6 AM and 4 PM, and at salinities from 0.1 to 15.6 ppt, although relatively few Delta Smelt were collected at temperatures above 23 °C or salinities above 8 ppt (Figure 1-3). There did not appear to be a pattern in detection of empty stomachs among each of the environmental variables, as empty stomachs occurred at low frequency across measurements, except a slightly higher frequency of empty stomachs occurred at warmer temperatures (20-21°C) and between 7 AM and 11 AM (Figure 1-3).

Juveniles of each year class were collected beginning in June (mean 36.7 mm FL), although 1 smelt at 32 mm FL was collected in May 2014 (Table 1-2). A general pattern of growth for each year class occurred with increased monthly mean lengths as each year progressed, with some individual months being variable or lower to the previous month due in part to small sample sizes. Adult Delta Smelt were collected through the May of the following year hatch (year class) with a mean length of 73.9 mm FL.

Gut Fullness. – A total of 1,925 Delta Smelt were included in analysis of the fullness. A subset of these fish included assignment of a rank of relative index of fullness (n = 1,200) that was used to place the calculated percent fullness relative to body weight in context of what was observed in stomachs (Figure 1-4). For example, stomachs that appeared “full” (rank 4) occurred over a range of calculated fullness (%) values with the median being 0.89% for “full” stomachs. Stomachs “half-full” had a median value of 0.25% and “3/4 full” were 0.52% (Figure 1-4). Application of this pattern to calculated fullness (%) data would be that Delta Smelt stomachs on average were ¾ to mostly full (Figure 1-5).

There was a significant difference in calculated stomach fullness (%) among years (Kruskal-Wallis = 20.507; $P < 0.003$; Figure 1-5). A post-hoc test revealed fullness was significantly lower in 2013 than 2011, 2012, 2014, 2015, and 2017, but other pairwise combinations of years were not found to be significantly different. There was a significant difference in stomach fullness among salinities (Kruskal-Wallis = 8.583; $P = 0.014$, df = 2; Figure 1-5), with post hoc test of significant differences between <0.5 and 0.5-6 ppt ($P = 0.009$) and also <0.5 and >6 ppt ($P = 0.0497$), but not between 0.5-6 and >6 ppt ($P = 0.661$). Seasonal fullness was significantly different among June-August, September-November, and December-May (Kruskal-Wallis = 15.649, $P = 0.0004$). Post hoc test results indicated significant differences between June-August and September-November ($P = 0.0004$) and significant differences between September-

November and December-May *(P* = 0.0002) due to higher September-November fullness, but there was not a significant difference between June-August and December-May (*P* = 0.761). Fullness (%) differed among hour of collection (Kruskal-Wallis = 202.264, *P* < 0.0001). Most of the 55 post hoc pairwise comparisons were significantly different, except between 4 PM and 6 AM or 7 AM or between 8 AM and 9 AM, 10 AM, 11 AM, or 1 PM and between 12 PM and 2 PM (Figure 1-5). The extreme high mean value at 3 PM was a small sample size (n=17) with the stomach contents all large prey of amphipods, mysids, cumaceans, and larval fish.

Mean (± SE) fullness (%) was 0.426 (± 0.011) and condition factor (K) was 0.726 (± 0.002). We found no linear relationship (R^2 = 0.0002; df = 1, 1923; *P* =0.572) between gut fullness and condition factor (Figure 1-6).

General Summary of Diet. – A total of 295,546 items were identified and counted from Delta Smelt stomachs. The number of prey averaged 156 per stomach for fish with food present in guts (n=1,896), with the highest prey count being 2,427 in a single stomach (Figures 1-7A and 1-7B). We found that the maximum number of prey consumed increased as Delta Smelt increased in size from small juveniles up through adults (~55 mm FL), but did not increase among adults, possibly a function of prey size and stomach capacity (Figure 1-7). The number of prey in stomachs appeared to be a function of the size of prey, the stomachs with the most numerous prey also had the lowest mean mass per individual prey item and some of the lowest frequency had the largest mean mass for prey (Figure 1-7A). Number of prey when scaled to stomach fullness saw that both stomachs with numerous small items and also stomachs with few large items had high stomach fullness, but the stomachs with fewer items had lower fullness per individual among fork lengths (Figure 1-7B).

Amphipods ranged in length from 0.5 to 6 mm and were mostly small *Corophium* spp., juveniles of *Americorophium stimpsoni* and *A. spinicorne* (53.7%), with *Gammarus* spp. including *Gammarus daiberi*, *Crangonyx* sp., and *Hyalella* sp. (7.0%) and unidentified amphipods (1.4%) (Figure 81-). Mysids *Hyperacanthomysis longirostris* and unidentified mysids (8.4%) had the widest range of body lengths 0.5-11 mm found in stomachs, with 2/3 of those mysids being 1-3 mm long. Only a few native *Neomysis kadiakensis* (n=5) and *N. mercedis* (n=5) mysids were found in stomachs, compared to hundreds of the introduced *H. longirostris* (n=431). Larval fish (6% of larger prey) ranged in length from 2.0 to 13.9 mm. Pacific Herring *Clupea pallasii* ranged from 5.0 to 10.5 mm, whereas Prickly Sculpin *Cottus asper* ranged from 3.5 to 7.0 mm, Longfin Smelt *Spirinchus thaleichthys* from 3.0 to 7.0 mm, and *Tridentige*r spp. from 2.0 to 3.0 mm (Figure 1-8).

Cyclopoid and calanoid copepods were the numerically dominant prey items in the stomachs of Delta Smelt during most years, salinity ranges, and seasons, with cladocerans dominant in the December-May period in fresh water (Tables 3-5). A pattern was evident that prey use was similar within seasons and salinities among years. During the June-August period juvenile Delta Smelt ate mostly *Pseudodiaptomus* spp. in freshwater (<0.5 ppt) among years (Table 1-3), while in the LSZ (0.5-6 ppt) *Limnoithona* spp. with *Pseudodiaptomus* spp. was also consumed in large numbers (Table 1-4). Juvenile Delta Smelt were less common above 6 ppt. Their diets were more variable, and included *Limnoithona* spp., *Acartiella sinensis*, and *Tortanus* spp. copepods and also demersal invertebrates, amphipods and cumaceans (Table 1-5). Diets during September-November were similar to the previous season with copepods numerically dominant,

but the variability in species of copepods increased in freshwater (<0.5 ppt) and LSZ (0.5-6 ppt), notably with an increase in *Pseudodiaptomus* spp. in the fall (Tables 3-4). The few Delta Smelt that were collected in September-November at >6 ppt which primarily consumed copepods and cladocerans with a reduced presence of demersal invertebrates (Table 1-5). During December-May in freshwater (<0.5 ppt), adults shifted to a majority of *Sinocalanus doerrii* with other calanoids, cyclopoids and cladocerans (Table 1-3). Delta Smelt in the LSZ (0.5-6 ppt) shifted consumption to higher percentages of *Eurytemora affinis*, *Acanthocyclops* spp., other cyclopoid copepods, and cladocerans among years (Table 1-4). The December-May high salinity (>6 ppt) diets also included high percentages of *E. affinis* in addition to *Limnoithona* spp. and other cyclopoid copepods and cladocerans (Table 1-5).

In terms of prey mass in the diet of Delta Smelt, cyclopoid and calanoid copepods were dominant for young juveniles during the summer period. Diet by weight for juveniles was more variable as the fish matured with larger prey items such as mysids, amphipods and larval fishes important during several years and the latter being important during the spring period only (Tables 6-8). Similar to diet by number, diet by weight had a pattern of generally consistent prey use among years within seasons and variable among salinity regions, with increased contribution of larger prey (Tables 6-8). Diet by weight for June-August in freshwater (<0.5 ppt) was mostly *Pseudodiaptomus* spp. and *Sinocalanus doerrii* (Table 1-6). During June-August in the LSZ (0.5-6 ppt) diets were more variable with *Pseudodiaptomus* spp., *A. sinensis*, *Tortanus* spp. *Limnoithona* spp, along with mysids and fish and some amphipods contribute by weight (Table 1-7). Diet for June-August at >6 ppt included a greater diversity of prey and larger prey types, such as *Tortanus* spp. copepods, cumaceans and fish (Table 1-8). The "Other" category of 68.5% for June-August 2014 in >6 ppt was due largely to isopods; one fish contained 8 of the total 20 isopods counted among all Delta Smelt stomachs. The September-November period had high percentages of calanoid copepods for diets by weight, *Pseudodiaptomus* spp. the dominant copepod in <0.5 and 0.5-6ppt, but mysids also contributed to diets in fresh water (<0.5 ppt) for several years (Table 1-6). For September-November 2017, we found a substantial amount (>96%) of diet by weight comprised of the amphipods *Gammarus* spp. and *Corophium* spp. in fresh water (<0.5 ppt). This is largely in contrast to prior data from 2011 to 2016, where amphipods were not a large biomass component of Delta Smelt diet even during the other wet year of 2011. Fish during September-November in the LSZ consumed more *Acartiella* sp., other cyclopoids (nearly all cyclopoid copepodites), but also mysids as in the lower salinities (Tables 6-7). The few fish in September-November caught in >6 ppt had variable diets with a mix of copepods, mysids and other items shifting among years as to larger percentages of diet by mass (Table 1-8). Adults during December-May in freshwater consumed high percentages by weight of *S. doerrii*, other copepods, cladocerans, amphipods and larval fish (Table 1-6). Like diet by number, *E. affinis*, *A. vernalis*, cladocerans were major food components by weight in the LSZ in December-May, as were larval fish in several years (Table 1-7). Larval fish identified in stomachs were mostly Pacific Herring (49%), Prickly Sculpin (7%), with a few Longfin Smelt (1%) and gobies of the genus *Tridentiger* spp. (1%), along with unidentified larval fish (41%) due to the state of digestion. Diet by weight during December-May in >6 ppt was highly variable with *E. affinis*, other cyclopoids (mostly unidentified cyclopoid copepodites), cladocerans, amphipods, and cumaceans all contributing differently among years.

Use of prey among individual fish within sample periods, reported as percent frequency of occurrence, revealed prey types contributing in large part to percent by number and by weight

were consumed by the majority of individuals (Tables 9-11). This measure of "presence-absence" of the prey types among fish was limited by small sample sizes for some periods. Among periods of large samples of Delta Smelt (n >10), *Pseudodiaptomus* spp. was the most commonly consumed prey among fish in salinities <0.5 and 0.5-6 ppt. There was similarity in prey use among years, but difference among seasons. The December-May period had a greater number of prey used among fish than the other seasons.

Nonmetric multidimensional scaling (NMDS) ordination plots revealed patterns among year, season, salinity, and agency for diet by number (Figure 1-9) and diet by weight (Figure 1-10). One-way ANOSIM statistical global-test showed a significant difference in diet by percent number between groups of months (seasons) (R = 0.357, P = 0.001) and salinity ranges (R = 0.332, P = 0.001). Post-hoc pairwise comparisons for seasons revealed December-May diets were strongly dissimilar from June-August (R = 0.623) and September-November (R = 0.546), whereas diets were similar among June-August and September-November (R = -0.035). Post-hoc pairwise comparisons for salinity ranges results appeared to follow a gradient, with significant differences among all pairs with the greatest difference between <0.5 and >6 (R = 0.6, P = 0.001), with decreasing difference between <0.5 and 0.5-6 (R = 0.281, P = 0.001) and lastly >6 and 0.5-6 (R = 0.19, P = 0.008). There was not a significant difference found in the global test in diet between year groups (R = -0.021, P = 0.292) or agencies (R = -0.081, P = 0.744). The SIMPER results revealed the dissimilarity among salinities due to mostly *P. forbesi* and *Limnoithona* spp., with other prey (*S. doerrii*, other cyclopoids, cladocerans, *E. affinis*) contributing differently among salinities. The SIMPER results for season dissimilarity was similar in many ways, but the importance of *E. affinis* increased for dissimilarity between December-May to the other seasons.

Diet by percent weight ANOSIM results were similar to that of diet by percent number with significant differences between seasons (R = 0.293, P = 0.001) and salinity ranges (R = 0.332, P = 0.001). Post-hoc pairwise comparisons of diet by weight for months revealed December-May diets were strongly dissimilar from June-August (R = 0.586) and September-November (R = 0.395), whereas diets were similar among June-August and September-November (R = -0.015). There was a significant difference between salinity ranges in diet by weight for all groups (<0.5 and 0.5-6 R = 0.248, 0.5-6 and > 6 R = 0.271, and <0.5 and >6 R = 0.546). No significant difference was found in the global test in diet between year groups (R = 0.042, P = 0.189).

Discussion

This study provides a comprehensive summary of Delta Smelt prey consumption among seasons that are informative of the life stages of Delta Smelt, and how diets vary with salinity across recent years of varying freshwater outflow conditions. We found Delta Smelt to have somewhat consistent and broad diets within seasons and salinities across years, but diets did vary significantly among salinities and seasons within years. This is attributed to the seasonal and regional abundance of zooplankton, most notable with high densities of *P. forbesi* in freshwater during summer and *E. affinis* high densities in LSZ during winter (Hennessy 2017). The most extreme seasonal pattern was consumption of larval fish by adult Delta Smelt in spring, a function attributed to Delta Smelt being large enough to capture and consume fish larvae. Larval fish, such as Pacific Herring and Prickly Sculpin in spring, would convey nutritional benefit as

large prey during the energetically demanding spawning period of Delta Smelt (Damon et al. 2016). The duration of spawning periods by native fishes and thus abundance of larvae over a period of time could bestow foraging benefit to adult Delta Smelt. Conditions that allow production of small larvae, thus prey, over longer periods would be advantageous to Delta Smelt. The comparison among years was influenced by variable inter-annual conditions in the Estuary and thus the resulting prey field available to Delta Smelt. For this study, our evaluation was one of several years including comparison of the wet water year of 2017 relative to the other water years that ranged from wet to critically dry.

Based on prior research it was not surprising that this study found copepods dominated the diet of Delta Smelt across years and seasons. Based on stomach contents from the 1970s and 1980s, Delta Smelt were found to rely heavily on copepods with mysids, cladocerans, and amphipods, with the copepods shifting from *E. affinis* in the 1970s to *P. forbesi* in the late 1980s (Moyle et al. 1992), a function of *P. forbesi* becoming dominant after introduction. Findings in the early- and mid-1990s were similar to ours, with seasonal and annual trends of copepods important to diet composition, mostly *Pseudodiaptomus* spp. (Lott 1998). Another similarity to previous findings was the presence of amphipods and larval fish (Lott 1998), but at higher levels for this study than previously found. Herbivorous calanoid copepods (*P. forbesi*, *S. doerrii*, and *E. affinis*) were important components to diet seasonally, consistently among years in freshwater and low salinity zone in the recent period (2011-2017). Smaller *Limnoithona* spp. also made up large portions of diet numerically in recent years, but was not a large contribution to stomach mass in most periods and areas. Seasonal shifts in prey consumed could also be a function of the increasing size of Delta Smelt, which may increase foraging capacity and success. Young Delta Smelt have shown selection against *S. doerrii* (Slater and Baxter 2014), but here we found *S. doerrii* to be a large component of Delta Smelt diet in winter (December-May) in freshwater, possibly a function of improved foraging ability by adults. Along with seasonal production, high mortality of young life stages could limit the numbers of adult *P. forbesi* available as prey. Kimmerer et al. (2018) showed *P. forbesi* nauplii and juveniles experience high mortality in Suisun Bay probably due to clam grazing and predatory copepods which was offset by subsidies from freshwater into Suisun Bay during summer and fall.

While copepods are an undoubtedly important staple of the Delta Smelt diet, prey items that are found to be numerically dominant may be smaller and not reflect the true relative importance of prey biomass to nutritional needs of the fish. The relative benefit of prey types to an organism should include biomass estimates of diet items versus only numerical-related estimates, and indeed larger prey types with more caloric potential are likely to influence the habitat use of an organism within its ecosystem. Conversely, larger items high in caloric value and seemingly of high importance may be uncommon in the environment, inconsistently represented in the diet, or inherently less numerous in the diet due to their size. This concept of size applied to Delta Smelt prey types would place high value on mysids and larval fish, as energetically or nutritionally superior. Smaller crustaceans (i.e. amphipods) have a lower volume of mass per individual with a greater ratio of external chitin relative to mass; chitin is not assimilated by predators (Vijverberg and Frank 1976). The frequency of stomachs with many small prey could be a signal of poor feeding conditions, with greater effort and possibly increased predation risk needed to acquire prey versus collection of a few large prey. The range of prey consumed and percent frequency of occurrence was high with most fish consuming the same types of prey.

Comparison across the seasons revealed that while copepods were still of importance to Delta Smelt diet with respect to biomass, it is clear other food items shared or dominated importance with respect to biomass during certain years and seasons, especially for adult Delta Smelt. Larval fishes were a dominant prey by weight in Delta Smelt diets when data is summed from 2011-2016 and *Gammarus* spp. by weight for 2017. However, a closer look reveals larval fishes were not present in diets of Delta Smelt across many sampling dates and was influenced by seasonal production of larval fish and size of adult Delta Smelt able to capture larger prey (larval fish in stomachs were up to 13.9 mm). That said, the data are suggestive that native fishes that spawn in winter (i.e. Pacific Herring and Prickly Sculpin) produce larvae of importance to the diet of adult Delta Smelt, when they are large enough to consume larval fish. There was evidence of the introduced gobies, *Tridentiger* spp., possibly Shokihaze Goby (*T. barbatus*) and Shimofuri Goby (*T. bifasciatus*) larvae, in stomachs of juvenile Delta Smelt. *Tridentiger* spp. goby larvae in summer have a pelagic period following hatch (~2-3 mm FL) before settling out to a demersal life history around 13-18 mm FL, based on CDFW 20-mm and STN catch patterns. The goby spawning period in summer occurs when the majority of Delta Smelt are juveniles and thus Delta Smelt might not be of size to take advantage of this and other larval fish as food in summer.

Delta Smelt diets did include what is traditionally considered "demersal" prey, such as amphipods and cumaceans. The dominance of *Gammarus* spp. by weight in diet was driven by their relatively high mass per length and numbers consumed in 2017 that was largely not seen in other years. Among amphipods consumed by Delta Smelt, by far the dominant prey was native *Americorophium* spp. of a narrow size range (i.e. copepod sized ~ 1-1.5 mm). *Americorophium* spp. are a tube building amphipod, but we did not observe evidence of tubes debris in stomachs. An interesting observation of gut contents was that there was little to no debris (e.g. sand, silt, and detritus) in Delta Smelt stomachs, as seen in other fishes that forage for benthos along the substrate such as Threadfin Shad *Dorosoma petenense* (Ingram and Ziebell 1983) or as tube or clam siphon nippers such as *Tridentiger* spp. goby (Slater 2005). The absence of debris in stomachs along with the types and size of amphipods found in stomachs are likely evidence of Delta Smelt taking advantage of epi-benthic prey or individuals available in the water column. Cumaceans are also regularly detected by CDFW meso-zooplankton (Clark-Bumpus; CB) nets towed obliquely through the water (CPUE data available at http://www.dfg.ca.gov/delta/data/Zooplankton/CPUE_ZooMap.asp) and provides evidence they would be available to Delta Smelt in the water column.

A surprise finding during the study was of terrestrial insects (e.g. chironomids, flies, aphids, ants, and spiders) in stomachs of Delta Smelt. They occurred in stomachs at a very low frequency and so were reported in the "Other" zooplankton category. Nearly all occurrences were from fish >54 mm FL collected by Kodiak Trawl which sampled adult fish oriented to the surface of the water.

The types of prey found in stomachs was found to be not significantly different among years, but the prey available as herbivorous calanoid copepods was higher in freshwater and the low salinity zone during the wet year of 2017. There was evidence that gut fullness of Delta smelt was higher in 2017 than some other years. It is unclear if this was due to increased availability of prey in the wet year of 2017, or a function of smaller sample sizes available in 2017 across salinities and seasons limiting comparisons. Gut fullness was actually higher in the low salinity

zone than other regions. This is similar to the fullness pattern observed in previous related work (Hammock et al. 2017). Our data showed no relationship between gut fullness and fish condition factor. While instantaneous gut fullness may be an indicator of short-term food availability or feeding success, it may not have direct relation to certain health and condition metrics of individuals as these measures are impacted more by a suite of prior conditions experienced by each fish. Fullness as a function of time was similar for two different measures, with higher frequency of empty stomachs and a lower fullness (%) in the early hours of the day. Juvenile and adult Delta Smelt are believed to be a visual predator (Sullivan et al. 2016). Our findings of low stomach fullness in the early hours and then reaching mostly full by late morning could be partly explained by foraging during daylight. Fullness as a measure is dynamic, as fewer items would be needed to reach fullness when eating larger prey or if smaller in size, thus having a smaller stomach to fill.

This study revealed patterns in Delta Smelt diet that were informed by zooplankton data. Zooplankton data can provide trends in prey type and densities relative to the habitat of Delta Smelt. The concurrent fish and zooplankton samples can also provide opportunities for selectivity analysis as to the densities biologically relevant to foraging by Delta Smelt. The importance of copepods was evident from stomach contents and there was associated zooplankton data to look at summer and fall trends for this study, that *Pseudodiaptomus* spp. abundant in summer and fall was a major food item of Delta Smelt. The lack of concurrent zooplankton data for adults during January-May does not allow for close comparison or analysis of selectivity. Added complication to understanding the prey field for adults is the lack of sampling of amphipods and mixed types of larval fish during spring (CDFW Smelt Larval Survey samples January-March). The meso-zooplankton data used is informative of adult copepod sized prey, but might be limited in effective collection of smaller prey (<0.5 mm), such as all life stages of *Limnoithona spp*. The CB net also does not appear efficient in collection of less numerous larger prey such as larval fish and macro-invertebrates. Additional examination of the mysid net for understanding larval fish and macro-zooplankton is warranted to help improve the information regarding the available prey field. Future efforts will look more closely at available prey data and how we might examine selectivity or preference measures by the various life stages of Delta Smelt.

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Tables

Table 1-1. Summary of Delta Smelt Collected by CDFW and USFWS Surveys Among Months and Salinity Ranges (<0.5, 0.5-6, and > 6 ppt) During the Period 2011-2017 that were Examined for Stomach Contents During this Study

Month	Salinity (ppt)	2011	2012	2013	2014	2015	2016	2017	Total
Jan	<0.5		45	40	10	9	4	14	122
	0.5-6		50	22	54	8	3	1	138
	>6		53		6				59
Feb	<0.5		30	11	11	18	3	7	80
	0.5-6		50	18	21	21	2		112
	>6		4						4
Mar	<0.5		65	26	34	4	6	8	143
	0.5-6		10	19	2	1	1		33
	>6								
Apr	<0.5		64	13	16	1	13	4	111
	0.5-6		28	2	2				32
	>6				1				1
May	<0.5		30	4	11	4			49
	0.5-6		3	5	1		1		10
	>6								
Jun	<0.5		49	28	19	4		1	101
	0.5-6		19	32	24			5	80
	>6			7					7
Jul	<0.5			8	2	10		1	21
	0.5-6				9			5	14
	>6			30	8			2	40
Aug	<0.5	42	18	6	30	1		(4)	101
	0.5-6	24	6	4	67			(17)	118
	>6	4		2	1			14	21
Sep	<0.5	8			3			(18)	29
	0.5-6	33		2	67	4		(9)	115
	>6		1	2	1			(2)	6
Oct	<0.5	34	14			1		(36)	85
	0.5-6	12	8	3	9			2 (4)	38
	>6								0
Nov	<0.5	17	2	2					21
	0.5-6	17	9	2			7	(6)	41
	>6	6							6
Dec	<0.5	57	6		38		21		122
	0.5-6	41	5	3	22	1	2		74
	>6	17		5		3	3		28
Total		**312**	**569**	**296**	**469**	**90**	**66**	**160**	**1962**

Notes: USFWS Samples in Parentheses

Table 1-2. Summary of Mean Fork Lengths (mm) of Delta Smelt Collected by CDFW and USFWS Per Month that Were Examined for Stomach Contents During the Period 2011-2017

Month	Salinity (ppt)	2011	2012	2013	2014	2015	2016	2017	Total
Jan	<0.5		63.4	67.3	65.9	61.4	67.0	65.7	65.1
	0.5-6		62.2	70.1	65.8	65.3	70.7	68.0	65.3
	>6		61.6		67.8				62.3
Feb	<0.5		63.3	65.2	66.7	64.7	74.3	68.6	65.2
	0.5-6		63.1	71.1	68.9	67.4	67.5		66.3
	>6		68.0						68.0
Mar	<0.5		65.9	71.2	67.3	69.8	66.3	68.5	67.5
	0.5-6		63.4	75.3	75.0	66.0	65.0		71.1
	>6								
Apr	<0.5		67.9	74.5	68.4	65.0	72.0	77.5	69.5
	0.5-6		68.1	76.0	74.5				69.0
	>6				67.0				67.0
May	<0.5		71.0	78.5	73.5	74.0			72.4
	0.5-6		67.7	77.2	69.0		32.0		71.3
	>6								
Jun	<0.5		35.5	36.0	35.3	31.0		36.0	35.4
	0.5-6		33.8	38.1	31.5			48.4	35.8
	>6			44.3					44.3
Jul	<0.5			46.5	35.5	47.7		46.0	46.0
	0.5-6				45.6			40.4	43.7
	>6			47.6	47.6			45.5	47.5
Aug	<0.5	44.1	51.9	42.0	48.0	47.0		51.5	46.9
	0.5-6	43.6	41.3	48.0	47.7			50.9	47.0
	>6	49.5		46.5	52.0			48.9	48.9
Sep	<0.5	59.4			62.3			50.2	53.8
	0.5-6	49.8		59.5	52.1	58.8		53.0	51.9
	>6		46.0	51.0	63.0			48.0	51.8
Oct	<0.5	54.8	61.4			52.0		57.0	56.8
	0.5-6	57.8	54.3	67.3	54.7			52.9	56.2
	>6								
Nov	<0.5	57.5	67.5	55.0					58.2
	0.5-6	56.9	64.3	64.0			61.9	55.2	59.5
	>6	54.3							54.3
Dec	<0.5	62.3	66.2		60.2		62.2		61.8
	0.5-6	59.4	63.4	62.0	57.5	69.0	65.0		59.5
	>6	57.6		64.2		70.0	68.3		61.3
Total		**54.5**	**60.4**	**58.7**	**55.8**	**61.9**	**65.8**	**55.4**	**58.0**

Note: A single 32 mm FL juvenile Delta Smelt was caught by the SKT in May 2016 and not included in calculation of the total May mean length.

Table 1-3. Diet by Percent Number of Major Prey Categories in Stomachs of Delta Smelt Collected in <0.5 ppt for Months June-August (J-A), September-November (S-N), and December-May (D-M) Among Years 2011-2017

	Diet by percent number (%N)																					
	J-A	J-A	J-A	J-A	J-A	J-A	J-A	J-A	S-N	S-N	S-N	S-N	S-N	S-N	S-N	S-N	D-M	D-M	D-M	D-M	D-M	D-M
	2011	2012	2013	2014	2015	2016	2017	2017*	2011	2012	2013	2014	2015	2016	2017	2017*	2012	2013	2014	2015	2016	2017
Prey Category	(42)	(66)	(38)	(47)	(15)	(0)	(2)	(4)	(59)	(16)	(2)	(3)	(1)	(0)	(0)	(53)	(286)	(99)	(81)	(73)	(26)	(51)
Calanoid copepods																						
Eurytemora spp.	0.0	0.0	0.0	0.0	0.1		0.7	0.0	0.0	0.0	0.0	0.0	0.0			0.0	4.1	3.6	3.7	1.0	14.2	2.3
Pseudodiaptomus spp.	71.3	63.0	52.7	59.0	61.5		92.8	22.0	63.5	52.4	18.6	70.2	65.3			63.4	7.4	8.0	6.7	8.5	5.6	0.9
Sinocalanus doerrii	1.7	10.6	5.4	5.9	5.8		0.0	4.1	5.0	8.1	3.5	3.6	2.0			0.0	43.4	26.7	36.2	1.3	54.5	10.8
Acartiella sinensis	0.2	0.0	0.0	0.2	0.0		0.0	4.6	15.4	1.4	0.0	0.0	0.0			6.6	0.3	0.0	0.0	0.0	0.0	0.0
Tortanus spp.	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0			0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other calanoids	17.4	7.3	8.9	8.0	6.5		0.0	2.3	3.2	0.5	0.0	3.6	26.7			8.3	6.1	11.7	4.9	5.0	10.2	5.4
Cyclopoid copepods																						
Limnoithona spp.	0.6	11.4	4.7	13.7	9.2		0.0	42.7	2.4	4.2	20.9	16.7	5.0			0.2	0.0	0.0	0.0	0.4	0.2	0.2
Acanthocyclops spp.	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0			0.0	2.5	5.8	5.0	3.3	2.0	1.2
Other cyclopoids	1.0	1.2	0.1	1.4	4.6		0.7	6.9	1.2	3.2	17.4	2.4	0.0			1.1	10.5	11.9	24.7	35.1	5.5	6.3
Other Copepods																						
Harpacticoids	1.1	0.1	7.1	0.4	0.1		0.0	10.1	1.2	4.6	29.1	0.0	0.0			1.4	1.8	0.2	0.2	0.9	0.6	0.0
Copepod nauplii	2.9	0.3	15.9	2.6	0.3		0.0	2.8	1.5	1.6	8.1	0.0	0.0			0.2	0.0	0.0	0.0	0.2	0.1	0.0
Cladocerans	0.8	2.5	0.9	6.0	6.4		4.6	2.8	1.0	0.0	0.0	2.4	0.0			0.9	13.2	28.9	14.6	41.9	6.1	68.3
Mysids	0.0	0.2	0.1	0.1	0.0		1.3	0.9	1.9	20.6	0.0	0.0	0.0			0.1	0.1	0.1	0.0	0.0	0.0	0.0
Amphipods																						
Gammarus spp.	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.5	0.0	0.0	0.0			13.4	0.3	0.2	0.7	0.2	0.4	0.6
Corophium spp.	0.0	0.1	0.0	0.0	2.2		0.0	0.5	1.7	1.8	1.2	0.0	1.0			2.6	9.1	0.4	0.6	0.3	0.1	0.7
Unidentified amphipods	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0			0.4	0.1	0.0	0.1	0.0	0.0	0.1
Cumaceans	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.2	0.0	0.0	1.2	0.0			0.0	0.2	0.0	0.4	0.1	0.0	0.4
Fish	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0			0.0	0.2	0.0	0.1	0.0	0.0	0.5
Other	3.0	3.2	4.1	2.7	3.4		0.0	0.5	1.9	1.2	1.2	0.0	0.0			1.3	0.7	2.6	2.2	1.8	0.5	2.0
Total	100	100	100	100	100		100	100	100	100	100	100	100			100	100	100	100	100	100	100

Note: Each year includes December from the preceding year (e.g. 2012 includes December 2011-May 2012). Number of stomachs with food present in parentheses. No samples (NS) occurred in some years and months reported as blank fields. Fields are shaded darker green with higher percentage values.
* Identifies samples collected by USFWS in 2017

Table 1-4. Diet by Percent Number of Major Prey Categories in Stomachs of Delta Smelt Collected in 0.5-6 ppt for Months June-August (J-A), September-November (S-N), and December-May (D-M) Among Years 2011-2017

	Diet by percent number (%N)																					
	J-A	J-A	J-A	J-A	J-A	J-A	J-A	J-A	S-N	S-N	S-N	S-N	S-N	S-N	S-N	S-N	D-M	D-M	D-M	D-M	D-M	D-M
	2011	2012	2013	2014	2015	2016	2017	2017*	2011	2012	2013	2014	2015	2016	2017	2017*	2012	2013	2014	2015	2016	2017
Prey Category	(24)	(21)	(32)	(88)	(0)	(0)	(10)	(17)	(61)	(17)	(6)	(75)	(4)	(7)	(1)	(19)	(177)	(71)	(83)	(52)	(8)	(3)
Calanoid copepods																						
Eurytemora spp.	0.0	0.0	6.2	0.0			0.0	0.0	0.3	0.1	0.0	0.0	0.0	0.0	25.0	5.5	3.8	9.9	61.8	14.1	47.7	47.6
Pseudodiaptomus spp.	4.3	20.7	31.2	9.7			90.0	1.1	11.2	67.2	3.6	42.9	78.7	19.9	75.0	3.7	1.1	0.4	1.5	2.2	1.6	0.2
Sinocalanus doerrii	0.0	1.2	0.0	0.0			0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0
Acartiella sinensis	12.9	1.1	2.3	8.7			0.2	1.9	8.5	10.1	15.9	6.2	1.3	8.0	0.0	1.0	3.3	0.3	1.9	0.4	0.3	0.2
Tortanus spp.	1.2	0.2	0.2	0.0			0.3	0.0	0.2	0.3	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Other calanoids	0.0	1.0	1.0	1.3			2.3	0.1	0.3	0.9	0.6	4.2	14.1	0.3	0.0	1.4	0.4	0.7	9.2	3.5	4.6	4.1
Cyclopoid copepods																						
Limnoithona spp.	76.8	73.7	52.0	65.8			5.6	90.2	4.5	8.1	69.9	38.9	4.0	64.4	0.0	51.6	1.4	0.2	0.6	3.7	12.0	0.2
Acanthocyclops spp.	0.0	0.0	1.0	0.0			0.0	0.0	3.8	0.0	0.0	0.0	0.0	0.0	0.0	4.5	19.7	19.4	5.2	16.7	7.4	10.9
Other cyclopoids	0.0	0.0	1.1	14.0			0.6	5.1	66.7	0.9	8.0	5.9	1.2	4.6	0.0	28.4	36.1	26.1	13.0	48.3	9.5	18.8
Other Copepods																						
Harpacticoids	3.2	0.1	0.2	0.1			0.1	0.7	0.3	0.4	0.4	0.2	0.0	1.1	0.0	0.7	1.1	0.9	0.2	0.2	0.5	0.0
Copepod nauplii	0.3	0.4	0.2	0.1			0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.2	0.3	0.1	0.6	0.0
Cladocerans	0.1	0.0	0.3	0.1			0.1	0.0	2.5	0.0	0.0	0.0	0.3	0.3	0.0	1.4	27.0	38.2	1.2	8.6	9.6	14.2
Mysids	0.4	0.8	1.6	0.1			0.1	0.0	0.5	3.4	1.4	0.2	0.2	0.2	0.0	0.1	0.3	0.3	0.0	0.0	0.1	0.0
Amphipods																						
Gammarus spp.	0.0	0.1	0.0	0.0			0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.3	0.0	0.0	0.1	0.3	0.1	0.0	0.3	0.0
Corophium spp.	0.3	0.1	0.2	0.0			0.4	0.2	0.8	7.4	0.0	0.1	0.2	0.5	0.0	0.1	3.5	0.4	0.2	0.1	2.6	0.2
Unidentified amphipods	0.1	0.0	0.0	0.0			0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0
Cumaceans	0.3	0.0	0.2	0.0			0.0	0.1	0.0	0.1	0.0	0.7	0.0	0.2	0.0	0.2	1.4	1.6	1.9	0.5	3.0	1.7
Fish	0.0	0.1	0.1	0.0			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.4	0.9	0.0	0.0	0.0
Other	0.3	0.5	1.7	0.1			0.2	0.4	0.3	0.5	0.1	0.7	0.0	0.0	0.0	0.9	0.2	0.5	1.7	1.5	0.1	2.1
Total	100	100	100	100			100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Note: Each year includes December from the preceding year (e.g. 2012 includes December 2011-May 2012). Number of stomachs with food present in parentheses. No samples (NS) occurred in some years and months reported as blank fields. Fields are shaded darker green with higher percentage values.
* Identifies samples collected by USFWS in 2017

Table 1-5. Diet by Percent Number of Major Prey Categories in Stomachs of Delta Smelt Collected in >6 ppt for Months June-August (J-A), September-November (S-N), and December-May (D-M) Among Years 2011-2017

	Diet by percent number (%N)																					
	J-A 2011	J-A 2012	J-A 2013	J-A 2014	J-A 2015	J-A 2016	J-A 2017	J-A 2017*	S-N 2011	S-N 2012	S-N 2013	S-N 2014	S-N 2015	S-N 2016	S-N 2017	S-N 2017*	D-M 2012	D-M 2013	D-M 2014	D-M 2015	D-M 2016	D-M 2017
Prey Category	(3)	(0)	(30)	(5)	(0)	(0)	(15)	(0)	(5)	(1)	(2)	(1)	(0)	(0)	(0)	(2)	(74)	(0)	(12)	(0)	(3)	(3)
Calanoid copepods																						
Eurytemora spp.	0.0		0.0	0.0			0.0		0.0	0.0	0.0	0.0				0.0	2.0		42.6		85.5	78.2
Pseudodiaptomus spp.	3.7		0.8	0.0			0.1		6.3	0.0	4.3	0.0				0.0	0.3		0.1		0.0	0.0
Sinocalanus doerrii	0.0		0.0	0.0			0.0		0.1	0.0	0.0	0.0				0.0	0.1		0.0		0.0	0.0
Acartiella sinensis	18.5		0.4	0.0			0.1		21.6	0.0	6.4	0.0				0.1	2.4		2.2		0.0	0.0
Tortanus spp.	3.7		3.3	11.1			0.2		1.0	0.0	14.9	69.2				0.0	0.6		2.1		0.0	0.0
Other calanoids	0.0		0.1	0.0			0.0		0.0	0.0	0.0	11.5				0.0	0.9		2.3		2.7	8.2
Cyclopoid copepods																						
Limnoithona spp.	3.7		89.4	0.0			86.5		3.1	100.0	63.8	3.8				91.1	2.5		12.1		0.9	0.4
Acanthocyclops spp.	0.0		0.0	0.0			0.0		1.2	0.0	0.0	0.0				0.0	12.6		6.0		4.2	2.7
Other cyclopoids	0.0		4.4	5.6			12.2		26.3	0.0	0.0	0.0				8.6	55.2		21.1		1.8	8.0
Other Copepods																						
Harpacticoids	3.7		0.2	0.0			0.2		0.2	0.0	8.5	0.0				0.0	2.0		0.5		0.0	0.0
Copepod nauplii	0.0		0.2	0.0			0.3		0.0	0.0	0.0	0.0				0.1	0.1		0.5		0.3	0.1
Cladocerans	0.0		0.0	0.0			0.0		35.0	0.0	0.0	0.0				0.0	19.1		1.2		0.0	0.0
Mysids	7.4		0.1	0.0			0.0		0.0	0.0	2.1	3.8				0.0	0.2		0.0		0.0	0.0
Amphipods																						
Gammarus spp.	0.0		0.0	0.0			0.0		0.0	0.0	0.0	0.0				0.0	0.0		0.1		0.0	0.0
Corophium spp.	25.9		0.1	16.7			0.1		4.4	0.0	0.0	0.0				0.0	0.3		0.6		2.7	0.1
Unidentified amphipods	0.0		0.0	16.7			0.0		0.1	0.0	0.0	0.0				0.0	0.0		0.0		0.0	0.0
Cumaceans	33.3		0.2	5.6			0.0		0.7	0.0	0.0	3.8				0.0	1.3		2.5		1.8	0.1
Fish	0.0		0.1	0.0			0.0		0.0	0.0	0.0	0.0				0.0	0.2		0.0		0.0	0.0
Other	0.0		0.6	44.4			0.3		0.1	0.0	0.0	7.7				0.0	0.2		6.2		0.0	2.0
Total	100		100	100			100		100	100	100	100				100	100		100		100	100

Note: Each year includes December from the preceding year (e.g. 2012 includes December 2011-May 2012). Number of stomachs with food present in parentheses. No samples (NS) occurred in some years and months reported as blank fields. Fields are shaded darker green with higher percentage values.
* Identifies samples collected by USFWS in 2017

Table 1-6. Diet by Percent Weight of Major Prey Categories in Stomachs of Delta Smelt Collected in <0.5 ppt for Months June-August (J-A), September-November (S-N), and December-May (D-M) Among Years 2011-2017

	Diet by percent weight (%W)																					
	J-A 2011	J-A 2012	J-A 2013	J-A 2014	J-A 2015	J-A 2016	J-A 2017	J-A 2017*	S-N 2011	S-N 2012	S-N 2013	S-N 2014	S-N 2015	S-N 2016	S-N 2017	S-N 2017*	D-M 2012	D-M 2013	D-M 2014	D-M 2015	D-M 2016	D-M 2017
Prey Category	(42)	(66)	(38)	(47)	(15)	(0)	(2)	(4)	(59)	(16)	(2)	(3)	(1)	(0)	(0)	(53)	(286)	(99)	(81)	(73)	(26)	(51)
Calanoid copepods																						
Eurytemora spp.	0.0	0.0	0.0	0.0	0.1		0.1	0.0	0.0	0.0	0.0	0.0	0.0			0.0	2.4	2.4	2.1	0.6	6.8	1.1
Pseudodiaptomus spp.	69.2	71.5	65.0	56.1	64.0		65.6	32.9	43.7	2.8	8.7	43.0	81.6			1.3	6.1	7.8	6.2	10.0	4.0	0.6
Sinocalanus doerrii	4.7	17.7	11.4	15.3	9.2		0.0	9.5	4.7	0.7	15.6	14.3	4.0			0.0	44.9	34.1	43.0	2.1	64.4	9.8
Acartiella sinensis	0.8	0.0	0.0	0.6	0.0		0.0	12.1	15.9	0.1	0.0	0.0	0.0			0.3	0.3	0.0	0.0	0.0	0.0	0.0
Tortanus spp.	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0			0.0	0.0	0.1	0.0	0.0	0.0	0.0
Other calanoids	17.2	3.8	7.0	6.9	4.6		0.0	2.0	1.1	0.0	0.0	6.8	13.2			0.1	4.2	9.3	3.6	5.9	8.5	3.7
Cyclopoid copepods																						
Limnoithona spp.	0.1	1.6	0.6	2.8	1.3		0.0	8.2	0.2	0.0	7.1	5.3	0.8			0.0	0.0	0.0	0.0	0.0	0.0	0.0
Acanthocyclops spp.	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0			0.0	1.5	4.1	3.3	3.0	1.3	0.6
Other cyclopoids	1.4	0.7	0.1	1.2	4.7		0.4	7.6	0.7	0.2	22.8	4.5	0.0			0.0	4.5	6.4	6.9	27.0	2.6	2.4
Other Copepods																						
Harpacticoids	1.1	0.1	5.9	0.4	0.1		0.0	8.0	0.4	0.1	41.8	0.0	0.0			0.0	0.7	0.1	0.1	0.5	0.2	0.0
Copepod nauplii	0.3	0.0	1.4	0.2	0.0		0.0	0.2	0.0	0.0	1.2	0.0	0.0			0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cladocerans	0.9	1.8	0.9	6.6	4.6		2.8	2.6	0.2	0.0	0.0	3.8	0.0			0.0	8.3	20.2	10.1	34.3	3.8	35.8
Mysids	0.0	0.6	0.6	2.0	0.0		31.2	9.7	30.6	93.8	0.0	0.0	0.0			0.1	1.9	4.4	0.6	0.1	0.0	1.6
Amphipods																						
Gammarus spp.	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	1.1	0.0	0.0	0.0			91.4	4.9	4.2	8.4	4.9	6.2	8.9
Corophium spp.	0.0	0.1	0.0	0.0	1.6		0.0	6.8	0.8	0.8	0.9	0.0	0.5			5.5	9.0	3.9	3.0	1.9	1.1	7.5
Unidentified amphipods	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0			1.2	1.4	0.1	1.2	0.2	0.0	0.2
Cumaceans	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.8	0.0	0.0	22.3	0.0			0.0	0.9	0.1	2.3	0.9	0.2	1.7
Fish	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0			0.0	7.8	1.2	3.8	0.0	0.0	20.3
Other	4.2	2.0	7.0	7.9	9.9		0.0	0.4	0.9	0.2	1.8	0.0	0.0			0.1	1.3	1.7	5.5	8.3	0.8	5.7
Total	100	100	100	100	100		100	100	100	100	100	100	100			100	100	100	100	100	100	100

Note: Each year includes December from the preceding year (e.g. 2012 includes December 2011-May 2012). Number of stomachs with food present in parentheses. No samples (NS) occurred in some years and months reported as blank fields. Fields are shaded darker blue with higher percentage values.
* Identifies samples collected by USFWS in 2017.

Table 1-7. Diet by Percent Weight of Major Prey Categories in Stomachs of Delta Smelt Collected in 0.5-6 ppt for Months June-August (J-A), September-November (S-N), and December-May (D-M) Among Years 2011-2017

	Diet by percent weight (%W)																					
	J-A 2011	J-A 2012	J-A 2013	J-A 2014	J-A 2015	J-A 2016	J-A 2017	J-A 2017*	S-N 2011	S-N 2012	S-N 2013	S-N 2014	S-N 2015	S-N 2016	S-N 2017	S-N 2017*	D-M 2012	D-M 2013	D-M 2014	D-M 2015	D-M 2016	D-M 2017
Prey Category	(24)	(21)	(32)	(88)	(0)	(0)	(10)	(17)	(61)	(17)	(6)	(75)	(4)	(7)	(1)	(19)	(177)	(71)	(83)	(52)	(8)	(3)
Calanoid copepods																						
Eurytemora spp.	0.0	0.0	6.8	0.0			0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	19.7	8.2	2.5	4.0	19.6	13.7	26.2	35.5
Pseudodiaptomus spp.	11.0	50.9	54.9	25.3			94.4	5.8	20.3	24.0	7.6	48.4	73.6	39.4	80.3	9.5	1.2	0.2	0.6	3.9	1.3	0.3
Sinocalanus doerrii	0.0	3.4	0.1	0.0			0.2	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.0	0.0	0.0
Acartiella sinensis	44.1	3.7	5.5	34.4			0.4	12.6	21.3	4.9	50.4	16.2	2.7	23.0	0.0	3.6	5.2	0.2	1.7	0.9	0.4	0.4
Tortanus spp.	11.2	2.4	1.8	0.0			1.5	0.0	1.3	0.4	0.0	0.0	0.0	1.4	0.0	0.3	0.1	0.2	0.1	0.0	0.0	0.0
Other calanoids	0.0	1.4	0.8	1.5			1.2	0.2	0.2	0.2	0.7	3.6	8.6	0.3	0.0	1.2	0.4	0.4	2.0	4.3	2.2	1.8
Cyclopoid copepods																						
Limnoithona spp.	19.4	16.8	9.4	19.3			0.7	48.0	0.8	0.3	14.9	7.6	0.6	13.6	0.0	16.3	0.2	0.0	0.0	0.7	1.1	0.0
Acanthocyclops spp.	0.0	0.0	1.3	0.0			0.0	0.0	4.9	0.0	0.0	0.0	0.0	0.0	0.0	9.8	16.0	8.1	2.5	22.1	4.6	12.9
Other cyclopoids	0.0	0.0	0.7	15.9			0.5	10.7	37.6	0.2	8.2	4.4	0.8	3.8	0.0	25.3	11.0	4.2	2.4	26.0	2.6	8.0
Other Copepods																						
Harpacticoids	3.4	0.1	0.2	0.1			0.0	1.5	0.2	0.1	0.4	0.2	0.0	1.0	0.0	0.9	0.5	0.2	0.1	0.1	0.2	0.0
Copepod nauplii	0.0	0.0	0.0	0.0			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Cladocerans	0.1	0.0	0.2	0.1			0.0	0.0	4.2	0.0	0.0	0.0	0.3	0.4	0.0	3.8	25.9	18.9	0.7	10.4	7.0	21.6
Mysids	5.0	10.1	8.2	2.1			0.8	0.4	6.2	67.2	14.7	9.7	13.4	0.9	0.0	6.2	1.8	7.0	0.8	0.5	7.0	0.0
Amphipods																						
Gammarus spp.	0.0	0.3	0.0	0.1			0.0	0.0	1.3	0.4	1.0	0.9	0.0	6.6	0.0	0.8	2.2	4.3	0.6	0.9	5.6	0.0
Corophium spp.	0.6	0.1	1.9	0.1			0.2	16.1	0.4	1.8	1.3	0.3	0.0	7.6	0.0	9.2	5.0	1.9	1.0	1.6	24.9	0.1
Unidentified amphipods	0.0	0.0	0.0	0.0			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.2	0.0	1.3	0.0
Cumaceans	4.0	0.0	2.7	0.5			0.0	2.9	0.3	0.3	0.0	7.8	0.0	2.1	0.0	3.7	9.8	5.6	7.7	5.9	15.8	17.8
Fish	0.0	5.8	0.3	0.0			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	17.4	44.3	56.3	0.0	0.0	0.0
Other	1.1	5.1	5.2	0.5			0.2	1.6	0.5	0.3	0.8	0.9	0.0	0.0	0.0	1.2	0.5	0.3	3.5	8.8	0.0	1.6
Total	100	100	100	100			100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Note: Each year includes December from the preceding year (e.g. 2012 includes December 2011-May 2012). Number of stomachs with food present in parentheses. No samples (NS) occurred in some years and months reported as blank fields. Fields are shaded darker blue with higher percentage values.
* Identifies samples collected by USFWS in 2017

Table 1-8. Diet by Percent Weight of Major Prey Categories in Stomachs of Delta Smelt Collected in >6 ppt for Months June-August (J-A), September-November (S-N), and December-May (D-M) Among Years 2011-2017

	Diet by percent weight (%W)																					
	J-A 2011	J-A 2012	J-A 2013	J-A 2014	J-A 2015	J-A 2016	J-A 2017	J-A 2017*	S-N 2011	S-N 2012	S-N 2013	S-N 2014	S-N 2015	S-N 2016	S-N 2017	S-N 2017*	D-M 2012	D-M 2013	D-M 2014	D-M 2015	D-M 2016	D-M 2017
Prey Category	(3)	(0)	(30)	(5)	(0)	(0)	(15)	(0)	(5)	(1)	(2)	(1)	(0)	(0)	(0)	(2)	(74)	(0)	(12)	(0)	(3)	(3)
Calanoid copepods																						
Eurytemora spp.	0.0		0.1	0.0			0.0		0.0	0.0	0.0	0.0				0.0	1.4		24.4		64.6	80.7
Pseudodiaptomus spp.	1.2		2.1	0.0			0.3		7.1	0.0	3.6	0.0				0.0	0.4		0.1		0.0	0.1
Sinocalanus doerrii	0.0		0.0	0.0			0.1		0.1	0.0	0.0	0.0				0.0	0.1		0.0		0.0	0.0
Acartiella sinensis	8.2		1.5	0.0			0.7		31.7	0.0	6.3	0.0				1.5	4.2		4.3		0.0	0.0
Tortanus spp.	4.8		28.0	3.0			0.7		4.5	0.0	37.8	30.6				0.0	2.6		12.0		0.0	0.0
Other calanoids	0.0		0.1	0.0			0.1		0.0	0.0	0.0	2.0				0.0	0.9		1.4		1.4	5.1
Cyclopoid copepods																						
Limnoithona spp.	0.1		25.6	0.0			59.1		0.4	100.0	4.7	0.0				72.0	0.3		1.7		0.1	0.1
Acanthocyclops spp.	0.0		0.0	0.0			0.0		1.0	0.0	0.0	0.0				0.0	11.5		6.0		3.5	4.5
Other cyclopoids	0.0		5.0	0.3			32.8		13.0	0.0	0.0	0.0				26.4	24.0		7.7		0.8	4.8
Other Copepods																						
Harpacticoids	0.5		0.3	0.0			0.6		0.1	0.0	2.5	0.0				0.0	1.1		0.3		0.0	0.0
Copepod nauplii	0.0		0.0	0.0			0.1		0.0	0.0	0.0	0.0				0.0	0.0		0.0		0.0	0.0
Cladocerans	0.0		0.0	0.0			0.0		33.9	0.0	0.0	0.0				0.0	21.1		1.4		0.0	0.1
Mysids	11.4		5.7	0.0			0.0		0.0	0.0	45.0	13.2				0.0	6.8		0.0		0.0	0.0
Amphipods																						
Gammarus spp.	0.0		0.0	0.0			0.0		0.0	0.0	0.0	0.0				0.0	0.0		0.2		0.0	0.0
Corophium spp.	9.3		0.7	6.6			4.6		2.7	0.0	0.0	0.0				0.0	1.2		11.4		16.4	0.4
Unidentified amphipods	0.0		0.0	17.6			0.0		0.0	0.0	0.0	0.0				0.0	0.2		0.4		0.0	0.0
Cumaceans	64.6		3.6	4.0			0.4		4.9	0.0	0.0	7.8				0.0	10.5		22.3		13.2	2.1
Fish	0.0		11.4	0.0			0.0		0.0	0.0	0.0	0.0				0.0	13.5		0.0		0.0	0.0
Other	0.0		15.9	68.5			0.6		0.6	0.0	0.0	46.5				0.0	0.2		6.4		0.0	2.2
Total	100		100	100			100		100	100	100	100				100	100		100		100	100

Note: Each year includes December from the preceding year (e.g. 2012 includes December 2011-May 2012). Number of stomachs with food present in parentheses. No samples (NS) occurred in some years and months reported as blank fields. Fields are shaded darker blue with higher percentage values.
* Identifies samples collected by USFWS in 2017

Table 1-9. Diet by Percent Frequency of Occurrence of Major Prey Categories in Stomachs of Delta Smelt Collected in <0.5 ppt for Months June-August (J-A), September-November (S-N), and December-May (D-M) Among Years 2011-2017

	Diet by percent frequency of occurrence (%FO)																					
	J-A 2011	J-A 2012	J-A 2013	J-A 2014	J-A 2015	J-A 2016	J-A 2017	J-A 2017*	S-N 2011	S-N 2012	S-N 2013	S-N 2014	S-N 2015	S-N 2016	S-N 2017	S-N 2017*	D-M 2012	D-M 2013	D-M 2014	D-M 2015	D-M 2016	D-M 2017
Prey Category	(42)	(66)	(38)	(47)	(15)	(0)	(2)	(4)	(59)	(16)	(2)	(3)	(1)	(0)	(0)	(53)	(286)	(99)	(81)	(73)	(26)	(51)
Calanoid copepods																						
Eurytemora spp.	0.0	3.0	0.0	2.1	6.7		50.0	0.0	0.0	0.0	0.0	0.0	0.0			0.0	32.2	50.5	49.4	42.5	88.5	29.4
Pseudodiaptomus spp.	97.6	92.4	97.4	100.0	86.7		100.0	100.0	94.9	62.5	100.0	100.0	100.0			100.0	62.9	62.6	59.3	32.9	80.8	31.4
Sinocalanus doerrii	57.1	72.7	68.4	66.0	66.7		0.0	25.0	33.9	12.5	50.0	33.3	100.0			0.0	55.2	64.6	49.4	13.7	76.9	19.6
Acartiella sinensis	4.8	1.5	2.6	2.1	0.0		0.0	50.0	62.7	25.0	0.0	0.0	0.0			58.5	9.4	1.0	0.0	1.4	0.0	2.0
Tortanus spp.	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0			0.0	0.3	1.0	0.0	0.0	0.0	0.0
Other calanoids	69.0	50.0	78.9	53.2	46.7		0.0	50.0	39.0	6.3	0.0	66.7	100.0			49.1	73.1	75.8	65.4	86.3	96.2	60.8
Cyclopoid copepods																						
Limnoithona spp.	9.5	47.0	55.3	72.3	53.3		0.0	75.0	25.4	18.8	100.0	66.7	100.0			3.8	2.8	3.0	6.2	12.3	19.2	13.7
Acanthocyclops spp.	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0			0.0	57.0	71.7	51.9	71.2	80.8	45.1
Other cyclopoids	45.2	24.2	7.9	29.8	66.7		50.0	75.0	45.8	37.5	100.0	66.7	0.0			24.5	80.4	83.8	66.7	89.0	96.2	58.8
Other Copepods																						
Harpacticoids	16.7	4.5	13.2	27.7	6.7		0.0	25.0	40.7	12.5	100.0	0.0	0.0			15.1	13.6	9.1	13.6	19.2	30.8	3.9
Copepod nauplii	33.3	9.1	26.3	34.0	20.0		0.0	50.0	1.7	6.3	100.0	0.0	0.0			5.7	0.3	1.0	0.0	15.1	7.7	2.0
Cladocerans	35.7	50.0	36.8	61.7	53.3		50.0	25.0	10.2	0.0	0.0	33.3	0.0			11.3	81.1	80.8	67.9	90.4	96.2	78.4
Mysids	0.0	9.1	13.2	14.9	0.0		50.0	25.0	57.6	100.0	0.0	0.0	0.0			3.8	4.5	4.0	4.9	1.4	0.0	3.9
Amphipods																						
Gammarus spp.	0.0	0.0	0.0	0.0	0.0		0.0	0.0	1.7	6.3	0.0	0.0	0.0			60.4	16.4	16.2	37.0	24.7	30.8	35.3
Corophium spp.	0.0	6.1	0.0	0.0	33.3		0.0	25.0	57.6	25.0	50.0	0.0	100.0			47.2	54.2	26.3	44.4	17.8	19.2	29.4
Unidentified amphipods	2.4	0.0	0.0	0.0	0.0		0.0	0.0	3.4	0.0	0.0	0.0	0.0			9.4	8.4	2.0	9.9	4.1	0.0	7.8
Cumaceans	0.0	0.0	0.0	0.0	0.0		0.0	0.0	15.3	0.0	0.0	33.3	0.0			0.0	5.6	1.0	13.6	12.3	3.8	17.6
Fish	0.0	1.5	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0			0.0	5.6	3.0	4.9	0.0	0.0	13.7
Other	38.1	39.4	36.8	38.3	46.7		0.0	25.0	32.2	18.8	50.0	0.0	0.0			24.5	27.3	38.4	56.8	45.2	34.6	39.2
Maximum	98	92	97	100	87		100	100	95	100	100	100	100			100	81	84	68	90	96	78

Note: Each year includes December from the preceding year (e.g. 2012 includes December 2011-May 2012). Number of stomachs with food present in parentheses. No samples (NS) occurred in some years and months reported as blank fields. Fields are shaded darker red with higher percentage values.
* Identifies samples collected by USFWS in 2017

Table 1-10. Diet by Percent Frequency of Occurrence of Major Prey Categories in Stomachs of Delta Smelt Collected in 0.5-6 ppt for Months June-August (J-A), September-November (S-N), and December-May (D-M) Among Years 2011-2017

	Diet by percent frequency of occurrence (%FO)																					
	J-A	J-A	J-A	J-A	J-A	J-A	J-A	J-A	S-N	S-N	S-N	S-N	S-N	S-N	S-N	S-N	D-M	D-M	D-M	D-M	D-M	D-M
	2011	2012	2013	2014	2015	2016	2017	2017*	2011	2012	2013	2014	2015	2016	2017	2017*	2012	2013	2014	2015	2016	2017
Prey Category	(24)	(21)	(32)	(88)	(0)	(0)	(10)	(17)	(61)	(17)	(6)	(75)	(4)	(7)	(1)	(19)	(177)	(71)	(83)	(52)	(8)	(3)
Calanoid copepods																						
Eurytemora spp.	0.0	0.0	40.6	0.0			0.0	0.0	16.4	5.9	0.0	0.0	0.0	0.0	100.0	26.3	62.7	80.3	90.4	75.0	87.5	100.0
Pseudodiaptomus spp.	87.5	71.4	87.5	89.8			90.0	47.1	85.2	82.4	83.3	97.3	75.0	100.0	100.0	78.9	22.6	15.5	32.5	50.0	50.0	33.3
Sinocalanus doerrii	0.0	38.1	3.1	1.1			20.0	0.0	1.6	0.0	0.0	1.3	0.0	0.0	0.0	0.0	5.6	5.6	3.6	0.0	0.0	0.0
Acartiella sinensis	95.8	19.0	21.9	61.4			20.0	82.4	83.6	58.8	66.7	78.7	50.0	100.0	0.0	57.9	28.2	9.9	24.1	28.8	25.0	33.3
Tortanus spp.	58.3	4.8	15.6	0.0			30.0	5.9	23.0	11.8	0.0	0.0	0.0	14.3	0.0	5.3	4.0	7.0	1.2	1.9	0.0	0.0
Other calanoids	0.0	19.0	21.9	30.7			50.0	23.5	24.6	23.5	33.3	56.0	50.0	28.6	0.0	36.8	37.3	35.2	74.7	67.3	75.0	33.3
Cyclopoid copepods																						
Limnoithona spp.	66.7	19.0	56.3	81.8			50.0	94.1	49.2	23.5	83.3	81.3	75.0	71.4	0.0	73.7	17.5	15.5	30.1	44.2	37.5	33.3
Acanthocyclops spp.	0.0	0.0	21.9	1.1			0.0	0.0	11.5	0.0	0.0	0.0	0.0	0.0	0.0	31.6	63.8	85.9	78.3	73.1	50.0	100.0
Other cyclopoids	0.0	0.0	28.1	50.0			50.0	35.3	27.9	23.5	66.7	37.3	25.0	42.9	0.0	78.9	79.1	74.6	85.5	76.9	87.5	100.0
Other Copepods																						
Harpacticoids	58.3	4.8	12.5	9.1			10.0	47.1	39.3	17.6	66.7	20.0	0.0	42.9	0.0	42.1	29.9	9.9	31.3	30.8	25.0	0.0
Copepod nauplii	8.3	9.5	9.4	10.2			0.0	29.4	4.9	0.0	16.7	2.7	0.0	0.0	0.0	36.8	1.7	8.5	18.1	17.3	25.0	0.0
Cladocerans	4.2	0.0	6.3	13.6			10.0	0.0	14.8	0.0	16.7	0.0	25.0	14.3	0.0	31.6	85.9	94.4	55.4	63.5	87.5	66.7
Mysids	20.8	23.8	46.9	8.0			10.0	5.9	42.6	47.1	50.0	14.7	25.0	14.3	0.0	10.5	15.8	12.7	10.8	9.6	12.5	0.0
Amphipods																						
Gammarus spp.	0.0	4.8	0.0	1.1			0.0	0.0	4.9	17.6	16.7	2.7	0.0	28.6	0.0	5.3	7.3	28.2	15.7	15.4	12.5	0.0
Corophium spp.	25.0	4.8	9.4	1.1			10.0	35.3	34.4	47.1	16.7	8.0	25.0	42.9	0.0	15.8	67.8	28.2	33.7	21.2	25.0	33.3
Unidentified amphipods	4.2	0.0	0.0	0.0			0.0	0.0	6.6	5.9	0.0	0.0	0.0	0.0	0.0	0.0	4.5	8.5	6.0	0.0	12.5	0.0
Cumaceans	12.5	0.0	12.5	4.5			0.0	5.9	8.2	5.9	0.0	38.7	0.0	14.3	0.0	26.3	62.7	52.1	69.9	69.2	62.5	66.7
Fish	0.0	4.8	9.4	0.0			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.2	12.7	19.3	0.0	0.0	0.0
Other	16.7	14.3	21.9	5.7			20.0	23.5	23.0	11.8	33.3	20.0	0.0	0.0	0.0	31.6	14.7	28.2	39.8	61.5	12.5	66.7
Maximum	96	71	88	90			90	94	85	82	83	97	75	100	100	79	86	94	90	77	88	100

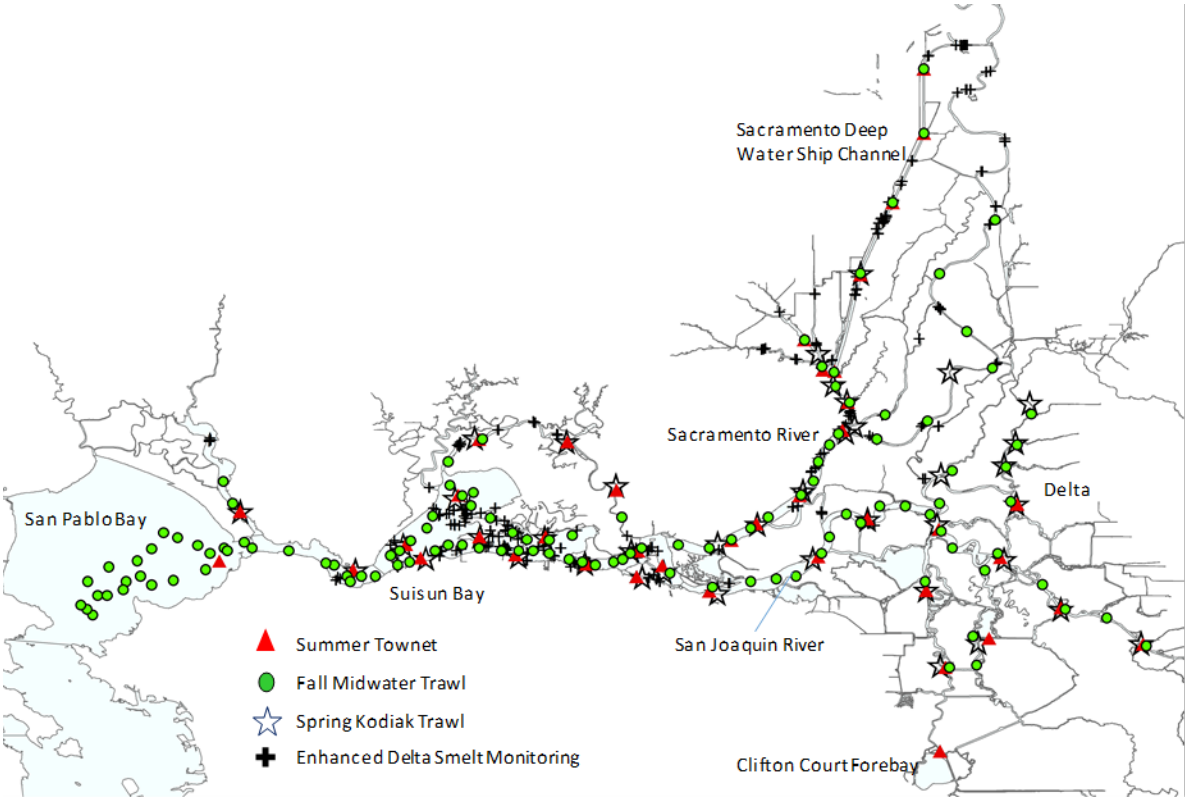
Note: Each year includes December from the preceding year (e.g. 2012 includes December 2011-May 2012). Number of stomachs with food present in parentheses. No samples (NS) occurred in some years and months reported as blank fields. Fields are shaded darker red with higher percentage values.
* Identifies samples collected by USFWS in 2017

Table 1-11. Diet by Percent Frequency of Occurrence of Major Prey Categories in Stomachs of Delta Smelt Collected in >6 ppt for Months June-August (J-A), September-November (S-N), and December-May (D-M) Among Years 2011-2017

	Diet by percent frequency of occurrence (%FO)																					
	J-A 2011	J-A 2012	J-A 2013	J-A 2014	J-A 2015	J-A 2016	J-A 2017	J-A 2017*	S-N 2011	S-N 2012	S-N 2013	S-N 2014	S-N 2015	S-N 2016	S-N 2017	S-N 2017*	D-M 2012	D-M 2013	D-M 2014	D-M 2015	D-M 2016	D-M 2017
Prey Category	(3)	(0)	(30)	(5)	(0)	(0)	(15)	(0)	(5)	(1)	(2)	(1)	(0)	(0)	(0)	(2)	(74)	(0)	(12)	(0)	(3)	(3)
Calanoid copepods																						
Eurytemora spp.	0.0		3.3	0.0			0.0		0.0	0.0	0.0	0.0				0.0	60.8		83.3		100.0	100.0
Pseudodiaptomus spp.	33.3		36.7	0.0			40.0		80.0	0.0	100.0	0.0				0.0	35.1		16.7		0.0	33.3
Sinocalanus doerrii	0.0		0.0	0.0			6.7		20.0	0.0	0.0	0.0				0.0	2.7		0.0		0.0	0.0
Acartiella sinensis	33.3		23.3	0.0			40.0		80.0	0.0	100.0	0.0				100.0	39.2		50.0		0.0	0.0
Tortanus spp.	33.3		60.0	40.0			53.3		80.0	0.0	100.0	100.0				0.0	36.5		33.3		0.0	0.0
Other calanoids	0.0		10.0	0.0			13.3		0.0	0.0	0.0	100.0				0.0	51.4		50.0		66.7	100.0
Cyclopoid copepods																						
Limnoithona spp.	33.3		73.3	0.0			86.7		40.0	100.0	100.0	100.0				50.0	41.9		58.3		66.7	100.0
Acanthocyclops spp.	0.0		0.0	0.0			0.0		20.0	0.0	0.0	0.0				0.0	63.5		83.3		100.0	100.0
Other cyclopoids	0.0		23.3	20.0			86.7		100.0	0.0	0.0	0.0				50.0	97.3		83.3		100.0	100.0
Other Copepods																						
Harpacticoids	33.3		16.7	0.0			46.7		20.0	0.0	50.0	0.0				0.0	63.5		50.0		0.0	0.0
Copepod nauplii	0.0		13.3	0.0			46.7		0.0	0.0	0.0	0.0				50.0	6.8		33.3		33.3	100.0
Cladocerans	0.0		0.0	0.0			0.0		40.0	0.0	0.0	0.0				0.0	93.2		66.7		0.0	33.3
Mysids	33.3		16.7	0.0			0.0		0.0	0.0	50.0	100.0				0.0	24.3		0.0		0.0	0.0
Amphipods																						
Gammarus spp.	0.0		0.0	0.0			0.0		0.0	0.0	0.0	0.0				0.0	0.0		16.7		0.0	0.0
Corophium spp.	33.3		6.7	40.0			26.7		40.0	0.0	0.0	0.0				0.0	40.5		50.0		33.3	66.7
Unidentified amphipods	0.0		0.0	20.0			0.0		20.0	0.0	0.0	0.0				0.0	8.1		8.3		0.0	33.3
Cumaceans	66.7		10.0	20.0			6.7		20.0	0.0	0.0	100.0				0.0	63.5		83.3		66.7	100.0
Fish	0.0		10.0	0.0			0.0		0.0	0.0	0.0	0.0				0.0	10.8		0.0		0.0	0.0
Other	0.0		33.3	40.0			53.3		40.0	0.0	0.0	100.0				0.0	20.3		58.3		0.0	100.0
Maximum	67		73	40			87		100	100	100	100				100	97		83		100	100

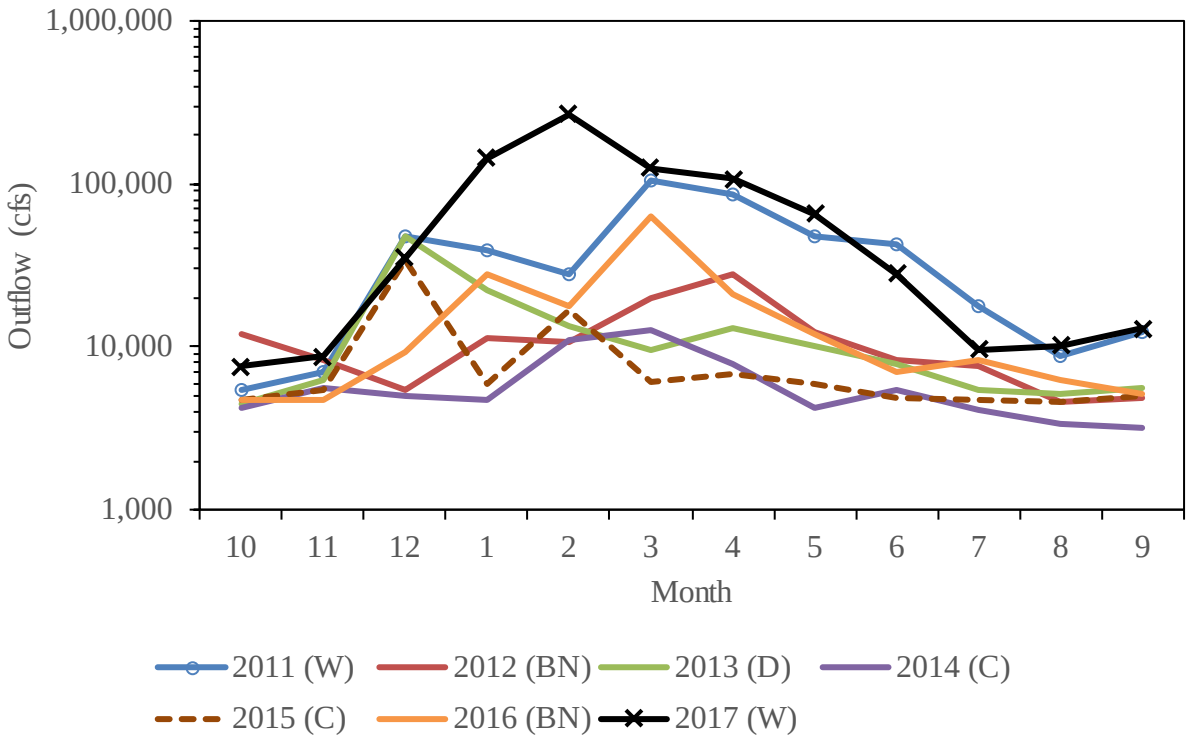
Note: Each year includes December from the preceding year (e.g. 2012 includes December 2011-May 2012). Number of stomachs with food present in parentheses. No samples (NS) occurred in some years and months reported as blank fields. Fields are shaded darker red with higher percentage values.
* Identifies samples collected by USFWS in 2017.

Figures



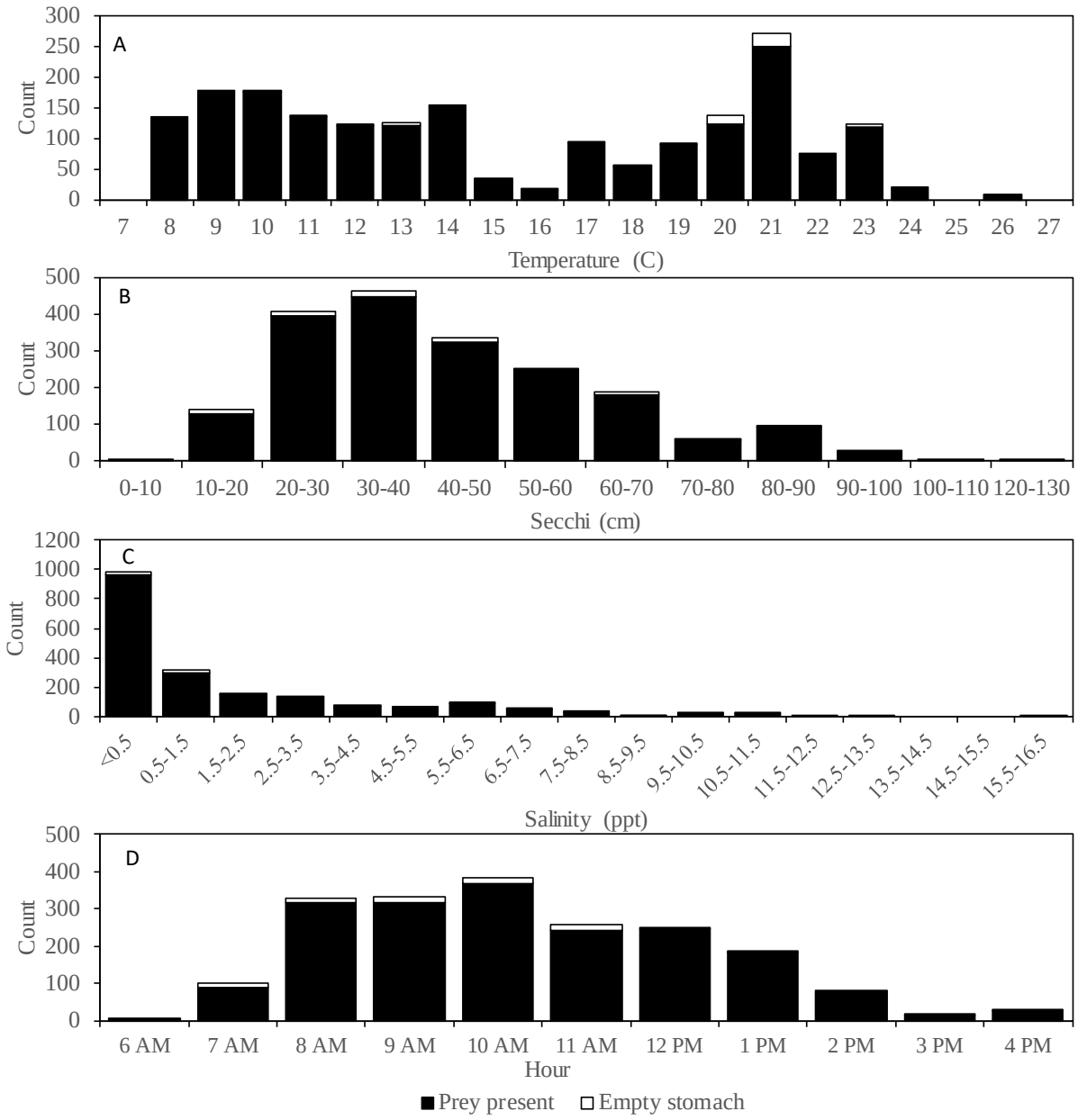
Note: Points include CDFW surveys Summer Townet (red triangle), Fall Midwater Trawl (green circle), and Spring Kodiak Trawl (blue star) with USFWS Enhanced Delta Smelt Monitoring (plus sign).

Figure 1-1. Map of CDFW and USFWS Sampling Locations in the Upper San Francisco Estuary



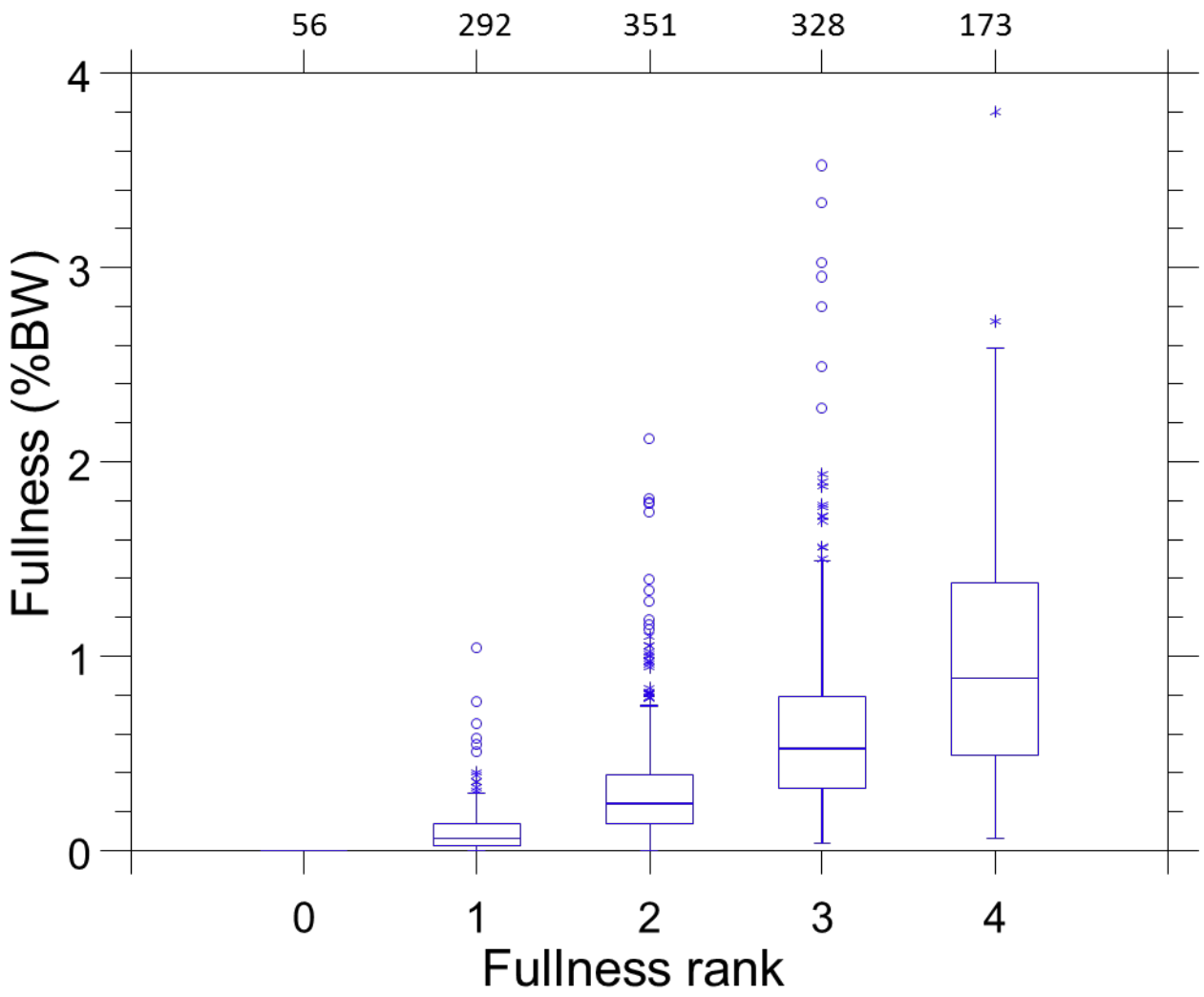
Note: Each water year is January-September, and the preceding October-December (e.g. water year 2011 is October 2010-September 2011). The Sacramento Valley water year index type is in parentheses in legend. Note, figure y-axis is log10 scale.

Figure 1-2. Monthly Mean Freshwater Outflow (cfs) Past Chipps Island for Water Years 2011-2017



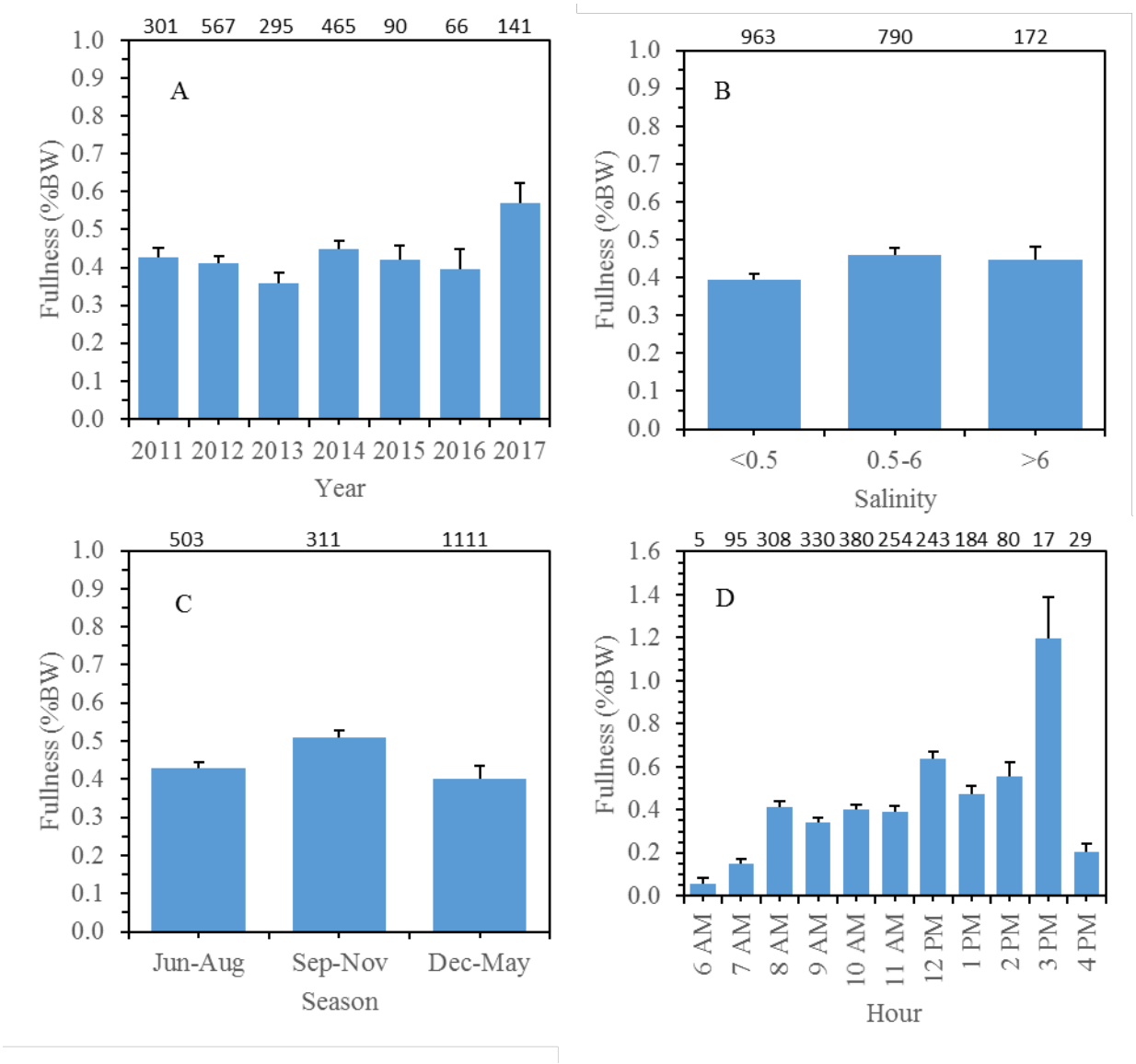
Note: A) Temperature (°C), B) Secchi Disk depth (cm), C) Salinity (ppt), and D) Hour of Collection During 2011-2017 Examined for this Study. Two temperature values were missing.

Figure 1-3. Count of Delta Smelt (N=1,962) with Prey Present in Stomachs or with Empty Stomachs Collected Among Environmental Variables



Note: Only a subset of study samples included visual rank (n = 1,200) with sample size included along top of boxplot. The central vertical line of each box is the median value. The box is the range of the central 50% of values between the 25% and 75% quartiles. The whiskers capture values within 1.5 times the upper 75% and lower 25% quartiles and values exceeding whiskers are asterisks or empty circles.

Figure 1-4. Boxplot of Delta Smelt Gut Fullness (%BW) Per Relative Index of Fullness Using the Scale 0 = Empty, 1 = 25% Full, 2 = 50% Full, 3 = 75% Full and 100% = Full



Note: Sample size included along the top of each bar chart.

Figure 1-5. Mean (±SE) Delta Smelt Gut Fullness (%BW) by A) Year, B) Salinity, C) Season, and D) Hour of collection During 2011-2017 CDFW and 2017 USFWS Surveys

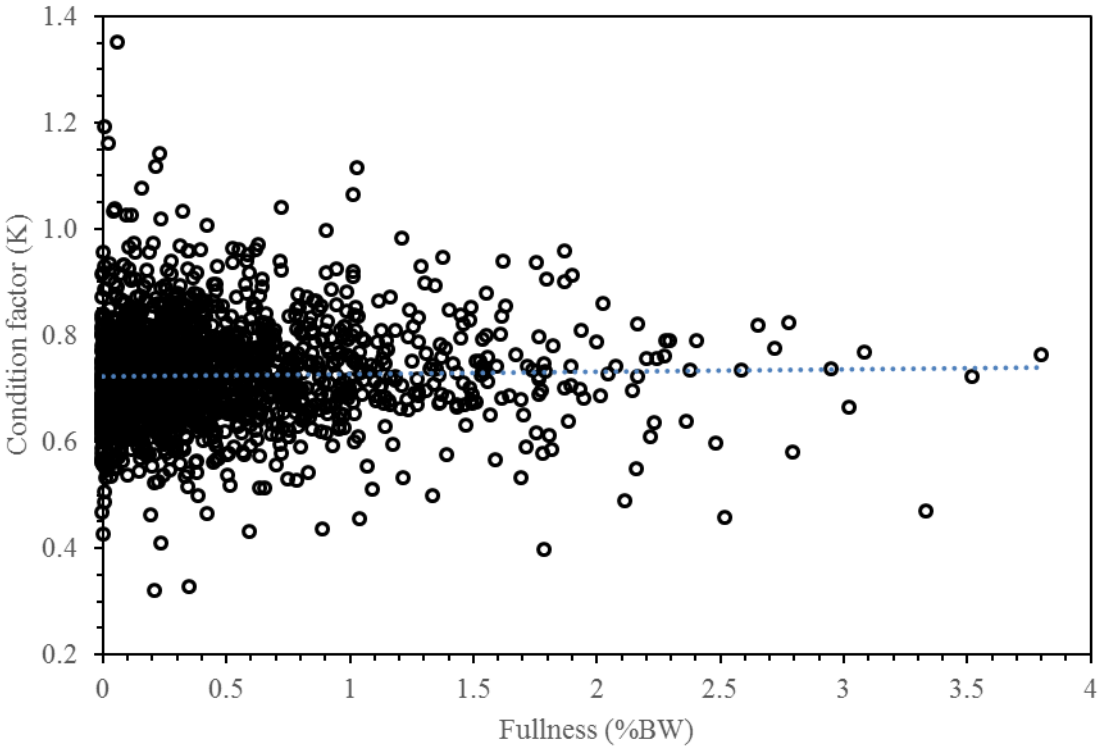
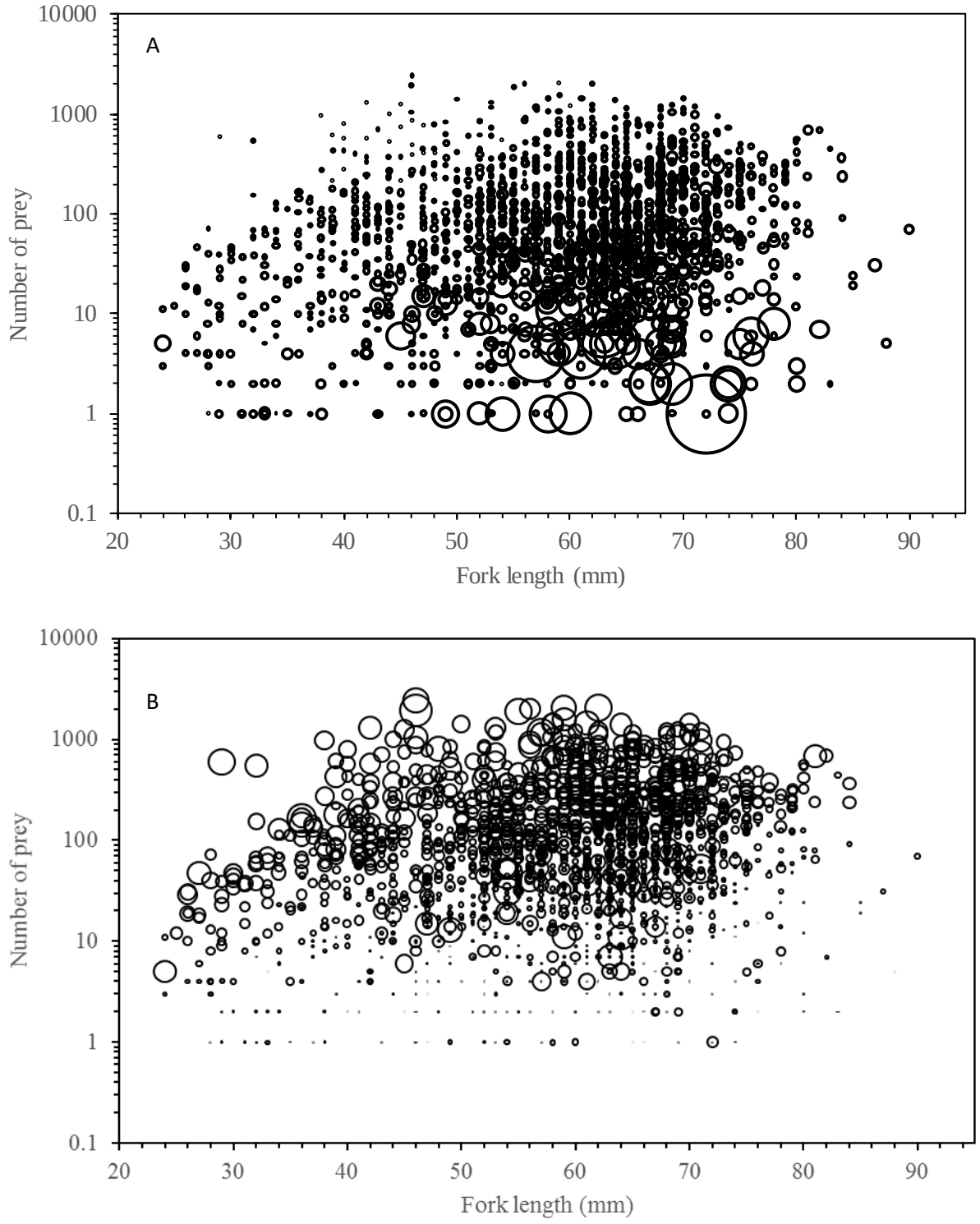
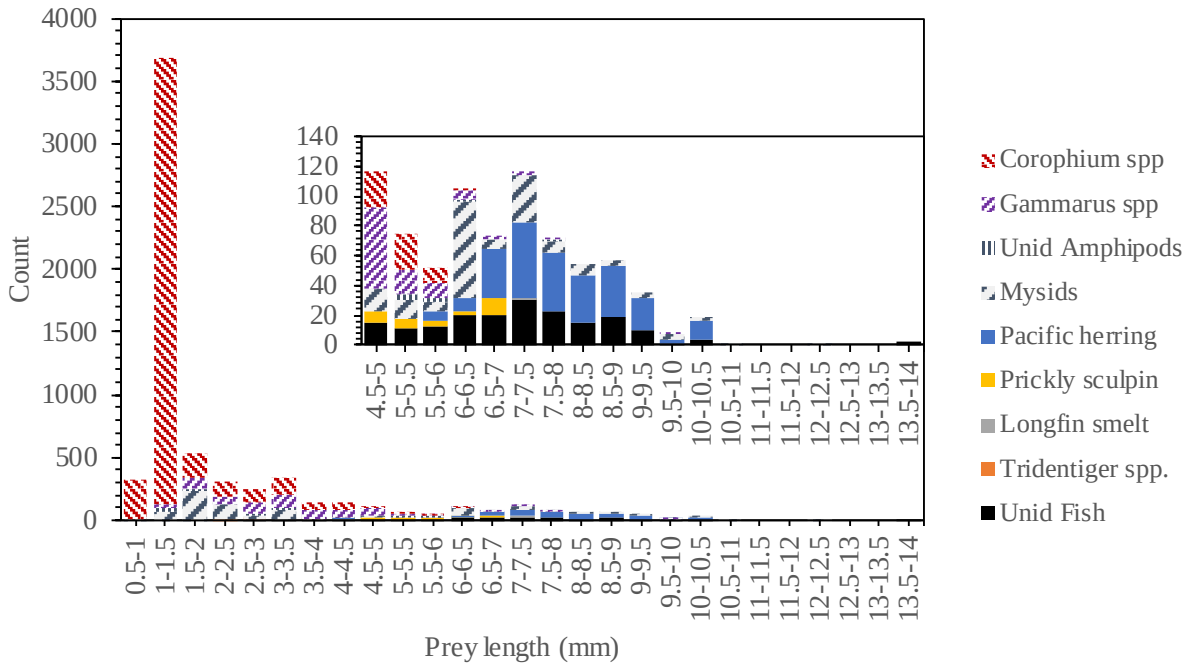


Figure 1-6. Condition Factor Plotted Against Stomach Fullness (%BW) with Linear Regression Fit Line y = -0.0028x + 0.7252, R^2 = 0.0002 for Delta Smelt (N = 1,925) Collected from 2011-2017 CDFW and USFWS Surveys



Note: (N=1,925) with size of bubble representing A) mean mass of prey (bubble scale is 0.00000238 to 0.01085000g) and B) stomach fullness (%BW) with bubble scale 0 to 4. y-axis is $\log_{10}$ scale.

Figure 1-7. Number of Prey in Stomachs Plotted Against Fork Length (mm) for Delta Smelt with Food Present in Guts Collected 2011-2017



Notes: Body lengths (±0.1 mm) were recorded for amphipods, mysids, cumaceans, and larval fish with counts grouped in 0.5 mm length bins. Inset figure is limited to length bins 4.5-14.0 mm to increase visibility of the y-axis scale. Delta Smelt were collected by CDFW and USFWS during 2011-2017.

Figure 1-8. Length-Frequency of Large Prey Found in Stomachs of Juvenile and Adult Delta Smelt During this Study

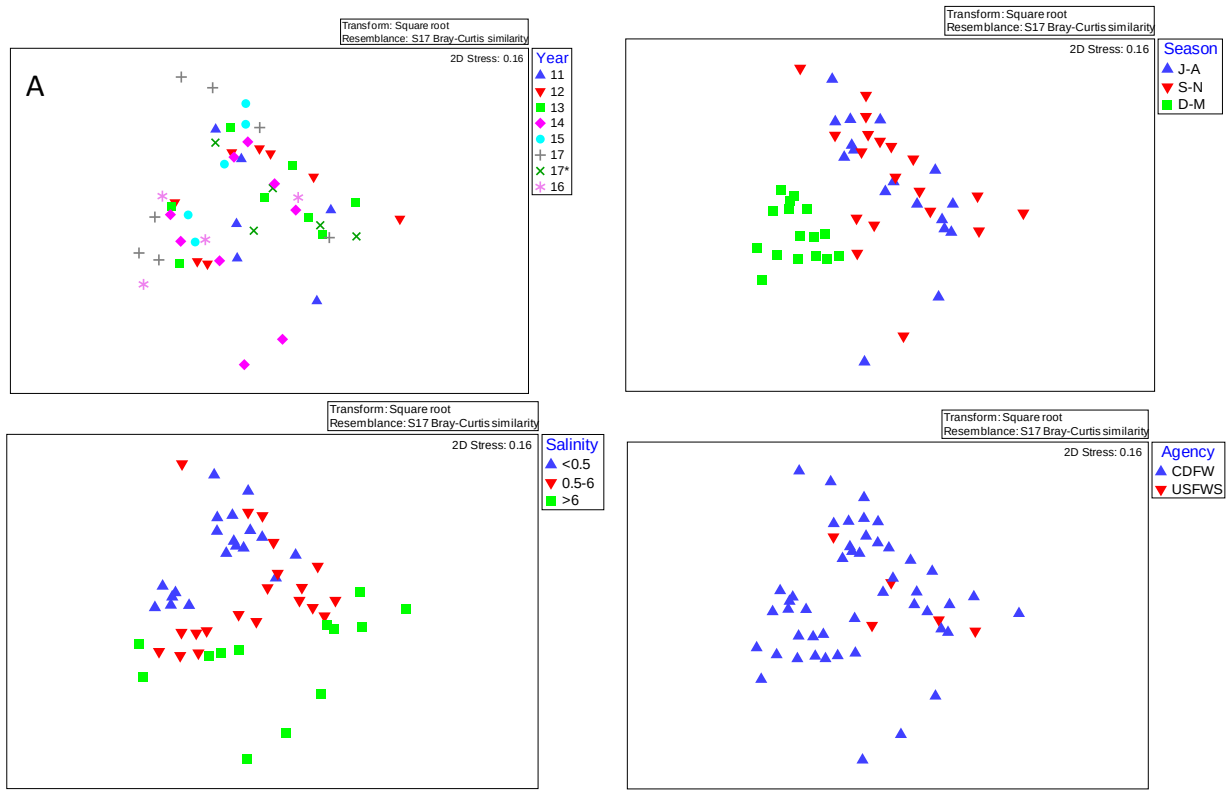


Figure 1-9. Nonmetric Multidimensional Scaling (NMDS) Ordination Plots of Delta Smelt Diet By Percent Number Among A) Year, B) Season, C) Salinity, and D) Agency for the Period 2011-2017

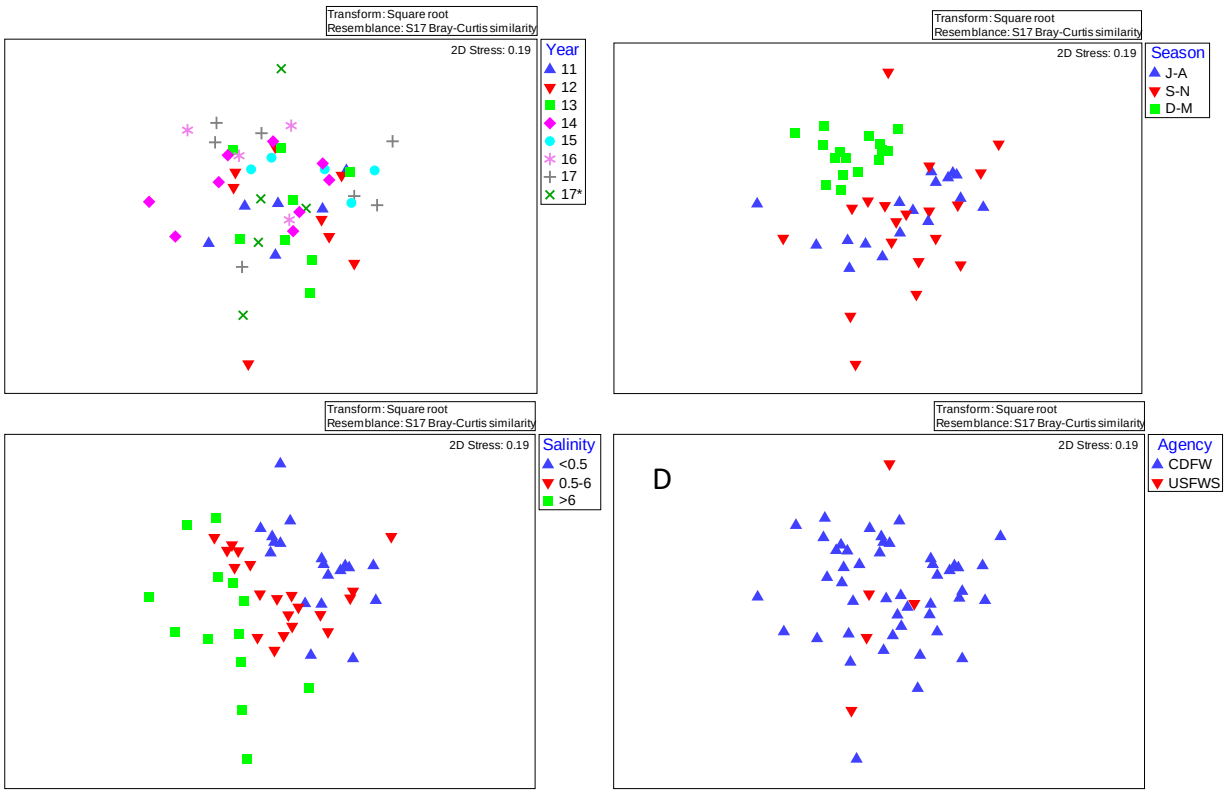


Figure 1-10. Nonmetric Multidimensional Scaling (NMDS) Ordination Plots of Delta Smelt Diet by Percent Weight Among Factors A) Year, B) Season, C) Salinity, and D) Agency

Supplemental Data: Tables

Table A1. Wet weight (µg) Estimates to Calculate Mass of Prey Types Found in Stomachs of Delta Smelt to Determine Diet by Percent Number and Stomach Fullness

Prey Category	Prey Type	Wet Weight (µg)	Source
Calanoid copepods			
Eurytemora spp.	*Eurytemora* spp. nauplii	1.8	Kimmerer 2006
Eurytemora spp.	*Eurytemora* spp. copepodite	10.1	Kimmerer 2006
Eurytemora spp.	*Eurytemora* spp. adult	40.3	Kimmerer 2006
Pseudodiaptomus spp.	*Pseudodiaptomus marinus*	73.3	Kimmerer 2006
Pseudodiaptomus spp.	*Pseudodiaptomus* spp. nauplii	1.8	Kimmerer 2006
Pseudodiaptomus spp.	*Pseudodiaptomus* spp. copepodite	13.7	Kimmerer 2006
Pseudodiaptomus spp.	*Pseudodiaptomus* spp. adult	19.4	CDFW unpublished
Pseudodiaptomus spp.	*Pseudodiaptomus forbesi*	54.9	Kimmerer 2006
Sinocalanus doerrii	*Sinocalanus doerrii* nauplii	2.7	CDFW unpublished
Sinocalanus doerrii	*Sinocalanus doerrii* copepodite	23.6	CDFW unpublished
Sinocalanus doerrii	*Sinocalanus doerrii* adult	70.7	CDFW unpublished
Acartiella sinensis	*Acartiella sinensis* copepodite	27.7	CDFW unpublished
Acartiella sinensis	*Acartiella sinensis* adult	75.3	CDFW unpublished
Tortanus spp.	*Tortanus* spp. copepodite	30.1	CDFW unpublished
Tortanus spp.	*Tortanus* spp. adult	219.6	CDFW unpublished
Tortanus spp.	*Tortanus dextrilobatus*	219.6	From Tortanus spp. adult
Other calanoids	*Acartia* spp. copepodite	11.4	Kimmerer 2006
Other calanoids	*Acartia* spp. adult	71.9	CDFW unpublished
Other calanoids	*Diaptomus* spp. copepodite	11.4	Kimmerer 2006
Other calanoids	*Diaptomus* spp. adult	73.3	Kimmerer 2006
Other calanoids	Unidentified calanoid	27.6	CDFW unpublished
Other calanoids	Calanoid copepodite	13.8	CDFW unpublished
Other calanoids	*Osphranticum* spp.	36.6	From Unidentified calanoid
Other calanoids	Other calanoid	36.6	Kimmerer 2006
Cyclopoid copepods			
Limnoithona spp.	*Limnoithona* spp. juvenile	0.5	Kimmerer 2006
Limnoithona spp.	*Limnoithona* spp. adult	5.6	CDFW unpublished
Acanthocyclops spp.	*Acanthocyclops* spp.	38.2	CDFW unpublished
Other calanoids	*Oithona davisae* adult	4.2	Kimmerer 2006
Other calanoids	*Oithona* spp. juvenile	1.1	Kimmerer 2006
Other calanoids	Other cyclopoid	44.4	CDFW unpublished
Other calanoids	UnID cyclopoid	21.7	CDFW unpublished
Other calanoids	cyclopoid copepodite	13.7	Kimmerer 2006
Other copepods			
Harpacticoid copepods	Harpacticoids	22.7	CDFW unpublished
Copepod nauplii	Copepod nauplii	2.4	CDFW unpublished
Cladocerans	*Bosmina* sp.	6.9	CDFW unpublished
Cladocerans	*Diaphanosoma* sp.	28.3	CDFW unpublished
Cladocerans	*Ceriodaphnia* sp.	32.3	CDFW unpublished
Cladocerans	*Daphnia* sp.	50.4	CDFW unpublished
Cladocerans	Other cladocera	30.1	CDFW unpublished
Cladocerans	UnID cladocera	22.5	CDFW unpublished

Prey Category	Prey Type	Wet Weight (µg)	Source
Cumaceans	Cumaceans	330.7	CDFW unpublished
Other	Unid copepods	24.7	Mean of Unidentified calanoid and cyclopoid
Other	Ostracods	48.1	CDFW unpublished
Other	Chironomid larvae	164	CDFW unpublished
Other	Terrestrial invertebrates	236.6	CDFW unpublished
Other	Other insect larvae	490.4	CDFW unpublished
Other	Rotifer *Keratella* spp.	1.3	CDFW unpublished
Other	Rotifer *Trichocerca* spp.	2.3	CDFW unpublished
Other	Rotifer *Synchaeta* spp.	3.6	CDFW unpublished
Other	Rotifer *Polyarthra* spp.	0.5	Kimmerer 2006
Other	Other rotifer	3.6	CDFW unpublished
Other	Unid rotifer	3.6	CDFW unpublished
Other	Barnacle nauplii	13.9	CDFW unpublished
Other	Other malacostraca	494	CDFW unpublished
Other	Crab zoea	29.6	CDFW unpublished
Other	Bivalve	33.4	CDFW unpublished
Other	Annelid worm pieces	13.9	From barnacle nauplii (similar size)
Other	Other zooplankton	93.9	CDFW unpublished
Other	Fish eggs	22.3	CDFW unpublished

Note: Prey types were grouped by prey category. Prey types include all life stages, unless noted otherwise. Wet weights were generated by CDFW or from conversion of carbon weight estimates in the literature (Kimmerer 2006). Conversion of carbon weight (µg) literature values to wet weight was conducted using ratios by Beers (1966) as: dry weight = carbon weight / 0.42 and wet weight = dry weight / 0.13.

Table A2. Length-Weight Relationships for Prey Types to Calculate Mass of Prey Found in Stomachs of Delta Smelt to Determine Diet by Percent Number and Stomach Fullness.

Prey Category	Prey Type	Length-weight relationship	Source
Mysids	*Hyperacanthomysis longirostris*	$W = 31.8 \times L\ 2.533$	CDFW unpublished
Mysids	*Acanthomysis aspera*	$W = 31.8 \times L\ 2.533$	From H. *longirostris*
Mysids	*Neomysis mercedis*	$W = 10.7 \times L\ 3.126$	CDFW unpublished
Mysids	*Neomysis kadiakensis*	$W = 10.7 \times L\ 3.126$	From N. *mercedis*
Mysids	Unid Mysids	$W = 31.8 \times L\ 2.533$	From H. *longirostris*
Amphipods			
Corophium spp.	*Corophium spp.*	$W = 9.3 \times L\ 3.401$	CDFW unpublished
Gammarus spp.	*Gammarus spp.*	$W = 16.5 \times L\ 3.076$	CDFW unpublished
Unid Amphipods	Unidentified Amphipod	$W = 9.3 \times L\ 3.401$	From *Corophium*
Fish	*Tridentiger spp.*	$W = 4.1 \times L\ 3.305$	CDFW unpublished
Fish	Longfin Smelt	$W = 1.7 \times L\ 3.374$	CDFW unpublished
Fish	Pacific Herring	$W = 4.1 \times L\ 3.205$	CDFW unpublished
Fish	Prickly Sculpin	$W = 24.3 \times L\ 2.778$	CDFW unpublished
Fish	Unidentified fish	$W = 24.3 \times L\ 2.778$	From Prickly Sculpin

Note: Length-weight relationships where body length (L) is in millimeters and wet weight (W) is micrograms.

Supplemental Data: Figures

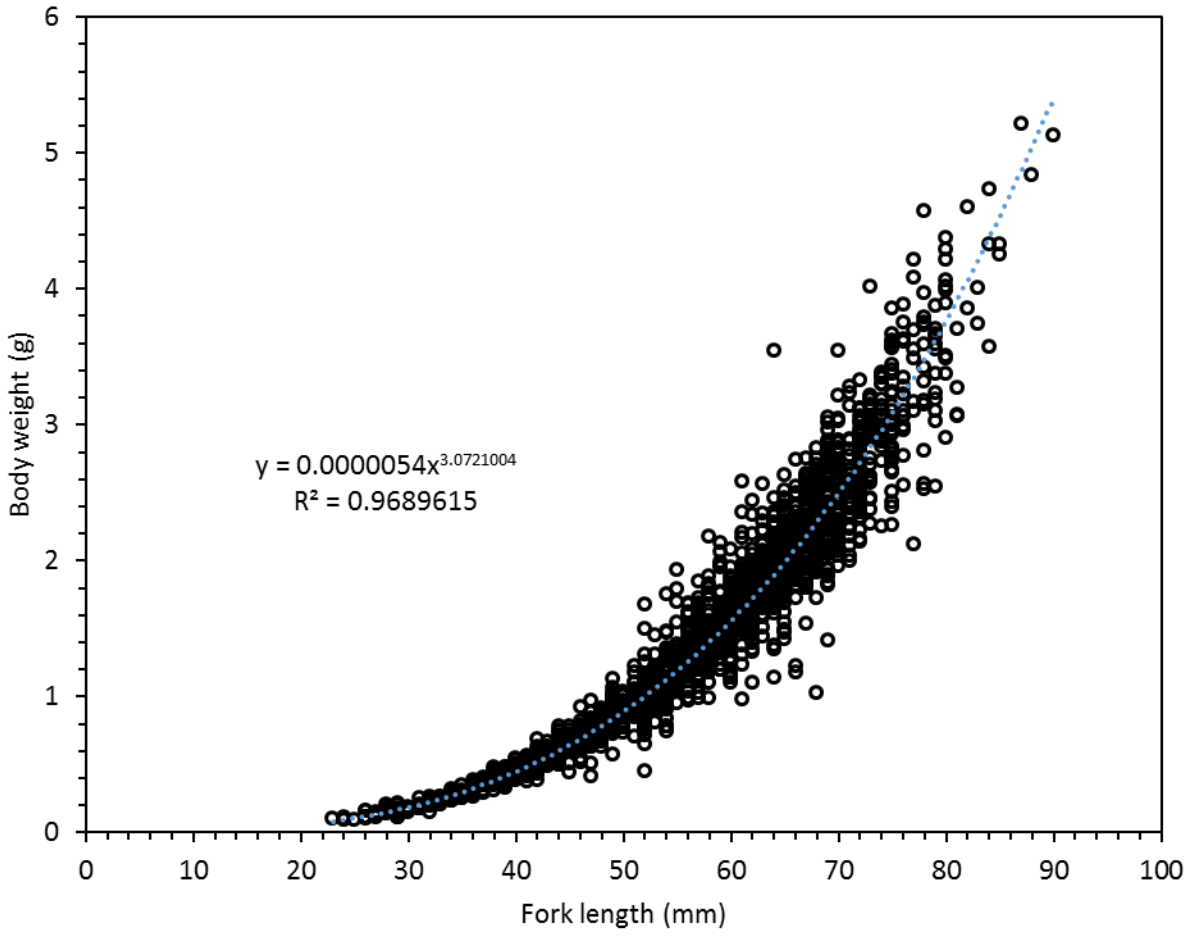
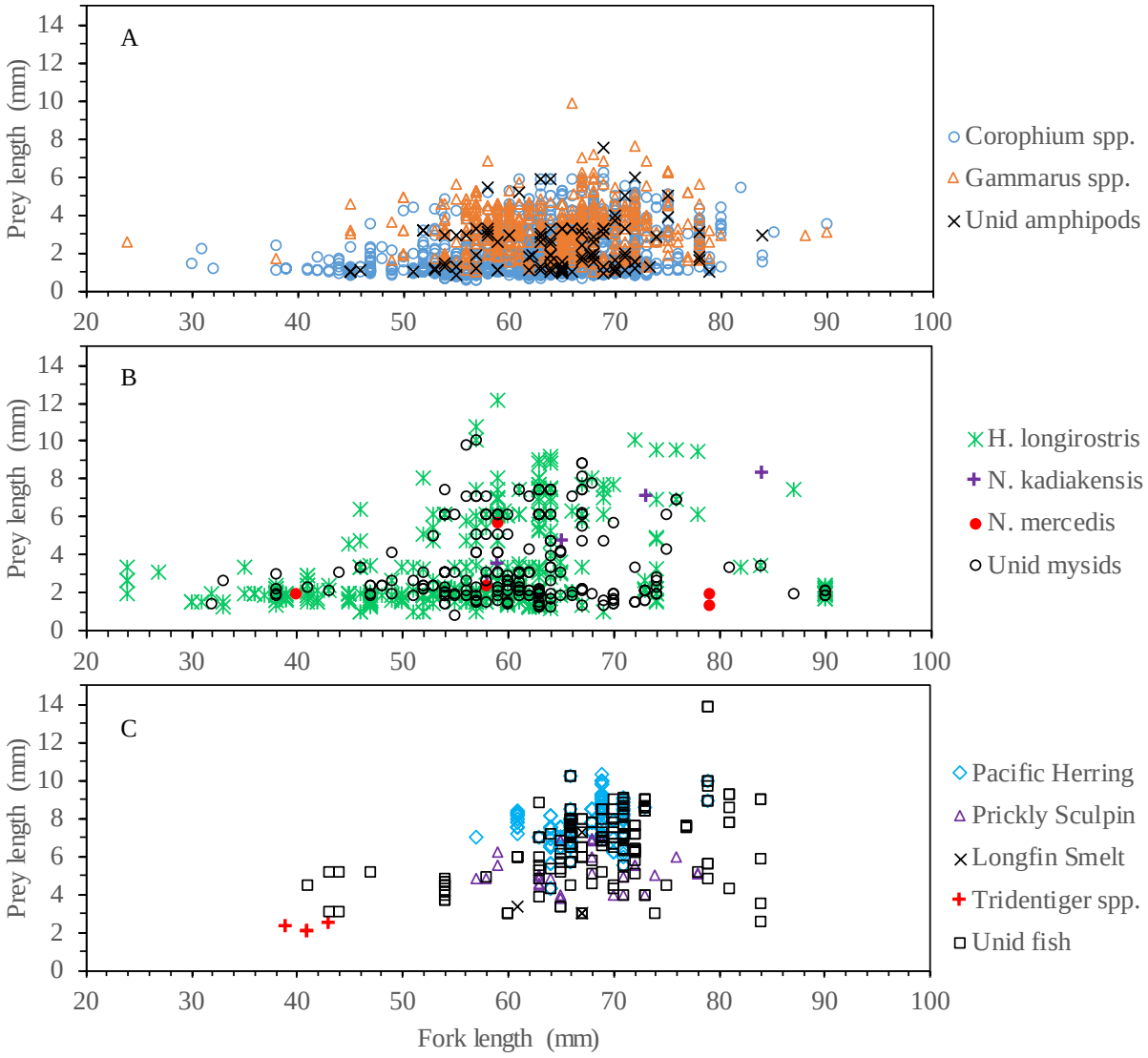
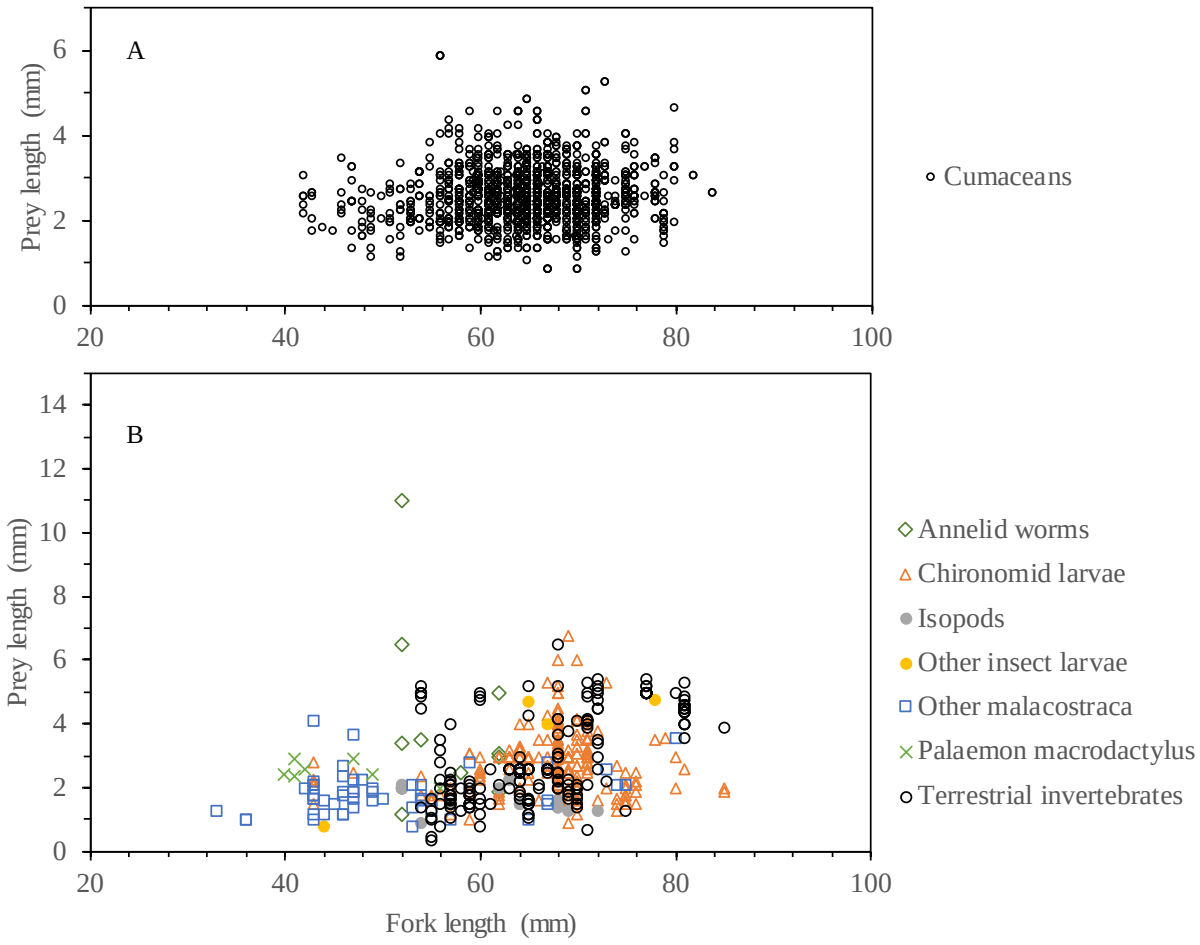


Figure B1. Scatterplot of Length-Weight Data with a Power Function for Delta Smelt (N = 1,925) Collected from 2011-2017 (CDFW and USFWS surveys)



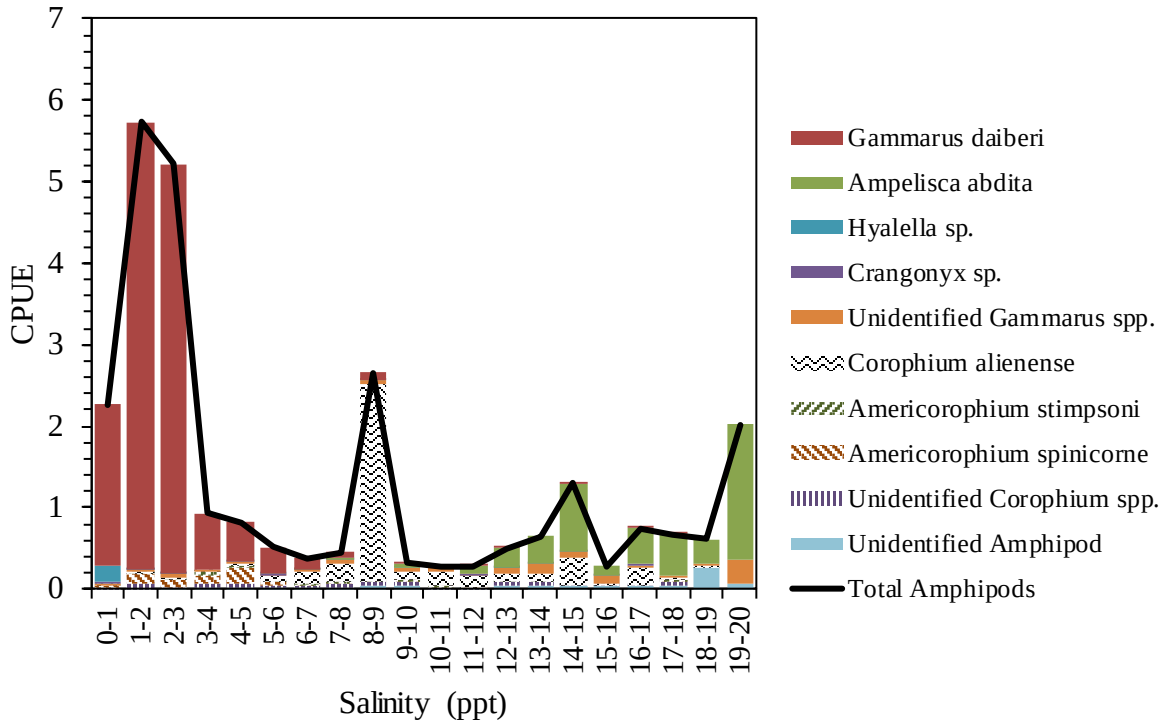
Note: Unidentified ("Unid") occurred for some prey types due to state of digestion or rare items that did not fit an existing identification category.

Figure B2. Scatterplots of Body Lengths (mm) of Large Prey Types A) Amphipods (n=5,310), B) Mysids (n=702) and C) Larval Fish (n=494) Found in Stomachs of Juvenile and Adult Delta Smelt by Fork Length (mm) Collected 2011-2017



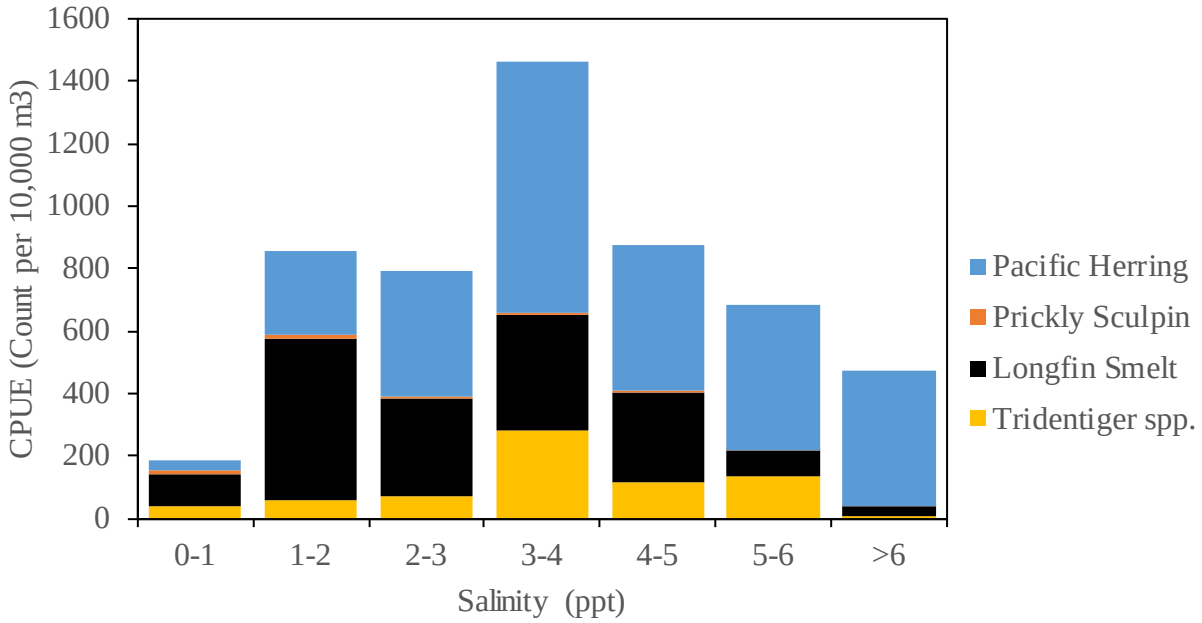
Note: Lengths were recorded for these prey types when intact that included cumaceans (n=1716 of 1765), annelid worms (n=11 of 18), chironomid larvae (n=198 of 213), other insect larvae (n=4 of 7), terrestrial invertebrates (Diptera: Chironomidae, Brachycera (flies), Homoptera (aphids), and Psocoptera (Barklice); Hymenoptera (ants), and Spiders) (n=248 of 953), shrimp *Palaemon macrodactylus* (n=7 of 7), and crab zoea and other malacostraca (n= 54 of 66) and isopods (n=20 of 20). Unidentified ("Unid") prey types occurred for some due to state of digestion or rare items that did not fit an existing category.

Figure B3. Scatterplots of Body Lengths (mm) of Prey Categories A) "Cumaceans" and B) "Other Zooplankton" Found in Stomachs of Juvenile and Adult Delta Smelt by Fork Length (mm) Collected 2011-2017



Note: The amphipod *Gammarus daiberi* was the most numerous amphipod in the salinity range common to Delta Smelt (<8 ppt), with *Corophium alienense* at salinities >6 ppt. Native *Corophium* amphipods *Americorophium stimpsoni* and *A. spinicorne* were also collected at salinity common to Delta Smelt, but at much lower CPUE than the introduced *G. daiberi*.

Figure B4. Mean CPUE (count per cubic meter) of Amphipods by Salinity (ppt) Collected by the CDFW FMWT Mysid Net During September-December Among Years 2013-2017



Note: For more information on CDFW 20-mm Survey visit: https://www.wildlife.ca.gov/Conservation/Delta/20mm-Survey.

Figure B5. Mean CPUE (count per 10,000 cubic meters) for Larval Fishes by Salinity (1 ppt) Collected by the CDFW 20-mm Survey at Core Stations During March-May Among Years 2011-2017

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Chapter 2: Histological Analysis of 7 Year-Classes of Delta Smelt

This chapter has been submitted to the Journal of Aquatic Animal Health.

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Keywords: histopathology, gill, liver, ionocyte, lipidosis, aneurysm

Abstract

This study examined the severity and incidence of lesions to the liver and gill of Delta Smelt (*Hypomesus transpacificus*) from 2011 through 2017 in the Sacramento-San Joaquin Delta and San Francisco Estuary (n=1053). The three most common lesions were gill ionocyte hyperplasia, liver lipidosis, and gill aneurysm. Model comparison was used to identify and quantify the drivers of the spatial and temporal patterns observed in gill and liver lesion scores, defined as summations of the severity scores of each lesion. Individuals with higher fork lengths exhibited increased gill and liver lesion score, indicating either that Delta Smelt accumulate lesions through their lives, or that larger individuals were more tolerant of liver and gill damage. Liver lesion score showed significant regional differences, while salinity was a better predictor of gill lesions than region, with increasing salinity decreasing gill lesion score. Regionally, Delta Smelt collected from the Confluence and Suisun Marsh had the lowest liver lesion score, while Delta Smelt collected from Cache Slough and Suisun Bay had the highest lesion scores, suggesting heterogeneous levels of environmental stressor exposure across regions. Gill and liver lesion score also varied significantly with year-class. The highest gill lesion score occurred in the 2015/16 year-class, and the lowest occurred in the 2017/18 year-class, a 2.8-fold difference. Individuals with comparatively high liver lesion scores persisted in the population until the

2014/15 year-class, when mean liver lesion score improved substantially. Given that the improvement in liver condition coincided with a decline in population associated with severe drought, our interpretation is that the least healthy individuals could not persist under stressful conditions, making the population appear healthier on average.

Introduction

Abundance-based fish monitoring programs, though useful, provide limited information on the potential influence of contaminants on fish populations. In recent years, increasing emphasis has been placed on fish health as an indicator of environmental stress since it provides a biological record of previous sub-lethal exposures (Stentiford et al., 2003, Ruiz-Picos et al. 2015). One method to examine fish health is histopathology, the microscopic study of abnormal structure of cells and tissue. Histopathology can be used to assess the influence of a variety of stressors, including pathogens, contaminants or unfavorable nutritional and water quality conditions (Teh et al. 1997, Handy et al. 2003, Stentiford et al., 2003). It is a powerful tool to detect and characterize the biological end points of previous exposure of organisms to environmental stressors such as contaminants and pathogens. In addition, it provides an important analytical link between biomolecular or biochemical assays and individual or population relevant endpoints (Adams et al. 1992, Johnson et al. 1993).

The two most widely used organs in fish histopathology studies are the liver and gills (Mallatt 1985, Hinton et al 1992, Myers et al 1998, Poleksic and Mitrovic-Tutundzic 1994, ICES 1997). These organs are sensitive to a variety of environmental stressors, and act as indicators of survival, growth and reproduction (Adams et al., 1992; Teh et al., 1997). In fish, the liver performs metabolic and detoxification functions, stores glycogen for short-term energy, and is the site of choriogenin and vitellogenin protein production used for egg chorion and yolk development, respectively. Therefore, impairment of liver functions has negative consequences for growth, survival and reproductive success of fish. Gills perform gas exchange, regulate internal osmolarity, and excrete ammonia, and as such are in constant, direct contact with water. As such, gills respond more rapidly than the liver to stressors and therefore represent an important and sensitive organ to assess water quality and contaminant exposure (Mallat 1985, Poleksic and Mitrovic-Tutundzic 1994, Au 2004). Gill lesions are therefore useful indicators of recent exposure of fish to stressors, such as recent migration of fish from fresh to brackish regions or exposure to contaminant stressors. The degree of morphological alterations in the gills indicates the degree of environmental contaminants and physicochemical (e.g., salinity) stressor exposure (Poleksic and Mitrovic-Tutundzic 1994, Schwaiger et al. 1997, Au 2004). Thus, morphological alterations of the liver and gills can indicate chronic and acute adverse effects of starvation, pathogens and environmental stressors, possibly leading to death (Brusle and Anadon, 1996, Adams et al., 1992).

The San Francisco Estuary (SFE; Figure 2-1) is formed by the convergence of the Sacramento and San Joaquin rivers and the Pacific Ocean. It is the largest estuary on the Pacific coast of the Americas (Moyle 2002). In addition to alterations to the geomorphology and hydrodynamics of the SFE to accommodate agriculture, urban development and water diversion, the estuary is also a major drainage for natural and anthropogenic contaminants. The Delta Smelt (*Hypomesus transpacificus*) is a small fish endemic to the SFE, and its population has been in decline for

decades (Sommer et al. 2007). This decline led to the listing of the species as threatened and endangered under the California and Federal Endangered Species Acts, respectively (USFWS 1993, CDFW 2014). Hypothesized causes for the decline in abundance include poor water quality, drought, altered habitat, climate change, food limitation, and diversion of fresh water (e.g., Sommer et al. 2007). However, these hypotheses are difficult to assess using only abundance estimates, without data collected below the scale of the individual.

The present study applies histopathological analysis to wild Delta Smelt collected during monitoring program sampling over a seven-year period (2011-2017), and uses data collected from individuals to characterize the temporal and spatial variability of fish histopathological condition. This period is ideal for examining the influence of river flow in particular on Delta Smelt condition because it encompasses the most severe drought in modern California history (~2012-2016), bracketed by wet years (2011 and 2017). Our previous work demonstrated that juvenile Delta Smelt collected from certain regions exhibited significantly depressed nutritional indices and elevated levels of histopathological lesions, suggesting that the species is, at a minimum, regionally stressed by contaminants and food limitation (Hammock et al. 2015). Specifically, Delta Smelt collected from Suisun Bay were under apparent nutritional stress during summer, while those collected from Cache Slough showed the most liver damage, and individuals from Suisun Marsh were in relatively good condition overall (Hammock et al. 2015). This study extends this health analysis, both from 2 to 7 years and across juvenile through adult life-stages, and examines whether previously reported variation in fish condition and nutritional status maintained their regional specificities. Using identical field and laboratory methodology to that of Hammock et al. (2015), we ask whether there are differences in histopathological condition associated with region, year-class, salinity, and freshwater outflow, a factor that is of interest to water managers.

Methods

Study Area and Sampling

Delta Smelt were collected from the SFE by the Interagency Ecological Program (IEP) fish monitoring studies conducted by the California Department of Fish and Wildlife (CDFW; n = 961; methods in Honey et al. 2004) and the United States Fish and Wildlife Service (USFWS) Enhanced Delta Smelt Monitoring Program (https://www.fws.gov/lodi/juvenile_fish_monitoring_program/jfmp_index.htm; n = 92; see Figure 2-1 in Hammock et al. 2015 for a map of the sampling regions). The CDFW fish were collected from Aug 2011 to Oct 2017 and the USFWS fish were collected from Aug 2017 to Nov 2017. Both agencies collected Delta Smelt in trawls, wrapped each fish live in aluminum foil, and placed in a dewar of liquid nitrogen kept on the boat (Teh et al. 2016). Conductivity and location data were collected at each sampling station and conductivity was converted to salinity for use in the analysis. Delta Smelt were transported to UC Davis while still submerged in liquid nitrogen.

Sample Preparation and Histopathology

Delta Smelt were stored in dewars until each individual was removed from liquid nitrogen and rapidly dissected as it thawed (5-10 min per fish; Hammock et al. 2015, Teh et al. 2016). Livers and gills were excised, preserved in 10% buffered formalin, and processed for histology

according to Teh et al. (1997, 2016). Briefly, tissues were embedded in paraffin, sectioned (3 µm thickness), and stained with hematoxylin and eosin (H&E stain; Teh et al. 1997). Histopathological analysis was conducted on gills and liver of each sampled fish following the methods of Teh et al (2004). Tissues were screened with a compound microscope for a variety of histopathological lesions and scored on an ordinal ranking system of 0 = none/minimal, 1 = mild, 2 = moderate, and 3 = severe. The seven liver lesions and eight gill lesions that were commonly observed are described in Table 2-1. These organs were also screened for other tissue alterations, including parasites, bacterial infection, preneoplastic foci and hepatocellular and gill neoplasms, but were not included in analyses because these abnormalities were never detected during the seven-year study. The same histologist read the slides through the entire study.

Table 2-1. Descriptions of Histopathological Lesions Observed in Delta Smelt

Lesion	Characteristics
Liver	
Macrophage aggregate (MA)	Macrophage is usually pigmented yellow brown to green brown, and were occasionally mixed with lymphocytes
Single cell necrosis (SCN)	Hepatocytes having hyperchromatic nuclei and eosinophilic (i.e., pink coloration) granular cytoplasm. Some necrotic cells have pyknotic nuclei and varying degrees of nuclear karyolysis and karyorrhexis
Lipidosis/fatty vacuolation or degeneration	Large lipid droplets that appear as clear, round, well demarcated, and cytoplasmic vacuoles in hepatocytes
Inflammation	Focal to multifocal aggregates of lymphocytes, occasionally mixed with other inflammatory cells (e.g., macrophage or eosinophil), infiltrating the connective tissue around bile ducts, blood vessels or parenchyma
Cytoplasmic inclusion bodies	Unknown materials in the cytoplasm of hepatocytes
Sinusoidal dilation/congestion/hemorrhage	Dilation of sinusoidal spaces due to congestion or hemorrhage
Glycogen depletion	Decreased size of hepatocytes, loss of the ‘lacy’, irregular, and poorly demarcated cytoplasmic vacuolation typical of glycogen, and increased cytoplasmic basophilia (i.e., blue coloration)
Gill	
Epithelial cell necrosis	Cells having hyperchromatic nuclei and eosinophilic (i.e., pink coloration) granular cytoplasm. Some necrotic cells have pyknotic nuclei and varying degrees of nuclear karyolysis and karyorrhexis
Aneurysm	Focal dilation of lamellar capillaries associated with epithelial and pillar cell necrosis and thromboses. Swollen lamellae packed with red blood cells
Secondary lamellar fusion	Fusion of lamellae resulting from epithelial, ionocyte, and mucus cell hyperplasia
Epithelial cell hyperplasia /hypertrophy	Proliferation of epithelial cells or enlarged epithelial cells in the lamellar epithelium
Secondary lamellar edema	Focal dilation or swelling of lamellae associated with hydropic vacuolation of epithelial cells
Ionocyte hyperplasia/hypertrophy	Proliferation of or enlarged ionocytes. Ionocytes (n=1-2) usually located at the junction between the filament and lamella have proliferated (n>5) and migrated to the tips of lamellae and occasionally cover the entire lamellae
Mucus cell hyperplasia	Proliferation of mucus cells. Mucus cells which are rarely seen in healthy gills have proliferated at the junction of filament and lamellae and occasionally cover the entire lamellae
Inflammation	Lymphocytes and eosinophils located in submucosal interstitial tissues near the tips of lamellae. May also be observed within epithelia of lamellae and gill arches

Notes: Liver lesion score included a summation of all of the liver lesions except glycogen depletion, which was analyzed separately. Gill lesion score included a summation of each of the gill lesions.

Statistical Analysis

Three response variables were examined in the study: liver lesion score, liver glycogen depletion, and gill lesion score. Liver and gill lesion scores were analyzed separately because gills are in direct contact with the water and therefore exhibit more rapid responses to stressors than livers (Mallatt, 1985). Liver lesion score was the summation of the scores of the six liver lesions that were observed during the study (each liver lesion in Table 2-1 except glycogen depletion), gill lesion score was the sum of the eight gill lesion scores in Table 2-1, and liver glycogen depletion was analyzed on its own. A common response of fish liver to toxicity is a loss of hepatic glycogen (Hinton and Laurén 1990; Wolf and Wolfe. 2005). However, glycogen depletion can also be indicative of food limitation or physicochemical stress, so glycogen depletion was analyzed separately from the liver lesions (Adams et al 1992). We analyzed each of the three variables using model comparison to identify and quantify the drivers of each response.

Six variables were used as predictors in the analysis: year-class, region, fork length, salinity, mean monthly outflow, and X2 (averaged from June 1 – Dec 31). X2 is defined as the tidally averaged distance from the Golden Gate Bridge (i.e., Pacific Ocean) to 2 bottom salinity isohaline (Kimmerer 2002; Figure 2-1). We were interested in year-class because Delta Smelt exhibits substantial variation in interannual abundance, related to environmental variation (e.g., Hamilton and Murphy 2018), which may be reflected in its histological condition. The region variable was included to ask whether the regional pattern detected in our previous work persisted through time (Hammock et al. 2015). Fork length was included because fish accumulate lesions through their lives and lesions are more frequent in older fish (Bernet et al. 1999). We included salinity because we expected that freshwater inputs to the SFE were more contaminated than the Pacific Ocean given our previous results (Hammock et al. 2015). Outflow was included because contaminants can both increase or decrease with flow, depending on the contaminant, substrate, and time since the last storm (e.g., Bertrand-Krajewski et al. 1998, Lee et al. 2002). We included X2 as a more stable indicator of water year type (wet vs. dry) than mean monthly outflow. It is akin to the year-class variable in that it groups all fish from the same year class together but is distinct from year-class because it is a continuous variable that describes the hydrodynamic conditions experienced by each year-class.

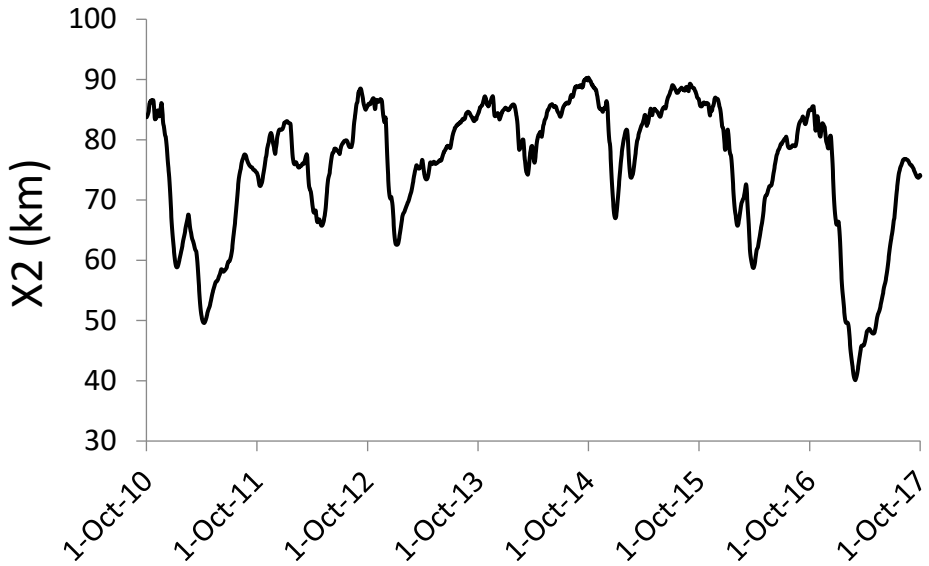
To make the year-class variable, we assigned individuals to year-classes based on the date of collection and fork length. There was little ambiguity assigning individuals to year-classes because Delta Smelt typically live for one year (Bennett 2005), and there is a large size difference between juveniles collected in June (~20-40 mm) and adults from the previous year-class (~60-80 mm). For example, the 2012/13 year-class included all Delta Smelt collected from June 2012 (juveniles) through May 2013 (adults), except for one 82 mm individual collected in June that was assigned to the 2011/12 year-class. To make the region variable, Delta Smelt were divided among five regions based on collection location. Regions included Cache Slough (C. Slough), the Sacramento River Deep Water Shipping Channel (SRDWSC), Confluence (Conf.), Suisun Bay (S. Bay), and Suisun Marsh (S. Marsh). These regions encompass different habitat types, salinities, distinct geographic regions, and stressors (map, descriptions, and justification in Hammock et al. 2015). Models included salinity as a continuous variable or as a dummy variable (<0.55 salinity: fresh, >0.55 salinity: brackish). Tidally averaged monthly flow at Chipps Island during the month that each fish was collected was used as the outflow variable (https://water.ca.gov/Programs/Environmental-Services/Compliance-Monitoring-And-

Assessment/Dayflow-Data). Finally, each fish in the same year-class was assigned the same X2 variable, consisting of mean daily X2 from June 1 through Dec 31 of each year. This period is meant to roughly encompass the bulk of the maturation period of Delta Smelt, from juvenile to adult.

The same set of 13 models fit to the liver lesion score data were fit to the liver glycogen depletion data (Tables 2-4 and 2-5, respectively). The structures of the first eight models fit to the liver and gill lesion scores were identical (Table 2-4, 2-6). The other three models fit to the gill lesion score results (models 9-11) were included based on a divergence in useful predictors of gill and liver lesion score. All liver lesion, gill lesion, and glycogen depletion models had negative binomial distributions to account for over-dispersion and because the response variables were integers from 0-10 (McElreath 2016). The models were fit using the 'glm.nb' command in the program R. The partial residuals for each response variable of highly-ranked models were plotted using the package 'visreg' to show the influence of each variable (Breheny and Burchett 2013). Effect sizes were calculated for selected models using the 'predict.glm' function in R. Continuous variables were set to their means to estimate the effect size of other variables set at their minimums and maximums. Discrete variables were set at mid-range levels to estimate effect sizes of other variables in the models (e.g., region and year-class).

Results

The study period included an extraordinarily dry period (2012-2015) that was bracketed by wet years in 2011 and 2017 (Figure 2-1). A total of 1,053 Delta Smelt were examined and 65.6% of the fish had at least one liver or gill lesion (Table 2-2).



Notes: X2 is the tidally averaged distance from the Golden Gate Bridge to the 2 ppt bottom salinity isohaline (Kimmerer 2002). The data reflect an extremely dry period in California bracketed by extremely wet years in 2011 and 2017. Data are from Department of Water Resources Dayflow website.

Figure 2-1. X2 Location Over the Study Period

Table 2-2. Sample Size of Delta Smelt by Region and Year-Class (n=1053).

Region	Year-class							Total
	2011/12	2012/13	2013/14	2014/15	2015/16	2016/17	2017/18	
Cache Slough	35	23	15	4	3	4	0	84
Confluence	22	82	13	134	11	14	64	340
SRDWSC	13	128	38	40	34	14	3	270
Suisun Bay	66	12	25	15	0	4	45	167
Suisun Marsh	29	67	51	25	7	5	8	192

Notes: SRDWSC is the Sacramento River Deep Water Shipping Channel

Table 2-3. Prevalence (%) and Mean Score of Delta Smelt Lesions Overall, by Region, and by Year-Class

		Region					Year-class						
Lesion	Prevalence (%)	C. Slough	SRDWSC	Conf.	S. Bay	S. Marsh	2011/12	2012/13	2013/14	2014/15	2015/16	2016/17	2017/18
Liver glycogen depletion	66.29	2.02	1.88	1.84	2.11	1.61	2.24	1.81	1.46	1.71	1.85	1.68	2.33
Gill ionocyte hyperplasia	31.72	0.83	0.99	0.68	0.22	0.63	0.42	0.95	0.47	0.77	1.09	1.49	0.03
Liver lipidosis	31.62	0.54	0.52	0.36	0.34	0.58	0.43	0.75	0.27	0.30	0.40	0.61	0.16
Gill aneurysm	18.71	0.19	0.23	0.25	0.26	0.19	0.28	0.29	0.16	0.22	0.22	0.17	0.15
Liver inflammation	6.74	0.13	0.07	0.09	0.14	0.02	0.14	0.02	0.09	0.15	0.09	0.02	0.03
Gill epithelial cell hyperplasia/hypertrophy	4.75	0.01	0.05	0.12	0.01	0.03	0.02	0.02	0.00	0.05	0.29	0.00	0.23
Liver macrophage aggregate	3.61	0.07	0.04	0.04	0.06	0.04	0.07	0.03	0.04	0.06	0.05	0.00	0.03
Liver sinusoid congestion	3.23	0.06	0.05	0.05	0.07	0.04	0.05	0.08	0.02	0.05	0.00	0.02	0.04
Liver single cell necrosis	3.04	0.06	0.04	0.04	0.03	0.01	0.07	0.04	0.01	0.01	0.04	0.00	0.04
Liver cytoplasmic inclusions or eosinophilic protein droplets	2.47	0.01	0.02	0.04	0.07	0.02	0.04	0.01	0.01	0.09	0.00	0.10	0.01
Gill fusion	0.95	0.02	0.01	0.00	0.01	0.02	0.02	0.03	0.00	0.00	0.00	0.00	0.00
Gill mucus cell hyperplasia	0.85	0.02	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.02	0.07	0.00	0.00
Gill secondary lamela edema	0.85	0.00	0.01	0.02	0.01	0.00	0.05	0.01	0.00	0.00	0.00	0.00	0.00
Gill inflammation	0.76	0.05	0.01	0.01	0.00	0.00	0.01	0.02	0.02	0.00	0.00	0.00	0.00
Gill epithelial cell necrosis	0.19	0.00	0.00	0.01	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.00

Notes: The first column is prevalence, all other columns are mean scores. Prevalence was calculated as the number of fish with scores >1 divided by the total number of fish for glycogen depletion and ionocyte hyperplasia (which can occur in response to very minor stress), all other lesions were calculated as >0.

Table 2-4. Model Comparison for Liver Lesion Score

Model #	Model	ΔAIC_c	df	AIC_c wt
11	~FL + Out + Reg + YC	0.0	14	0.6
12	~FL + Reg + YC	0.8	13	0.4
10	~FL + Out + Reg	61.4	8	<0.001
9	~FL+ Out + SalDum	69.0	5	<0.001
8	~FL+ Out	70.1	4	<0.001
7	~FL + Out + Sal	71.7	5	<0.001
4	~FL + Reg	75.5	7	<0.001
13	~FL + Reg + X2	77.1	8	<0.001
5	~FL + Reg + SalDum	77.3	8	<0.001
2	~FL	82.3	3	<0.001
3	~FL + SalDum	83.9	4	<0.001
6	~FL + Sal + Out	84.0	4	<0.001
1	~Intercept	178.4	2	<0.001

Notes: FL is fork length, Out is mean monthly outflow at Chipps Island (log_{10}-transformed), Reg is region (C. Slough, SRDWSC, Conf, S. Bay, S. Marsh), SalDum is salinity as a dummy variable (fresh/brackish), Sal is salinity as a continuous variable, YC is year-class as a factor, and X2 is mean X2 from June 1 to Dec 31 for each year class (continuous).
ΔAIC_c difference between model of interest and top-ranked model in Akaike Information Criterion Units corrected for small sample size, *df* degrees of freedom, *AIC_c wt* Akaike weight

Table 2-5. Model Comparison for Liver Glycogen Depletion

Model #	Model	ΔAIC_c	df	AIC_c wt
13	~FL + Reg + X2	0.0	8	0.58
12	~FL + Reg + YC	1.2	13	0.31
11	~FL + Out + Reg + YC	3.3	14	0.11
4	~FL + Reg	23.4	7	<0.001
5	~FL + Reg + SalDum	24.1	8	<0.001
10	~FL + Out + Reg	25.4	8	<0.001
2	~FL	32.4	3	<0.001
1	~Intercept	32.6	2	<0.001
3	~FL + SalDum	34.4	4	<0.001
6	~FL + Sal + Out	34.4	4	<0.001
8	~FL+ Out	34.4	4	<0.001
9	~FL+ Out + SalDum	36.4	5	<0.001
7	~FL + Out + Sal	36.4	5	<0.001

Notes: FL is fork length, Out is mean monthly outflow at Chipps Island (log_{10}-transformed), Reg is region (C. Slough, SRDWSC, Conf, S. Bay, S. Marsh), SalDum is salinity as a dummy variable (fresh/brackish), Sal is salinity as a continuous variable, YC is year-class as a factor, and X2 is mean X2 from June 1 to Dec 31 for each year class (continuous).
ΔAIC_c difference between model of interest and top-ranked model in Akaike Information Criterion Units corrected for small sample size, *df* degrees of freedom, *AIC_c wt* Akaike weight

Table 2-6. Model Comparison for Gill Lesion Score

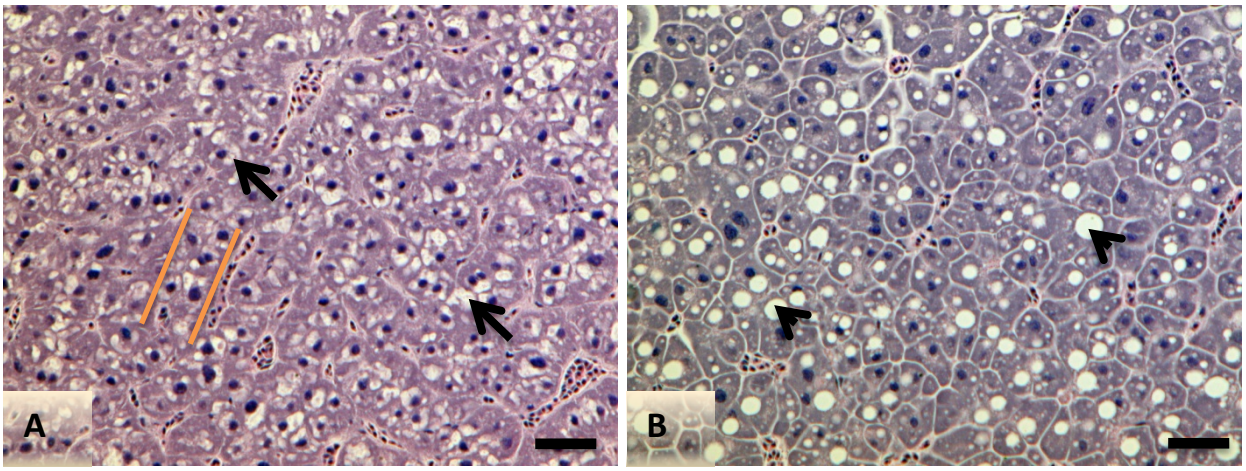
Model #	Model	ΔAICc	df	AIC_c wt
9	~FL + Sal + YC	0.0	10	0.9924
10	~FL + Sal + X2	9.8	5	0.0075
6	~FL + Sal	21.3	4	<0.001
11	~FL + Sal + Reg	22.7	8	<0.001
7	~FL + Sal + Out	22.8	5	<0.001
4	~FL + Reg	35.6	7	<0.001
5	~FL + Reg + SalDum	36.3	8	<0.001
3	~FL + SalDum	48.2	4	<0.001
2	~FL	72.4	3	<0.001
8	~FL + Out	73.2	4	<0.001
1	~Intercept	138.0	2	<0.001

Notes: FL is fork length, Out is mean monthly outflow at Chipps Island (log_{10}-transformed), Reg is region (C. Slough, SRDWSC, Conf, S. Bay, S. Marsh), SalDum is salinity as a dummy variable (fresh/brackish), Sal is salinity as a continuous variable, YC is year-class as a factor, and X2 is mean X2 from June 1 to Dec 31 for each year class (continuous).

ΔAIC$_c$ difference between model of interest and top-ranked model in Akaike Information Criterion Units corrected for small sample size, *df* degrees of freedom, *AIC$_c$ wt* Akaike weight

Liver Histopathology

The normal structure of the liver of Delta Smelt is lined with sinusoids and double rows of glycogen-rich hepatocytes organized into a tubular liver structure, the same as other teleosts (Akiyoshi and Inoue, 2004). 12.4% of individuals collected in this study presented normal livers exhibiting regular cells with a translucent, virtually unstained cytoplasm in which inclusions were absent. These clear-type hepatocytes observed in healthy livers stained with hematoxylin and eosin indicate good storage of glycogen (Figure 2-2, Panel A). Some level of glycogen depletion was observed in 85.2% of individuals, and 66.6% of individuals exhibited moderate or severe glycogen depletion (Table 2-3). Lipidosis/fatty vacuolation in hepatocytes was the most common liver lesion observed (31.6%; Figure 2-2, Panel B), followed by liver inflammation (6.7%; Table 2-3). Macrophage aggregates, single cell necrosis, cytoplasmic inclusions, and sinusoidal congestion were occasionally observed, with prevalence of <5% (Table 2-3).



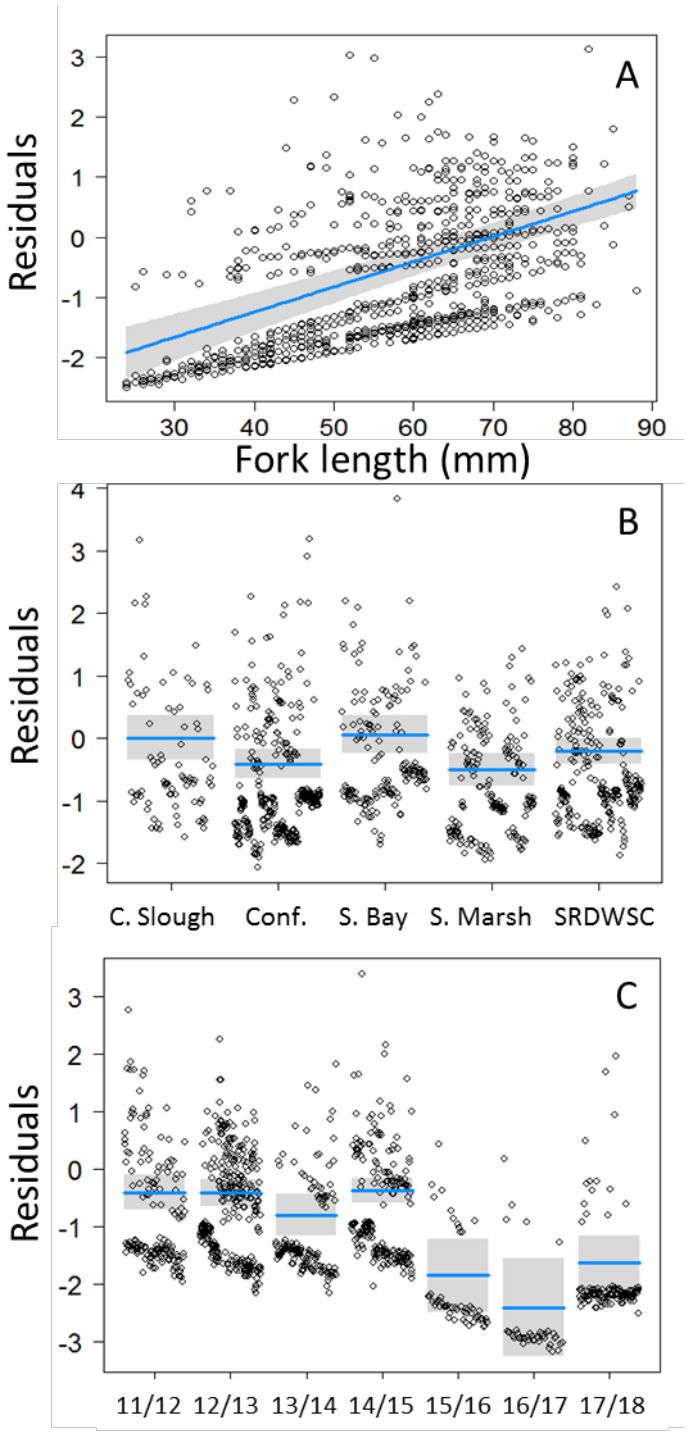
Notes: Liver Morphology is Lined with Rows of Organized tubular LIVER Structure (orange outlines). Panel B Shows a Glycogen Depleted Liver with Hepatic Lipidosis (intracellular lipid storage in large vacuoles, arrowheads) in Liver of a 2017 Wild Delta Smelt. The liver cells are smaller and more basophilic (bluish coloration) and the well-organized tubular liver structures are lost. H&E stain. Bar = 50µm

Figure 2-2. Panel A Shows a Normal Glycogen-Rich Liver (arrows) of 2017 Wild Delta Smelt

Liver Lesions Model Comparison

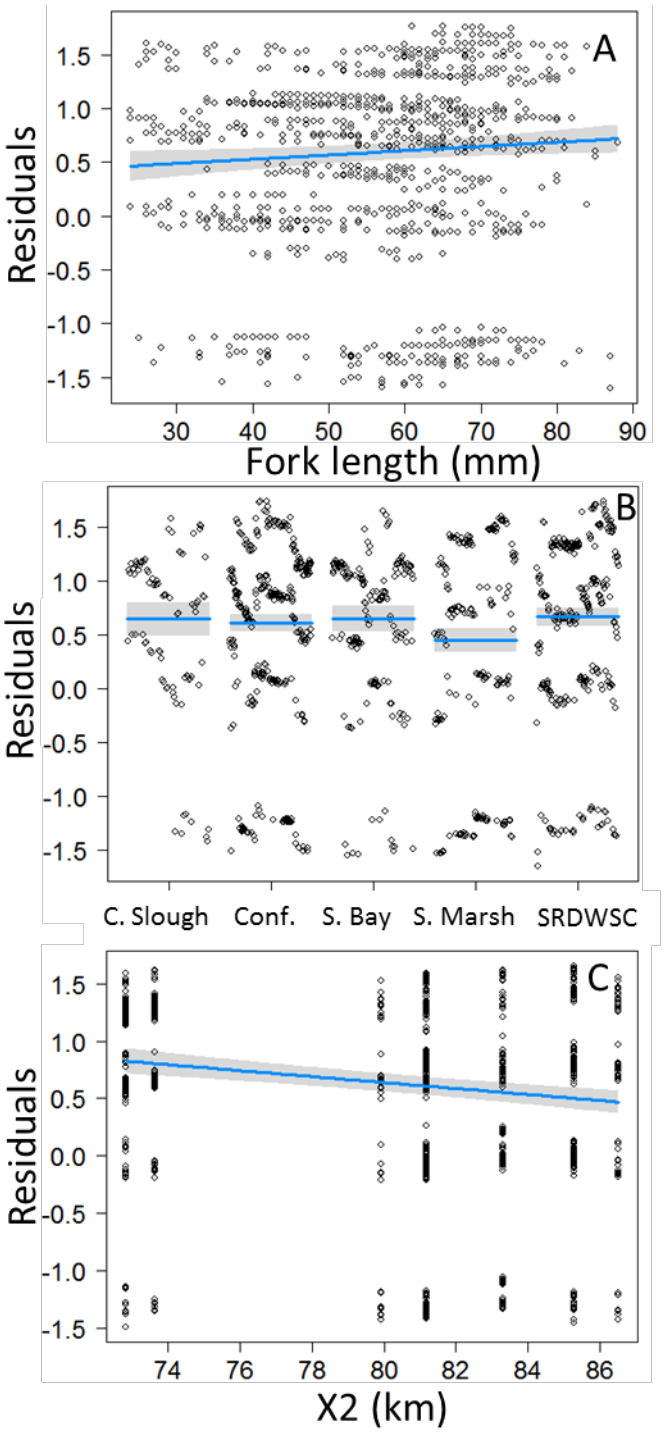
The top-ranked liver lesion model included fork length ($P < 0.0001$), outflow ($P = 0.09$), year-class ($P<0.0001$), and region ($P = 0.002$; Table 2-4). However, because the second-ranked model had very similar AIC_c weight than the top-ranked model but was more parsimonious, and outflow was non-significant, we selected the second-ranked model (although we note that the parameter estimate for outflow was negative [-0.33], Table 2-4). Based on the second-ranked model, liver lesion score increased with increasing fork length (Figure 2-3A), was highest in C. Slough and S. Bay and lowest in S. Marsh and the Conf. (Figure 2-3B). Liver lesion score peaked during the 2014/15 year-class and was lowest during the 2016/17 year-class (Figure 2-3C). Based on model predictions, as fork length increased from the minimum (24 mm) to the maximum (88 mm), liver lesion score increased 15-fold, from 0.181 to 2.636. Also based on model predictions, the highest mean year-class liver lesion score was 0.742 and occurred in the 2014/15 year-class; the lowest was 0.097 and occurred in the 2016/17 year-class, a 7.6-fold difference. Model predicted liver lesion score was highest in S. Bay (0.941) and C. Slough (0.894), and lowest in S. Marsh (0.532).

The top-ranked liver glycogen depletion model included fork length ($P = 0.022$), X2 ($P < 0.0001$), and region ($P = 0.039$; Table 2-5). Glycogen depletion increased with increasing fork length (Figure 2-4A), was lowest in S. Marsh (Figure 2-4B), and decreased with increasing X2, meaning that Delta Smelt exhibited livers that were richer in glycogen under drier conditions (Figure 2-4C). Increasing fork length from the minimum to the maximum increased glycogen depletion 1.29-fold, from 1.72 to 2.22. The lowest model estimated glycogen depletion was 1.58 in S. Marsh, while all the other regions had higher estimates of glycogen depletion. These estimates ranged from 1.85 in the Conf. to 1.96 in the SRDWSC. As X2 increased from the minimum to the maximum, model estimated glycogen depletion decreased 1.43-fold, from 1.67 to 2.39.



Notes: The grey bands show the 95% confidence interval.

Figure 2-3. The Partial Residuals from the Second-Ranked Liver Lesion Model by Fork Length (A), Region (B), and Year Class (C [e.g., 11/12 refers to the 2011-12 year-class]; Table 2-4)

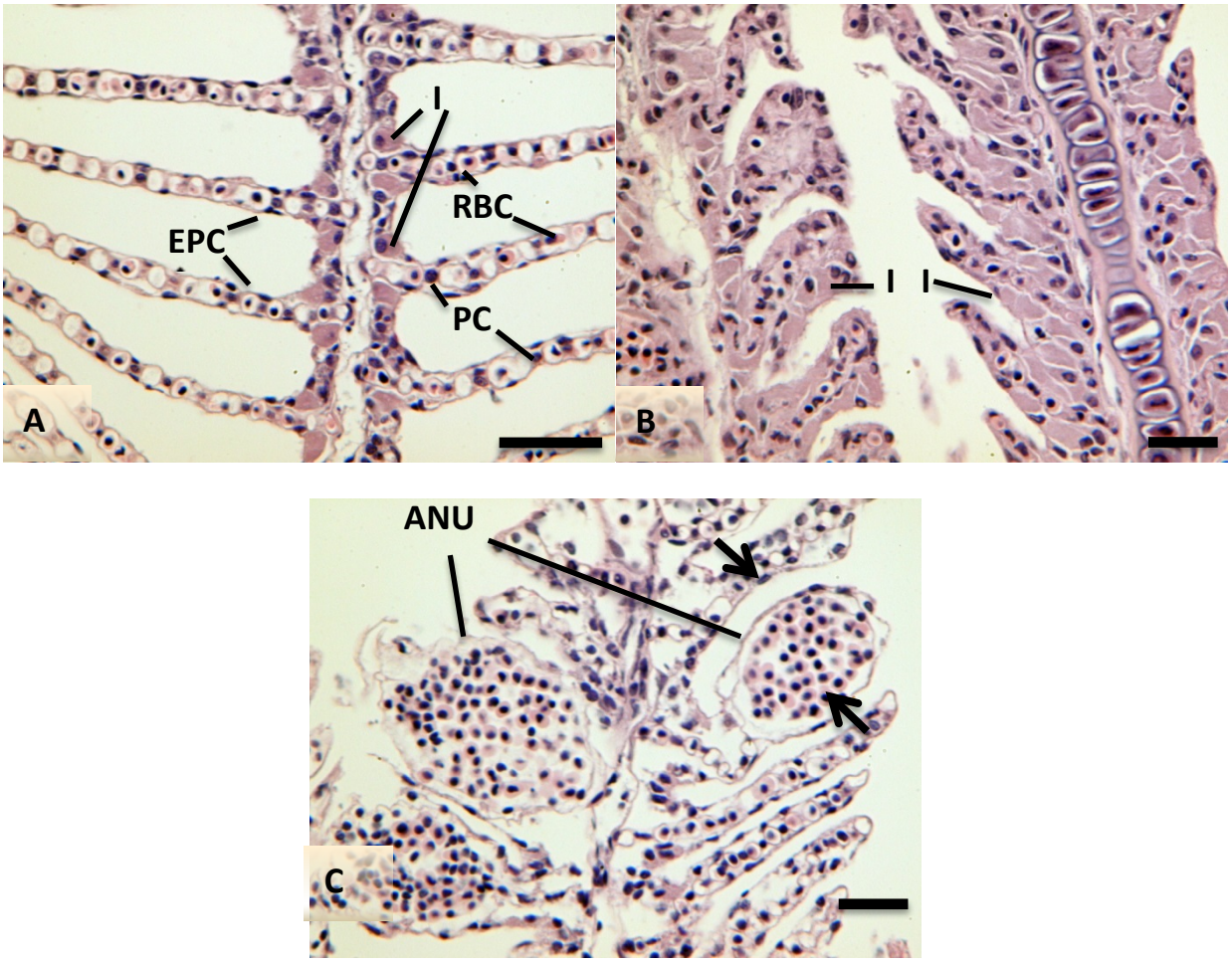


Notes: X2 is the tidally averaged distance from the Golden Gate Bridge to the 2 ppt bottom salinity isohaline (Kimmerer 2002). The grey bands show the 95% confidence interval.

Figure 2-4. The Partial Residuals from the Top-Ranked Liver Glycogen Depletion Model by Fork Length (A), Region (B), and X2 (C; Table 2-5)

Gill Histopathology

The gill structure of Delta Smelt is comparable to that of most teleosts, consisting of a filament and double row of thin leaf-like secondary lamella (Wilson and Lauren 2002). The secondary lamellae are mainly composed of two epithelial sheets joined together by pillar cells. Ionocytes, leukocytes, mucus and epithelial cells are usually located at the junction between the filament and secondary lamellae (Figure 2-5A). In this study, the most common gill lesions in individual fish were ionocyte hyperplasia/hypertrophy (31.7%, Figure 2-5B) and gill aneurysm (18.7%; Figure 2-5C, Table 2-3). Ionocyte hyperplasia was most prevalent in the 2012/13 (43.9%), 2014/2015 (37.6%), 2015/2016 (52.7%), and 2016/17 (73.2%) year classes (Table 2-3). Gill epithelial cell hyperplasia (25.5%) was most prevalent in the 2015/2016 year-class.

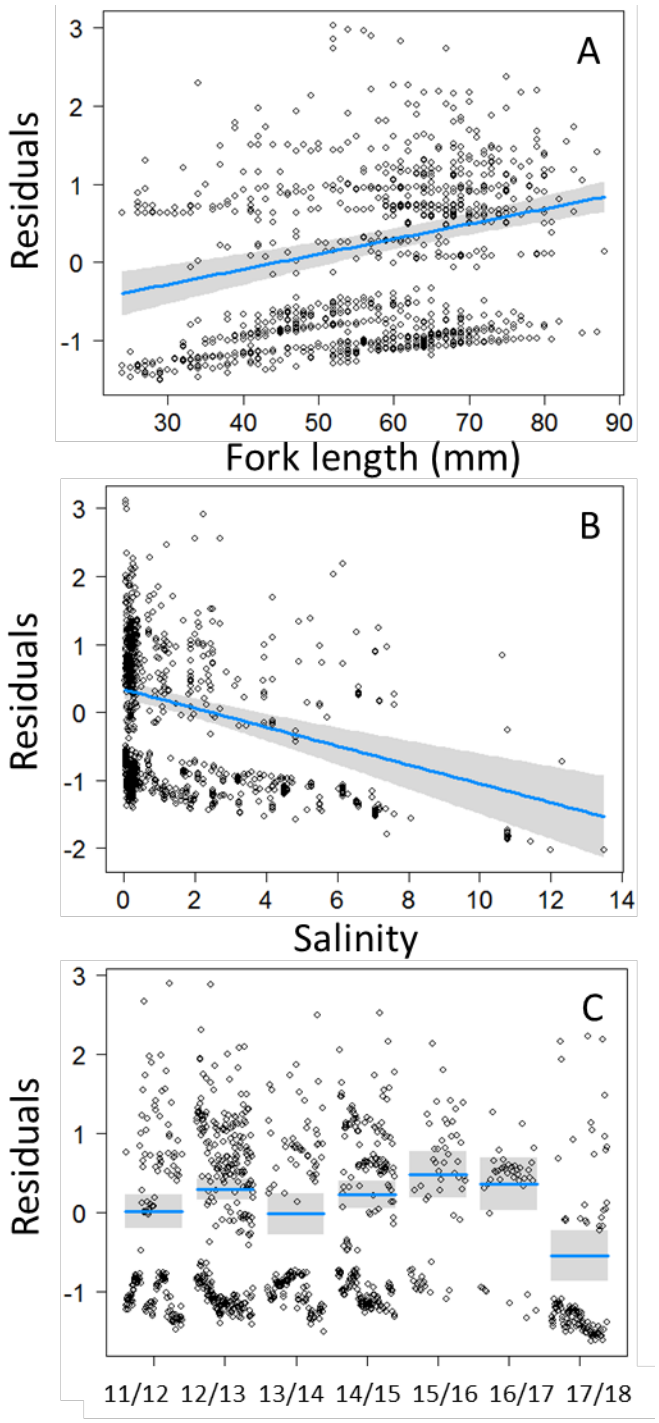


Notes: One or two ionocytes (I) are usually located at the junction between the filament and lamella. The secondary lamellae of the gill filaments are the sites of gas exchange and are mainly composed of two epithelial sheets (EPC) joined together by pillar cells (PC). Red blood cells (RBC) flow through channels formed by walls of pillar and epithelial cells where gas exchange occurs. Panel B shows severe ionocyte hyperplasia in a Delta Smelt collected in 2017. Note that the entire secondary lamella is covered by proliferated ionocytes. Panel C shows severe aneurysm (ANU) in the secondary lamellae of 2017 wild Delta Smelt. Aneurysm is caused by pillar cells necrosis resulting in excessive localization of blood cells in the secondary lamallae. Note that the epthelial cell wall (arrows) remain intact. H&E stain. Bar = 30µm.

Figure 2-5. Panel A Shows a Normal Gill Morphology of a Delta Smelt Collected from the 2017-18 Year-Class

Gill Model Comparison

The top-ranked gill lesion model included fork length ($P < 0.0001$), salinity ($P < 0.0001$), and year-class ($P < 0.0001$; Table 2-6). Gill lesion score increased with increasing fork length (Figure 2-6A) and decreased with increasing salinity (Figure 2-6B). The lowest lesion score, after accounting for fork length and salinity, occurred in the 2017/18 year class (Figure 2-6C). Based on model predictions, increasing fork length from the minimum to the maximum increased gill lesion score 3.4-fold, from 0.56 to 1.94. Increasing salinity from the minimum to the maximum decreased gill lesion score 6.5-fold, from 1.32 to 0.20. After accounting for the influence of fork length and salinity, the highest gill lesion score (1.28) occurred in the 2015/16 year-class, and the lowest (0.46) occurred in the 2017/18 year-class, a 2.8-fold difference.



Notes: The grey bands show the 95% confidence interval.

Figure 2-6. The Partial Residuals for the Top-Ranked Gill Lesion Score Model by Fork Length (A), Salinity (B), and Year Class (C [e.g., 11/12 refers to the 2011-12 year-class]; Table 2-6)

Discussion

This seven-year study is the longest-term health assessment of the endangered Delta Smelt. Typically, the goal of histological studies is to identify environmental stressors and to quantify the spatial and temporal extent of their effects. As such, the health of the study species itself tends to be secondary in importance to what its health tells researchers about the ecosystem. Ideally, these 'indicator species' are widespread, abundant, occupy high trophic levels, are relatively sessile (so that an individual's health reflects local conditions), and are tolerant of a wide range of environmental stressors (contaminants, water quality, and pathogens; e.g., Goede and Barton 1990; Adams et al. 1990; Teh et al. 1997; Schwaiger et al. 1997). The latter point is especially important so that stressors manifest themselves as sublethal, observable effects, rather than killing the fish and causing the information on its condition to be lost. Thus, as a small, vagile, rare, delicate fish that feeds on zooplankton and larval fish, the Delta Smelt is not an ideal indicator species. It was selected for this study because of interest in conserving and recovering the species itself, not for its traits as an indicator species. Consequently, the histological data are relatively difficult to interpret, and the traits of the species must be considered when interpreting its histopathology.

Overall, the majority of Delta Smelt in the study exhibited at least one gill or liver lesion, providing evidence that contaminants are one of the multiple stressors (Sommer et al. 2007) affecting Delta Smelt. Delta Smelt showed a marked improvement in liver health as a severe drought progressed in California, albeit with an apparent time lag in the response (2012-2016: Figure 2-1, 2-3C). The improvement in liver condition occurred both because individuals with unhealthy livers were less prevalent than during previous years, and because individuals with livers in the best condition exhibited improved liver condition compared to previous years (Figure 2-3C). We propose two nonexclusive interpretations. Given that the individuals with the highest liver lesion scores were absent during the latter years of the drought, we suggest that the least healthy individuals were not able to persist under the stressful conditions (e.g., high temperatures, scarce prey, high salinities), and were therefore not sampled. For the healthiest individuals, liver health may have improved due to improved water quality due to reduced runoff (e.g., Sansalone and Buchberger 1997). Whatever the causes, improving liver health was not a positive sign for the population as the severity of the drought increased, since the population reached historical lows even as liver health improved (http://www.dfg.ca.gov/delta/data/fmwt/indices.asp).

Fork length was the most important predictor of gill and liver condition (Tables 2-4 through 2-6), with larger individuals exhibiting gills in the poorest condition, and the most lesion laden and glycogen depleted livers. We suggest two non-exclusive possibilities to explain this pattern. Larger individuals may be less likely to succumb to poor health, and therefore better able to persist in sub-optimal condition (e.g., Capkin et al. 2006). In addition, larger, presumably older individuals have had a longer period of exposure and therefore more time to accumulate lesions than smaller, younger individuals. These relationships are consistent with previous work showing that larger fish exhibit a higher prevalence and severity of lesions (Bernet et al. 1999).

The major function of fish gill ionocytes (also known as chloride or mitochondria-rich cells), is ionic regulation and ammonia excretion (Perry 1997). Salinity was a better predictor of gill lesions than region, and the gill lesion with the highest prevalence was ionocyte hyperplasia

(32% of fish had moderate or severe ionocyte hyperplasia, Table 2-3). Ionocyte hyperplasia has been reported in fish migrating from saltwater to freshwater (Hirai et al 1999), freshwater to saltwater (Evans 1984) and following exposure to pollutants (Evans 1987). Thus, the relationship between salinity and gill lesion score suggests that some ionocyte hyperplasia represents a natural response of the fish to the salinity of its environment as it attempts to maintain its internal osmolarity (e.g., Hirai et al. 1999). Unlike pavement (respiratory) cells which are squamous, ionocytes are generally quite large. Therefore, ionocyte proliferation /hypertrophy on the lamellae (Figure 2-5B) results in the thickening of the epithelium, and, consequently, an increase in the blood to water diffusion distance. A gill aneurysm (Figure 2-5C) is formed due to weakening or necrosis of pillar cells resulting in blockage and excessive stagnation of blood. Ionocyte hyperplasia does not appear to be an entirely normal response of movement into freshwater (e.g., Hirai et al. 1999) as most instances of the proliferation resulted in the gill structure appearing abnormal, indicating that the organ may be impaired. This may suggest that contaminants may be more of an issue in freshwater. Gill aneurysm is a common response of fish exposed to aquatic pollutants (Meyers and Hendricks 1985; Evans 1987). Both gill ionocyte proliferation and aneurysm can result in hypoxia, respiratory failure, and problems with ionic and acid base balance and thus affect the general health of fish. Finally, mobilization of energy reserves for repair and maintenance of gill and liver function in turn might contribute to reduced growth, survival, and reproduction and an increased susceptibility to disease (Adams et al. 1992).

Our previous work on juvenile Delta Smelt demonstrated that individuals collected from C. Slough had significantly more liver and gill damage than fish collected from other parts of the SFE, especially S. Marsh (Hammock et al. 2015). In this far larger study, which included 809 more individuals and five more year-classes, a similar pattern was found (Figure 2-3). Accounting for the strong influence of fork length and year-class, Figure 2-3 shows that the fish with the most damaged livers occurred in C. Slough (as before) and S. Bay, while the healthiest fish occurred in S. Marsh (as before) and the Confluence. Liver fatty degeneration observed as lipidosis was one of the most recurrent alterations found in the livers of Delta Smelt (Table 2-3). Hepatocellular lipidosis is associated with exposure to chlorinated hydrocarbons and other contaminants (Hinton et al. 1992), including PCBs (Teh et al. 1997; Anderson et al. 2003), crude oil extracts (Solangi and Overstreet 1982), metals (Arellano et al. 1999; Giari et al. 2007) and in feral fish from sites contaminated by mixtures of xenobiotics (Greenfield et al. 2008; Triebskorn et al. 2008). Driving mechanisms of lipidosis include toxic injury causing impaired lipid oxidation or protein synthesis, resulting in accumulation of triglycerides in hepatocytes. Alternatively, malnutrition may increase fat mobilization and impair apoprotein synthesis (Hinton and Laurén 1990). This study provides further evidence that the contaminants in C. Slough are affecting Delta Smelt (Hammock et al. 2015), though the habitat may provide mitigating benefits allowing the Delta Smelt population to persist there despite the contaminant inputs (Werner et al. 2000, Kuivila and Moon 2004; Weston et al. 2014).

In addition to having fish with the lowest liver lesion score, fish collected from S. Marsh showed the most glycogen rich livers. The loss of hepatic glycogen can occur as a direct toxic effect of contaminants (Schwaiger et al. 1997, Teh et al. 1997), or as a result of reduced health condition caused by nutritional or physicochemical stress. Thus, the presence of fish with glycogen rich livers in S. Marsh suggests some combination of the following: relatively low metabolic rates, low contaminant exposure, low environmental stress, and abundant food. The latter point is possibly related to the quantity of tidal wetlands in the region (Matern et al. 2002, Hammock et

al. 2019), which are generally productive habitats (Shaffer and Sullivan 1988, Beck et al. 2001). Given the relatively good liver condition of fish collected from S. Marsh, and population collapse during the drought when the region became too saline, access to the S. Marsh region appears to be important for the persistence of Delta Smelt (Feyrer et al. 2011).

Overall, regional patterns of lesions suggest that S. Marsh was less stressful because Delta Smelt exhibited reduced lesion prevalence. However, by the same rationale, the drought years of 2015 and 2016 were less stressful because liver lesion scores were low, but there is reason to suggest an alternative interpretation. Due to the reduced abundance and stressful ambient conditions (e.g., high salinity) one possibility is that the reduction in lesion score may indicate that conditions were too stressful for individuals with even moderate cellular damage (as detailed by histopathology) to persist. This difficulty—is an unhealthy fish a good sign for a region or year-class because it shows that an unhealthy fish can persist, or a bad sign because it is unhealthy—suggests that lesion severity and prevalence requires a more thorough evaluation of other factors to draw more definitive conclusions. Regardless of the interpretation, the prevalence of lesions suggests that contaminant impacts are harming Delta Smelt, with multiple instances of lesions that likely reduce survival.

We conclude that histopathology is a useful tool for assessing the health of the Delta Smelt, given that the results are consistent with contaminant exposure. Consistent with our previous work, S. Marsh continues to appear to be favorable habitat when available to Delta Smelt (i.e., not too saline), as fish show relatively low liver lesion scores and rich liver glycogen, combined with relatively full stomachs (Hammock et al. 2015). The livers of fish in C. Slough and S. Bay were damaged, suggesting contaminant exposure. However, despite the intense interest in conserving the species, the traits of Delta Smelt (rare, vagile, delicate) are not ideal for its use as an indicator species for monitoring water quality in the highly altered SFE. Multiple variables should therefore be considered, including the condition of the indicator species, its population dynamics, additional indicator species if possible (e.g., *Tridentiger bifasciatus, Gasterosteus aculeatus*), and the ambient and antecedent environmental conditions. Ideally, future analyses will add to this continuous dataset, will account for the complex interactions of biotic and abiotic factors involved, and include a true indicator species in the study.

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Chapter 3: Exploring Life History Diversity of Delta Smelt During a Period of Extreme Environmental Variability

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Abstract

The Delta Smelt (*Hypomesus transpacificus*) was once an abundant pelagic forage fish endemic only to the tidal reaches of the Sacramento-San Joaquin Delta and served as the proverbial "Canary-in-the-Coal-Mine" for ecosystem health, but is now rarely observed in long-term monitoring surveys, signifying its trajectory towards extinction in the wild. Its demise has been attributed to numerous human-caused impacts that have befuddled resource managers and stakeholder alike. The species has been described as semi-anadromous, rearing in brackish waters from summer through late fall before migrating to freshwaters in the winter months prior to spawning in the spring, however; individuals have also been observed rearing in freshwater year-round and hatching into low-salinity habitats. The spawning period occurs from March through June and is dictated by the duration of suitable temperatures for maturation and hatching. In this study, we use otolith microstructure and otolith microchemistry to explore variability in life history attributes (hatch-date, natal origin, dispersal history and life history phenotype) over a period of extreme environmental variability to further our understanding of the adaptive response of Delta Smelt life histories to climate change and management actions. Delta Smelt hatch-dates and dispersal dates from freshwater to the low-salinity zone were driven by water temperature, with hatching ceasing and dispersal occurring when temperatures exceeded 20°C in freshwater habitats. Hatch-dates and dispersal dates occurred earlier during the 2012-2016 and the wet year of 2017. Despite years with extreme flow and temperature variability, the majority of Delta Smelt hatched into freshwater habitats with isotopic signatures consistent with natal origins in the North Delta, the semi-anadromous form of the life history dominated the population structure and the freshwater resident form persisted despite warm summer water temperatures. While Delta Smelt exhibited significant life history diversity in this study, this diversity appeared to have not resilience in 2017.

Introduction

Diverse life history strategies in fishes have evolved to optimize reproduction and survival in response to spatially and temporally variable environments (Stearns 1992). Species with complex life cycles produce many small, vulnerable offspring that disperse from spawning sites to nursery

habitats of variable and often unpredictable quality. Moreover, prey availability can be temporally variable, thus species with complex life cycles are burdened with the responsibility of placing young in the right place at the right time to ensure population persistence. However, predicting when and where nursery habitats will be productive is challenging, thus mortality is naturally high in early life stages, leading to stochastic recruitment to adulthood (Hjort 1914). Species have developed a diversity of life history strategies to contend with spatially and temporally variable nursery habitats, such as spreading reproductive effort over a prolonged season ensuring some offspring hatch during productive periods or spawning across multiple habitats insuring some offspring will be dispersed or occur in productive nurseries. Such strategies can occur due to reproductive phenotypic plasticity in response to environmental variability or as a bet-hedging strategy.

The Delta Smelt (*Hypomesus transpacificus*) was historically an abundant pelagic, euryhaline species, endemic to the tidal freshwaters of the Sacramento-San Joaquin Delta and brackish habitats of the upper San Francisco Estuary (Moyle et al. 1992). The population declined in the 1980s, and was first listed as threatened under the California (CESA) and Federal Endangered Species Acts (FESA) in 1993 (USFWS 1993); it was subsequently up-listed to Endangered under the CESA in 2009 and warranted but precluded for federal endangerment in 2010 (CFG Commission 2009; USFWS 2010) following a second step-change decline in the early 2000s (Mac Nally et al. 2010; Thomson et al. 2010). While the ultimate cause of this continued decline is not known with certainty, multiple proximate factors have been considered important in causing the Delta Smelts decline including, reduced freshwater flows into the estuary, alteration of the historic food web by invasive species and declining preferred herbivorous zooplankton, loss of turbid low-salinity nursery habitat, contaminants and entrainment into the massive pumping facilities in the South Delta (Moyle et al. 2016a). To protect the species from entrainment into the State Water Project (SWP) and Central Valley Project (CVP), pumping is limited at times when Delta Smelt are near the intakes which frequently interrupts freshwater deliveries to a multi-billion-dollar agriculture industry and urban sector exceeding 25 million people, making the protection of the species highly controversial (Moyle et al. 2016).

Delta Smelt have a complex life cycle adapted to take advantage of high spatial and temporal variability that occurred in the historic Delta (Moyle et al. 2016). The Delta was once a mosaic of river channels, backwater sloughs, tidal wetlands, floodplains, and riparian forests formed at the confluence of two major tributaries of the Central Valley, one flowing from the north, the Sacramento River and from the south the San Joaquin River (Whipple et al. 2012). The hydrographs of these two rivers varied providing spatial and temporal heterogeneity. The Sacramento River typically peaked earlier in the year due to precipitation as runoff, while the San Joaquin peaked later due to snowmelt-based runoff due to the higher elevation of the Sierra Nevada mountains in the southern Central Valley. The region occurs within a Mediterranean climate and is subject to extreme environmental variability, with cool wet winters and warm dry summers. Precipitation is highly variable, the Delta experiences greater inter-annual variability than the rest of the United States, with most of the precipitation arriving as atmospheric rivers (Dettinger 2011; Dettinger 2013). In years of extreme precipitation, much of the Delta and San Francisco Bay was fresh for extended periods of time, while in dry periods brackish water would move upstream as far as the city of Sacramento in the fall. However, this variability no longer occurs due to construction and operations of several large dams that capture much of the runoff, eliminating peak flows during wet periods and regulating inflows during dry periods to limit

saltwater intrusion into the Delta, largely to protect the two large water projects pumping facilities located in the South Delta (Hutton et al. 2017a; Hutton et al. 2017b).

Delta Smelt have been described as semi-anadromous, spawning in tidal freshwater regions of the Delta in spring and rearing in the LSZ from juvenile to sub-adult life stages in the summer-fall months before migrating back to freshwater in the late-fall and winter (Bennett 2005; Moyle et al. 2016a; Moyle et al. 1992). However, Delta Smelt have also been found in the tidal freshwaters of the North Delta year-round in recent monitoring surveys (Sommer and Mejia 2013; Sommer et al. 2011), and studies using otolith strontium isotope ratios have discovered freshwater and brackish water year-round residents in addition to the traditional semi-anadromous life history, suggesting Delta Smelt may spawn both in freshwater and brackish water and utilize freshwater nursery habitats found in the Delta in addition to the LSZ (Bush 2017).

Historically the LSZ was a zooplankton rich area of the estuary, and it was thought the migratory behavior of Delta Smelt was an adaptation to the spatial variability in prey availability (Moyle et al. 1992). However, the LSZ is no longer productive, phytoplankton blooms are now rare due in part to the invasion of a voracious filter feeder, the overbite clam (*Potamocorbula amurensis*), and rising ammonia concentrations from wastewater thought to be further suppressing phytoplankton growth (Dugdale et al. 2007; Glibert et al. 2014; Kimmerer et al. 1994). In addition, the food web for Delta Smelt has been altered by several invasive species of copepod that may be less nutritious or more difficult for Delta Smelt to capture (Kimmerer and Orsi 1996; Slater and Baxter 2014). The LSZ was also an area of high turbidity, described as the estuarine turbidity maxima (Bennett et al. 2002; Kimmerer et al. 1998). Turbidity is an important environmental attribute that explains the distribution of Delta Smelt in monitoring surveys, and is thought to provide refuge from predation and visual contrast for foraging on small semi-transparent zooplankton (Bever et al. 2016; Feyrer et al. 2007; Moyle et al. 2016b). However, turbidity has also declined (Bever et al. 2018) in the LSZ, thus this habitat may no longer provide benefits to Delta Smelt.

Delta Smelt are predominately an annual species with a relatively protracted reproductive period (February-July), often lasting 4-6 months (Bennett 2005), thought to be an adaptation to the spatial and temporal variability in the river flows (Moyle et al. 1992). Females can produce multiple clutches of eggs within a spawning season (Damon et al. 2016; Kurobe et al. 2016), thus biotic and abiotic conditions encountered during maturation in the fall and winter can have large effects on total annual egg production and ultimately recruitment success. Water diversions have caused greater intrusion of saline water into the Delta, shifting the location of the LSZ upstream, into the deep channelized confluence region of the Sacramento and San Joaquin Rivers, reducing nursery habitat volume and quality for Delta Smelt (Kimmerer et al. 2009; Kimmerer et al. 2013). This erosion of abiotic habitat quality in the fall may limit growth and maturation of fish and lead to poor recruitment. Feyrer et al. (2007) found fall habitat suitability to be an important covariate in stock-recruitment models predicting successful recruitment to the next generation, however; more recent analyses do not provide support for the importance of fall habitat quality (ICF 2017).

Given the Delta Smelt's complex life history, environmental variability including fall abiotic habitat conditions may have significant effects on the many life history attributes. Temperature

in the fall has been hypothesized to influence maturation in Delta Smelt (Brown et al. 2016; IEP-MAST 2015). Several studies have documented acute lethal temperature and thermal stress thresholds for juvenile and adult Delta Smelt, establishing their thermal sensitivity to temperatures above 24 °C (Brown et al. 2016; Jeffries et al. 2016a; Jeffries et al. 2018; Komoroske et al. 2015) However; Delta Smelt experience high mortality when cultured for extended periods of time at or above 20 °C (Tien Chieh-Hung personal communication) and otolith growth studies (Hobbs CH 5 DOP report) suggests Delta Smelt grow poorly when inhabiting habitats above 20 °C, thus we defined the "Maturation Window" as the duration of time when the estuary was below 20 °C in the fall and 12 °C in the spring (Figure 3-1). The 12 °C cutoff for the Maturation Window was based on the mean temperature when first yolk-sac (~4-5 mm) Delta Smelt were encountered during larval surveys (CDFW 20-mm Survey). We also define a "Hatching Window", based on the duration between 12 °C and 20 °C in the spring, assuming poor survival for fish hatching into habitats above 20 °C (Figure 3-1). Summer temperatures are also likely to influence the life history of Delta Smelt. Summer temperatures can approach sub-lethal stress inducing levels causing fish to seek thermal refuge. Temperatures in freshwater habitats are typically warmer than in Suisun Bay in the summer and fall, thus Delta Smelt dispersal from freshwater natal habitats to the LSZ may be cued when temperatures exceed 20 °C.

In this study we used otolith microstructure and microchemistry to quantify key life history attributes (hatch dates, natal origins, dispersal and life history phenotypes) for Delta Smelt collected from 2011 to 2017. This time period captures a wet-cool year (2011) and a wet-warm year (2017) bracketing a period of extreme drought (2012-2016) and allows us to explore how environmental variability influences Delta Smelt life history. We predict the duration of maturation window and hatching window will have a positive effect on the duration of hatching, the thermal phenology (dates when temps surpass 12 and 20 °C) will correspond with hatch phenology, and the Julian date when spring temperatures exceed 20 °C dispersal will correspond with dispersal phenology. Lastly, we predict high flow years will have longer maturation windows and hatch windows due to the associated weather conditions during the winter and spring of high flow years, causing cooler air temperatures and water temperatures, and these patterns will result in overall greater life history diversity in wet years.

Methods

Sample Collection

Delta Smelt were collected by several agency partners from 2011 to 2017 for this study (Table S1). For each survey, fish were given a unique serial number upon capture, measured for fork-length and frozen in liquid nitrogen or preserved in 95% ethanol. Sagittal otoliths were dissected from the heads of Delta Smelt and stored dry in Thermo Scientific Cell Culture Plates. Before mounting, the membrane remains surrounding the otoliths were removed by soaking in 95% ethanol for a minimum of 24 hours. Once the membrane was removed, otoliths were mounted onto microscope glass slides with Crystal Bond® thermoplastic resin in the sagittal plane. Otoliths were sanded sulcus side up until the outermost rings were visible, turned and sanded with wet-dry sandpaper (Buehler 800 and 1200 grit) until the core rings were visible and then polished with a polishing cloth and 0.3-micron polishing alumina. Otoliths were digitized with a 12 Megapixel digital camera attached to an Olympus CH30 compound microscope at a

magnification of 20X, using AM Scope (*www.amscope.com*). Otolith increments were enumerated and the increment width and radial distance (µm) from the core to each daily ring was measured using Image-J NIH software *(http://imagej.nih.gov/ij/*). Aging transects occurred in the dorsal plane of the otolith approximately 90 degrees from the anterior-posterior axis. Each otolith was aged by a minimum of two independent readers and assessed for age agreement using an average percent error (APE) of <10% for quality assurance. If two age readings were greater than 10% APE, the otolith was read by a third, more senior age reader and reassessed. Hatch dates were calculated by subtracting the age from the capture date. Hatch dates where not calculated for fish collected from the SKT survey because fish at this time of year are forming an annulus, thus daily increments are no longer reliable.

Sample Analysis

Polished otoliths were mounted on petrographic slides (~20 per slide) for otolith microchemistry. Otolith strontium isotope ratios ($^{87}Sr/^{86}Sr$) were analyzed using established protocols at the UC Davis Interdisciplinary Center for Plasma Mass Spectrometry (Hobbs et al. 2010b; Hobbs et al. 2005b). In brief, a multi-collector inductively coupled plasma mass spectrometer (*Nu Plasma HR* from Nu Instrument Inc.) was interfaced with a Nd:YAG 213 nm laser (New Wave Research UP213) for *in situ* strontium isotopic measurement by laser ablation (LA-MC-ICP-MS). Helium was used as the carrier gas to maximize sensitivity and minimize sample deposition at the ablation site and was mixed with Argon gas between the laser sample cell and the plasma source, for better plasma stability. Gas blank and background signals were monitored until ^{84}Kr and ^{86}Kr stabilized after the sample change (i.e. exposing sample cell to the air) and were measured for 30 seconds and subtracted from the raw ratios. Strontium isotope ratios ($^{87}Sr/^{86}Sr$) were internally normalized by the measured $^{86}Sr/^{88}Sr$ ratio relative to assumed ratio of 0.1194, which corrects for mass discrimination. Rubidium on mass 85 was monitored to account for any ^{87}Rb interference on ^{87}Sr.

A laser beam of 40 µm diameter traversed across the otolith from ~100 µm before the core to the dorsal edge at 10 µm per second, with the laser pulsing at 10-Hz frequency resulting in 5-10 J/cm^2 photon output. Digital images of aging transects were used to place laser profiles along the transect used for age increment measurement to facilitate merging age and laser profiles to create an $^{87}Sr/^{86}Sr$ chronology. Processing of otolith chemistry data was performed offline using the IsoFishR application (https://github.com/MalteWillmes/IsoFishR) (Willmes et al. 2018). To minimize error in merging age increment and $^{87}Sr/^{86}Sr$ profiles, we transformed age and $^{87}Sr/^{86}Sr$ laser profiles from distance to core (µm) to proportional distances. Then we fit a cubic spline (df = 10) to the $^{87}Sr/^{86}Sr$ profile and predicted $^{87}Sr/^{86}Sr$ onto the age transect, resulting in a time series of $^{87}Sr/^{86}Sr$. An aragonite coral from the South China Sea, and Pacific Ocean-caught fish otolith (White Seabass) were analyzed with the same laser parameters at the beginning, middle, and end of each day with at least three replicates per standard. The analytical accuracy was evaluated by comparing the results of replicate analyses of the coral and otolith reference materials to the modern global mean seawater $^{87}Sr/^{86}Sr$ ratio of 0.70918 (McArthur 2010). All $^{87}Sr/^{86}Sr$ ratios measured on standards during the analyses in this study were within ± 0.0001 of the mean seawater value and thus no corrections were made to the otolith data.

Salinity Life History Reconstructions

The relationship between salinity and $^{87}Sr/^{86}Sr$ ratios of ambient water has been previously characterized (Hobbs et al. 2010b; Ingram and DePaolo 1993; Phillis et al. 2011), and the 1:1

incorporation of ambient $^{87}Sr/^{86}Sr$ into Delta Smelt otoliths has been established (Hobbs et al. 2005a). We used a strontium isotope ratio of $^{87}Sr/^{86}Sr = 0.7077$ to represent a threshold value between fresh (<0.5 psu) and low-salinity habitats in the upper San Francisco Estuary (for details see Supplemental Materials). Distinct life history patterns (phenotypes) were readily apparent upon visual inspection of $^{87}Sr/^{86}Sr$ chronologies. Freshwater resident fish had relatively flat chronologies with $^{87}Sr/^{86}Sr$ values <0.07077 from core to edge, semi-anadromous fish had chronologies that exhibited a sigmoid shape with $^{87}Sr/^{86}Sr$ <0.7077 in the early-life before exhibiting a distinct increase in $^{87}Sr/^{86}Sr > 0.7080$ and brackish-resident fish had chronologies that had high $^{87}Sr/^{86}Sr > 0.7080$ values across the entire profile. Classification of life history phenotypes was done only for adult life stage fish captured from January-May during the CDFW SKT-Survey. We did not classify sub-adult life stages collected in the fall months as in some years, fish were found to be moving from freshwater natal habitats to the LSZ in these months and classification of life history phenotype during this period could lead to incorrect classifications. The natal origins of juveniles and sub-adults were classified based on the salinity-Sr isotope relationships described above.

Environmental Data

We used water temperature recorded at 15-min intervals from five stations (Antioch-ANH, Deepwater Ship Channel-DWS, Mallard-MAL, Martinez-MRZ, Rio Vista-RVB) monitored by the California Department of Water Resources (CDWR) and archived on the California Data Exchange Center (https://cdec.water.ca.gov/) to calculate the mean daily temperature in the Delta Smelt's primary habitat. Freshwater flows to the estuary (Delta Outflow) was accessed from the DAYFLOW model, available (https://water.ca.gov/Programs/Environmental-Services/Compliance-Monitoring-And-Assessment/Dayflow-Data) which provides daily net Delta Outflows in cubic feet per second (CFS). Here we converted CFS to a volume using standard conversion procedures.

Results

Delta Smelt abundance rebounded to the highest levels since 2001 during the wet and relatively cool year of 2011, however; this recruitment success was wiped out during the summer of 2012 (Figure 3-2). Delta Smelt abundance remained low following 2012, which coincided with a prolonged and unprecedented level of drought conditions. Despite a return of wet conditions in 2017, the Delta Smelt population failed to rebound as it did in 2011.

Hatch

Delta Smelt collected during the study exhibited a prolonged period of hatching lasting on average ~ three months and began approximately 2-3 weeks following the last peak in Delta Outflow in 2011, 2016 and 2017. The earliest fish hatched was on Julian date 44 (Feb 13) in 2015 and the latest fish hatched was on Julian date 195 (July 14) in 2011, with mean hatching occurring from Julian date 78 to 137 (Table 3-1). Hatch distributions appeared to be multi-modal during several years (e.g. 2011) (Figure 3-3), but otherwise hatching was relatively continuous during the spring. Hatching phenology shifted earlier in time during the drought years and occurred on average 13-days earlier per year from 2011 to 2015 (Figure 3-3). Unfortunately, very few fish (N=13) were collected in 2016, precluding a reliable assessment of hatch distributions in that year (Table S1), however; in 2017, the mean date was approximately two

weeks later than the 2013-2015-time period (Table 3-1). The hatching phenology tracked the Julian date when temperatures surpassed 12 °C (Table 3-2), which occurred earlier during the drought years, and later during the wet years. However, from 2014-2016 hatching began 9-25 days after temperatures exceeded 12 °C (Figure 3-4). Warm summer conditions also persisted later during the drought, shortening the maturation windows in 2015 and 2016 by approximately 1-month (Table 3-2) corresponding with delayed hatching in those years (Figure 3-4.).

Dispersal

The phenology of temperature also appeared to be associated with the timing of Delta Smelt dispersal from freshwater to the LSZ (Figure 3-4). As with hatching, dispersal began earlier during the drought years, beginning approximately when temperatures exceeded 20 °C. Delta Smelt exhibited relatively broad distributions for dispersal dates, ages and lengths (Figure 5A-C). The mean Julian date of dispersal ranged from day 171 in 2014 to 221 in 2011 (Table 3-3) and occurred earlier during the drought (Figure 3-5A). The mean age at dispersal (aka, residence time in freshwater) varied from 71 days in 2014 to 116 days in 2016, the next longest freshwater residence time being 103 days in 2015 (Table 3-3). The mean lengths at dispersal ranged from 29-mm in 2014 to 44-mm in 2016, the next largest mean occurring in 2015 (42-mm).

Predictions

The Maturation Window had a strong positive effect on the hatch-date duration (Figure 3-6A), while there was no clear trend with the Hatch Window (Figure 3-6B). Hatching phenology was correlated with temperature, the Julian date when hatching began was positively correlated with the Julian date when temperatures exceeded 12 °C (Figure 3-6C). The Julian date when hatching ended was positively correlated with the Julian date of 20 °C (Figure 3-6D). Interestingly, the Julian date of hatch beginning was also positively correlated with the Maturation Window (Figure 3-6E), suggesting more complex interactions during the reproductive period may be influencing hatch phenology. Dispersal phenology was associated with temperature, the beginning of dispersal corresponded positively with the Julian date water temperatures exceeded 20 °C (Figure 3-6F). The years of high outflow (2011, 2017) corresponded with longer Maturation Windows and Hatch Windows, in part due to temperatures exceeding 12 °C later in those years.

Natal Origins

The vast majority of fish in all survey years had freshwater natal origins (Figure 3-7). The distributions in most years appeared to be continuous, however; in 2017 there appeared to be several modes of natal origin. Few fish hatched in habitats with very low-salinity (0.5 to 1 psu), and the LSZ (1-6 psu) in all years except 2015 and 2016. There did not appear to be a strong difference between Julian hatch-dates for fish with different natal origins, although hatching did appear to begin slightly earlier in freshwater habitats (Figure 3-8).

Life History Phenotype

The migratory phenotype was the dominant life history for adult Delta Smelt collected during the CDFW Spring Kodiak Trawl Survey between 2011-2017, but the freshwater resident life history type contributed 48% in the wet year of 2011 and 39% during the dry year of 2013, thus freshwater flow likely did not have a strong effect on the life history phenotype composition (Fig. 9). In each year, brackish resident fish were found but consistently contributed the fewest individuals to the adult population (Figure 3-9).

Discussion

Life history diversity within populations can have a stabilizing effect in species with complex life cycles by spreading the risk of catastrophic mortality across habitats and time (Hilborn et al. 2003; Kerr and Secor 2010; Schindler et al. 2010). Life history diversity in Delta Smelt appeared to be improved during the wet years, however; severe drought conditions from 2012-2016 appear to have eroded demographic resilience in 2017. From 2012 to 2017, abundance indices in long-term monitoring survey reached successively all-time lows, causing researchers and resource managers to question whether extinction was inevitable for Delta Smelt (Hobbs et al. 2017). Freshwater flows in the early part of 2017 were extremely high, leading to a wide spatial distribution of adults; in February fish were caught from the Sacramento Deepwater Ship Channel in the north Delta (CDFW Spring Kodiak Trawl data) and for the first time, as far west as the Petaluma River (Hobbs unpublished data), reducing the probability of fish finding mates during the spawning season. This wide dispersal of adults was evident in offspring being found in the Napa River from March through June of 2017 (CDFW-20mm Survey data), however, this wide spatial distribution did not translate into high recruitment. The spatial distribution of larvae in 2011 was similar to 2017, but the larval abundance index was several fold greater in 2011, further supporting the role of demography in explaining poor recruitment in 2017.

The natural flow regime for the Sacramento-San Joaquin Delta has been highly modified to maximize water delivery for agriculture and urban use (Hutton et al. 2017a; Hutton et al. 2017b). Massive reservoirs along the Central Valley tributaries capture the bulk of runoff from precipitation events reducing the frequency of pulse flows and capping the peak of flow events in the winter to deliver water later in the spring and summer when demand is high. The life history of Delta Smelt evolved to take advantage of this hydrograph; fish migrate from brackish water to freshwater following the first pulse flows of the winter when turbidity is high (Bennett and Burau 2015), however; spawning typically occurs in the spring, thus fish hold over in freshwater for several months prior to spawning (Moyle et al. 1992, Bennett 2005). In this study fish began hatching approximately 2-3 weeks following the last peak in Delta Outflow during the wet years of 2011 and 2017, suggesting declining flows in the late winter-spring period cue Delta Smelt to spawn. Delta Smelt larvae are generally poor swimmers (Bennett 2005), and due to their small size are likely to be dispersed during high spring flows, thus Delta Smelt likely migrate upstream and spawn after peak flows to optimize downstream dispersal during the larval stage. This hypothesis is consistent with recent observations of increased abundance of adult Delta Smelt in the Yolo Bypass during the drought (Mahardja et al. 2019). In the wetter years, we saw some evidence of elevated numbers of fish with natal origins in very low-salinity and the LSZ, however; the vast majority of fish were hatched and reared in freshwater habitats for 1 to 8 months prior to dispersing into the LSZ, thus flows in 2011 and 2017 did not appear to result in significant downstream dispersal. This may be due to fish spawning and rearing in the Sacramento Deepwater Ship Channel where high residence time and relatively protected conditions from river flows occurs.

Temperature has also been identified as a cue for spawning in laboratory cultured Delta Smelt. Damon et al. (2016) found spent female Delta Smelt occurred at temperatures from 8 to 18 °C in the wild, with the majority of ripe fish occurring above 11°C. Meanwhile in laboratory cultures, spawning occurs when water temperatures are increased above 12 °C and the duration of spawning can be extended beyond the range observed in the wild by maintaining temperatures

below 16-18 °C (Tien Chieh-Hung- Personal Communication). Furthermore, hatching success is optimal when temperatures are maintained between 14-18 °C in lab cultures (Bennett 2005). In this study, Delta Smelt hatching began when daily mean temperatures exceeded 12 °C, with the majority of fish hatching when temperatures were below 20 °C, which we termed the Hatching Window. However, the duration of this Hatching Window (12 °C – 20 °C) was not correlated with the range of hatch-dates in this study. This was likely due to the extremely low adult abundance during the drought, limiting the ability of the population to fill the suitable hatching niche. This was most evident in 2015 and 2016 when hatching began weeks after temperatures exceeded 12 °C and concluded well before temperatures exceeded 20 °C.

During the drought, maturation windows were dramatically reduced due to shifting thermal phenology. Regional down-scaled climate models predict temperatures to cool later and warm earlier, compressing available suitable thermal habitat by reducing the amount of time Delta Smelt have to mature, which was similar to observations from 2013-2015 (Brown et al. 2016). Delta Smelt hatching phenology tracked thermal phenology, hatching earlier during the drought, however; hatching was also delayed relative to the day of year when the estuary surpassed 12 °C from 2014-2016. This suggests, thermal habitat compression delayed maturation and spawning, and may have led to poor recruitment in those years. Hatching early in annual species can be an advantage, giving individuals more time to reach a maximum length before maturity. Delta Smelt fecundity increases exponentially above 65-mm (Damon et al. 2016), and individual-based models suggests maximizing length prior to maturity can have large effects on population dynamics (Rose et al. 2013a; Rose et al. 2013b). However, Delta Smelt growth was also poor during the drought and fish sizes were similar between wet and dry years (Hobbs, unpublished data). Thus, hatching earlier did not provide an advantage to the population as fish abundance collapsed during drought.

Temperature also appeared to influence when Delta Smelt dispersed from freshwater natal habitats to the LSZ. Water temperatures exhibit a longitudinal gradient in the summer months, habitats in Suisun Bay are typically 1-2 °C cooler than the Delta due to proximity to cooler air temperatures. Fish entered the LSZ when daily mean temperatures exceeded 20 °C but exhibited a wide range in dispersal dates. The importance of this thermal threshold was apparent during the drought when water temperature warmed early and entered the LSZ correspondingly earlier. However, an earlier dispersal date could also be explained by the location of the LSZ being further upstream during the drought, presumably resulting in a closer proximity to natal habitats and a more rapid dispersal. Fish entered the LSZ at a wide range in age and lengths during the drought, thus a geographic proximity explanation is less likely. The wide range in dispersal dates, age and lengths was somewhat surprising, further emphasizing the diversity in movements of the species. Importantly, these data shed light on the significance of managing for habitat quality in freshwater as well as the LSZ. Moreover, the data highlight the need to account for the prior history of individuals when conducting studies focused solely on habitat conditions in the LSZ.

Delta Smelt have a complex life history which can be comprised of freshwater and brackish water residents as well as the more common semi-anadromous phenotype (Bush 2017). In this study, the proportion of freshwater residents contributing to the adult population varied but did not appear to correspond with variability in freshwater outflow at the annual timescale. For example, freshwater residents comprised more than 40% of fish caught in 2011, a wet year and

2013 a dry year. Thus, another factor may be important in driving the relative recruitment of freshwater resident and semi-anadromous individuals. Summer water temperature is a likely factor that limits recruitment of freshwater resident Delta Smelt. During the summer months, the freshwater portions of the Delta often reaches or exceeds levels that cause physiological stress. (Jeffries et al. 2016b; Jeffries et al. 2018; Komoroske et al. 2013), yet a portion of the population remains in freshwater year-round and in some years the freshwater resident contingent can be a significant fraction of the spawning fish. This phenomenon raises several questions regarding the biology and ecology of the species. For example, are freshwater resident Delta Smelt more 'tolerant' of warm waters or do freshwater residents find thermal refuge in freshwater that is currently not sampled by monitoring surveys? Regardless of how Delta Smelt live in the Delta during the summer-fall, the fact that in some years a large number of adults are freshwater residents suggests that current management of critical habitat and flow actions to maintain fall habitat may need to be expanded to include freshwater habitats, particularly in the North Delta. The North Delta food web action may provide a food subsidy to the freshwater resident fish, however; if these increased flows to the North Delta are warm, this could have a detrimental effect, thus we urge caution in executing such actions.

This study was conducted to facilitate a more thorough understanding of how freshwater flows influence life history diversity in Delta Smelt during period of high flows and extreme drought. Life history diversity, in terms of hatching distribution, natal origins, dispersal history, and life history phenotypes was high during wet years, however; we found that temperature was an equally important environmental driver of Delta Smelt life history, dictating maturity schedules, hatching and dispersal phenology. Unfortunately, this life history diversity did not provide population resilience during the drought period and the population abundance may now be limiting recruitment when seemingly good environmental conditions occur.

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Table. 3-1. Summary of Delta Smelt Hatching from 2011-2017.

	Beginning Hatch		Mean Hatch		Ending Hatch		Hatch IQR	Hatch Percentile
Year	**JD**	**date**	**JD**	**date**	**JD**	**date**	**n days**	**n days**
2011	74	15-Mar	137	17-May	195	14-Jul	29	78
2012	50	19-Feb	109	18-Apr	160	9-Jun	22	63
2013	59	28-Feb	99	9-Apr	158	7-Jun	25	55
2014	54	23-Feb	97	6-Apr	145	25-May	21	53
2015	44	13-Feb	78	18-Mar	116	26-Apr	19	44
2016	79	20-Mar	91	31-Mar	102	12-Apr	14	21
2017	60	1-Mar	108	18-Apr	168	17-Jun	33	71

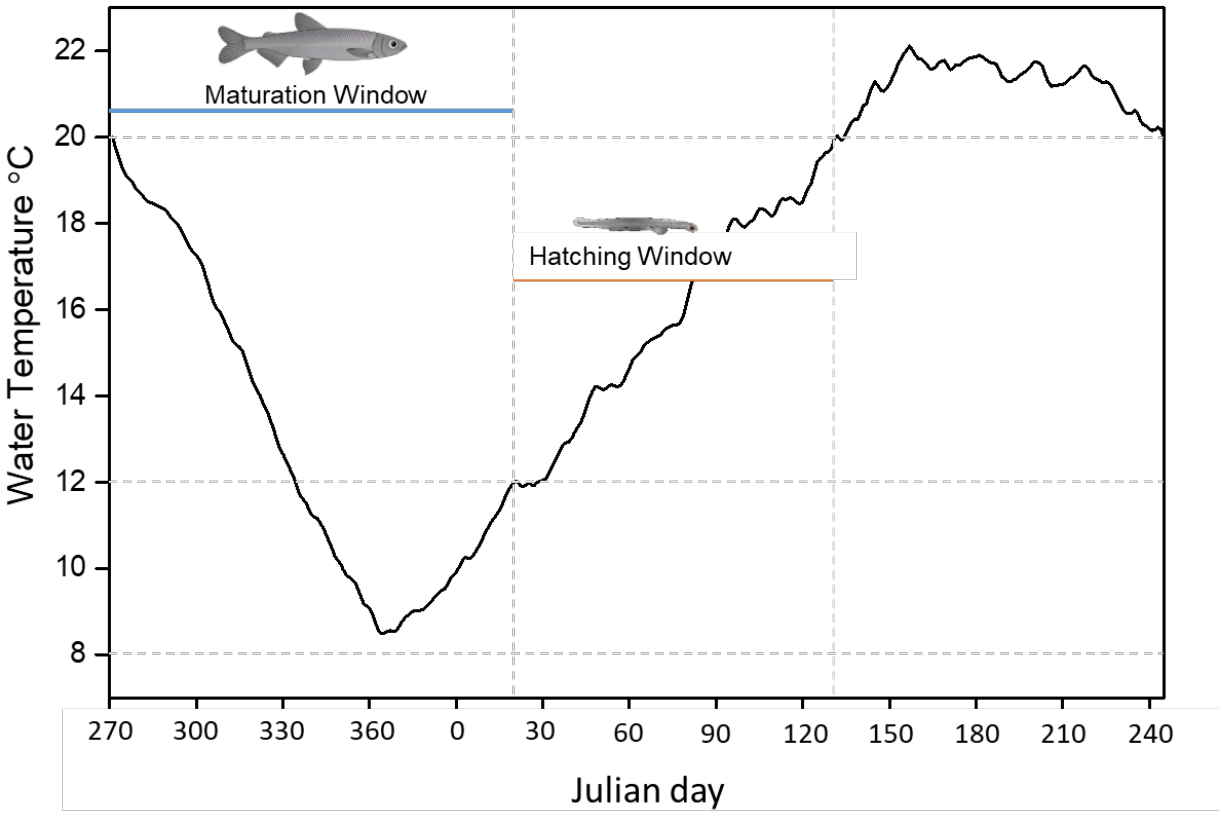
Notes: Hatch Range was Calculated Both as the Interquartile Range (IQR) and as the Percentile Range (95%-5%).

Table 3-2. Summary of Thermal Phenology from 2011-2017

Year	JD 12 C	JD 20 C	Hatch Window	Maturation Window
2011	71	172	101	160
2012	65	153	88	156
2013	62	134	72	150
2014	44	135	91	142
2015	35	156	121	112
2016	44	139	95	118
2017	69	168	99	161

Table 3-3. Summary of Dispersal Phenology from 2011-2017

Year	Mean Dispersal Date			Mean Dispersal Age	Mean Dispersal Length
2011	222	-	11-Aug	78	36
2012	195	-	14-Jul	83	36
2013	188	-	7-Jul	87	35
2014	171	-	20-Jun	71	28
2015	184	-	4-Jul	103	42
2016	207	-	27-Jul	114	44
2017	193	-	12-Jul	83	35



Note: The Maturation Window, modified from Brown et al. 2016 begins in the fall when daily estuary wide temperatures drop below 20 °C and ends when hatching occurs in the spring at 12 °C. The Hatching Window ends in the late-spring when temperatures exceed 20 °C.

Figure 3-1. Conceptual Model Describing the Relationship Between Water Temperature, Timing and Duration of the Maturation Window and Hatching Window for Delta Smelt

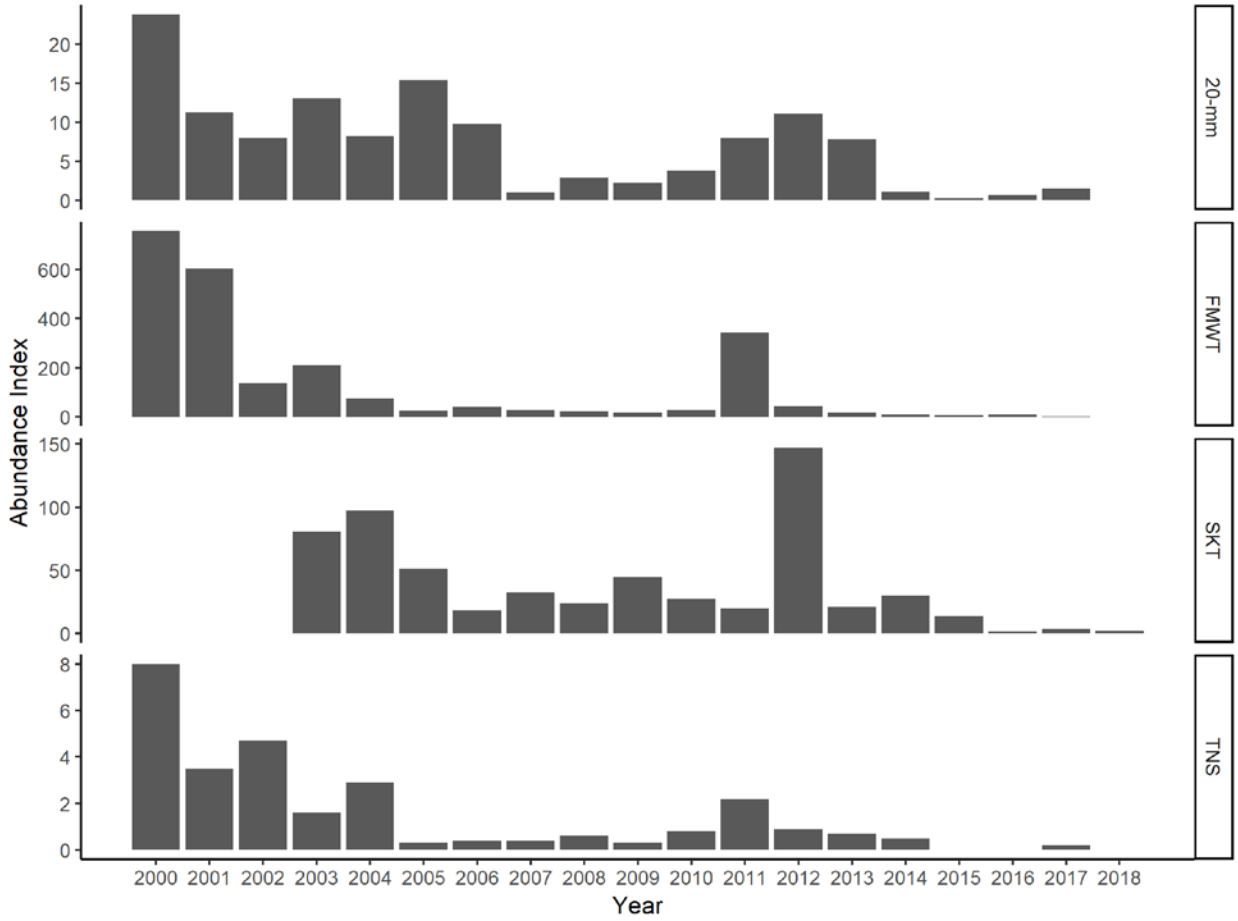
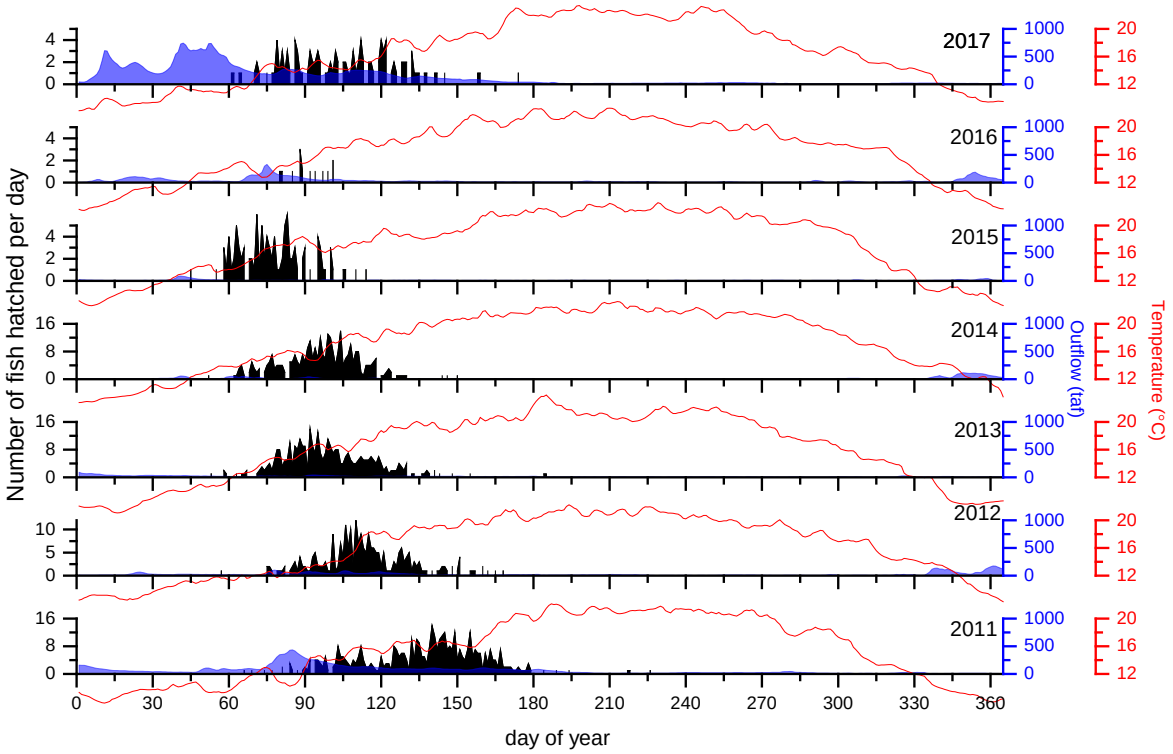
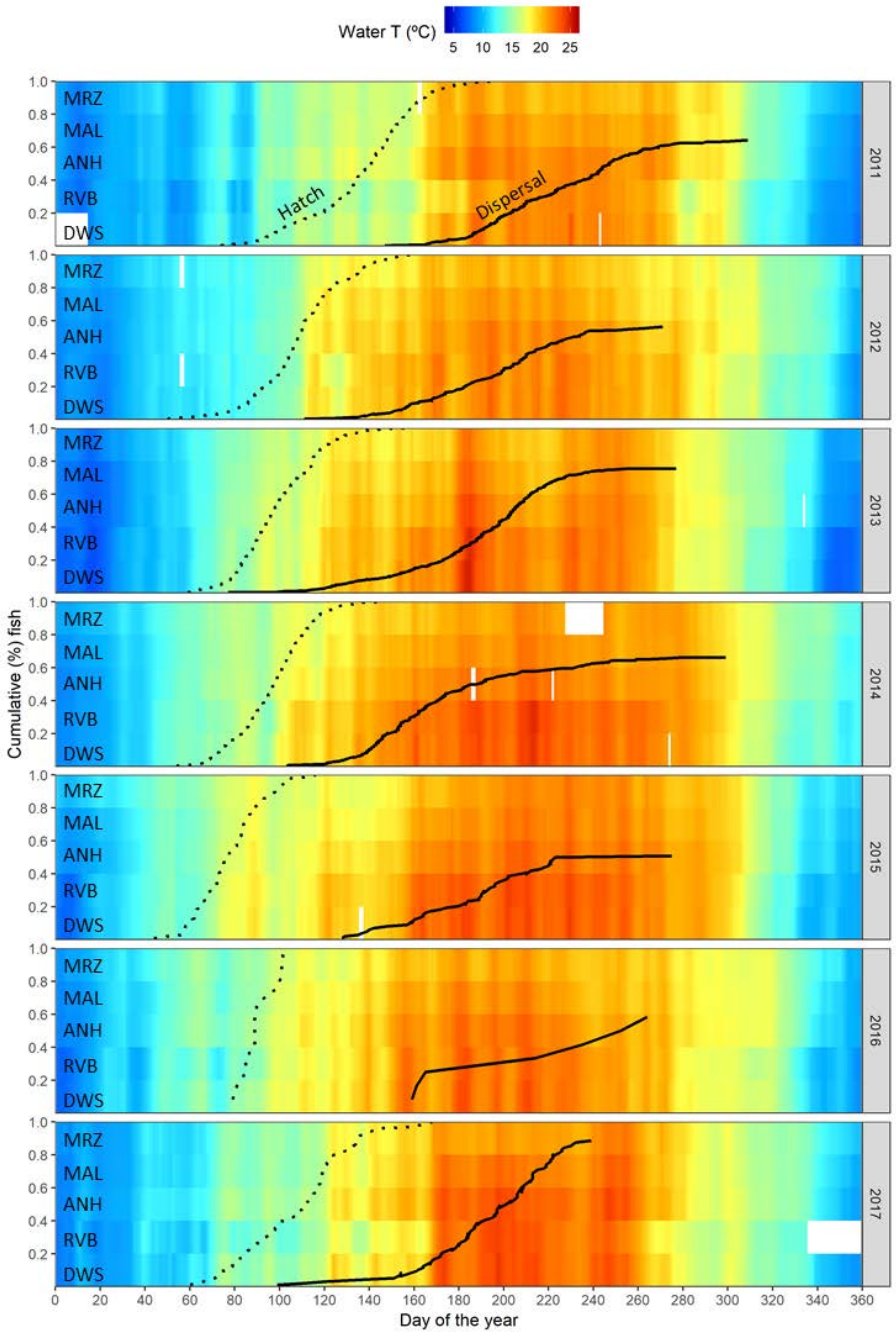


Figure 3-2. California Department of Fish and Wildlife Delta Smelt Abundance Indices from 2000 to 2018



Note: Filled blue polygons represent Delta Outflow, red lines depict daily mean water temperatures at the 5-index stations. Note the temperature is scaled from 12 to 20 °C to emphasize the period of suitable maturing and hatching.

Figure 3-3. Julian Hatch-Date Distributions (black vertical bars) for Delta Smelt from 2011-2017



Note: The heatmap represents daily mean water temperature from the five sonde stations arranged vertically from the North Delta (DWS-bottom) to far western Suisun Bay (MRZ-top). Station names are Deepwater Ship Channel (DWS), Rio Vista (RVB), Antioch (ANH), Mallard (MAL), and Martinez (MRZ).

Figure 3-4. Cumulative Distribution of Hatch-Date (dotted line) and Dispersal-Date (solid line) for Delta Smelt from 2011 to 2017

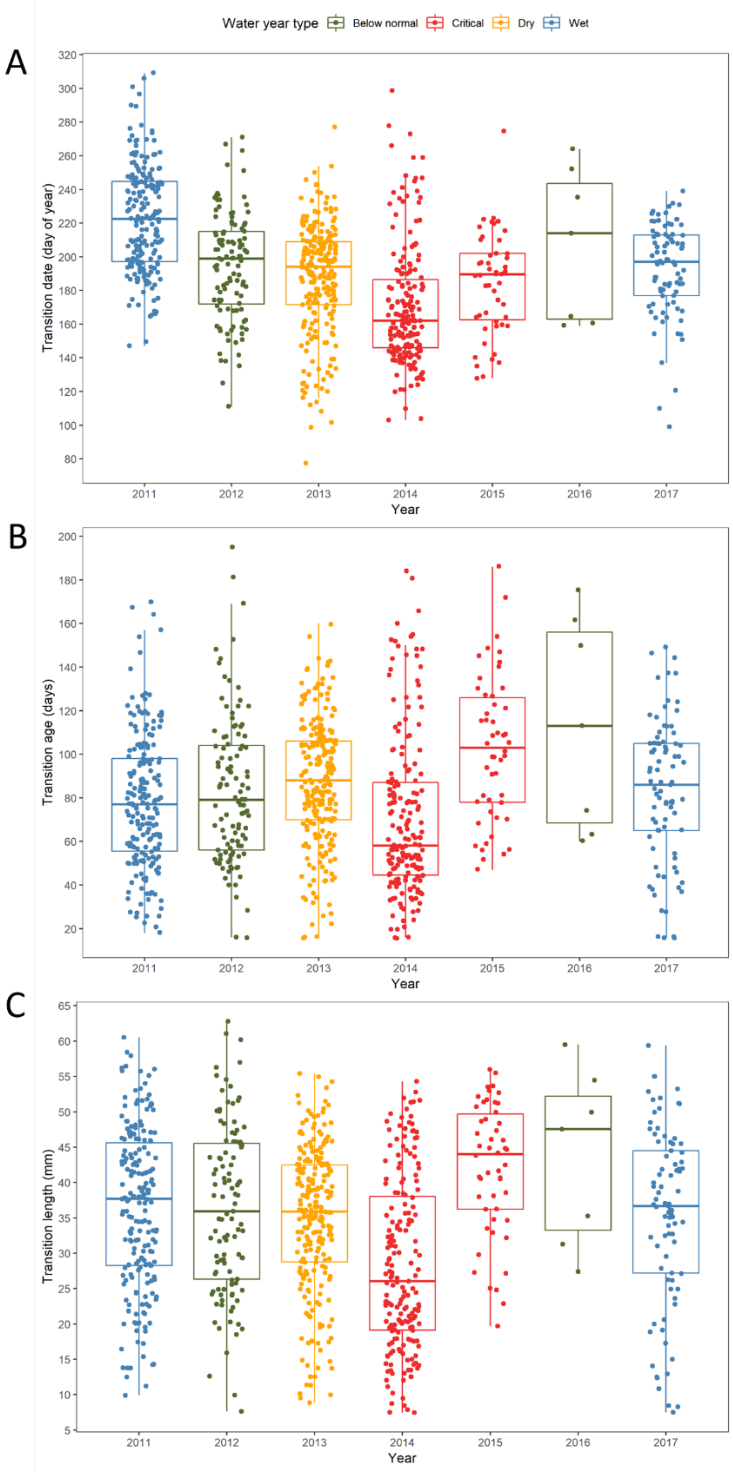
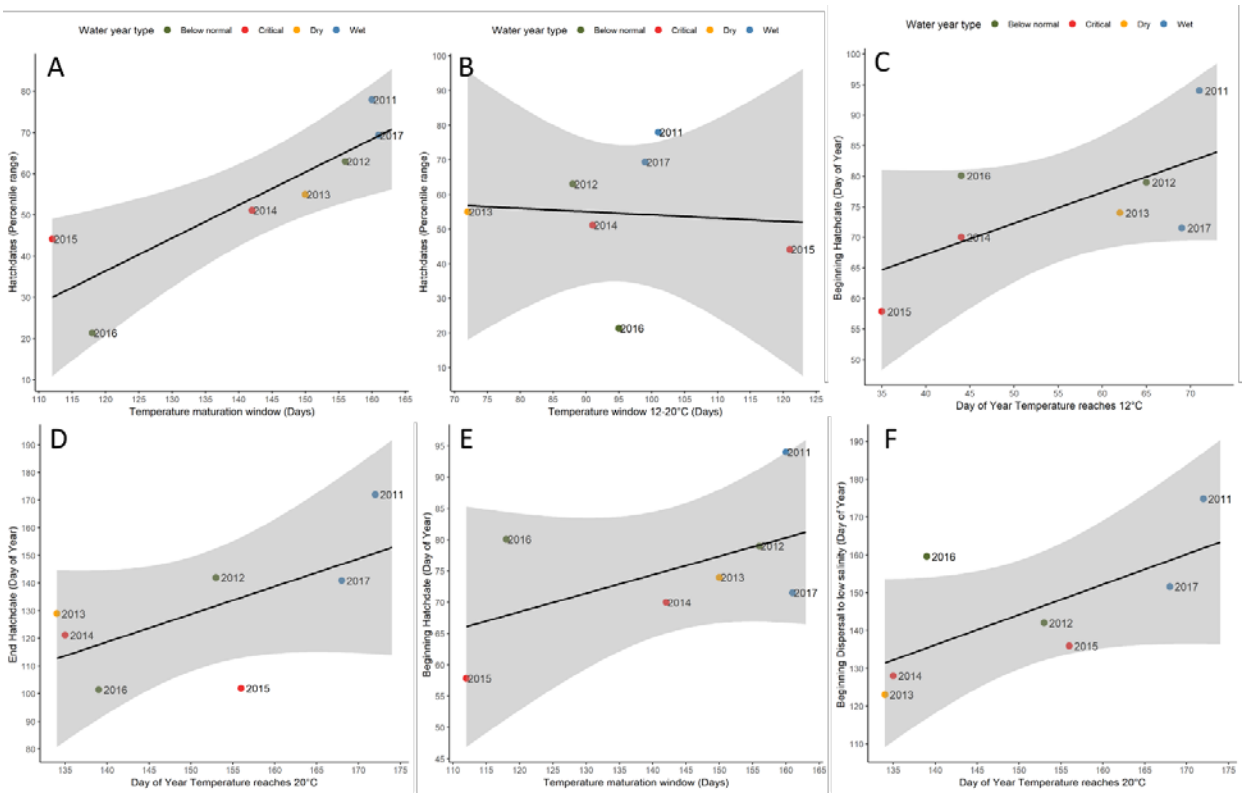
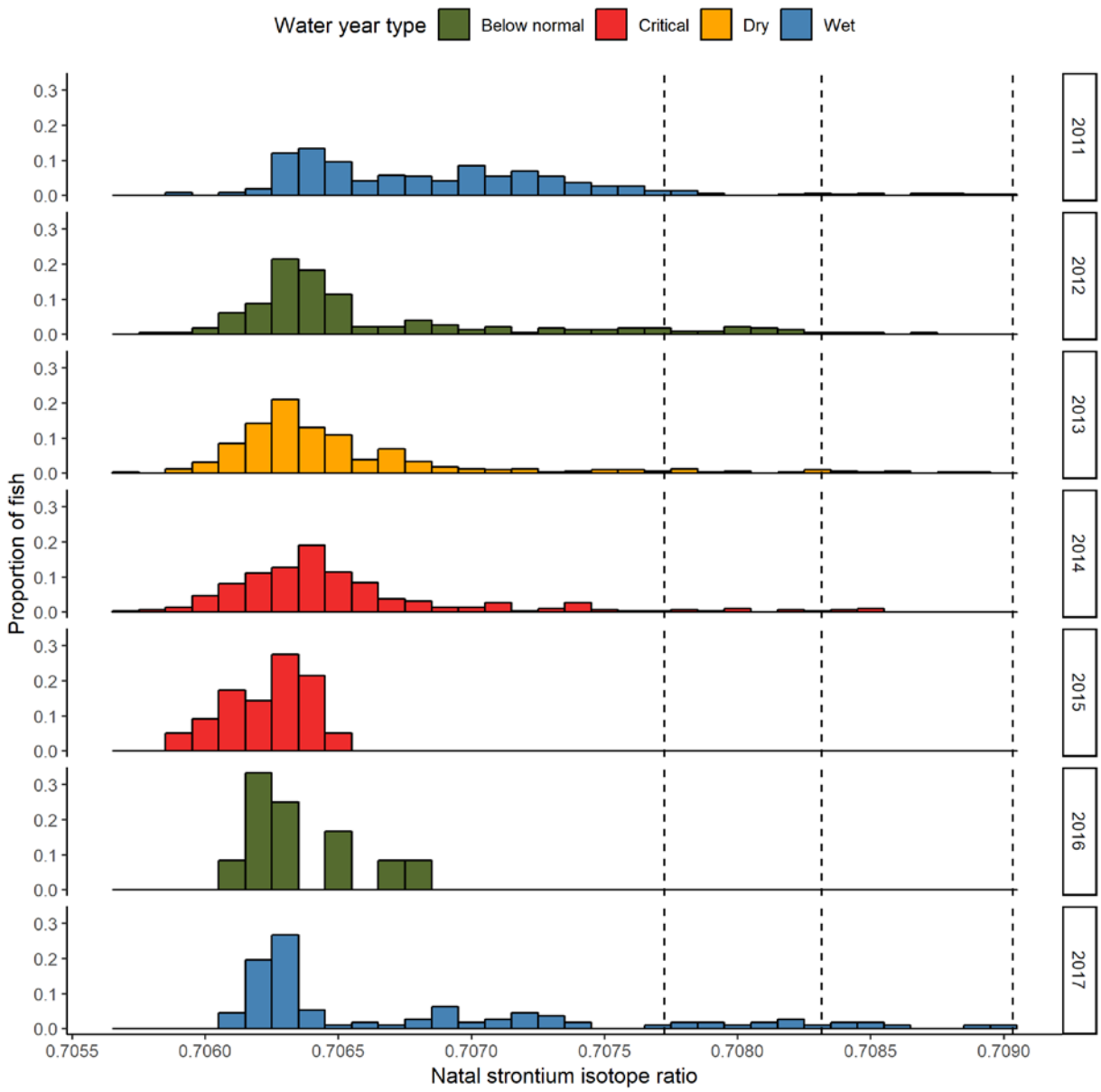


Figure 3-5. Boxplots of Julian Date (A), Age (B) and Length (C) when Delta Smelt Dispersed from Freshwater to the LSZ from 2011-2017



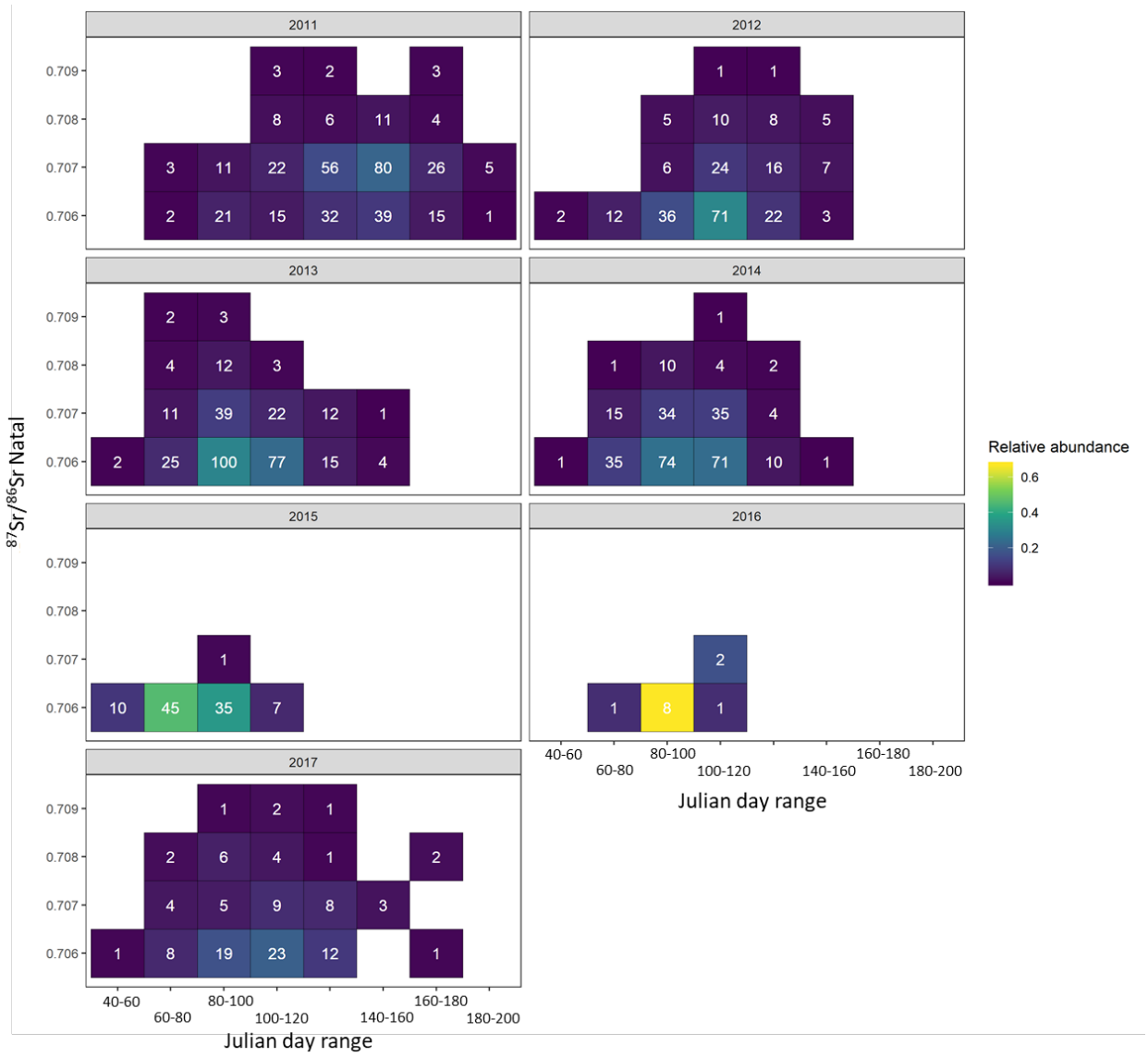
Note: A) Effect of the Matuation Window Hatch-Date Durations, B) Effect of Hatch Window on Hatch Duration, C) Effect of Julian day 12 °C on Beginning of Hatch, D) Effect of Julian Day 20 °C on end of hatch, E) Effect of the Maturation Windown on Beginning of Hatch, F) Effect of Julian day 20 °C on Beginning of Dispersal to the LSZ.

Figure 3-6. Trends in Hatch-Date Duration and Phenology



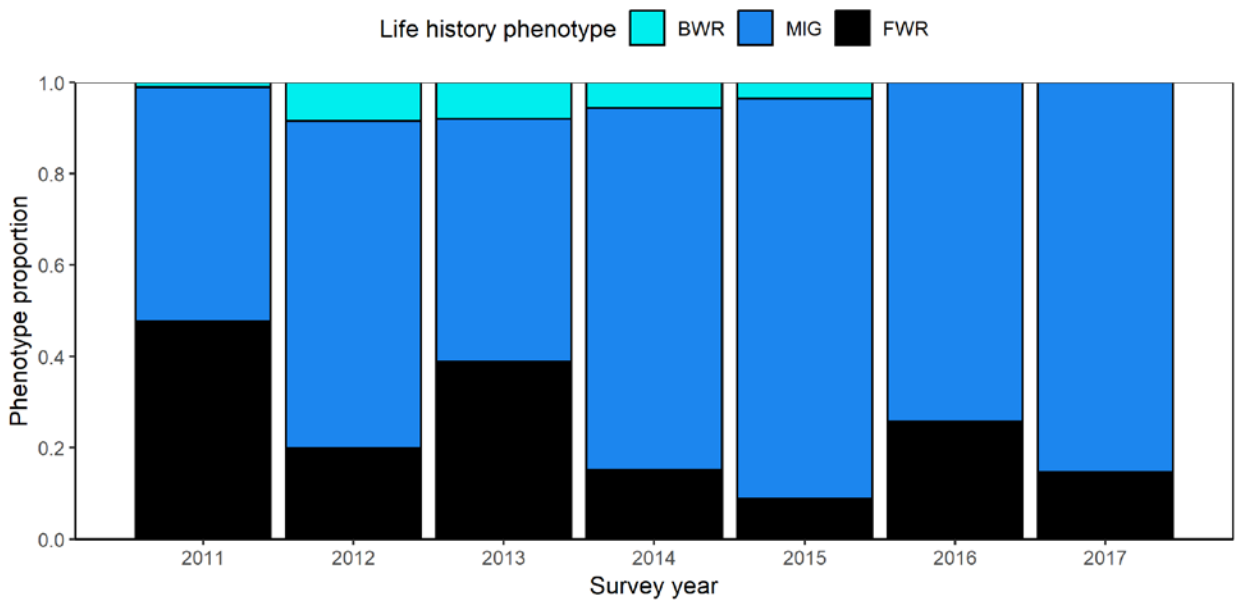
Note: Vertical bars depict the isotope ratio corresponding to thresholds between fresh (<0.5 psu) , 0.5 to 1 psu and 1-6 psu.

Figure 3-7. Natal Origins (strontium isotope ratios) for Delta Smelt from 2011-2017



Note: The colors and numbers inside boxes depict the number of fish with the corresponding combination of Julian hatch-date and natal $^{87}Sr/^{86}Sr$.

Figure 3-8. Heatmap of Julian Hatch-Date and Natal Origins ($^{87}Sr/^{86}Sr$)



Notes: BWR = Brackish Water Resident, MIG = Semi-Anadromous and FWR = Freshwater Resident

Figure 3-9. The Proportion of Different Life History Phenotypes Contributing to Adult Abundance in the California Department of Fish and Wildlife's Spring Kodiak Trawl Survey, which Samples Maturing Adults from January-May

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Supplemental Material

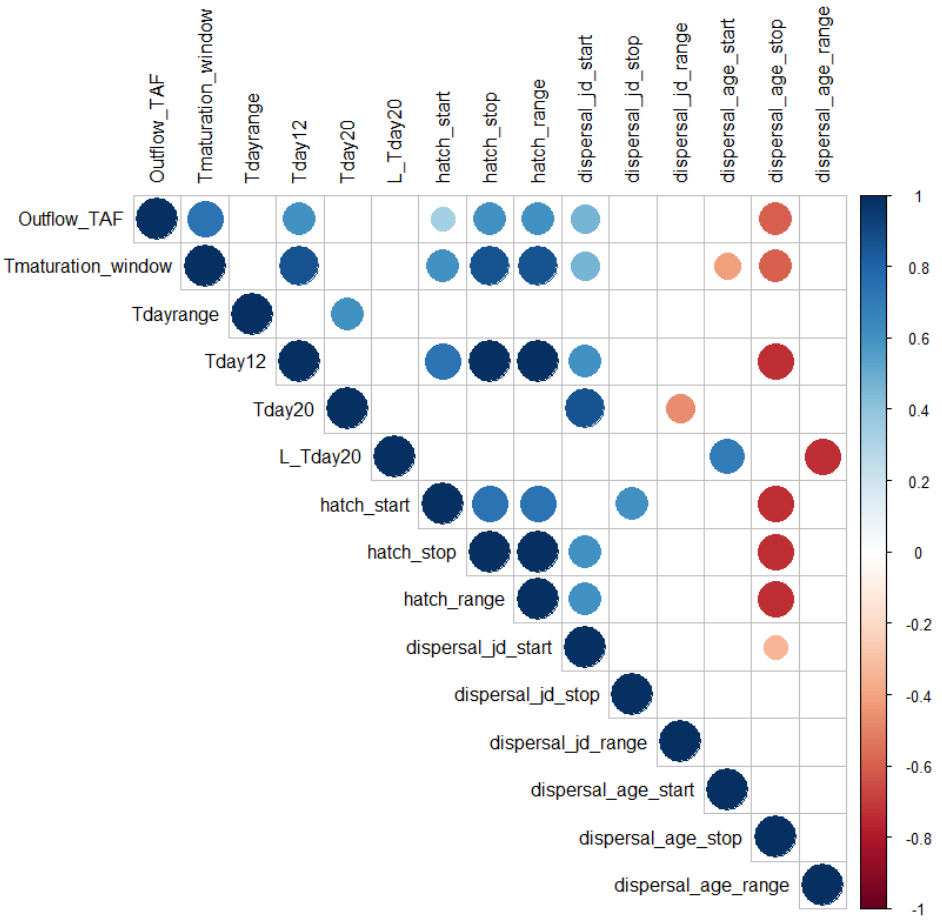


Figure S1. Kendall-Tau Correlation Matrix for Outflow, Temperature, Hatch Metrics and Dispersal Metrics

Table S1. Sample Sizes by Survey

Year	EDSM	FMWT	TNS	GES	GES-CCE	GES-TNS	SFBS	YB	Total
2011	0	191	171	0	0	0	2	1	365
2012	0	36	87	101	0	0	0	5	229
2013	0	10	107	200	0	0	0	15	332
2014	0	28	71	0	159	30	6	3	297
2015	0	4	12	0	0	0	49	33	98
2016	0	6	0	0	0	0	1	5	12
2017	87	0	25	0	0	0	0	0	112

Notes: EDSM = USFWS Enhanced Delta Smelt Monitoring, FMWT-CDFW Fall Midwater Trawl, TNS = CDFW Summer Townet Survey, GES = CDFW Gear Evaluation Study, GES-CCE CDFW Gear Evaluation Covered Cod End Study, GES-TNS CDFW Gear Evaluation Summer Townet Survey, SFBS CDFW San Francisco Bay Study, YB DWR Yolo Bypass Juvenile Fish Monitoring.

The Strontium ISOSCAPE of the San Francisco Estuary

Strontium isotope ratios ($^{87}sr/^{86}Sr$) have been used in a variety of provenance studies as a natural tracer of heterogeneous landscapes (Walther and Limburg 2012). Spatial variation in $^{87}Sr/^{86}Sr$ ratios within watersheds is derived from bedrock age and rock type, which dictates the rate of radiogenic in-growth of ^{87}Sr and composition of the underlying bedrock as different types of rock influence the weathering of Sr into the watershed (Bataille and Bowen 2012; Bataille et al. 2014). $^{87}Sr/^{86}Sr$ ratios in biogenic carbonates, such as fish otoliths are largely derived from the surrounding water, with very small influence from diet contributing to otolith $^{87}Sr/^{86}Sr$ ratios (Walther and Limburg 2012). Thus, otolith $^{87}Sr/^{86}Sr$ ratios can be used to reconstruct origins and migratory history for fishes in freshwater. Since the half-life of ^{87}Sr is on the order of 4.5 billion years, the ocean exhibits a relatively stable modern $^{87}Sr/^{86}Sr$ globally mean value of 0.70918 (Faure and Mensing 2005; Hodell et al. 2007). When fresh river waters flow into enclosed bays, consistent spatial/longitudinal gradients in salinity occur and the mixing of fresh and ocean $^{87}Sr/^{86}Sr$ follows a conservative linear mixing process, such that $^{87}Sr/^{86}Sr$ of mixers of fresh and saltwater can be used to estimate salinity with relatively high precision (Ingram and DePaolo 1993; Shao et al. 2018; Walther and Nims 2015). However, this mixture can be largely dependent on the bulk concentration of Sr and $^{87}Sr/^{86}Sr$ ratios of freshwater endmember (rivers) entering the bay.

The strontium isotopic composition of the San Francisco Estuary and the Sacramento-San Joaquin Delta has been characterized previously in several studies (Hobbs et al. 2010a; Ingram and Sloan 1992; Ingram and DePaolo 1993; Phillis et al. 2011) and shown to reconstruct low-salinity conditions (<6 psu) with high precision, but at higher salinities (>6 psu) the concentration of ocean Sr dominates the mixing process resulting in poorer salinity resolution (Hobbs et al. 2010a). However, these studies utilized only a single freshwater endmember in their mixing models, derived from water samples collected by Ingram and Slough (1992). The Delta receives freshwater from multiple source rivers including the Sacramento River from the north, the Cosumnes River and Mokelumne River from the east and the San Joaquin River from the south, each having relatively unique Sr concentrations and $^{87}Sr/^{86}Sr$ ratios which could influence the mixing process and subsequent estimates of low-salinity values from water $^{87}Sr/^{86}Sr$ ratios. The Sacramento River is the dominant source of freshwater to the estuary in most years, since much of the San Joaquin River flows are diverted in the South Delta before reaching the confluence of the Sacramento and San Joaquin Rivers.

The hydrology of the Delta is of great importance for managing freshwater distribution to in-Delta and south Delta water projects which provides water to approximately 25 million people and supports one of the largest agricultural areas in the world. Thus, flows from the tributaries entering the Delta are highly regulated, monitored and tracked using a variety of modelling approaches (MacWilliams et al. 2016; MacWilliams et al. 2015). Flows exiting the Delta (hereinafter Outflow) and mixing with bay and ocean water are estimated using a 1-D hydrodynamic model rather than measured flows due to the inherent challenges with accurately accounting for tidal flows and in-delta consumption and discharge from agriculture. The California Department of Water Resources maintains this database and data are available from (http://water.ca.gov/dayflow/). This model also includes flow estimates from tributaries entering the Delta and water diverted by the CVP and SWP (Exports).

Analysis of Water for Strontium Isotopes

To advance our understanding of the mixing properties of freshwater endmembers entering the Delta we collected water samples in over three months of 2012. Water samples and water quality parameters including electrical conductivity (µs/cm), temperature (Celsius °C) were collected from surface water grabs at select stations during California Department of Fish and Wildlife's Spring Kodiak Trawl survey (Fig. S1). The water samples were filtered through a 0.45-µm filter (WhatmanTM Puradisk) into a 250-mL polypropylene container and acidified by adding 1-mL of 3% nitric acid. Samples were then transported to a class 100 laboratory at the UC Davis Interdisciplinary Center for Plasma Mass Spectrometry. Element concentrations were measured using an Agilent 7500ce quadrupole inductively coupled plasma mass spectrometer. For strontium isotopic analysis an aliquot of each water sample was made at volume totaling 1 ng of total strontium. These samples were evaporated to dryness and reconstituted in double-distilled nitric acid (8M) for Sr chromatographic separation using micro-column packed with Sr spec resin (Eichrom Inc.). After separation the samples were dried and reconstituted in 2% double-distilled nitric acid and analyzed with the Nu Plasma HR (MC-ICP-MS). Samples were introduced into the mass spectrometer with a desolvating nebulizer system (DSN-100). Replicate analyses of NIST SRM 987 (strontium carbonate) were conducted bracketing every six samples and normalizing for instrument drift between sessions. An in-house modern coral reference material was powdered and processed in parallel with each water sample set and resulted in an $^{87}Sr/^{86}Sr$ ratio of 0.709182±0.000017 (2σ, n=8) showing high precision and accuracy.

Mixing Model Formulations

The mixing dynamics of freshwater and seawater in the San Francisco Estuary have been previously studied by Ingram and Sloan (1992) and Phillis et al. (2011). In these studies, the estimated salinity of a mixture assuming a simple two-endmember mixing model derived from Faure and Mensing (2005) was used, however; in Phillis et al. (2011) a simplifying assumption of zero salinity for freshwater was used to simplify algebraic derivation from the original mixing model. Here, we derived the equation for estimating salinity form the two-endmember mixing model without this assumption.

Term Definitions:

R_A = Strontium ratio of endmember A

R_B = Strontium ratio of endmember B

R_m = Strontium ratio of the mixed water (for a given mix F)

C_A = Strontium concentration of endmember A

C_B = Strontium concentration of endmember B

C_M = Strontium concentration of the mixed water (for a given mix F)

S_A = Salinity of endmember A

S_B = Salinity of endmember B

S_M = Salinity of the mixed water (for a given mix F)

F = Proportion of endmember A in the mixture (from 0 to 1)

We used a simple two-endmember mixing model (Faure, 1986). The salinity of a mixture is the salinity of each endmember multiplied by its proportional contribution to the sample:

$$S_M = S_A * F + S_B(1 - F)$$

Where the proportion of endmember A can be estimated from:

$$F = \frac{S_M - S_B}{S_A - S_B}$$

Similarly, the strontium concentration mixing equation for two endmembers:

(3) $$C_M = C_A * F + C_B(1 - F)$$

Combining these two mixtures provides the simple two-endmember model in Faure (1986). Strontium ratio mixing equation:

(4) $$R_M = R_A * C_A * \left(\frac{F}{C_M}\right) + R_B * C_B * \left(\frac{1-F}{C_M}\right)$$

Sub out C_M:

(5) $$R_M = R_A * C_A * \left(\frac{F}{C_A * F + C_B(1-F)}\right) + R_B * C_B * \left(\frac{1-F}{C_A * F + C_B(1-F)}\right)$$

Solve for F:

(6) $$R_M = \left(\frac{R_A C_A F}{C_A * F + C_B(1-F)}\right) + \left(\frac{R_B C_B - R_B C_B F}{C_A * F + C_B(1-F)}\right)$$

$$R_M = \frac{R_A C_A F + R_B C_B - R_B C_B F}{C_A F + C_B - C_B F}$$

$$R_M(C_A F + C_B - C_B F) = R_A C_A F + R_B C_B - R_B C_B F$$

$$R_M C_A F + R_M C_B - R_M C_B F = R_A C_A F + R_B C_B - R_B C_B F$$

$$R_M C_A F - R_M C_B F - R_A C_A F + R_B C_B F = R_B C_B - R_M C_B$$

$$F(R_M C_A - R_M C_B - R_A C_A + R_B C_B) = R_B C_B - R_M C_B$$

$$F = \frac{R_B C_B - R_M C_B}{R_M C_A - R_M C_B - R_A C_A + R_B C_B}$$

Substitute F for the salinity mixing equation:

(7) $$\frac{S_M - S_B}{S_A - S_B} = \frac{R_B C_B - R_M C_B}{R_M C_A - R_M C_B - R_A C_A + R_B C_B}$$

$$S_M - S_B = \frac{R_B C_B - R_M C_B}{R_M C_A - R_M C_B - R_A C_A + R_B C_B} * (S_A - S_B)$$

$$S_M - S_B = \frac{S_A R_B C_B - S_A R_M C_B - S_B R_B C_B + S_B R_M C_B}{R_M C_A - R_M C_B - R_A C_A + R_B C_B}$$

Final equation for the salinity of a mixture:

(8) $$S_M = \left(\frac{S_A R_B C_B - S_A R_M C_B - S_B R_B C_B + S_B R_M C_B}{R_M C_A - R_M C_B - R_A C_A + R_B C_B}\right) + S_B$$

Validation of the mixing model for 2011-2012

Flow data from October 1, 2011 to May 31, 2012 are shown in Fig. S2. 2011 was a wet year in the Central Valley of California but transitioned into a dry year in 2012. Sacramento River flows comprised the bulk of the inflows (76%) to the Delta and Delta Outflow (92%) during this time period except for a brief flow pulse from the Yolo Bypass, a large man-made floodplain engineered to protect the city of Sacramento from flooding. The rivers flowing into the Delta from the east, Cosumnes and Mokelumne River comprise a very small proportion (~3%) of flows into the Delta. In 2011, the San Joaquin River was the second largest contributor to flows to the Delta comprising about 22% of flows into the Delta from January to August but only 12% during this period in 2012 when Exports at the time exceeded San Joaquin River flows. These data would suggest that using the Sacramento River freshwater endmember would provide a reasonable approximation to the mixing of freshwater and ocean $^{87}Sr/^{86}Sr$ ratios. However, since Delta Smelt can spawn in the south Delta and potentially in the lower reaches of the San Joaquin River, we included the San Joaquin River in mixing models.

The majority of water samples generally followed the strontium mixing line with two endmembers, our sample with the lowest Sr concentration and salinity (sample D56 collected at station 712 on May 2nd 2012) and a sample collected in the Pacific Ocean at Muir Beach just north of the entrance to San Francisco Bay on April 23rd 2007 with a Sr concentration of 6819-ppt and salinity of 31.8-psu (Fig S3A). However, there were a few samples that fell below the line, these being from the Napa River which consists of contributions from the Napa River as well as the Delta and samples from Suisun Bay. Upon closer inspection of these data in Fig. S3B it is apparent that the majority of the samples fell above this mixing line suggesting these two simple endmembers do not completely represent the complex mixing dynamics of the Delta with the ocean. There was greater variability for samples collected below 0.5-psu reflecting the spatial and temporal variability in contributions of different freshwater endmembers to the mixing dynamics of the system (Fig S3B). Regardless, there was strong correlation between overall Sr concentration and salinity suggesting that the mixing properties for samples above 0.5-psu could be reliably reconstructed using a weighted average of freshwater endmembers.

There was a linear relationship between the inverse of Sr concentration and the $^{87}Sr/^{86}Sr$ ratio of water samples, however, freshwater samples varied spatially and temporally (Fig S4). This variability could be partially explained by examining variability at the individual stations and survey months. During the first survey (March 1-3, 2012) flows from the Sacramento River were low (~33,000 CFS) while flow from the Yolo Bypass increased from ~90 CFS to ~1,900 for the 10-days prior to sampling. Samples collected in the North Delta fell along a gradient of increasing Sr concentration and salinity from Station 711 at the intersection of the mainstem Sacramento River with the north Delta to station 719 in the Sacramento Deepwater Ship Channel. Meanwhile in the Lower Sacramento River from station 707 to 704 this north-south

gradient in Sr concentration flipped reflecting the influence of Sacramento River water to the lower Sacramento channel (Fig. 5). This pattern is evident in Fig. S4, where samples for this month in the north Delta were generally shifted left along the x-axis, but samples more influenced by the Sacramento River had lower Sr concentrations. During the April survey flows from the Sacramento River increased to ~80,000 CFS but flows from the Yolo Bypass jumped to 67,000 CFS, thus contributing more water to the north Delta than the March survey. Sr concentration and salinity followed a similar north-south gradient from the northern stations to the confluence with the Sacramento River where both salinity and Sr concentration dropped (Fig 6). Despite the increased Yolo Bypass flows, the $^{87}Sr/^{86}Sr$ ratio below the Sacramento River confluence was still isotopically lighter (~0.7059) than above the confluence (0.7063) reflecting the larger influence of Sacramento River water flowing south into the Lower Sacramento channel. The stations within the Sacramento River and confluence were right shifted (lower Sr concentration) in Fig. 4, representing this spatial variation. During the May survey flows declined to ~43,000 CFS from the Sacramento and only 600 CFS from the Yolo Bypass. The north-south gradient in Sr concentration and salinity above and below the Sacramento River confluence remained (Fig 7), however; the $^{87}Sr/^{86}Sr$ ratio of Sacramento River water appeared to be isotopically heavier than in March and April suggesting the Sacramento River freshwater endmember likely changes seasonally with flows for the major tributaries to the Sacrament River (American River, Feather River and Upper Sacramento River) changing, altering the $^{87}Sr/^{86}Sr$ ratio and Sr concentration entering the north Delta (Fig 7).

The three surveys conducted in spring 2012 provide valuable insight on how freshwater endmembers contribute to the mixing of ocean water with freshwater. Freshwaters were variable in the north Delta for Sr concentration and $^{87}Sr/^{86}Sr$ ratio, however; the Sr concentration of bay water appeared to dominate the mixing dynamics, reducing the influence of spatial and temporal variability in the contribution of the freshwater endmember. While the Sacramento River flows dominated the overall contribution to Delta outflow during this time period, samples collected in the Lower San Joaquin at station 804 shed additional light on how San Joaquin River flows could influence the mixing dynamics. During the March survey when Sacramento River flows were low, the Sr concentration, salinity and $^{87}Sr/^{86}Sr$ ratio appeared to reflect the influence of San Joaquin River water (Fig. S5). In previous collections along the San Joaquin River we found that San Joaquin River water was isotopically $^{87}Sr/^{86}Sr$ heavier (0.7073) had higher Sr concentration (~1,000 ppt) and was saltier (0.4-psu), largely due to the influence of agricultural water in the south Delta and the older geology found in the San Joaquin Basic (Barnett-Johnson et al. 2008). In subsequent surveys when Sacramento River water increased this site had a lower $^{87}Sr/^{86}Sr$ ratio, Sr concentration and salinity reflecting of the contribution of Sacramento River water. Thus, in future reconstructions of salinity from $^{87}Sr/^{86}Sr$ ratio, the contribution of San Joaquin River flows will likely be needed to provide reliable salinity predictions.

Theoretical mixing curves using only Sacramento River or San Joaquin River water demonstrated the range of $^{87}Sr/^{86}Sr$ ratios for water with salinity values less than 0.5-psu, with the San Joaquin threshold value being isotopically heavier (0.7077) compared to the scenario with only Sacramento River (0.7066) (Fig. S8). The majority of our water samples collected in spring 2012 fell close to a 70:30 mixture of Sacramento River with San Joaquin River water. As reported in our previous study (Hobbs et al. 2010), $^{87}Sr/^{86}Sr$ ratios can provide fine scale resolution for salinity below 6-psu, but due to differences in Sr concentrations between fresh and

ocean waters and resultant strong non-linear relationship between salinity and isotope ratios (Fig S8), estimates of salinities above 6-psu were less reliable.

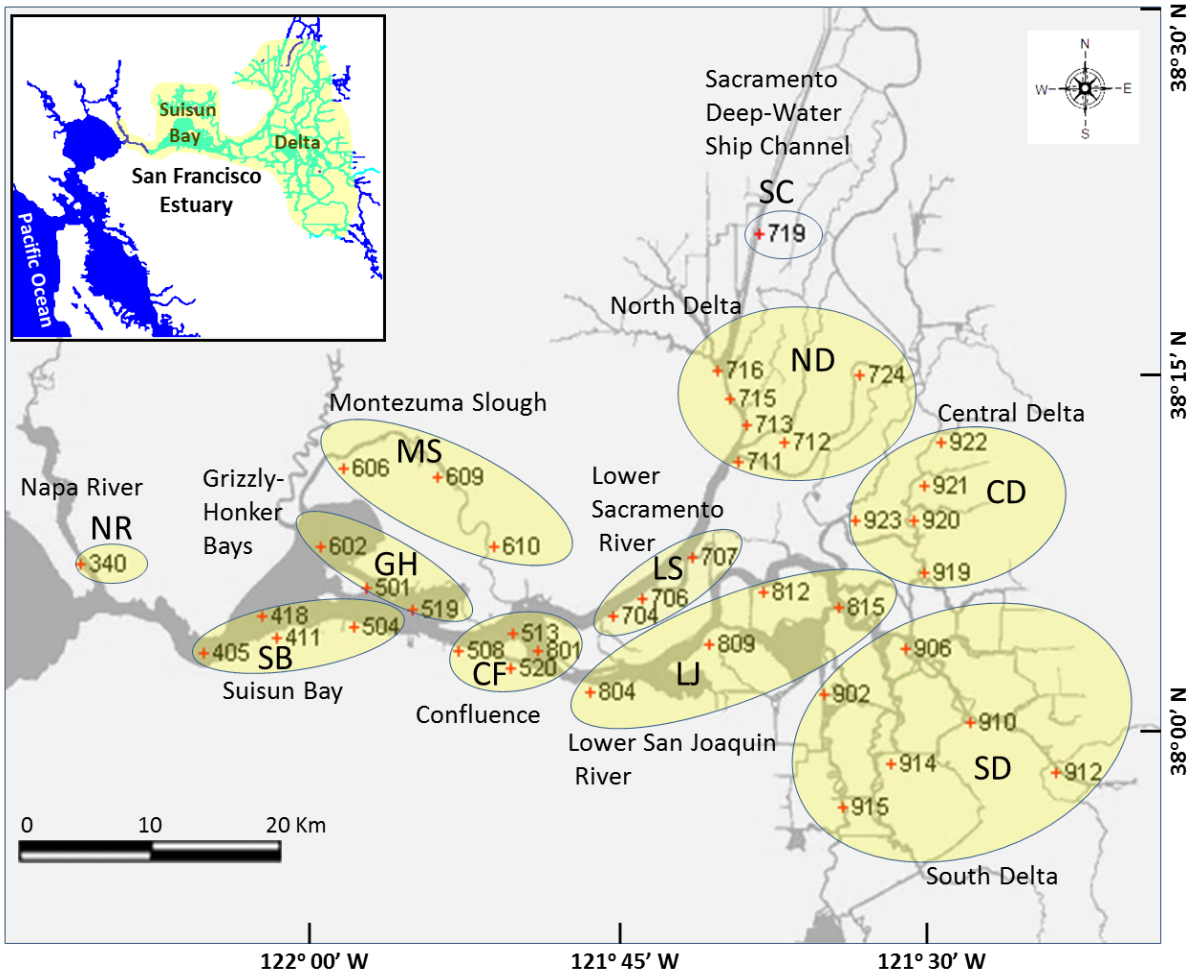


Figure S1. Map of Spring Kodiak Trawl Survey Stations and Regions

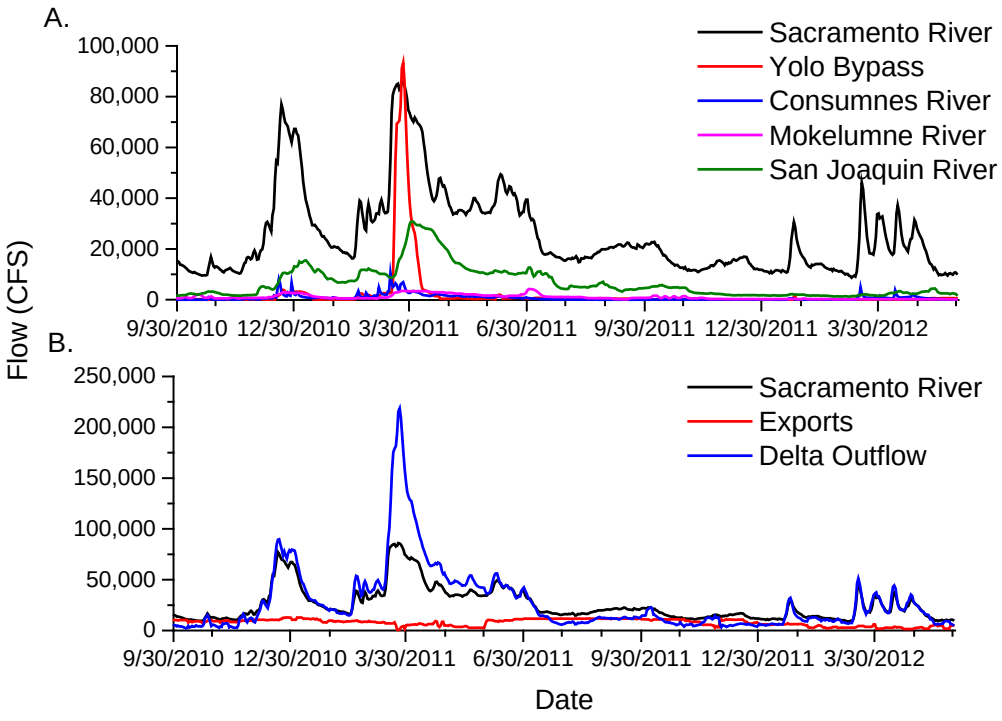
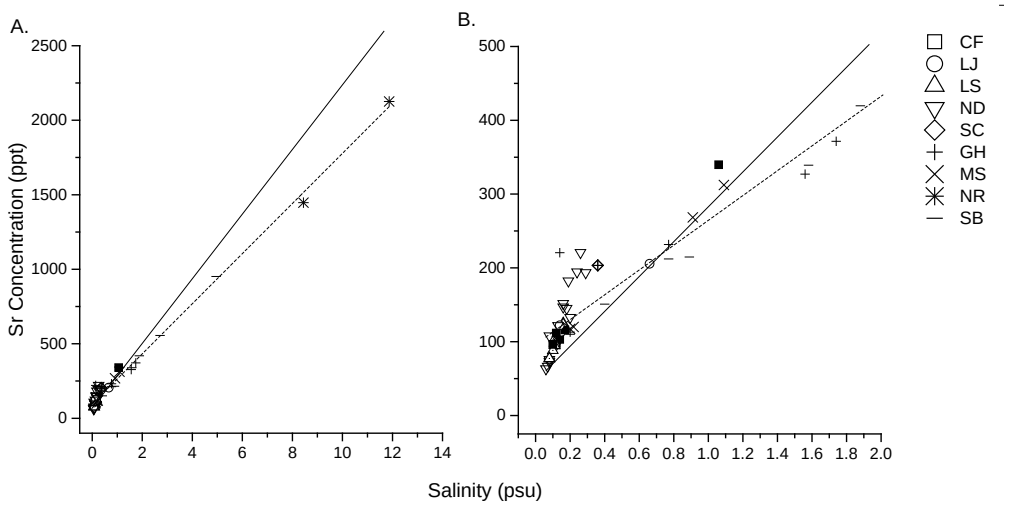
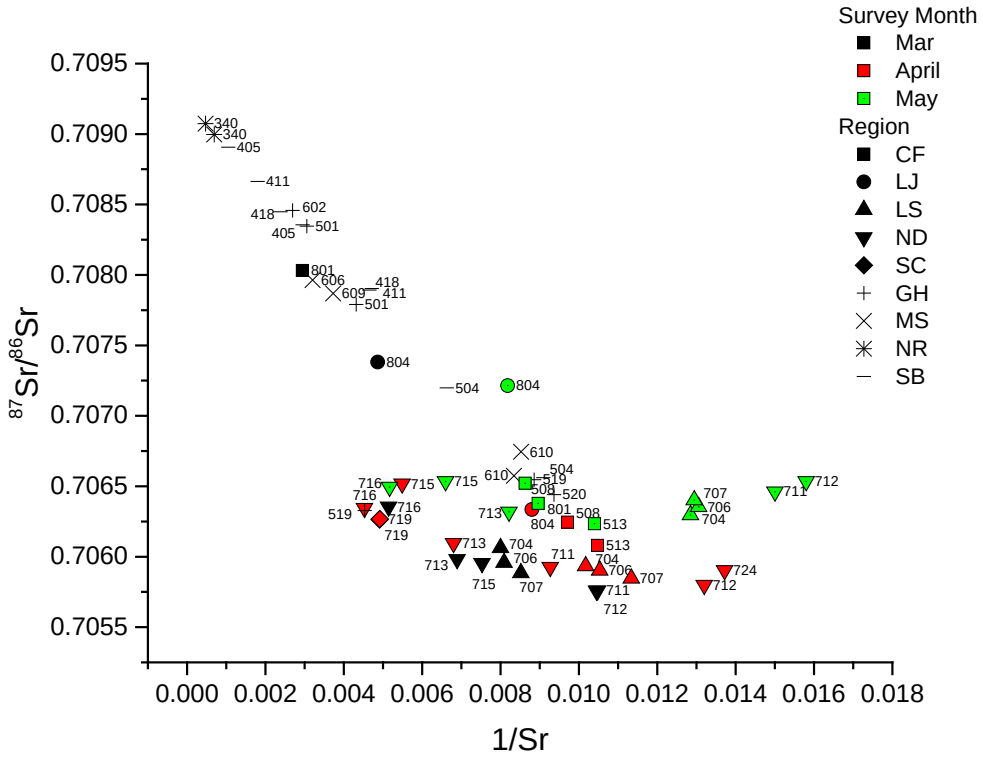


Figure S2. A. Daily Mean Flows (cubic feet per second-CFS) into the Delta from Oct 1, 2011 to Jun 1, 2012. B. Sacramento River Flows Entering the Delta, Export Volume Pumped Out of the Delta and Flows Exiting the Delta into the Bay



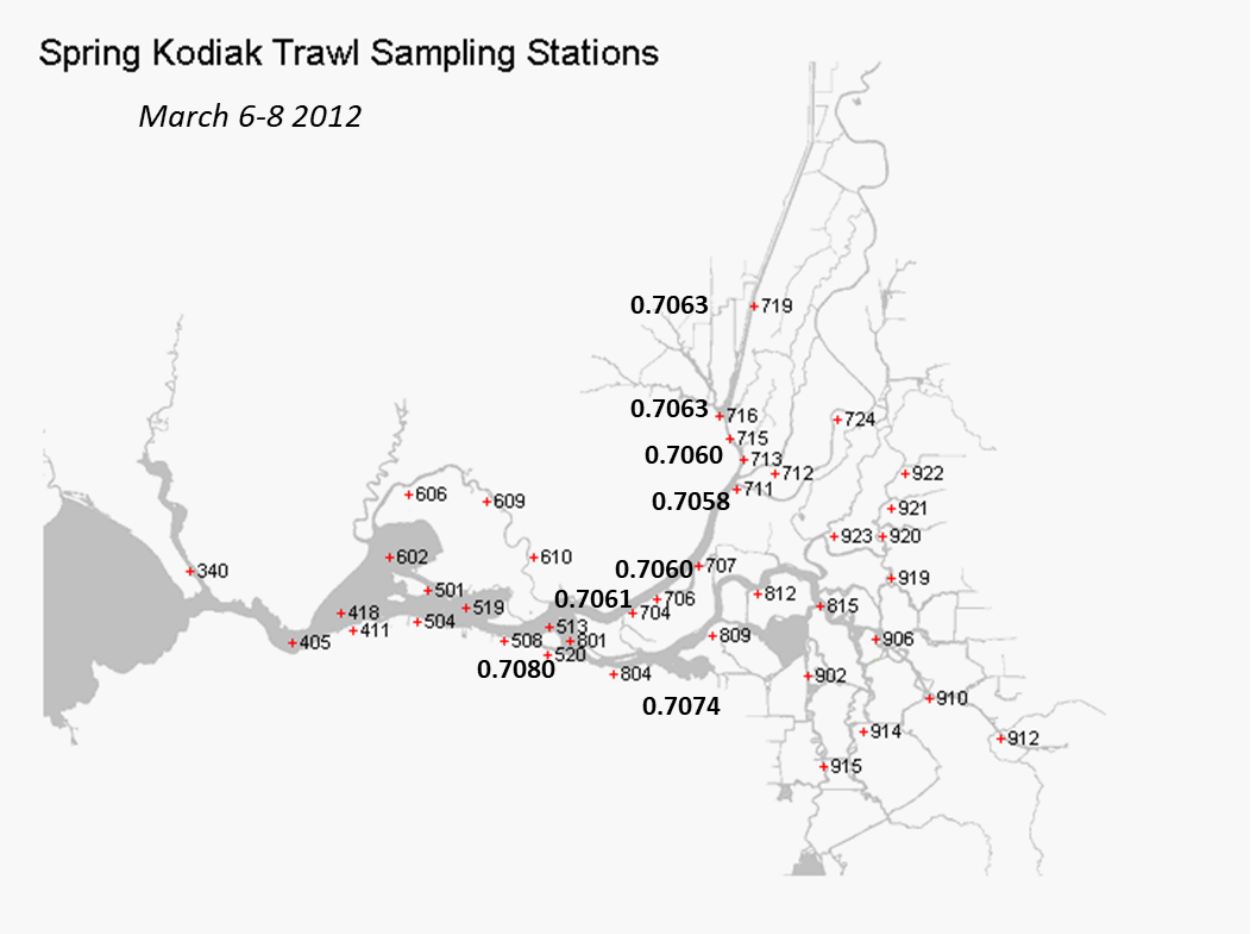
Note: The dashed line represents a linear regression of water samples while the solid line represent the mixing line which ties the lowest freshwater endmember collected in this study (station 712 5/2/12 Sr = 63 ppt and salinity = 0.06 psu) with an ocean water sample we collected during a previous survey (collected in at Muir Beach 4/23/07; Sr = 6819 and salinity = 31.8 psu). (B), is the same data focusing on the Sr concentration range up to 500 ppt and salinity of 2-psu. Symbols represent the regions of the Delta.

Figure S3. (A) Sr Concentration (parts per thousand-ppt) of Water Samples Versus Salinity (practical salinity units-psu)



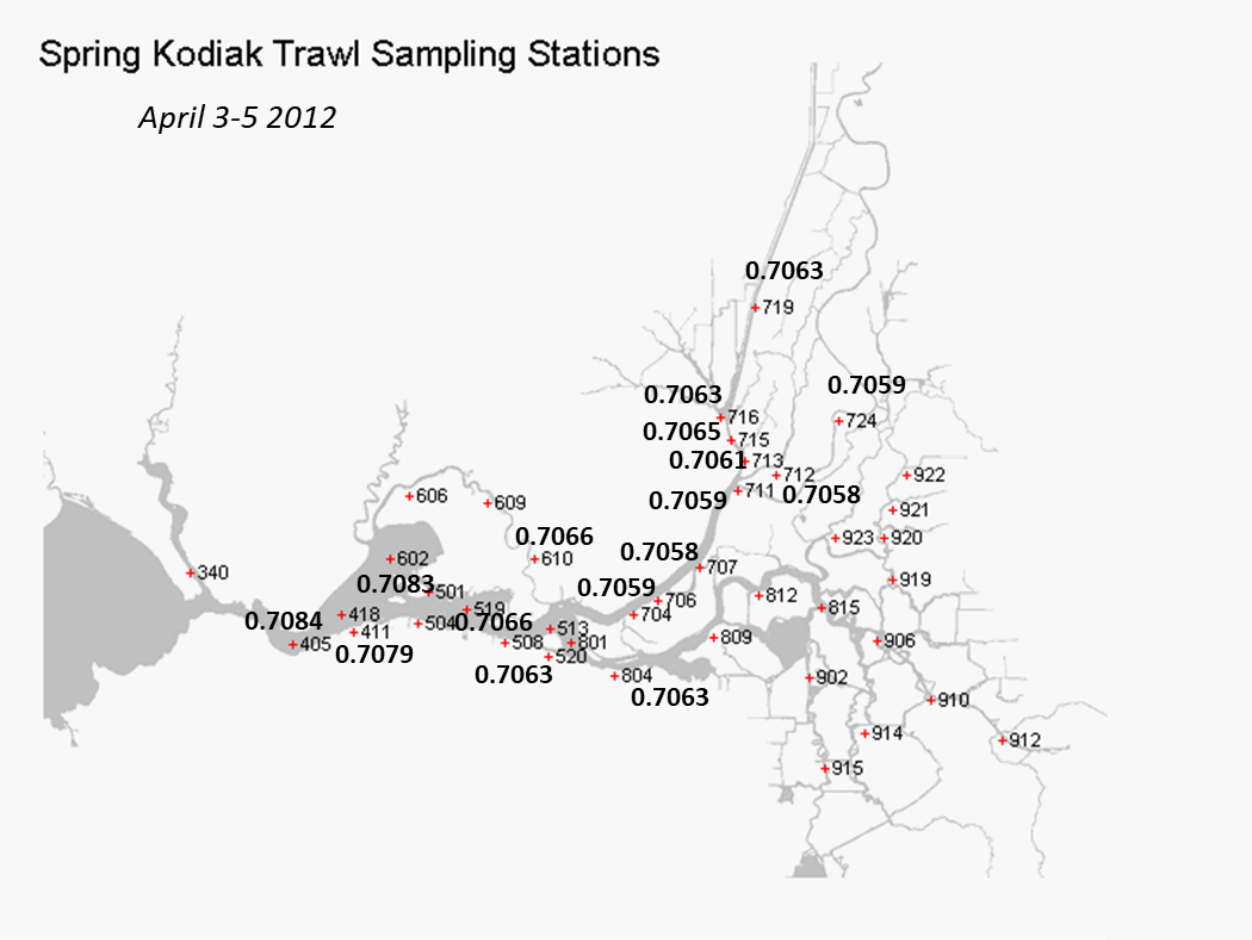
Note: Labels for individual samples are the CDFW station numbers reference in figure S1.

Figure S4. Strontium Isotope Ratio of Water Samples Versus the Inverse of Sr Concentration. Symbols Represent the Regions and Colors the Survey Month



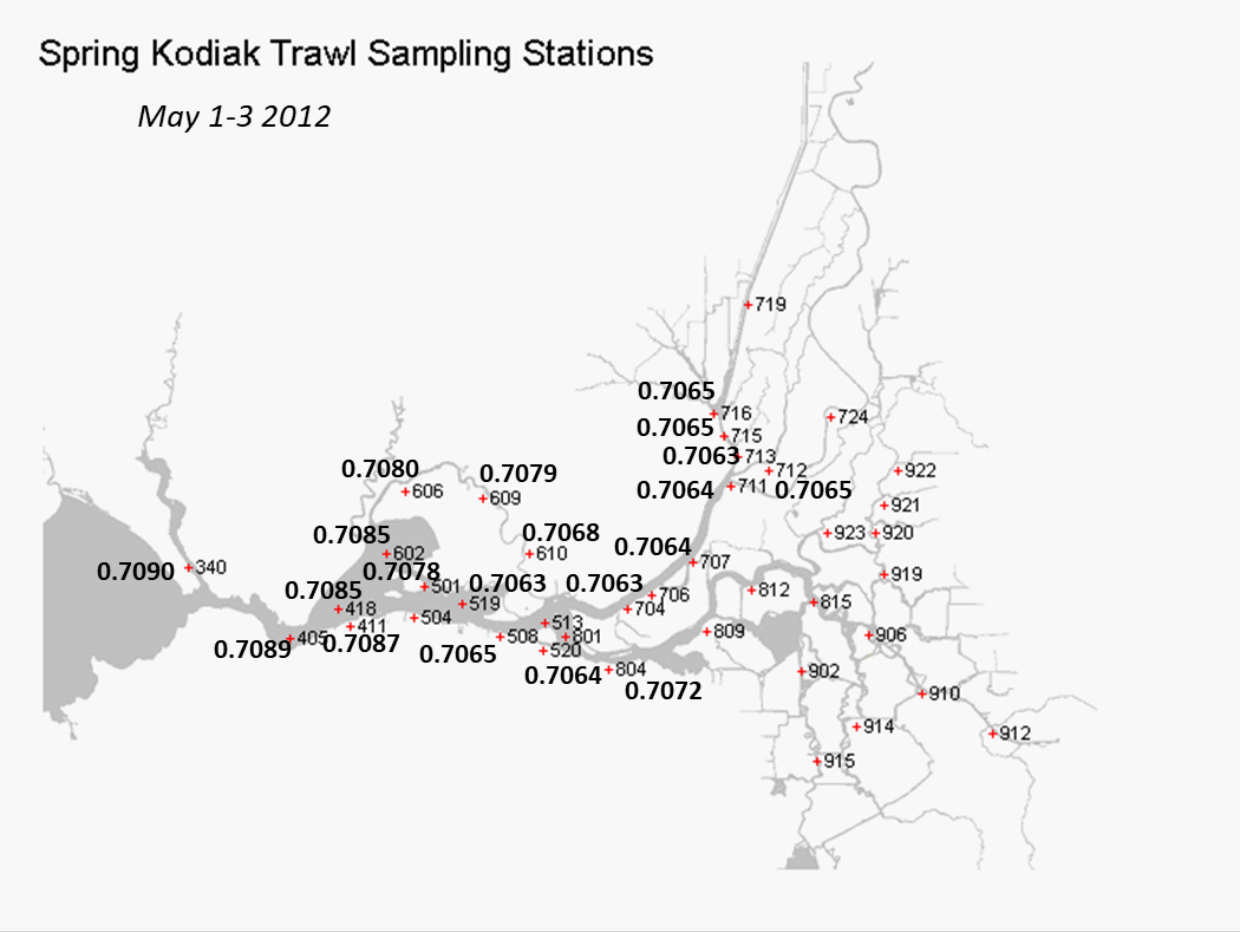
Sample ID	StationCode	Regions	SampleDate	Salinity (psu)	Sr_ppm	$^{87}Sr:^{86}Sr$		2 SE
D13	801	CF	3/6/2012	1.06	340	0.708031	±	1.35E-05
D14	804	LJ	3/6/2012	0.66	206	0.707381	±	1.26E-05
D4	704	LS	3/7/2012	0.16	125	0.706064	±	1.13E-05
D5	706	LS	3/7/2012	0.16	124	0.705959	±	1.33E-05
D6	707	LS	3/8/2012	0.15	117	0.705885	±	1.23E-05
D7	711	ND	3/8/2012	0.11	95	0.705758	±	2.22E-05
D8	712	ND	3/8/2012	0.12	96	0.705758	±	2.02E-05
D9	713	ND	3/8/2012	0.18	145	0.705981	±	1.35E-05
D10	715	ND	3/8/2012	0.20	133	0.705954	±	1.17E-05
D11	716	ND	3/8/2012	0.24	195	0.706354	±	1.06E-05
D12	719	SC	3/8/2012	0.36	204	0.706264	±	1.26E-05

Figure S5. March Survey of Water Strontium Isotopes and Water Quality at Select SKT Stations



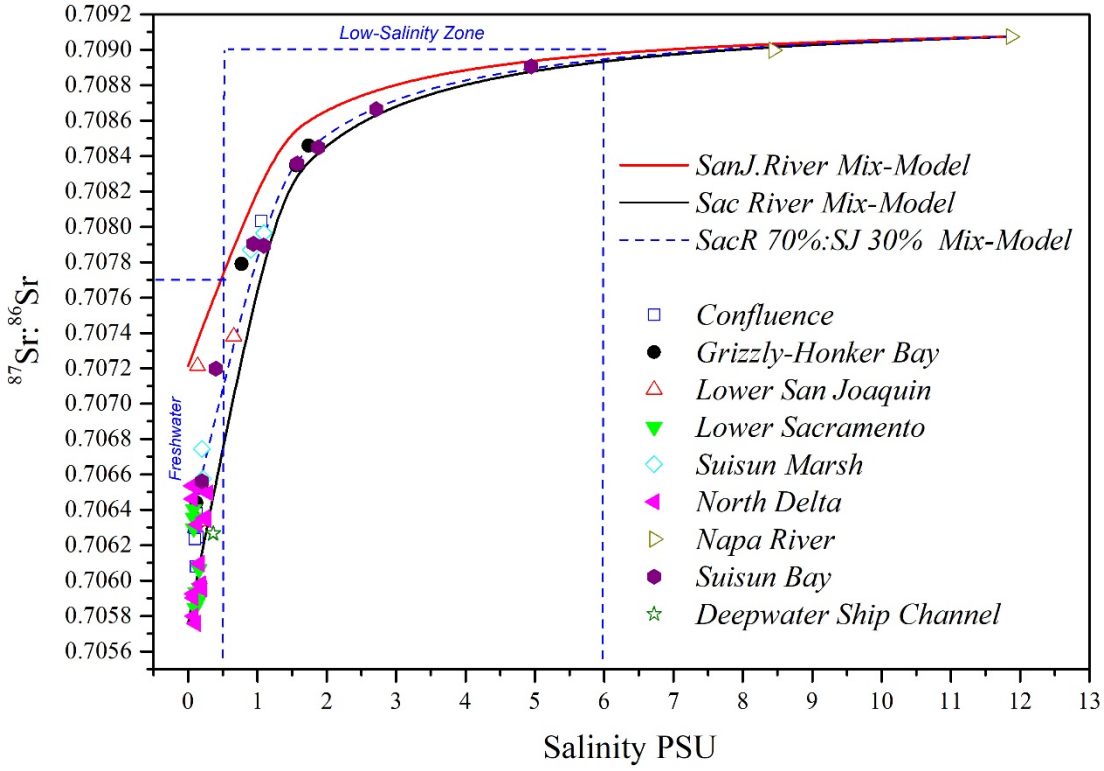
Sample ID	StationCode	Regions	SampleDate	Salinity (psu)	Sr_ppm	$^{87}Sr:^{86}Sr$		2 SE
D19	501	GH	4/3/2012	1.56	327	0.708347	±	1.22E-05
D21	508	CF	4/3/2012	0.14	103	0.706245	±	1.02E-05
D22	513	CF	4/3/2012	0.12	95	0.706081	±	1.30E-05
D23	519	GH	4/3/2012	0.20	113	0.706547	±	1.35E-05
D37	804	LJ	4/3/2012	0.15	114	0.706335	±	1.13E-05
D27	704	LS	4/4/2012	0.11	98	0.705935	±	1.68E-05
D28	706	LS	4/4/2012	0.11	95	0.705903	±	1.37E-05
D29	707	LS	4/4/2012	0.10	88	0.705846	±	1.47E-05
D30	711	ND	4/4/2012	0.08	108	0.705926	±	1.22E-05
D31	712	ND	4/4/2012	0.08	76	0.705799	±	1.28E-05
D32	713	ND	4/4/2012	0.16	147	0.706096	±	1.57E-05
D33	715	ND	4/4/2012	0.19	182	0.706518	±	1.39E-05
D34	716	ND	4/4/2012	0.26	221	0.706345	±	1.11E-05
D35	719	SC	4/4/2012	0.36	203	0.706267	±	9.62E-06
D36	724	ND	4/4/2012	0.08	73	0.705903	±	1.58E-05
D15	340	NR	4/5/2012	8.44	1447	0.708997	±	1.31E-05
D16	405	SB	4/5/2012	1.58	339	0.708357	±	1.16E-05
D17	411	SB	4/5/2012	0.77	212	0.707904	±	1.06E-05
D18	418	SB	4/5/2012	0.89	215	0.707893	±	1.05E-05
D20	504	SB	4/5/2012	0.20	110	0.706561	±	1.00E-05
D26	610	MS	4/5/2012	0.22	120	0.706574	±	1.18E-05

Figure S6. April Survey of Water Strontium Isotopes and Water Quality at Select SKT Stations



Sample ID	StationCode	Regions	SampleDate	Salinity (psu)	Sr_ppm	$^{87}Sr:^{86}Sr$		2 SE
D44	508	CF	5/1/2012	0.17	116	0.706521	±	1.90E-05
D45	513	CF	5/1/2012	0.10	96	0.706235	±	1.47E-05
D46	519	GH	5/1/2012	0.14	221	0.706328	±	1.57E-05
D47	520	GH	5/1/2012	0.12	107	0.706441	±	1.17E-05
D61	801	CF	5/1/2012	0.12	112	0.706379	±	1.38E-05
D62	804	LJ	5/1/2012	0.14	122	0.707214	±	5.46E-05
D52	704	LS	5/2/2012	0.08	78	0.706296	±	1.13E-05
D53	706	LS	5/2/2012	0.08	77	0.706355	±	1.15E-05
D54	707	LS	5/2/2012	0.08	77	0.706403	±	1.03E-05
D55	711	ND	5/2/2012	0.07	67	0.706462	±	1.36E-05
D56	712	ND	5/2/2012	0.06	63	0.706535	±	1.48E-05
D57	713	ND	5/2/2012	0.13	122	0.706318	±	1.45E-05
D58	715	ND	5/2/2012	0.16	152	0.706535	±	1.18E-05
D59	716	ND	5/2/2012	0.29	193	0.706495	±	1.62E-05
D38	340	NR	5/3/2012	11.87	2126	0.709074	±	1.83E-05
D39	405	SB	5/3/2012	4.95	952	0.708906	±	9.04E-06
D40	411	SB	5/3/2012	2.71	556	0.708664	±	1.51E-05
D41	418	SB	5/3/2012	1.88	420	0.708449	±	1.24E-05
D42	501	GH	5/3/2012	0.77	232	0.707791	±	1.43E-05
D43	504	SB	5/3/2012	0.40	151	0.707199	±	1.11E-05
D48	602	GH	5/3/2012	1.74	372	0.708458	±	1.35E-05
D49	606	MS	5/3/2012	1.09	312	0.707963	±	1.45E-05
D50	609	MS	5/3/2012	0.91	268	0.707869	±	1.28E-05
D51	610	MS	5/3/2012	0.20	117	0.706745	±	1.49E-05

Figure S7. May survey of Water Strontium Isotopes and Water Quality at Select SKT Stations



Note: Based on the average proportional flows from the Sacramento and San Joaquin contributing to the estuary, the dashed line depicts the mixing dynamics in winter 2012. Individual water samples are demarked by symbols, shapes and colors corresponding to locations.

Figure S8. Strontium Isotope Mixing Relationship Between the Sacramento River, San Joaquin River and the Ocean, Solid Lines

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Chapter 4: Environmental and Ontogenetic Drivers of Growth in a Critically Endangered Species

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Abstract

The Delta Smelt (*Hypomesus transpacificus*) was once an abundant fish, endemic only to the tidal reaches of the Sacramento-San Joaquin Delta and served as the proverbial "Canary-in-the-Coal-Mine" for ecosystem health, but is now rarely observed in long-term monitoring surveys, signifying its trajectory towards extinction in the wild. Its demise has been attributed to numerous human-caused impacts that have befuddled resource managers and stakeholders alike. The availability of high quality, low-salinity rearing habitat in the late-summer and fall has been considered an important driver of growth and survival. To improve rearing conditions for Delta Smelt, freshwater flows into the estuary in the fall are mandated in years following above-normal and wet years, however; the direct benefits of such actions to Delta Smelt have yet to be determined. Here, we used otolith microstructure techniques to quantify growth rates of Delta Smelt collected in summer and fall encompassing two wet years with fall flow-actions (2011 and 2017) and the five intervening years of drought. Somatic growth was high in 2011, and generally declined during the drought. Somatic growth distributions were elevated in 2015 and 2016, although sample size precluded clear differences in these two years. Somatic growth remained low in 2017 despite a return of high flows. Recent otolith growth (14-days prior to capture) was driven by the interactions of the three water quality attributes and age, with the poorest growth occurring when fish occupied warmer-saltier habitats with higher water clarity. When accounting for the ontogenetic age-effect on recent otolith growth, the years 2011, 2012 and 2014 had higher growth than the other years of study and when we accounted for abiotic water quality attributes, differences between years were reduced, suggesting inter-annual patterns were largely driven by differences in water quality. Regional comparisons of recent growth were complicated by the limited number of samples caught among regions and years, but generally wen did not find consistent regional pattern in growth rates. Our results indicate water temperature is the primary abiotic driver of growth and recent years have been excessively warm in the estuary resulting in poor growth and likely contributing low abundance.

Introduction

The Delta Smelt (*Hypomesus transpacificus*) was historically an abundant pelagic, euryhaline species, endemic to the tidal freshwaters of the Sacramento-San Joaquin Delta (Delta) and brackish habitats of the upper San Francisco Estuary(SFE) (Hobbs et al. 2017; Moyle et al. 2016b; Moyle et al. 2018). The population declined in the 1980s, and was listed as threatened under the Federal Endangered Species Act in 1993 (USFWS 1993) and was up-listed to endangered under the California Endangered Species Act in 2009 as a result of continued decline in the early 2000s (Mac Nally et al. 2010a; Thomson et al. 2010). While the ultimate cause of Delta Smelts demise is not known with certainty, multiple proximate factors have been considered important in contributing to the decline, including reduced freshwater flows into the estuary and loss of low-salinity habitat, alteration of the historic food web by invasive species and declining herbivorous zooplankton prey, pervasive legacy contaminants and modern herbicides, and direct mortality incurred by entrainment into small agricultural water diversions and the massive pumping facilities located in the South Delta (Bennett 2005; Fong et al. 2016; Moyle et al. 2016b).

To protect the species from entrainment into the State Water Project (SWP) and Central Valley Project (CVP) facilities, pumping is limited at times when Delta Smelt are found in close proximity to the South Delta intakes which frequently interrupts freshwater deliveries (Exports) to 25 million people and a multi-billion-dollar agriculture industry causing significant economic stress and political tension (Moyle et al. 2016a; Moyle et al. 2018; Scoville 2019). Limiting exports has been highly controversial because estimated impacts of direct mortality by SWP and CVP on the population have been highly variable and relatively small in most years and not found to have been a driving force on population trends (Kimmerer 2008; Kimmerer 2011; Mac Nally et al. 2010b; Maunder and Deriso 2011), although at times the impacts can be large (Kimmerer and Rose 2018).

In addition to limiting exports, freshwater flows are required to position the Low Salinity Zone (LSZ), a volume of low-salinity habitat (1 to 6-psu) within the shallow shoals and marshes of Suisun Bay and Marsh, (Cloern and Jassby 2012; Jassby et al. 1995) during the months of September and October of years when the preceding precipitation and runoff period was Wet or Above Normal as defined by the Sacramento Basin 40-30-30 index (hereinafter Fall X2 Action)(USFWS 2008). Delta Smelt inhabit the LSZ during summer and fall months before dispersing to tidal freshwaters of the Delta in winter to spawn (Bennett 2005; Bennett and Burau 2015; Moyle et al. 1992) and prefer turbid habitats with low tidal velocities, conditions commonly found in the shallows of Suisun Bay (Bever et al. 2018; Bever et al. 2016; Feyrer et al. 2007; Nobriga et al. 2008). Freshwater flows entering the estuary have declined during the fall months as a result of increased exports at SWP and CVP (Hutton et al. 2017a; Hutton et al. 2017b) resulting in greater intrusion of saline water into the Delta, shifting LSZ upstream into the deeper more channelized confluence region of the Sacramento and San Joaquin Rivers and reducing nursery habitat quantity and quality for Delta Smelt (Bever et al. 2016; Kimmerer et al. 2013). This reduction in habitat in the fall was found to be an important covariate in stock-recruitment models predicting successful recruitment to the next generation (Feyrer et al. 2011; Feyrer et al. 2007); however, this trend has weakened with additional years of data (ICF 2017). When the LSZ is located within Suisun Bay, the volume of suitable habitat for Delta Smelt is

greatly expanded (Kimmerer et al. 2013), likely improving conditions for growth and survival (Brown et al. 2014; IEP-MAST 2015)

Rapid growth in the early life of fishes is considered a critical vital rate, with rapid growth resulting in increased survival probability due to greater ability to avoid predation and capture prey (Cushing 1990; Hjort 1914). Furthermore, subtle differences in growth and subsequent mortality in the larval stage can lead to large differences in recruitment and year-class strength (Anderson 1988; Houde 1989a; Leggett and Deblois 1994), thus understanding the biotic factors the affect growth in the early life is critical for managing fisheries. Environmental variability can also lead to significant variability in early life growth and recruitment. Water temperature has a direct effect on metabolic rates in poikilotherm fishes, where growth rates are generally higher in warmer temperature (Houde 1989b), however; when species are found at temperatures near thermal limits growth rates can be reduced significantly (Neuheimer et al. 2011; Neuheimer 2019; Wenger et al. 2016).

Otoliths have long been used to determine growth rates in fishes. Otoliths are small bone-like structures found in the inner ear of fishes and are formed by secretion of calcium carbonate and proteins into the endolymph of the inner ear creating layers of light and dark bands on the otolith. These layers have been validated to infer daily age and Delta Smelt otoliths grow in direct proportion to fish growth (Hobbs et al. 2007). The width of otolith increments allows assessment of growth, analogous to tree-ring based dendrochronology, assuming increment width is a good proxy for fish growth. Otolith size for cultured Delta Smelt has been shown to be a good proxy for fish size and growth (Hobbs et al. 2007), thus we can use daily otolith increments widths as our primary variable for examining effects of environmental conditions on Delta Smelt growth.

To gain a better understanding of how freshwater flow management influences Delta Smelt growth, we used otolith age and increment widths as a proxy for fish growth in this study. Our primary research objective was to determine if Delta Smelt grew faster when fall flows were managed to maintain the LSZ within Suisun Bay? The years 2011 and 2017 were “Wet-Years” resulting in implementation of the Fall X2 Action, and the LSZ was located within the Suisun Bay/Marsh region in September and October. However, in 2011 the vast majority of Delta Smelt collected by monitoring surveys were from Suisun Bay precluding a regional comparison in that year, while in 2017 fish in low numbers were collected in Suisun Bay and the Lower Sacramento River. Since Delta Smelt are pelagic mobile species, and have been observed to migrate towards freshwater in the fall, we could not be certain fish collected in the Lower Sacramento River represented a distinct group of fish from those collected in Suisun Bay, therefore we included fish collected from 2012-2016, a period of drought and much reduced overlap of the LSZ with Suisun Bay to compare inter-annual growth variability to address the question,

Did Delta Smelt Grow Faster in Wetter Years When the LSZ Occurred in the Suisun Bay/Marsh Region in the Fall?

This approach relaxes the assumption that measured growth was attributable to the region and associated habitat attributes where a fish was captured. In this approach we also address the growth response of Delta Smelt to drought conditions. While, this approach provides a reasonable means to hypothesis test, this analysis does not provide a model for predicting Delta Smelts growth response to flows or abiotic habitat attributes the respond to flow management. Therefore, we used recent otolith growth (14-Day otolith margin increments) and abiotic water

quality attributes, salinity, temperature and turbidity, the primary habitat variables that determine habitat quality to address the question,

How does Abiotic Habitat Attributes Salinity, Temperature and Turbidity Influence Delta Smelt Growth?

Previous otolith growth studies have demonstrated the strong ontogenetic (age) effect that occurs in Delta Smelt (Hobbs unpublished report). Therefore, we included age as an intrinsic factor that controls otolith growth, which provides for better model predictions for extrinsic effects (Morrongiello and Thresher 2015). With the marginal increment model we also address question 1. and determine if regional differences in growth occur and if regional difference are determined by abiotic water quality attributes.

Methods

Collection of Delta Smelt

Delta Smelt were collected by several agency partners from 2011 to 2017 for this study (Table S1). For each survey, fish were given a unique serial number upon capture, measured for fork-length to the nearest 1-mm and frozen in liquid nitrogen. Sagittal otoliths were dissected from the fish cranium using ultra-fine forceps (Dupont® SE140, stainless steel) and stored dry in tissue culture trays. Before mounting, otoliths were "cleared" by soaking in 95% ethanol for up to 24 hours. Otoliths were then mounted onto glass slides with Crystal Bond® thermoplastic resin in the sagittal plane, ground to the core on both sides with 1,200 grit wet-dry sandpaper and polished with a polishing cloth and 0.3-micron polishing alumina on polishing wheel (MIT Corp). Otoliths were digitized with a 12-Megapixel digital camera (AM Scope) at a magnification of 20X with an Olympus CH30 compound microscope. Digital images at 20X magnification were merged into a complete image of a transect from the core to the dorsal edge (Adobe Photoshop) at a 90° angle from the primary axis of the otolith.

Otolith daily increments were enumerated and increment widths where measured using calibrated images in Image-J 4.0 (United States National Institutes of Health; https://imagej.nih.gov/ij/). Image clarity for daily increments at the edge of the otoliths were evaluated by visual inspection and scored from 1 to 3, 1 being highest quality and 3 lowest. Samples scored 3 were excluded from growth models.

Age was determined for each fish using multiple independent readings of age for each fish, where mean age of at least two age readings was used when age readings of an individual was less than 10% of average percent error (APE). If consensus was not achieved for the multiple age readings, the age reading with the greatest difference from the mean was discarded and APE was recalculated. If consensus was not achieved after removing outlier age readings that sample was removed from further analysis.

Somatic Growth Rate (G)

EQ 1. $$G = {Length_{capture} - Length_{hatch}} \Big/ {Age}$$

Where age was the mean number of otoliths increments from the multiple age readings length at hatch was assumed to be 5.2 mm based on the captive-reared Delta Smelt population maintained by the UC Davis Fish Conservation and Culture Laboratory (Bennett 2005). We used Somatic Growth Rates to determine how Delta Smelt growth varies by month and year.

Recent "Marginal Increment Growth" was quantified using the mean width of the last 3 ,7, 14, and 30 daily increments prior to capture. Otolith increment widths have been shown to be proxy of somatic growth in fishes including Delta Smelt (Hobbs et al. 2007). This approach was employed to test hypotheses regarding growth responses to capture regions, survey month, years and abiotic habitat attributes. We used generalized additive models (GAMs) to model growth response to abiotic habitat attributes measured at capture (salinity [PSU], temperature [°C] and Secchi depth [cm]), which were assumed to be a reasonable proxy for the abiotic conditions experienced by each fish. GAMs were preferred over linear models (e.g., GLMs) as they use nonparametric smooth splines which can describe any complex non-linear functional shape between response and predictor variables (Wood 2011). Splines for multiple independent predictor variables can be used additively and/or interactively to predict the response variable. GAMs were conducted using restricted marginal likelihood (REML) and generalized cross validation (GCV) in the mgcv package in R version 3.2.2. Growth was modeled using a Gaussian distribution with an identity link function since growth data was normally distributed.

GAMs were constructed (Table S2) to first examine intrinsic drivers of growth or ontogenetic effects (age and prior growth rate) which can explain a majority of growth variation in young fishes. Delta Smelt undergo multiple ontogenetic growth transitions, occurring at the larval to early juvenile stage and juvenile to sub-adult life stage (Hobbs et al. 2007). Moreover, growth in young fishes is highly auto-correlated, thus fish growing fast during a period of investigation is likely to have been growing fast prior to the period of investigation. We tested five intrinsic models; models with age (at the beginning of the of the marginal growth period), prior growth rate only, a model with age, a model with the additive effects of age and prior growth and the interaction term for age and prior growth, and prior growth additively and a model with an interaction term for age and prior growth

Extrinsic models were developed using model residuals from the best-fitting intrinsic model to assess the additional deviance explained by including extrinsic variables. Extrinsic effect models included abiotic habitat attributes (salinity, temperature and water clarity) individually, additively and with interactive terms in the models, and were evaluated as with the intrinsic model procedure. Model smooth complexity was limited to a basis dimension of $k = 6$ to prevent over-parameterization and the thin-plate regression spline was used for additive models and the tensor product smooth (*ti*) was used for interactive models. The model structure with the highest, R^2, deviance explained, and lowest estimated degrees of freedom and AIC was chosen as the best model.

To facilitate regional comparisons of growth, we assigned sample stations from the California Department of Fish and Wildlife's long-term monitoring surveys to regions defined by the U.S. Fish and Wildlife Services Enhanced Delta Smelt Monitoring Program. We examined capture region (Fig.1), month and year as categorical dummy variables in GAMs using the random basis function "re", to examine growth response to spatial and temporal variability. The random

smoother “re” used on categorical variables provides a similar statistical analysis to linear models such as ANOVA.

Results

We aged a total of 1,445 Delta Smelt collected from 2011 to 2017 with an overall mean average percent error between multiple age-readings of 3.7%. Catch and subsequent archival of Delta Smelt for this study was heterogeneous, precluding detailed statistical hypothesis testing of growth across fully-factoral year, month, and region factors (Fig. 2). Despite this heterogeneity in sample distributions, we explored variation in growth rates using box-plots of growth representing the median and the 25^{th} and 75^{th} quartile range (Fig. 3 and Fig. 4). Somatic growth rates of Delta Smelt appeared to vary by year, with 2011 and 2015 exhibiting higher median growth, and generally declining during the drought, and remaining relatively low during the 2017 wet year (Fig. 3). Growth rates also varied by survey month, being higher during the summer months than fall months and were higher in September and October of 2011 compared to other years (Fig. 4A).

Our first study question pertained to Delta Smelt occupying the Suisun Bay and Suisun Marsh region in September and October however; due poor sample dispersion among regions, months and years we could not make direct comparisons within years between regions, even when aggregating Suisun Bay and Marsh into a single region, save for 2017 (Fig. 4B). In this wet year (2017) there appeared to be no difference in somatic growth rates between Suisun Bay/Marsh and the Lower Sacramento River.

For the recent “marginal growth” (14-Days prior to capture) analysis we selected a sub-set of samples to provide better dispersion of samples by survey months (September to November) from 2011 to 2017 and survey regions whereby a random draw of 10 individuals was taken from the full dataset. In addition, digital images of otolith marginal otolith increments were scored for image quality and interpretation of the last 3-30 increments and only otoliths with high certainty were retained. The final dataset included a total of 282 fish collected by the FMWT, TNS and EDSM. We examined four intrinsic growth models including age, prior growth rate only, the additive age + prior growth and interactive effect of age and prior growth rate on the marginal otolith growth. Results for the 3, 7, 14 and 30-Day mean increment widths were similar, thus we chose to use the 14-Day mean increment width for the remainder of the analysis. Models were assessed for model performance and fit, and stronger models were indicated by higher deviance explained given the effective degrees of freedom (edf), a measure of model complexity, and model R^2 and overall lower AIC values (Fig. 5). Intrinsic model results were similar for models including age and prior growth, explaining 61 to 66% of deviance, an R^2 of 0.64 and having similar AIC from 672.7 to 643.3. However, model edf was double for models including prior growth, thus we chose the age-only intrinsic model when evaluating extrinsic factors. Recent otolith growth was strongly driven by ontogeny (age), where young fish <125-Days old grew faster than older fish (Fig. 6). The age-only intrinsic model was highly statistically significant $p < 0.0001$, explained 61% of the deviance and an $R^2 = 0.62$ and there was no pattern in the model residuals that would suggest poor model fit.

We evaluated 8 models including categorical extrinsic factors (month, year and region) that accounted for the intrinsic age-only effect by using model residuals, and 7 models that included the age-only intrinsic effect and abiotic habitat attributes (temperature, salinity and Secchi depth) and 7 models using residual growth from the intrinsic age-only effect with abiotic habitat attributes (Table S2). All models including region as a factor were generally weaker than models with year and month factors (Fig. 5). The year effect was the strongest of the categorical models with lower edf, and AIC and higher R^2 and deviance explained. Year explained 32% of model deviance and had an $R^2 = 0.26$ and was the only term that had a significant p-value. Age-corrected marginal growth over the last 14-days was higher in 2011, 2012 and 2014 and lower in with poor growth 2013, 2015, 2016 (Fig. 7). Growth during the wet year of 2017 was generally low for the study period, but exhibit greater variation, likely due to the larger sample size acquired by the EDSM. Median growth for the North Delta, specifically the Cache Slough/Liberty Island and Sac. Deepwater Shipping channel was elevated relative to the other regions, but these differences were not statistically significant (Fig. 7).

Models for extrinsic abiotic habitat attributes only (i.e. residual models) explained 1 to 32% of model deviance, R^2 ranging from 0.02-0.22 and when combined with the intrinsic age-only effects, explained 66 to 72% percent of model deviance, R^2 ranging from 0.63-0.77 (Fig. 5). The model including the three abiotic habitat attributes and their interactions (tsc) had the highest model deviance explained, but was also the most complex model with an edf of 32, which was approximately 2-times greater than the tc model (Fig. 5). The tsc explained ~5% more deviance than the tc model and only had a slightly lower AIC than tc. In all the models, the effect of temperature was the strongest driver of marginal otolith growth and in general, trends for all three variables varied little among model structures. Growth declined with increasing temperature and salinity, and was slightly reduced when fish were caught in areas with Secchi depths less than 0.2-m (Fig. 8).

Lastly, when accounting for both the ontogenetic effect and abiotic effect on growth, inter-annual differences in growth were reduced relative to the models with only the ontogenetic effect (Fig. 7), further suggesting the abiotic water quality attributes were the principle drivers of Delta Smelt growth where regional differences would likely be driven by the abiotic attributes encountered by fish (Fig. 9)

Discussion

Despite the wet year in 2017 positioning the LSZ within Suisun Bay for much of the year, growth rates for fish collected within Suisun Bay/Marsh did not appear to be elevated relative to fish caught in the Lower Sacramento River. However, growth did appear to be high for fish in Suisun Bay during the fall of 2011. While we did not include prey availability in this study, herbivorous zooplankton including *Psuedodiamptomus forbesi* appeared to have been abundant in 2017 in most regions of the estuary including Suisun Bay and Suisun Marsh (see Schultz CH 10 DOP report and Hennessey IEP-MAST report). Turbidity was also elevated in 2017 and similar to the other wet year (2011) within Suisun Bay and Suisun Marsh suggesting the LSZ habitat was suitable for Delta Smelt feeding and growth. The principle difference between 2011 and 2017 was summer water temperatures within Suisun Bay and Suisun Marsh (see Schultz CH 10 DOP report and MacWilliams IEP-MAST report). Summer daily mean water temperatures

where 1-2 °C higher in 2017 than 2011 and was likely an important driver of growth differences between these two wet years (Fig. 10). Small increases in water temperature can have large effects on growth in young poikilothermic fishes such as Delta Smelt. Moreover, several studies have indicated Delta Smelt are particularly sensitive to warm water (Jeffries et al. 2016; Komoroske et al. 2015; Komoroske et al. 2014).

Variability in growth over the lifetime of a fish can vary due to myriad factors as the fish disperses from natal habitats and searches for suitable rearing habitat. The majority of Delta Smelt were born in freshwater habitats, although some appear to hatch into low-salinity habitats according to natal otolith strontium isotope ratios (Bush 2017). Furthermore, we found that Delta Smelt exhibited a wide range of residency times in freshwater during the early-life (Hobbs CH4 DOP report), thus habitat conditions in freshwater and the LSZ need to be accounted for when assessing growth patterns. To minimize variability created by prior history, we used otolith increment widths at the otolith margin (14-Days) in this study, which reflects recent somatic growth (Hobbs et al. 2007). Assuming fish capture location represented variability in habitat conditions encountered during formation of increments over the last 14-Days, we found little evidence for region as an important factor contributing to Delta Smelt growth, however; sample biasing in space and time can confound any meaningful assessments of a regional effect when samples size vary considerably by survey month, year and region. However, otolith growth was influenced by water quality conditions measured at capture, the poorest growth occurring when fish were captured in waters with high water clarity, high salinity and warm temperatures. Recent physiological and behavioral studies on Delta Smelt suggests the species is very sensitive to multi-stressor conditions supporting our observations on the interactions of these three abiotic habitat attributes effect on growth (Davis et al. 2019).

Our results suggest temperature is an important abiotic factor that could be limiting recruitment success for Delta Smelt. Laboratory studies examining the biochemical and molecular response to thermal stress show that the species elicits a suite of sub-lethal biochemical responses at temperatures 4-6 °C below their critical thermal maximum, which would correspond to acute exposures ranging from 22-23 °C (Komoroske et al. 2015; Komoroske et al. 2014). During the drought, water temperatures throughout the estuary were frequently near this threshold and at times higher (Fig. 10). Our growth model would indicate that much of the estuary was marginally suitable for growing Delta Smelt in the summer and early fall months. Poor growth likely results in higher mortality and could further explain the extremely low abundance during the drought. Poor growth in the sub-adult stage fish may also contribute to overall lower egg production by producing smaller fish at maturity, delaying maturity or limiting the fishes capacity to produce multiple batches of eggs within a season (Damon et al. 2016). The drought also had a significant impact on the maturation window, a theorized period of time when temperatures are suitable for growth and fish are capable of investing energy into gonad maturation (Brown et al. 2016). During the peak of the drought in 2014 and 2015 the maturation window was approximately one month shorter precluding the potential production of multiple batch-spawns in those years (Hobbs CH4 DOP report).

Growth was generally higher for fish caught in freshwater to salinity of approximately 4-psu, after which growth declined. (Komoroske et al. 2016) discovered that while Delta Smelt are capable of living in salinity up to 32-psu for short durations, fish experiencing salinity below 18-psu exhibited significant molecular impairment suggesting prolonged exposure to salinity greater

than 6-psu could be energetically detrimental. Meanwhile the trend with salinity may be indicative of poor feeding conditions in higher salinity habitats. Several studies have documented lower herbivorous zooplankton prey density and biomass in more brackish areas than freshwater (Hammock et al. 2015; Kimmerer et al. 2018). However, despite differences in zooplankton density, feeding success was higher for Delta Smelt occupying the LSZ (see Slater DOP report). Turbidity is thought to be an important habitat attribute, providing refuge for predators while also being important for detecting transparent prey for larval Delta Smelt (Hasenbein et al. 2013) and the combination of higher turbidity and available herbivorous zooplankton prey in the LSZ likely explains the trend in feeding success.

The management of Delta Smelt and their habitat has largely been focused on freshwater flows in the fall to maintain suitable nursery habitat in the geomorphic complex Suisun Bay and Suisun Marsh. While this management strategy does increase the volume of the LSZ, this study and the associated studies conducted by the Directed Outflow Project suggests that the Fall X2 Action may not provide increased habitat quality. This may be due to the deleterious impacts of invasive species on foodweb productivity or further erosion of physical habitat quality within the Suisun Bay and Suisun Marsh region. This study and the report on life history diversity (Hobbs DOP CH4 “Exploring Life History Diversity in a Critically During Endangered Species During Extreme Environmental Variability”) indicates temperature is an additional important abiotic stressor limiting the recovery of Delta Smelt. Furthermore, the population abundance may have surpassed a level where increased abundance during years of “good” conditions may no longer be feasible due egg limitation of difficulty in finding mates.

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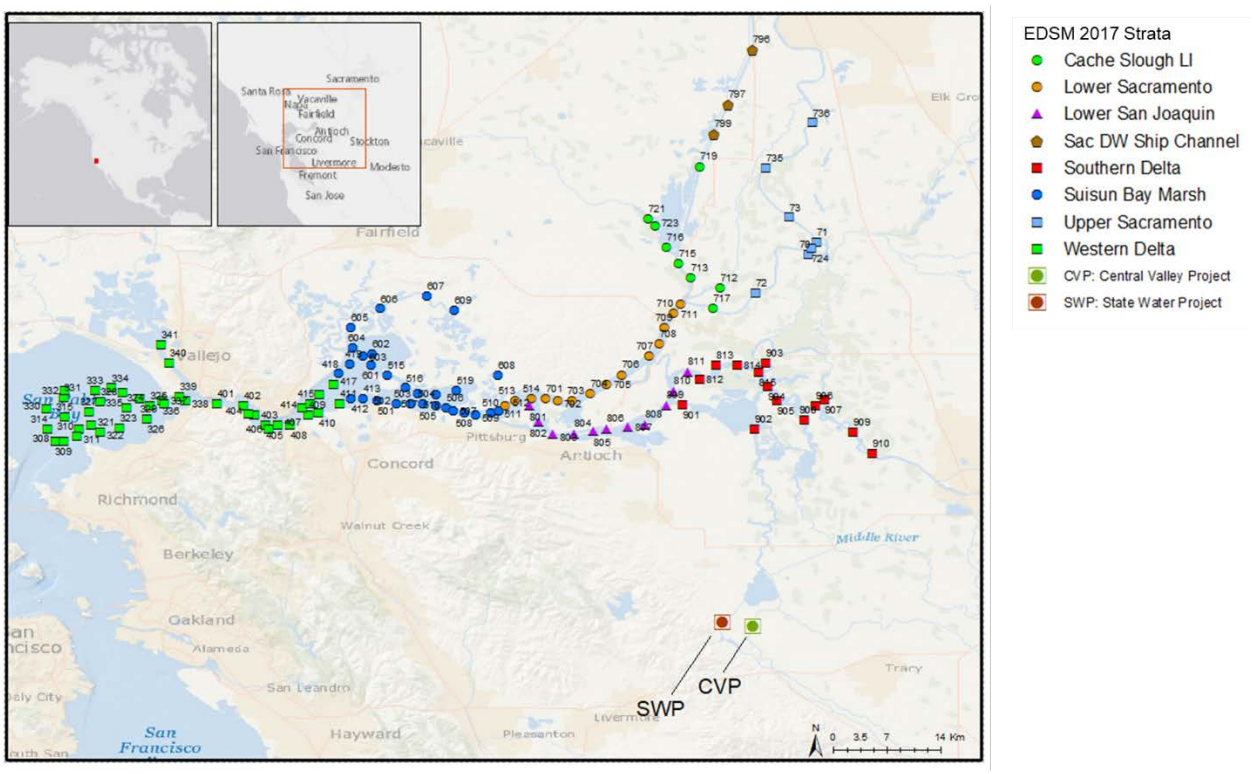
Table 4-1. Sample Sizes Available to Compare Suisun Bay/Marsh with the Lower Sacramento Strata by Year and Month

	JUN		JUL		AUG		SEP		OCT		NOV		DEC	
Year	**LS**	**SB**	**LS**	**SB**	**LS**	**SB**	**LS**	**SB**	**LS**	**SB**	**LS**	**SB**	**LS**	**SB**
2011	1	20		18	3	36	1	22		30	6	3	71	30
2012	19	11			2	5	60		19	42	8			3
2013	8	33		17	1	3	182	2	1		19		2	3
2014	25		9	8	72	1	58	1	11				35	1
2015			3		1	1	24		1				2	
2016											6			
2017		3		6	3	27	17	10	31	5	2	3		



EDSM Region	EDSM Strata
Far west	Western Delta
West	Lower Sacramento
	Lower San Joaquin
	Suisun Bay/Marsh
North	Lower Sacramento
	Upper Sacramento
South	Lower San Joaquin
	Southern Delta

Notes: Station numbers are the CDFW station numbers and the symbol and colors are the EDSM stratum definition used in 2017. Table depicts the EDSM Regions, a high order regional aggregation of EDSM Strata. Note that the Lower Sacramento EDSM Strata is split between stations 705 and 706 for inclusion in the West or North Region.

Figure 4-1. Map of the Upper San Francisco Estuary

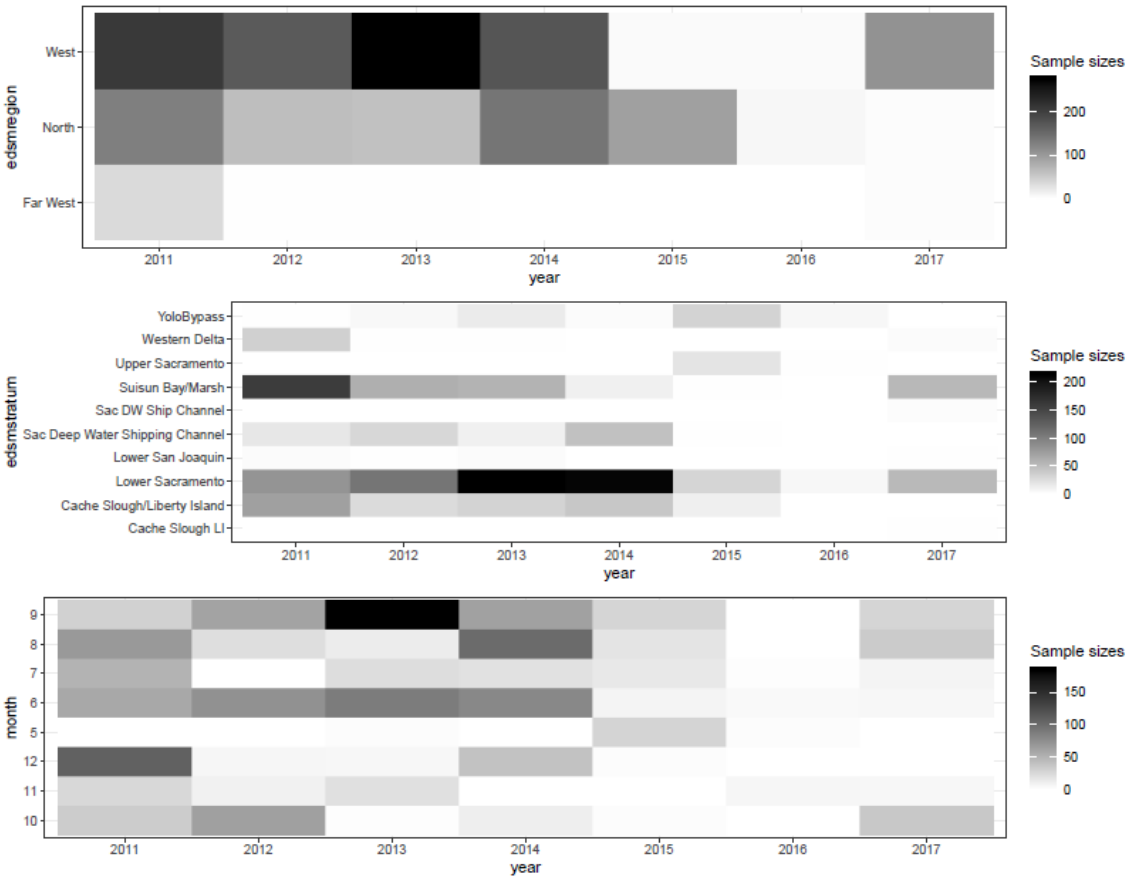
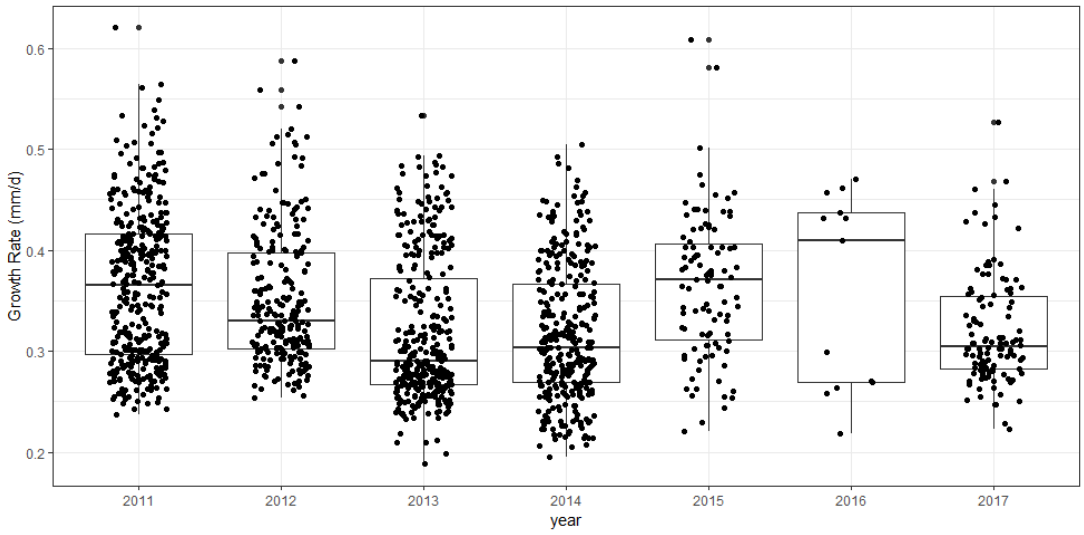
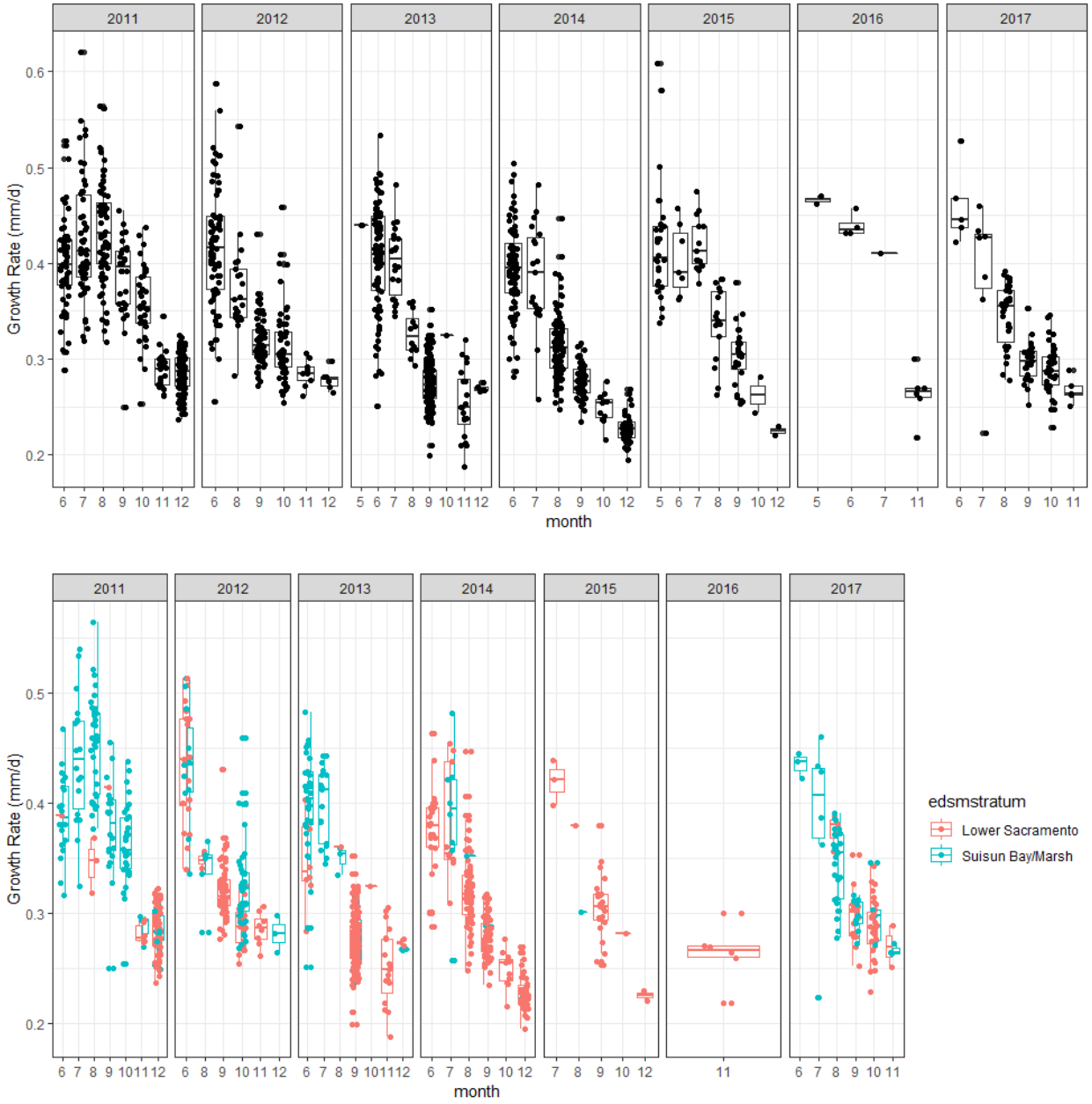


Figure 4-2. Heatmap of Sample Sizes by EDSM Region, Strata and Survey Month by Year



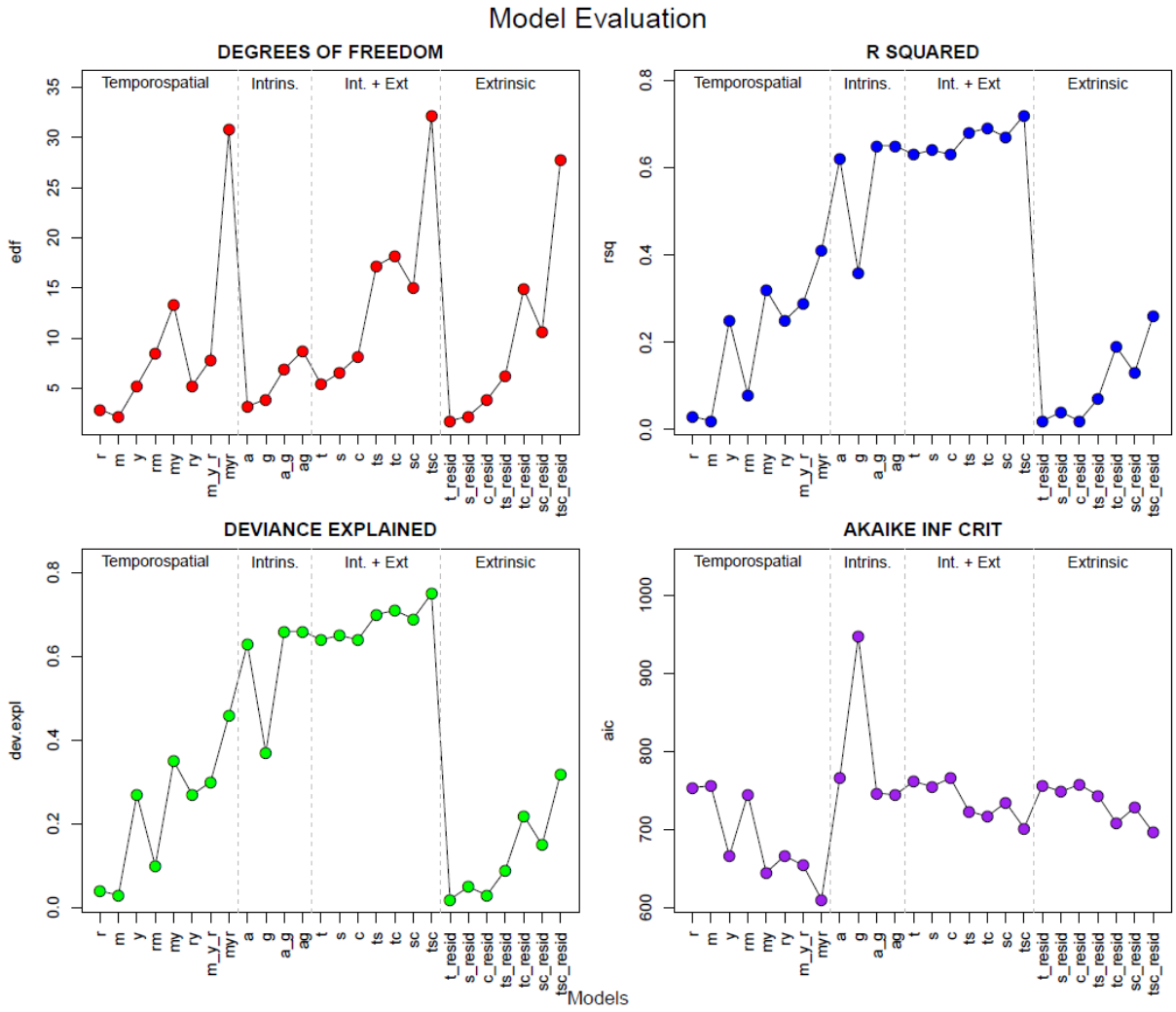
Note: Data represents fish collected throughout the estuary from a variety of surveys from May through December (Table S1).

Figure 4-3. Somatic Growth Rates (G) of Delta Smelt Collected from Surveys Conducted from 2011 to 2017



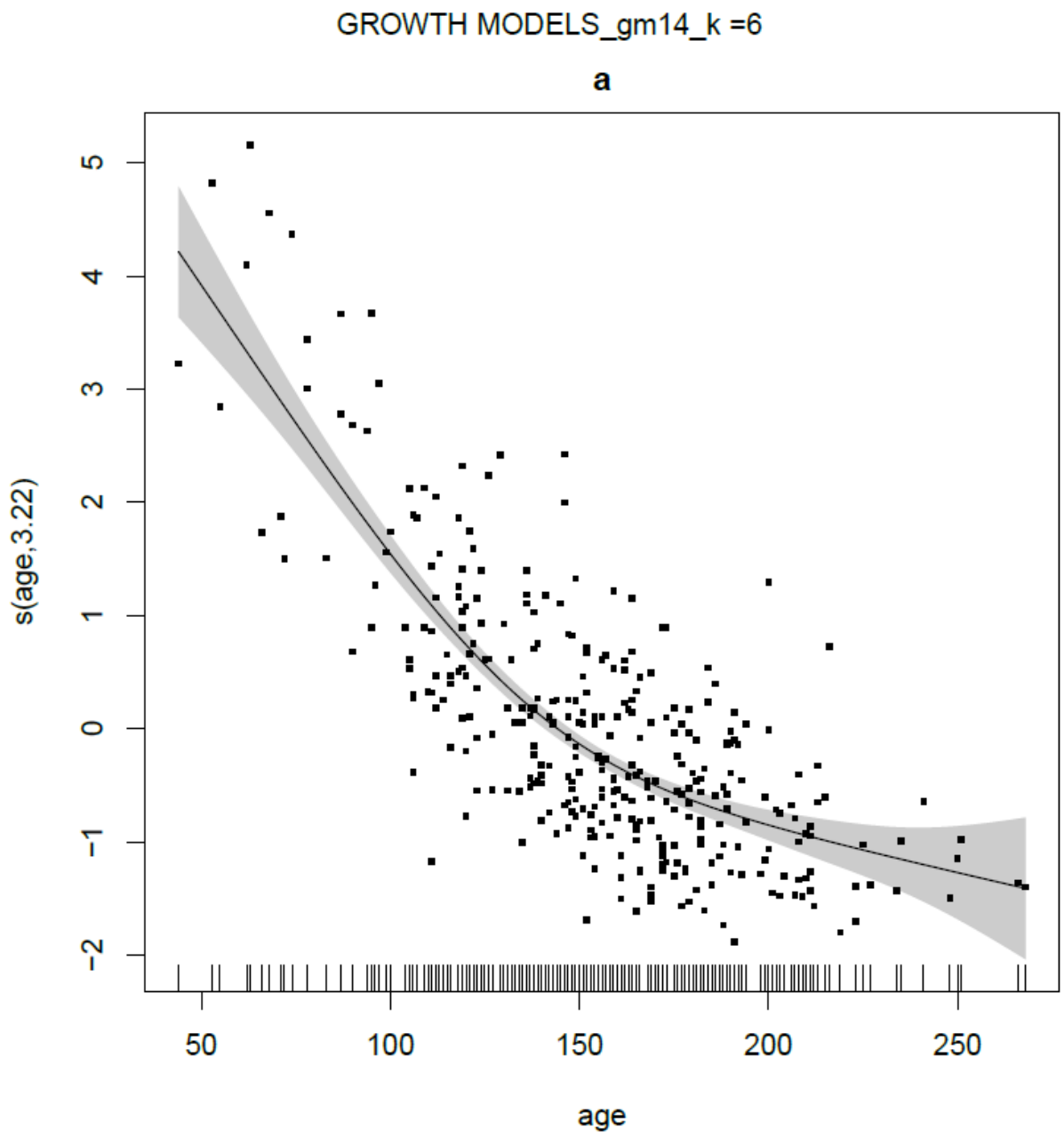
Note: (Top) by Survey Year and Month for all EDSM Stratum, (Bottom) survey year and month for the Suisun Bay/Marsh and Lower Sacramento EDSM stratum.

Figure 4-4. Boxplots of Somatic Growth Rates (G)



Notes: Categorical factors; r=region, m= month, y= year; abiotic habitat attributes including the intrinsic effect a=age, g=prior growth rate, t=temperature, s=salinity, c=clarity (Secchi depth), and abiotic habitat attributes using residuals from intrinsic model. Top left, model effective degrees of freedom-edf, top right, model R^2, bottom left= model deviance explained and bottom right, AIC.

Figure 4-5. Model Results of Marginal Otolith Growth Rates



Note: Age-corrected residuals from intrinsic models were used to compare additional factors.

Figure 4-6. Intrinsic Effect of Age on Marginal Otolith Growth (14-day) Rate from 2011-2017

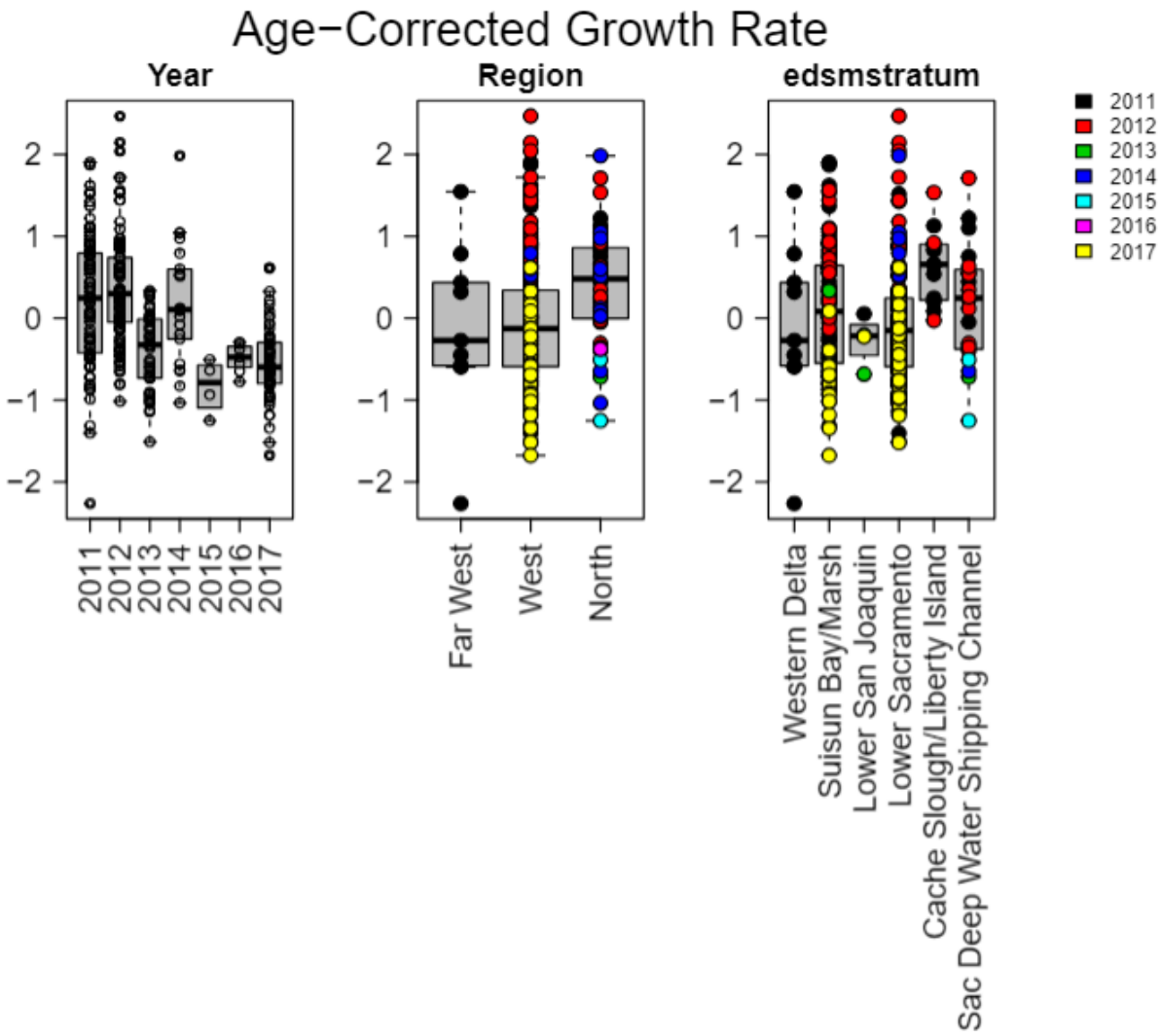


Figure 4-7. Ontogenetically Corrected Growth Response by Year, EDSM Region and EDSM Strata for 2011-2017

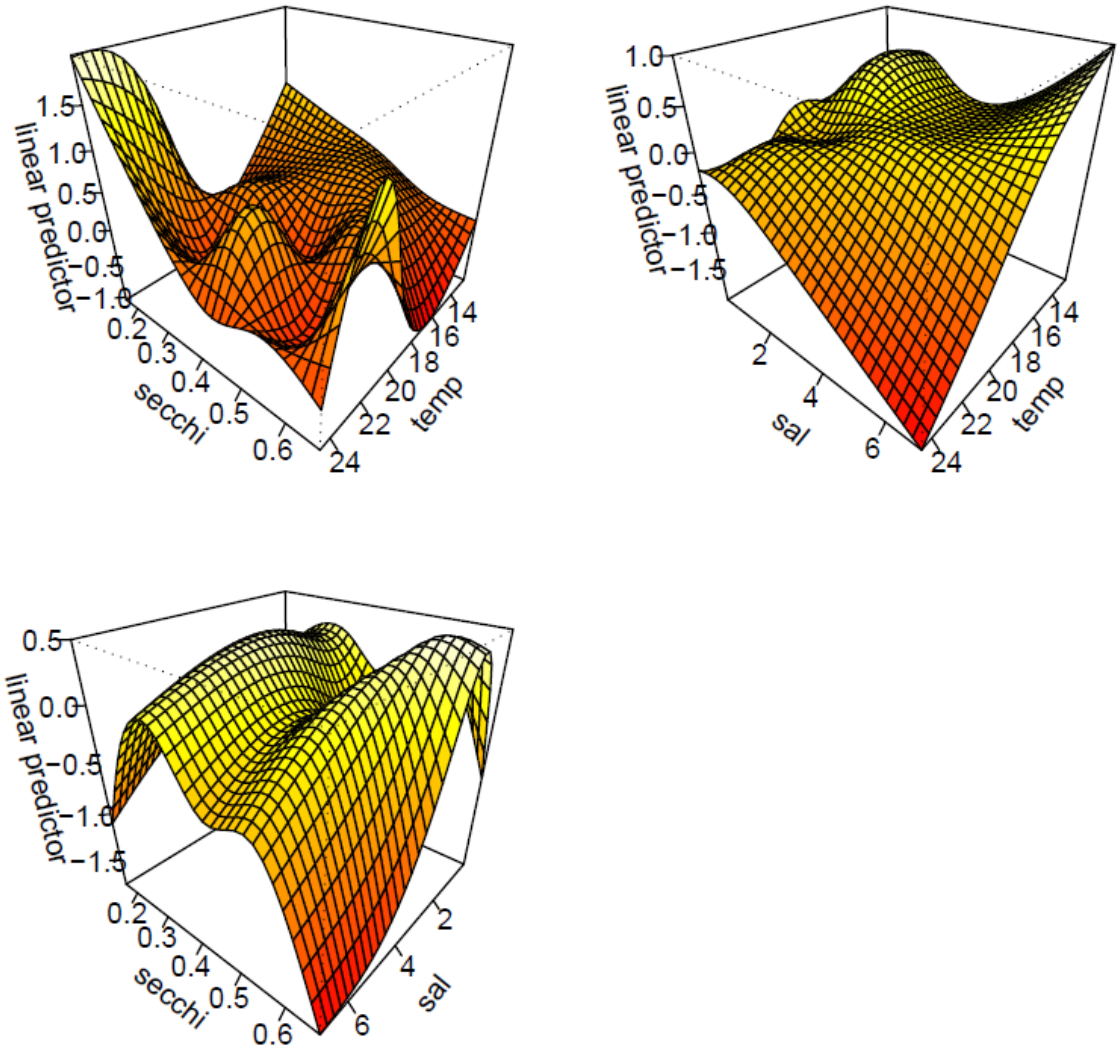


Figure 4-8. Tensor Product Smooths 3-d Plots for Water Temperature (temp), Salinity (sal) and Water Clarity (secchi) at Capture on 14-day Age Corrected Marginal Otolith Growth Rate

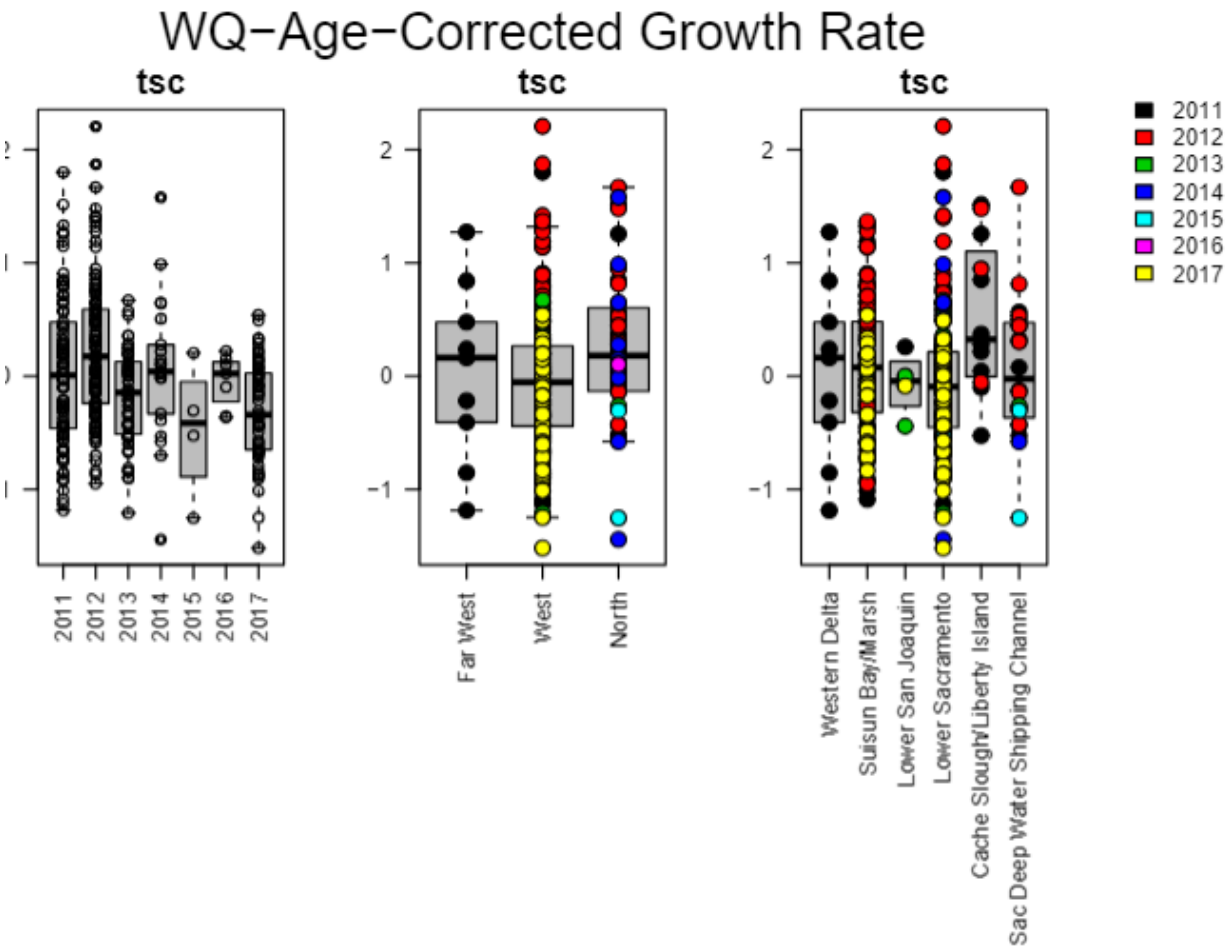
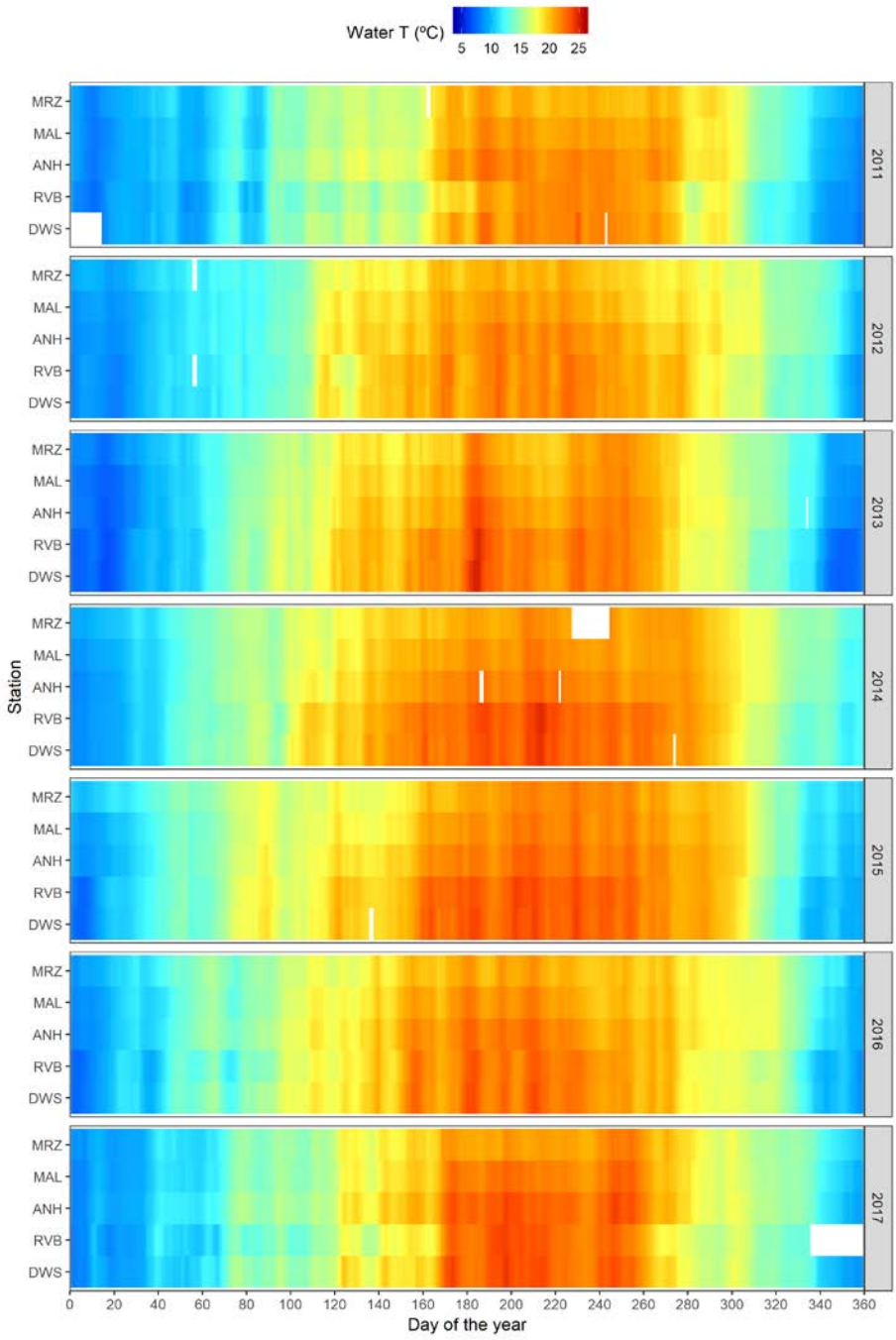


Figure 4-9. Ontogenetic and Water Quality Corrected Marginal Otolith Growth Response by Year, EDSM Region and EDSM Strata for 2011-2017



Note: Sites are arranged vertically, the upstream-most site at the bottom and downstream-most site at the top of each graph. North Delta (DWS-bottom) to far western Suisun Bay (MRZ-top). Station names are Deepwater Ship Channel (DWS), Rio Vista (RVB), Antioch (ANH), Mallard (MAL), and Martinez (MRZ), operated by California Department of Water Resources.

Figure 4-10. Heatmap of Daily Mean Water Temperature from Five Continuous Water Quality SONDES

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Supplemental Material

Table S1. Sample Sizes by Survey

Year	EDSM	FMWT	TNS	GES	GES-CCE	GES-TNS	SFBS	YB	Total
2011	0	191	171	0	0	0	2	1	365
2012	0	36	87	101	0	0	0	5	229
2013	0	10	107	200	0	0	0	15	332
2014	0	28	71	0	159	30	6	3	297
2015	0	4	12	0	0	0	49	33	98
2016	0	6	0	0	0	0	1	5	12
2017	87	0	25	0	0	0	0	0	112

Notes: EDSM = USFWS Enhanced Delta Smelt Monitoring, FMWT-CDFW Fall Midwater Trawl, TNS = CDFW Summer Townet Survey, GES = CDFW Gear Evaluation Study, GES-CCE CDFW Gear Evaluation Covered Cod End Study, GES-TNS CDFW Gear Evaluation Summer Townet Survey, SFBS CDFW San Francisco Bay Study, YB DWR Yolo Bypass Juvenile Fish Monitoring.

Table S2. Overview of the Different Candidate GAMs

Model	Description	Formula
r	stratum, random	gm14 ~ s(edsmstratum, bs = "re")
m	month, random	gm14 ~ s(month, bs = "re")
y	year, random	gm14 ~ s(year, bs = "re")
rm	region x month	gm14 ~ s(edsmstratum, bs = "re") + s(month, bs = "re") + ti(edsmstratum, month, bs = c("re", "re"))
my	month x year	gm14 ~ s(month, bs = "re") + s(year, bs = "re") + ti(month, year, bs = c("re", "re"))
ry	region x year	gm14 ~ ti(edsmstratum, bs = "re") + ti(year, bs = "re") + ti(edsmstratum, year, bs = c("re", "re"))
m_y_r	month + year + region	gm14 ~ s(edsmstratum, bs = "re") + s(month, bs = "re") + s(year, bs = "re")
myr	month x year x region	gm14 ~ te(edsmstratum, month, year, bs = c("re", "re", "re"))
a	age	gm14 ~ s(age, k = 6)
g	previous growth	gm14 ~ s(gp14, k = 6)
a_g	age + previous growth	gm14 ~ s(age, k = 6) + s(gp14, k = 6)
ag	age x previous growth	gm14 ~ ti(age, k = 6) + ti(gp, k = 6) + ti(age, gp)
t	temperature	gm14 ~ s(age, k = 6) + s(temp, k = 6)
s	salinity	gm14 ~ s(age) + s(sal, k = 6)
c	clarity	gm14 ~ s(age) + s(secchi, k = 6)
ts	temp x sal	gm14 ~ ti(age) + ti(temp, k = 6) + ti(sal, k = 6) + ti(temp, sal, k = c(6, 6))
tc	temp x clar	gm14 ~ ti(age, k = 6) + ti(temp, k = 6) + ti(secchi, k = 6) + ti(temp, secchi)
sc	sal x clar	gm14 ~ s(age) + ti(sal, k = 6) + ti(secchi, k = 6) + ti(sal, secchi)
tsc	temp x sal x clar	gm14 ~ s(age, k = 6) + s(temp, k = 6) + s(sal, k = 6) + s(secchi, k = 6) + ti(temp, secchi) + ti(temp, sal) + ti(secchi, sal) + ti(temp, sal, secchi)
t_resid	t on residuals of a	rrm ~ s(temp, k = 6)
s_resid	s on residuals of a	rrm ~ s(sal, k = 6)
c_resid	c on residuals of a	rrm ~ s(secchi, k = 6)
ts_resid	ts on residuals of a	rrm ~ ti(temp, k = 6) + ti(sal, k = 6) + ti(temp, sal)
tc_resid	tc on residuals of a	rrm ~ ti(temp, k = 6) + ti(secchi, k = 6) + ti(temp, secchi)
sc_resid	sc on residuals of a	rrm ~ ti(sal, k = 6) + ti(secchi, k = 6) + ti(sal, secchi)
tsc_resid	tsc on residuals of a	rrm ~ ti(temp, k = 6) + ti(sal, k = 6) + ti(secchi, k = 6) + ti(temp, secchi) + ti(temp, sal) + ti(secchi, sal) + ti(temp, sal, secchi)

Chapter 5: RNA-DNA Ratio as Proxy of Short-Term Growth in Juvenile Delta Smelt in the Sacramento-San Joaquin Delta and San Francisco Estuary

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Abstract

The purpose of this study is to determine drivers of short-term growth of juvenile Delta Smelt, using RNA-DNA ratio in skeletal muscle as proxy. Data for this study were gathered from juveniles collected from June through September from 2011 through 2016 in the Sacramento-San Joaquin Delta and San Francisco Estuary (n=414). Several Gaussian linear models were fit and compared with Akaike's Information Criterion corrected for small sample size (AICc), using RNA-DNA as the response. As shown for other juvenile fish, RNA-DNA ratio in Delta Smelt decreases as fish mature, indicating that younger fish have higher growth rates than older fish. After accounting for fish size differences, the influence of other variables on RNA-DNA ratio was analyzed. Smaller RNA-DNA ratios were observed at higher temperatures suggesting that Delta Smelt cannot fully compensate for increased metabolic demand at higher temperature by eating more, causing growth to decline. Additionally, significant differences in Delta Smelt growth were observed among regions and years. Delta Smelt collected at Suisun Marsh had the highest recent growth rate (relatively high RNA-DNA ratio) while those collected at Confluence and Suisun Bay had the lowest recent growth rate (relatively low RNA-DNA ratio). Analysis of RNA to DNA data by year at each month indicates that Juvenile Delta Smelt grew the fastest in 2011, then slowed down in 2012 and 2013, and was the slowest in 2014. The low number of fish collected in 2015 does not allow for conclusions about this year. This study suggests that low temperatures in combination with high percent gut fullness (as an indicator of foraging success) positively affect short-term growth in Delta Smelt. A laboratory experiment to establish RNA-DNA ratio levels indicative of good and poor growth in Delta Smelt is needed.

Introduction

RNA-DNA ratio in skeletal muscle is a useful indicator of recent growth rate (Bulow *et al*., 1978) and nutritional status in fish (Buckley, 1979; Wright and Martin, 1985). Total RNA is comprised of rRNA (75 to 94% of the total RNA), mRNA and tRNA. The number of ribosomes fluctuates in response to food availability and the demand for protein synthesis and somatic growth (Elaine *et al*., 2003). In contrast, DNA concentration represents an index of cell number and is insensitive to environmental changes; hence RNA-DNA ratio is not affected by the number of cells in a tissue sample. Moreover, RNA-DNA ratio correlates with growth rate within the last few days before sampling, which facilitates analyzing relationships between growth rate and environmental conditions measured during sampling (Buckley, 1984). For example, Wright and Martin (1985) found that changes in RNA-DNA ratio are closely related to growth with a lag time of 0.81 days in larval Striped Bass (*Morone saxatilis*) while in juvenile Atlantic Salmon (*Salmo salar*) RNA-DNA ratio decreased within 6 days after a decrease in ration level (Duguid *et al*., 2018). Buckley (1979) showed a significantly positive correlation between RNA-DNA ratio and protein growth rate in Atlantic Cod (*Gadus morhua*) larvae and suggested that a single RNA-DNA ratio measurement may be enough to assess the rate of growth (rapid or slow) at the time of capture. In addition, lower RNA-DNA ratios have been observed when fish are subjected to stressful conditions such as feed deprivation, diseases, temperatures outside the optimal range (Elaine *et al*., 2003; Spigarelli and Smith, 1975; Steinhart and Eckmann, 1992; Ueberschär and Clemmesen, 1992) or high heavy metal concentrations (Kearns and Atchison, 1979). Significant changes in RNA-DNA can be observed just 1-2 days after a change in food availability in larval and juvenile fish (Malloy and Targett, 1994). A decrease of 57% in the RNA-DNA ratio was observed in Herring (*Clupea harengus*) larvae after 3 days of starvation (Ueberschär and Clemmesen, 1992).

Fish size has a strong influence on RNA-DNA ratio. At earlier life stages when fish are growing faster the RNA-DNA ratio is higher in skeletal muscle due to a higher energy investment on synthesis of body mass. However, as fish mature more energy is used for reproduction and in locomotion during migration in the case of migratory fish, decreasing the RNA-DNA ratio. A significant correlation between RNA-DNA ratio and growth rate has been observed in several fish species (Duguid *et al*., 2018; Haines, 1973; Wright and Martin, 1985). These two parameters are more strongly correlated in younger individuals (Haines, 1973). However, during spawning season that correlation breaks down due to energy use in reproduction instead of body tissue synthesis (Haines, 1973). Chicharo and Chicharo (2008) suggest that it is very important not to directly compare growth rates or RNA-DNA ratio values of individuals with very different ages, as RNA-DNA ratio decreases with age. A slower growing older larvae or juvenile may be in equally good condition to a faster growing, younger larvae or juvenile and a decrease in RNA-DNA ratio with age most likely reflects a decrease in growth rate but not necessarily a decrease in condition (Chicharo and Chicharo 2008).

In this study, we will focus on juvenile Delta Smelt collected for between 2011 and 2016. Our previous work found that juvenile Delta Smelt collected from Suisun Bay were under apparent nutritional stress during summer and individuals from Suisun Marsh were in relatively good condition overall (Hammock *et al*. 2015). We used RNA-DNA ratio in skeletal muscle tissue to identify drivers of growth in juvenile Delta Smelt, including the identification of regions in the Sacramento-San Joaquin Delta (SSD) and the San Francisco Estuary (SFE) with more favorable

overall environmental conditions for growth. These regions with better conditions for fish growth can be targeted then as restoration or conservation areas to benefit Delta Smelt.

Methods

Study Area and Sampling

Juvenile Delta Smelt were collected from June to September 2011 to 2016 at 40 sampling stations within the SSD and the SFE by the Interagency Ecological Program (IEP) fish monitoring studies conducted by the California Department of Fish and Wildlife (CDFW; n = 961; methods in Honey *et al*. 2004). Because of the limited number of fish at some locations and the similarities among stations in terms of habitat the sampling stations were grouped into five geographical regions based on habitat type and proximity: Cache Slough (C. Slough), Sacramento River Deep Water Ship Channel (SRDWSC), Confluence, Suisun Bay, and Suisun Marsh (see Fig. 1 in Hammock *et al*. 2015 for a map of the sampling stations and regions). Delta Smelt were collected in trawls, wrapped live in aluminum foil, and immediately stored in liquid nitrogen until they were dissected at the UC Davis Aquatic Health Program Laboratory (Teh *et al*. 2016). Conductivity, temperature and location data were collected at each sampling station. Conductivity was converted to salinity for use in the analysis. Water samples were also collected and processed at UC Davis for pH, ammonia, nitrate and nitrite.

Sample Preparation

At the laboratory fish were removed from liquid nitrogen, fork length and body weight were measured, and then fish were rapidly dissected (methods in Teh *et al*. 2016). Dorsal muscle samples were dissected and preserved at -80°C until it was used to measure RNA–DNA ratio using the ethidium bromide fluorometric technique (Caldarone *et al*., 2001). Gastrointestinal tracts were preserved in 95% ethanol and sent to the CDFW Diet Study laboratory (Stockton, CA) for gut content analysis. Gut fullness was calculated for each fish with the following equation: gut fullness = (prey weight in gut / fish body weight) × 100 [Carruthers *et al*., 2005]). Based on the assumption that if fish are successful foraging, there will be a positive correlation between food abundance and gut fulness at each region.

Statistical Analysis

Five variables were used as predictors in the analysis: fork length, region, year-class, water temperature, and time of the day at collection. Other variables such as salinity, nitrite, nitrate, ammonia, sex, and gut fullness were also evaluated but did not have a significant effect on the RNA–DNA ratio. We were interested in year-class to evaluate the influence of drought because 2011 was a wet year while 2012 to 2015 were particularly dry and warm. The region variable was included to evaluate regional patterns detected in previous works (Hammock *et al*. 2015) and to assess the most suitable areas for juvenile Delta Smelt growth for ecosystem conservation purposes. Fork length was analyzed because RNA–DNA ratio decreases as fish get older (Chicharo and Chicharo, 2008). Water temperature is a well-known factor to influence fish growth and for that reason was included in the analysis (Sogard & Olla, 2001; Handeland *et al* 2008). Time of the day at collection was included as shown to influence the RNA–DNA ratio in our previous work (Hammock *et al* 2015).

For the year-class variable, individuals were assigned to year-classes based on the date of collection and fork length. Due to the large size differences between juveniles collected in June for one year (~20-40 mm) and adults from the previous year/class (~60-80 mm) there was no ambiguity assigning individuals to year/classes. Regions included the Sacramento River Deep Water Shipping Channel (SRDWSC), Cache Slough (C. Slough), Confluence (Conf.), Suisun Marsh (S. Marsh) and Suisun Bay (S. Bay). These regions represent different habitat types, salinities, distinct geographic regions, and stressors.

Different Gaussian linear models were fit and compared with the corrected Akaike's Information Criterion (AIC_c) to identify factors that influence RNA-DNA ratio (Burnham and Anderson 2002). The analysis was performed with data from juveniles collected from June to September from 2011 to 2016. Differences in RNA-DNA ratio among regions were analyzed with an ANCOVA followed by a *post hoc* Tukey test using Fork length and time of the day when fish were captured as covariables. Variations in RNA-DNA ratio among years at a month level and in gut fullness and water temperature among regions and years were analyzed with a One-Way ANOVA followed by a post-hoc Tukey test. Significant differences were established at $P<0.05$.

Results

The top ranked fit model (Table 5-1) indicates that fork length, the region where the fish was captured, year/class, water temperature and time of the day when fish were collected had the most significant influence ($F_{[12, 339]}= 42.87$, $P<0.0001$, adjusted $R^2=0.5887$) on RNA-DNA ratio. RNA-DNA ratio of juvenile Delta Smelt decreased with increasing fork length (Figure 5-1A) water temperature (Figure 5-1B), and Time of Day (Figure 5-1C).

Table 5-1. Model Comparison for the Influence of Standard Length (L), Region (R), Year/Class (YC), Water Temperature (WT), Time of the Day When Fish Were Captured (T) and Gut Fullness as Percent of Body Weight (SC).

Model	ΔAIC_c	df	AIC_c wt
8~ L + L2 + R + YC + WT + T	0.0	14	0.5274
7~ L + L2 + R + YC + T	1.8	13	0.2170
12~ L + L2 + R + YC + SC + WT + T	2.2	15	0.1774
10~ L + L2 + R + YC + SC + T	3.9	14	0.0746
6~ L + L2 + R + YC + WT	10.8	13	0.0024
11~ L + L2 + R + YC+ SC + WT	12.2	14	0.0012
9~ L + L2 + R + YC + SC	19.8	13	<0.001
5~ L + L2 + R + YC	20.0	12	<0.001
4~ L + L2 + R	126.2	8	<0.001
3~ L + L2	165.3	4	<0.001
2~ L	183.0	3	<0.001
1~	299.8	2	<0.001

ΔAIC_c is the change in Akaike information criterion corrected for small sample size, df is degrees of freedom, AIC_c wt is AIC_c weight expressed as a proportion.

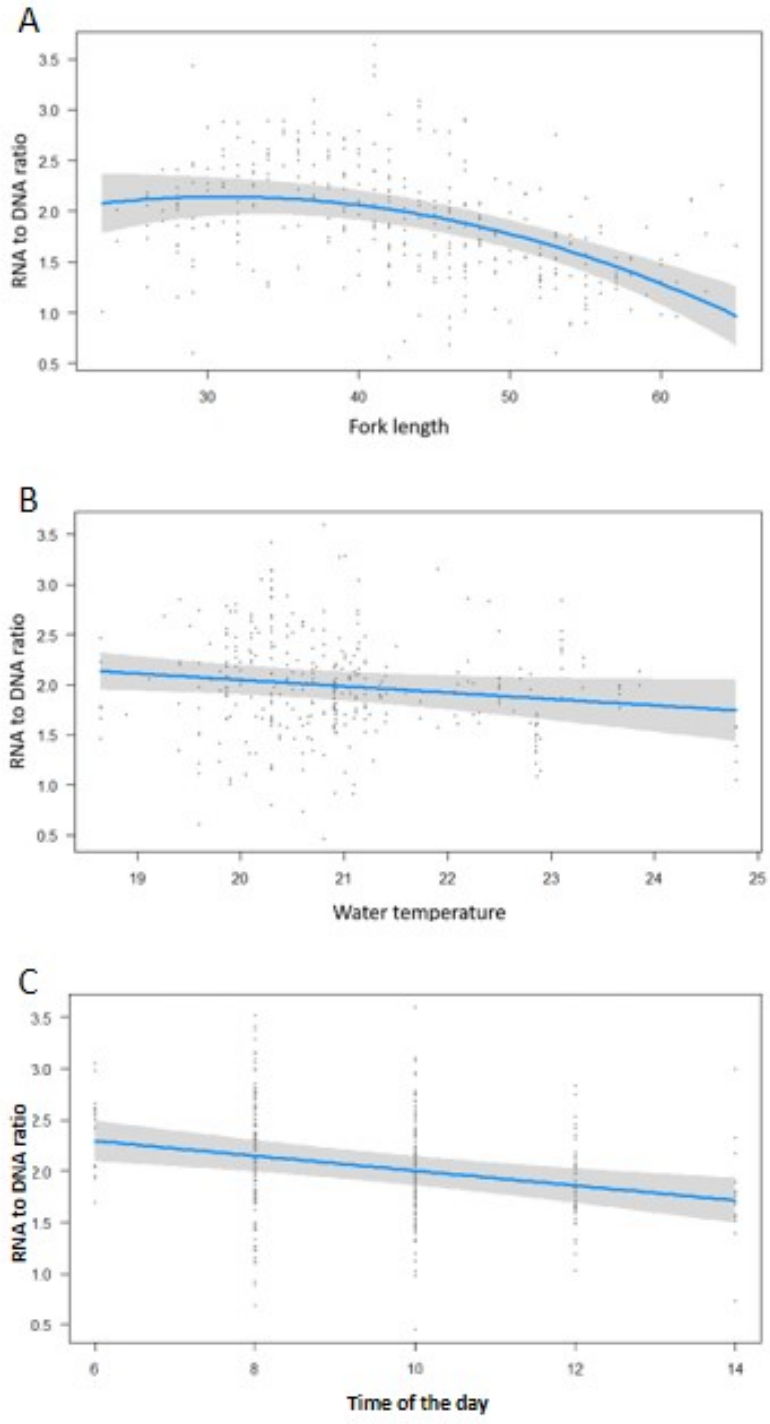

Figure 5-1. RNA-DNA Ratio (recent growth) of Juvenile Delta Smelt by Fork Length (A), Temperature (B),and Time of the Day (C)

1. Regional Differences in RNA-DNA Ratio for All Years (2011-2016)

There were significant differences in fish growth among regions ($F_{[19,313]}$=12.1393, P<0.0001, Table 5-2) for data collected between 2011 and 2016. The effect of Fork length and Time of the day were P=0.0516 and P=0.0028, respectively. The highest RNA-DNA ratio was observed at S.Marsh while the lowest at S. Bay.

Significant differences in gut fullness among regions were found ($F_{[4,389]}$=6.4395, P<0.0001, Table 5-3) being significantly higher at SRDWSC and Confluence, and lower at Suisun Bay.

Diffrences in water temperature at time fish were captured were also analyzed. Significant differences among regions were found ($F_{[4,458]}$=65.7349, P<0.0001, Table 5-4) being significantly higher at SRDWSC and Confluence but lower at Cache Slough, Suisun Bay and Suisun Marsh.

Table 5-2. Regional Differences in RNA-DNA Ratio in Juvenile Delta Smelt Collected in 2011-2016 After Controlling for Differences in Fork Length and Time of the Day

Region	N	RNA-DNA ratio
C. Slough	32	1.98 ± 0.15^{AB}
S. Marsh	37	2.35 ± 0.36^{AB}
SRDWSC	94	2.14 ± 0.09^{A}
S. Bay	95	1.78 ± 0.08^{B}
Conf.	74	1.93 ± 0.08^{AB}

Notes: Different letters among regions indicate significant differences (P<0.05).

Table 5-3. Regional Differences in Percent of Gut Fullness Relative to Fish Weight (arcsin square root transformed)

Region	N	Arcsin square root of % gut fullness
C. Slough	65	0.058 ± 0.004AB
S. Marsh	76	0.058 ± 0.006AB
SRDWSC	103	0.068 ± 0.003A
S. Bay	36	0.043 ± 0.004B
Conf.	112	0.059 ± 0.004A

Notes: Different letters among regions indicate significant differences (P<0.05)

Table 5-4. Regional Differences in Water Temperature at Time Delta Smelt Were Captured

Region	N	Temperature
C. Slough	69	20.44 ± 0.08^{C}
S. Marsh	40	20.44 ± 0.10^{C}
SRDWSC	136	22.01 ± 0.11^{A}
S. Bay	116	20.30 ± 0.05^{C}
Conf.	100	21.10 ± 0.10^{B}

Notes: Different letters among regions indicate significant differences (P<0.05)

2. RNA-DNA Ratio, Water Temperature and Percent of Gut Fullness Among Years

There were significant differences in RNA-DNA ratio among years at each month for data collected between 2011 and 2015 (June: $F_{[2,170]}=64.7613$, $P<0.0001$; July: $F_{[1,56]}=4.2034$, $P=0.045$; August: $F_{[3,72]}=3.9326$, $P=0.0117$; September: $F_{[1,27]}=0.4778$, $P=0.4953$, Table 5-5). RNA-DNA ratio progressively decreased over time being higher at 2011 and lower at 2014. There were no enough data from 2015 for the statistical analysis.

Significant differences in water temperature among year-classes were also found ($F_{[4,458]}=22.2684$, $P<0.0001$, Table 5-6) with 2015 the highest and the lowest in 2011. Significant differences in gut fullness were not found among years ($F_{[4,389]}=1.4192$, $P=0.2266$).

Table 5-5. RNA-DNA Ratio, Water Temperature and Gut Content Among Years by Month in Juvenile Delta Smelt

Month	Year	RNA-DNA ratio		Water temperature (°C)		Gut fullness (Arcsine square root transformed data)	
		N		N		N	
June	2012	72	2.41 ± 0.05^{A}	91	20.84 ± 0.09	66	0.059 ± 0.005^{A}
June	2013	77	2.53 ± 0.05^{A}	80	20.52 ± 0.09	65	0.059 ± 0.005^{A}
June	2014	24	1.41 ± 0.09^{B}	49	21.14 ± 0.12	42	0.058 ± 0.006^{A}
July	2013	39	1.60 ± 0.05^{A}	39	21.05 ± 0.15	37	0.047 ± 0.007^{A}
July	2014	19	1.43 ± 0.07^{B}	19	22.07 ± 0.21	19	0.056 ± 0.010^{A}
August	2011	32	1.91 ± 0.12^{A}	68	20.81 ± 0.16	67	0.048 ± 0.003^{C}
August	2012	23	1.55 ± 0.14^{AB}	23	22.10 ± 0.28	23	0.053 ± 0.005^{BC}
August	2013	12	1.49 ± 0.19^{AB}	12	22.38 ± 0.39	11	0.077 ± 0.007^{B}
August	2014	9	1.10 ± 0.23^{B}	9	22.38 ± 0.45	9	0.119 ± 0.008^{A}
September	2011	25	0.95 ± 0.09^{A}	43	19.97 ± 0.10		
September	2015	4	1.12 ± 0.23^{A}	4	22.7 ± 0.31		

Notes: Different letters among year/classes indicate significant differences ($P<0.05$).

Table 5-6. Water Temperature Among Year-Class

Year/Class	N	Temperature
2011	111	20.5 ± 0.10D
2012	114	21.1 ± 0.12BC
2013	135	20.9 ± 0.10CD
2014	82	21.5 ± 0.09B
2015	19	22.6 ± 0.14A

Notes: Different letters among year/classes indicate significant differences ($P<0.05$).

There were significant differences in RNA-DNA ratio among months ($F_{[3,328]}=69.2952$, $P<0.0001$, Table 5-7), which shows RNA-DNA ratio values progressively decrease as fish get older.

Table 5-7. Differences in RNA-DNA Ratio Among Months for Juvenile Delta Smelt

Month	N	RNA-DNA Ratio
June	173	2.32 ± 0.04^{A}
July	58	1.54 ± 0.07^{B}
August	76	1.64 ± 0.06^{B}
September	25	0.95 ± 0.11^{C}

Different letters among months indicate significant differences (P<0.05)

3. Variation in Gut Fullness (Arcsine square root transformed data) Over the Course of the Day

Gut fullness significantly increased over the course of the day ($F_{[1,350]}$=39.72, P<0.0001, Figure 5-2). Water quality data from the different regions are shown in Table 5-8.

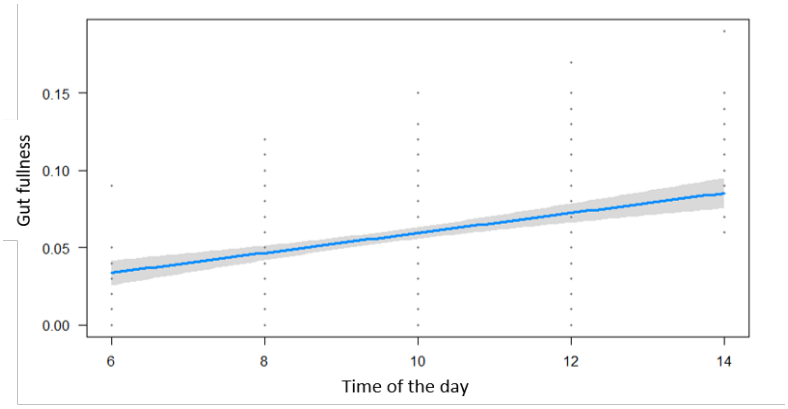

Figure 5-2. Changes in Gut Fullness Over the Course of the Day for All Regions at the Sacramento San Joaquin Delta and San Francisco Estuary from 2011 to 2016

Table 5-8. Water Quality Data at Regional Level

	C. Slough	Conf.	S. Bay	S. Marsh	SRDWSC
Salinity	0.08 ± 0.00	1.19 ± 0.09	5.55 ± 0.30	3.98 ± 0.20	0.27 ± 0.01
Total ammonia	0.21 ± 0.02	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.00	0.06 ± 0.01
Nitrate	0.92 ± 0.05	1.02 ± 0.03	0.70 ± 0.02	0.83 ± 0.02	0.64 ± 0.03
Nitrite	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
pH	8.00 ± 0.09	7.90 ± 0.01	7.88 ± 0.01	7.67 ± 0.01	8.18 ± 0.01

Discussion

RNA-DNA ratios have been used widely as an index of nutritional condition (Chicharo and Chicharo 2008). RNA-DNA ratio can provide estimates of growth rate over periods as short as one day and up to about one week (Bulow *et al*., 1978). This short time frame opens up the possibility of linking environmental conditions at the time of sampling to variability in growth. If growth affects survival, since smaller fish are more susceptible to predation, survival rates would increase as fish grows, while more vulnerable, slow growing individuals would be eliminated, as suggested in hypotheses about size-dependent mortality (Peterson and Wroblewski 1984).

Moreover, slower growth rate of juvenile fish may delay sexual maturity which would compromise population stability (Arendt, 1997). Our top ranked fit model, which used RNA-DNA ratio as proxy for recent growth, indicate that recent fish growth is driven by the fish size, region, year/class, water temperature and time of the day. We propose RNA-DNA ratio as a good proxy for short term growth, as it is affected by daily changes in water temperature and time of the day when fish was captured.

Results of this study show that Juvenile Delta Smelt at S. Marsh and C. Slough had the highest RNA-DNA ratio while Suisun Bay had the lowest RNA-DNA ratio, gut fullness, and lower water temperature at time of capture among all the regions. On the other hand, Delta Smelt at SRDWSC had the highest gut fullness and water temperature but low RNA-DNA ratio.

The top ranked fit model (Table 5-1) shows temperature plays a slightly significant (P=0.051) role as a driver of recent fish growth. We postulate that lower temperature may benefit ectothermic fish such as Delta Smelt on growth when food availability increases as is shown in fish collected from Suisun Marsh. Low temperatures in the range of 18-25°C found in this study promote higher RNA-DNA ratio. At a regional level the highest RNA-DNA ratio were registered at S. Marsh and C. Slough which also had some of the lowest temperatures and relatively medium values of gut fullness. This observation agrees with the role of low temperatures as driver of fish growth. However, S. Bay, which had the lowest temperature among the regions, also showed the lowest RNA-DNA ratio and gut fullness. Primary and secondary productivity at S. Bay has been low in part because of the particularly strong influence of the invasive species *Potamocorbula amurensis* (Alpine and Cloern, 1992, Kimmerer *et al*., 1994, Sobczak *et al*., 2002, Greene *et al*., 2011). These results highlight the interaction between water temperature and food availability on fish growth. Food availability below the energetic demands is associated to a slower growth, higher susceptibility to pathogens, less adaptability to environmental stressors and higher risk of predation (Arendt, 1997; Sogard & Olla, 2001). Moreover, even though the highest water temperature and gut fullness were found at SRDWSC, this region did not show the highest RNA-DNA ratio. A similar situation was observed at Confluence where water temperature and gut fullness were some of the highest and RNA-DNA ratio was one of the lowest. As shown for other fish species, temperature increments are associated to increased metabolic rate and a proportionally higher energy requirement for maintenance functions and growth (Sogard & Olla, 2001; Handeland *et al* 2008). We propose two nonexclusive interpretations: 1) that juvenile Delta Smelt are not able to cope with increased temperatures with the insufficient food resources available in some regions of the SSD and SFE such as in SRDWSC and Confluence, and 2) that other environmental stressors (*e.g*. contaminants or pathogens) are playing a role and deviate energy to other processes than growth for instance biotransformation of contaminants.

Influence of time of the day on RNA-DNA ratio is probably due to its relationship with water temperature and/or fish locomotion. As can be expected and observed in Figure 5-2, water temperature increases over the course of the day during the morning and it is during earliest hours when fish RNA-DNA ratios are higher. Response of RNA-DNA ratio to water temperature and time of the day highlights the sensitivity and usefulness of this parameter as indicator of recent growth in Delta Smelt. In larvae and juveniles of other fish species a lag time in the response of RNA-DNA ratio to food availability has ranged from 0.81 days to within 6 days (Wright and Martin, 1985; Ueberschär and Clemmesen, 1992; Malloy and Targett, 1994; Duguid

et al., 2018). The association between RNA-DNA ratio and water temperature and time of the day found in this study indicates that RNA-DNA ratio responds in less than one day. An association between time of the day and food availability was also investigated to explain the relationship between RNA-DNA ratio and time of the day. However, we observed higher gut fullness at later hours, which opposes to the higher RNA-DNA ratio observed in the morning (Figure 5-2). Moreover, fish locomotion also increases as the temperature increases during the day, which likely the cause of reduction in RNA-DNA ratio observed over the course of the day due to higher energy consumption in fish movement. These results indicate that the influence of time of the day on RNA-DNA ratio was driven by changes in water temperature and, probably, in fish locomotion over the course of the day.

Besides food abundance and water temperature, environmental stressor such as contaminants and salinity can also be affecting fish growth. Contaminants have been considered an important factor in the decline of Delta Smelt population in the SFE (Kuivila and Moon, 2004; Sommer *et al*., 2007; Brooks *et al*., 2012; Hammock *et al* 2015). After analyzing fish collected at the SSD and SFE from August 2011 to November 2017 (Teh *et al* submitted) showed that fish at S. Bay had the highest incidence of liver lesions and the lowest abundance of liver glycogen, the major short-term energy storage in fish (Heath, 1995), which was the result of contaminant exposure. Salinity could be a contributing factor to the poor nutritional fish status at S. Bay. Salinity at S. Bay (5.55 ±0.30) was the highest among all the regions during the evaluated period. Considering Delta Smelt is more likely captured in regions with a salinity range of 0.6-3 (Nobriga *et al*, 2008), the high salinity at S. Bay likely imposes an extra energy spend on ion regulation which can affect fish growth. Therefore, high load of contaminants and salinity plus the low food abundance could be contributing factors to the lowest RNA-DNA ratio found at S. Bay in this study. Relatively high salinity was also found at S. Marsh where we found the highest RNA-DNA ratio. One potential explanation is that the relatively high food availability and low temperature found at S. Marsh mitigate the effect high energy demand imposed by salinity. Moreover, Teh *et al* (submitted) also reported that S. Marsh consistently had the lowest incidence of glycogen depletion and liver lesions indicating low load of contaminants in this region. S. Marsh is relatively well connected to wetlands, a habitat type known to remove pollutants from water (Kivaisi, 2001). These results in combination with the relatively high food abundance and low temperature may explain the highest RNA-DNA ratio at S. Marsh found in this study. Additionally, C. Slough showed similar values of water temperature and gut fullness to S. Marsh. However, RNA-DNA ratio values were much lower. Contaminants may be playing an important role in the low RNA-DNA ratio values observed in C. Slough as it was precisely at this region where one of the highest incidences of liver lesions and glycogen depletion has been reported (Teh *et al*., submitted). Moreover, several studies have indicated high concentrations of pollutants in C. Slough (Werner *et al*., 2000; Kuivila & Moon, 2004; Weston *et al*., 2014;) which receives extensive urban runoff from West Sacramento and agricultural and secondary wastewater runoff (Weston *et al*., 2014) just upstream from the sampling sites in our study. However, to our knowledge there are no studies evaluating the impact of contaminant exposure on RNA-DNA ratio. Results of this study indicate that besides water temperature and food abundance, contaminants are also playing an important role as drivers of fish growth. Results of this study indicate that S. Marsh provides the more appropriate area for preservation of Delta Smelt in the SSD and SFE.

There are different combinations of factors affecting RNA-DNA ratio at each region. RNA-DNA ratio at the Confluence was one of the lowest, like the one observed at S. Bay. However, values of food abundance found in this study and incidence of liver lesions at the Confluence (Teh *et al*, submitted) were similar to those in S. Marsh, where the highest RNA-DNA ratio were found. Although water temperature was higher at the Confluence compared to S. Marsh it is unlikely that temperature by itself is driving the lowest RNA-DNA ratio observed at this region. Therefore, it is not clear what other factors could be driving the low RNA-DNA ratio observed at the Confluence. On the other hand, as stated before, fish from SRDWSC showed relatively good RNA-DNA ratio but not as high as in S. Marsh even though food abundance was the highest at this region. Contributing factors to lower RNA-DNA ratio at SRDWSC may include high water temperature, which was about 1.6 °C over the mean registered at S. Marsh, and the relatively high incidence of liver lesions indicating high exposure to contaminants according to Hammock *et al* (2015) and Teh *et al* (submitted). It is possible that some other factors that were not considered in this study are playing a more important role on the low RNA-DNA ratio observed at some regions in the SSD and SFE.

As shown for fish species that complete their life cycles in seasonal environments, like Delta Smelt, slow growth would delay sexual maturity, prolong the period of susceptibility to size-limited predators, decrease fitness, and increase the risk of mortality (Hutchings & Jones, 1998; Utrilla & Lobon-Cervia, 1999; Koops *et al*., 2004; Garvey *et al*., 2004). These effects combined may compromise population stability and abundance. Since smaller fish are more susceptible to predation, survival rates would increase as fish grows, while more vulnerable, slow growing individuals would be eliminated (Peterson and Wroblewski. 1984). This agrees with our hypothesis that at the regions mostly affected by stressors (*e.g*. low food abundance, contaminants, high temperature) only the more robust and healthiest fish are being analyzed since the unhealthy ones are not surviving.

Our top ranked fit model also showed that year/class significantly influenced the RNA-DNA ratio (Table 5-1). Changes in RNA-DNA ratio over the years can be partially explained by changes in water temperature. Coldest years also showed the highest RNA-DNA ratio values as indicated by our top ranked fit model showing an inverse, statistically significant relationship (P=0.0246) between RNA-DNA ratio and water temperature. Comparisons in RNA-DNA ratio were done on a monthly basis because there were big differences in the number of fish among months and there is a clear relationship between age and RNA-DNA ratio as shown in Table 5-7. Significant relationships among RNA-DNA ratio and gut fullness over the years were not found. These data reinforce our finding that relatively high temperatures have a negative impact on fish growth.

Given that food supply is not limited, fish growth rate strongly depends on fish size and water temperature, being higher at smaller sizes and at relatively high temperature (Jobling 1983; Austreng *et al* 1987). Relationship between growth rate and water temperature is positive until the optimal temperature. Further increments will reduce fish growth. We hypothesize that Delta Smelt would grow faster at higher temperatures if more food would be available. The highest growth rate and RNA-DNA ratio that Delta Smelt can reach under optimal feeding and temperature conditions are not known nor the optimal water temperature for Delta Smelt juveniles. However this study shows that at the temperature range of 18-25°C fish have relatively slow growth rates in some regions in the SSD and SFE. Contributing factors to the relatively

slow growth rate of Delta Smelt, based on this and previous studies from our research group, include the interaction between high temperature and low food abundance and environmental stressors (*e.g.* contaminants).

In conclusion, this study found that RNA-DNA ratio could be a good indicator of Delta Smelt growth as shown by its response to daily changes in water temperature and time of the day when fish is captured. Moreover, regional differences in fish growth indicate that Suisun Marsh is the most appropriate region in the SSD and SFE for nurturing Delta Smelt while Suisun Bay seems to be the least adequate for Delta Smelt growth. These results highlight the susceptibility of Delta Smelt population to warmer climate regimes in ecosystems with low food availability and high exposure to contaminants. Future research should include additional laboratory experimentations to determine the effects of salinity, temperature, and food on larval and juvenile Delta Smelt growth rate to establish RNA-DNA ratio levels indicative of good and poor growth in Delta Smelt.

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Chapter 6: Evaluation of Delta Smelt Health with Respect to Regional Delta Contaminant Levels

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Abstract

As part of the larger Directed Outflow Project, ambient samples were collected from six sites within five sub-strata: 1) Toe Drain, 2) Cache/Lindsey Sloughs, 3) Sacramento River at Isleton, 4) Sacramento River at Decker Island, 5) Montezuma Slough, and 6) Grizzly Bay, for use in Delta Smelt toxicity testing. Toxicity testing was conducted every two weeks from October to December 2017, with surviving Delta Smelt preserved for biomarker analyses. Biomarkers included those evaluating health, condition, and contaminant exposure, and mirrored those utilized in the wild Delta Smelt histopathology study within the larger DOP study. Lesion scores from histopathological analyses indicate potential metals, pesticide, and mixed contaminants exposure in the Cache Slough region, and enzymatic antioxidant assays indicate that fish exposed to water from Grizzly Bay and the Sacramento River at Isleton were being exposed to a higher load of unspecified organic compounds and were producing more reactive oxygen species. However, these changes are in general mild and do not indicate severe responses against contaminants. Taken together, data for the contaminant exposure study indicate that fish were potentially exposed to a higher load of organic contaminants during Exposures 3 and 4, which were initiated in the month of November 2017. However, it must be highlighted that most of the changes observed were mild and were not associated with biologically relevant changes in tissue morphology. Results from this study indicate that the water quality of the augmented Delta outflow in 2017 had little negative health effects on Delta Smelt. As 2017 was a wet year, the good water quality observed during this study period may be due to dilution or depletion of contaminants in the Delta outflow water, based on the results of the chemical analyses. Results of this study indicate that augmented Delta outflow is generally beneficial to Delta Smelt health and condition. We have conducted Delta Smelt toxicity testing in 2018 wet year and will incorporate the findings into this study.

Introduction

Within the larger scope of the Directed Outflow Project, the potential benefits of flow augmentation are being evaluated under the following hypotheses:

- *Hypothesis 1.* Will augmentation of the Sacramento River outflow significantly benefit growth, health, and condition vital rates of the Delta Smelt population?
- *Hypothesis 2.* Will augmentation of flow through the Yolo Bypass Toe Drain significantly benefit vital rates (e.g., growth and survival) of the Delta Smelt population?

However, given that the Sacramento-San Joaquin Delta is polluted from numerous anthropogenic sources such as industrial, urban, and agricultural contaminants (e.g., Thompson et al. 2007 and Smalling et al. 2013), there is some uncertainty regarding the exclusively beneficial effects of a higher flow augmentation. For instance, the Deep Water Ship Channel moves urban runoff from West Sacramento to the eastern portion of Cache Slough, and several studies have indicated that Cache Slough has relatively high concentrations of anthropogenic contaminants (Weston et al. 2014; Werner et al. 2000; Kuivila and Moon, 2004; Hammock et al. 2015). Juvenile Delta Smelt collected in 2012 and 2013 during the California Department of Fish and Wildlife's Summer Townet Surveys indicate exposure to contaminants, especially from the Deep Water Ship Channel and Cache Slough (Hammock et al. 2015), as histopathology revealed that high lesion rates and liver damage were prevalent in juvenile Delta Smelt sampled from these two locations.

Through toxicity testing and coordination with the Directed Outflow Project, our goal was to provide a complementary data set that can be used in the overall evaluation and characterization of the potential benefits of a Delta outflow augmentation. We applied the same histopathological and biomarker characterization of Delta Smelt as those used in the wild caught Delta Smelt (see Chapter 2 of current technical report). Integration of these proposed biomarkers will allow us to assess if Delta outflow alteration water poses risks to fish health in the Delta.

Methods

Sampling Design

Generalized random-tessellation stratified sampling (Stevens and Olsen 2004) used by the current EDSM program, took place every two weeks from October – December 2017. Fixed sampling locations in each of five sub-strata within the full study area were selected for use in the Delta Smelt contaminant study in order to make temporal comparisons over the duration of the study: 1) Toe Drain, 2) Cache/Lindsey Sloughs, 3) Sacramento River at Isleton, 4) Sacramento River at Decker Island, 5) Montezuma Slough, and 6) Grizzly Bay. These sites were selected because they were within the range of the larger DOP study and have been sources of Delta Smelt in the wild. Ambient sample collection was timed during the 'alternative-weeks' from the larger DOP project over the course of two days, due to the volume of sample required for toxicity testing and sample collection limitation during the DOP project. The use of fixed sampling locations rather than following the generalized random tessellation stratified sampling, was chosen in order to make spatial and temporal comparisons over the course of multiple project years.

Sample Collection

Twenty gallons of ambient water was collected from each site for use in Delta Smelt toxicity tests. Samples were collected by ICF staff by boat and were collected via bilge pump as sub-surface grabs, in four 5-gallon LDPE cubitainers (I-CHEM, Fisher Scientific). Sub-samples for chemical analyses were collected in addition to the 20 gallons.

Toxicity Testing

Juvenile Delta Smelt were obtained from the UC Davis Fish Culture and Conservation Laboratory (FCCL) and tested in-house at the FCCL (Byron, CA) to minimize transport and acclimation stressors to the fish. Toxicity tests were 96-hours in duration, using a static water exchange system. Tests consisted of four 5-gallon plastic buckets (Encore Plastics, Lowe's) as replicates, with 2 gallons of sample per replicate, and five Delta Smelt each. Ambient water from the California Aqueduct (used for routine fish culturing practices after basic water treatment processes including solids removal and UV disinfection) was used as the control. A secondary, "High Salinity" control was included, adjusted to match the sub-region with the highest salinity, in order to elucidate salinity stressors on Delta Smelt, if present. Fish were fed daily using formulated diet, and water quality parameters were kept within the optimal physiological parameters for the fish during the exposures. Mortality and abnormal swimming behavior were visually monitored daily (i.e., by technician, not by software or video recording), with dead fish removed when observed. Lethargic and/or moribund fish were preserved and archived. At the end of the 96-hours exposure, surviving fish were flash-frozen in liquid nitrogen and preserved according to procedures outlined in Teh et al. (2016) for biomarker analyses.

Biomarker Analyses

Biomarker analyses were performed on surviving Delta Smelt from all toxicity tests and evaluated 1) general condition, 2) growth, and 3) exposure to and effects of contaminants of fish health (Hammock et al. 2015 and 2017; Teh et al. 2016). Biomarkers have been optimized for Delta Smelt and followed all applicable standardized protocols.

Quality Assurance

For all the biomarkers of the exposure/effect to contaminants, one sample was used, which was aliquoted and analyzed over the course of the biochemical assays to check the quality and repeatability of the assays. In previous analyses conducted in our laboratory, we have obtained a coefficient of variation in the assays of less than 5%.

Statistics

Data was checked for normality using Shapiro-Wilk W test and for homogeneity of variances by the Bartlett's test, and analyzed by one-way ANOVA with a post hoc Tukey's test. The five regions (Cache Slough, Deep Water Ship Channel, Sacramento River, Suisun Bay/Marsh) was compared in terms of indices (condition factor, endocrine and biomarkers of contaminant stressors, hepatosomatic index, summed lesion score, and glycogen depletion) using six one-way analysis of variances (ANOVAs) and a one-way analysis of covariances.

Chemical Analyses

Organic/Inorganic Compounds

Water samples collected for organic/inorganic analyses were delivered to the Thomas Young lab at UC Davis, Department of Civil and Environmental Engineering, for each event during the project period. Analyses included GC-QTOF-MS and LC-QTOF-MS for targeted and non-target suspect analyses. Upon receipt, 1L water samples were divided into two extractions, polar and non-polar for analyses.

Metals

For each sample collected during the project period, two sub-samples (one whole water for total, one filtered for dissolved) were delivered to the California Animal Health and Food Safety Laboratory at UC Davis for trace metals analysis.

Results

Toxicity Test Exposures

A total of five contaminant experiments were carried out at the UCD Fish Conservation and Culture Facility in Byron, CA. Grizzly Bay was included as a sixth site beginning with the November 10 initiation date and was included in subsequent toxicity tests through the duration of the project period. High salinity controls were included to match the highest salinity of the sites tested; typically, this was the Grizzly Bay site. Water quality was measured on all ambient samples with each event. Samples were tested blind, therefore site IDs differed slightly among the exposures. Specific site codes are outlined with each collection date for each event. There were no statistically significant reductions in survival observed in any of the exposure treatments.

Analytical Chemistry

Non-target suspect analyses did not detect any compounds above the limit of quantitation, with the exception of the herbicide Azoxystrobin, which was detected at 4.73 ng/L in the Sacramento River at Isleton in Exposure 4. In the targeted analyses, Fipronil, Fipronil-sulfide, Fipronil-desulfinyl, and Fipronil-sulfone were detected at every site in every exposure during the project period, with concentrations ranging from 0.05 to 0.64 ng/L. Chlorpyrifos was detected in the Sacramento River at Isleton site in Exposure 3, and at the Toe Drain, Cache Slough, and Sacramento River at Decker Island sites in Exposure 4. Concentrations of Chlorpyrifos in both exposures ranged from 0.11 to 0.12 ng/L. Samples were also submitted for trace metals analyses. Barium (both total and dissolved fractions) was detected at every site in every exposure during the project period, with concentrations ranging from 16 to 42 µg/L. Iron, manganese, and nickel were detected sporadically throughout the project period, with concentrations ranging from 11 to 100 µg/L.

Biomarker Analyses

General Fish Condition

Gross measurements and weights were used to determine condition factor (CF) and hepatosomatic (HSI) index in fish. Changes in CF specifically reflect alterations in growth and

nutritional status while differences in HSI in fish at early life-stages may reflect general health and nutrition.

There were no significant differences observed in CF or HSI in any exposure (P=0.05). Sites Grizzly Bay and Montezuma Slough were statistically compared to the High Salinity Control. CF and HSI values are outlined below in Table 6-1.

Table 6-1. Summary of Condition Factor and Hepatosomatic Index of Delta Smelt

Toxicity Test	Treatment	Condition Factor		Hepatosomatic Index	
		Mean	SD	Mean	SD
Exposure 1 10/13/17	Control	0.877	0.159	0.843	0.232
	Site 1 – Lindsey Slough	0.823	0.194	0.956	0.564
	Site 2 – Cache Slough	0.808	0.227	0.996	9.235
	Site 3 – Grizzly Bay	0.964	0.301	0.972	0.362
	Site 4 – Montezuma Slough	0.888	0.114	1.260	0.439
	High Salinity Control	0.857	0.098	1.078	0.286
Exposure 2 10/27/17	Control	0.941	0.147	1.024	0.288
	Site 1 – Montezuma Slough	0.870	0.169	0.845	0.214
	Site 2 – Sacramento River at Isleton	0.938	0.260	1.034	0.405
	Site 3 – Cache Slough	1.088	0.370	0.984	0.444
	Site 4 – Sacramento River at Decker Island	0.925	0.213	0.984	0.424
	Site 5 – Toe Drain	1.023	0.142	0.925	0.354
	High Salinity Control	0.926	0.178	0.912	0.202
Exposure 3 11/10/17	Control	1.110	0.129	1.288	0.514
	Site 1 – Montezuma Slough	1.030	0.149	1.327	0.481
	Site 2 – Toe Drain	1.141	0.128	1.276	0.323
	Site 3 – Cache Slough	1.076	0.163	1.095	0.352
	Site 4 – Sacramento River at Isleton	1.140	0.078	1.282	0.478
	Site 5 – Sacramento River at Decker Island	1.083	0.087	1.020	0.479
	Site 6 – Grizzly Bay	1.086	0.097	0.879	0.245
	High Salinity Control	1.124	0.109	1.030	0.221
Exposure 4 11/24/17	Control	1.106	0.189	0.998	0.299
	Site 1 – Montezuma Slough	1.076	0.096	1.194	0.399
	Site 2 – Toe Drain	1.037	0.112	1.001	0.313
	Site 3 – Cache Slough	1.032	0.117	0.897	0.170
	Site 4 – Sacramento River at Isleton	1.102	0.146	1.120	0.449
	Site 5 – Sacramento River at Decker Island	1.103	0.120	1.081	0.306
	Site 6 – Grizzly Bay	1.060	0.087	1.086	0.450
	High Salinity Control	1.070	0.110	1.229	0.566
Exposure 5 12/8/17	Control	1.034	0.110	1.108	0.441
	Site 1 – Montezuma Slough	1.044	0.131	1.014	0.434
	Site 2 – Toe Drain	1.079	0.141	1.222	0.509
	Site 3 – Cache Slough	1.071	0.113	1.126	0.407
	Site 4 – Sacramento River at Isleton	1.054	0.121	1.076	0.582
	Site 5 – Sacramento River at Decker Island	1.096	0.087	1.135	0.411
	Site 6 – Grizzly Bay	1.095	0.102	0.970	0.222
	High Salinity Control	1.079	0.109	1.074	0.532

Histopathology

Histological analyses were performed on the liver and gill tissues from Delta Smelt exposed to water from: FCCL, FCCL water at salinity equivalent to Grizzly Bay (High Salinity Control), Toe Drain, Cache/Lindsey Slough, Sacramento River at Isleton, Sacramento River at Decker Island, Montezuma Slough, and Grizzly Bay.

Exposure 1

Most of the examined livers and gills had normal histological structure, without major pathological alterations. In livers, no single cell necrosis (SCN), macrophage aggregate (MA), sinusoidal congestion (SC) or inflammation (INF) were observed in any treatment. Moderate (rank 2) and severe (rank 3) Glycogen depletion (GD) were more prevalent in fish exposed to water collected from Site 3 – Grizzly Bay (4 of 8 fish), High Salinity Control (5 of 6 fish) and moderate and severe lipidosis (LIP) were more prevalent in fish from Site 2 – Cache Slough (3 of 8 fish), Site 3 – Grizzly Bay (6 of 8 fish), Site 4 – Montezuma Slough (6 of 8 fish), and High Salinity Control (3 of 6 fish) when compared to the standard control. In gills, there was no gill secondary lamella edema, mucus cell hyperplasia or fusion observed in any treatment. Several fish had mild lesions such as epithelial cell necrosis (GCN), inflammation (GINF), parasitic infection and gill aneurysm or telangiectasia (ANU). Moderate chloride cell or ionocyte hyperplasia (CCH) was observed in most of the fish in all treatments. One fish in Site 2 – Cache Slough had moderate ANU.

Exposure 2

In livers, there were no SCN, MA, SC or INF observed in any treatment. Prevalence of GD and LIP were observed but scores were not different among treatments. In gill, except moderate ANU in 1 fish of Site 1 – Montezuma Slough and moderate GCN and severe MCH and lamella fusion (Fig. 1) in 1 fish of Site 3 – Cache Slough. There were no lesions observed in any treatment.

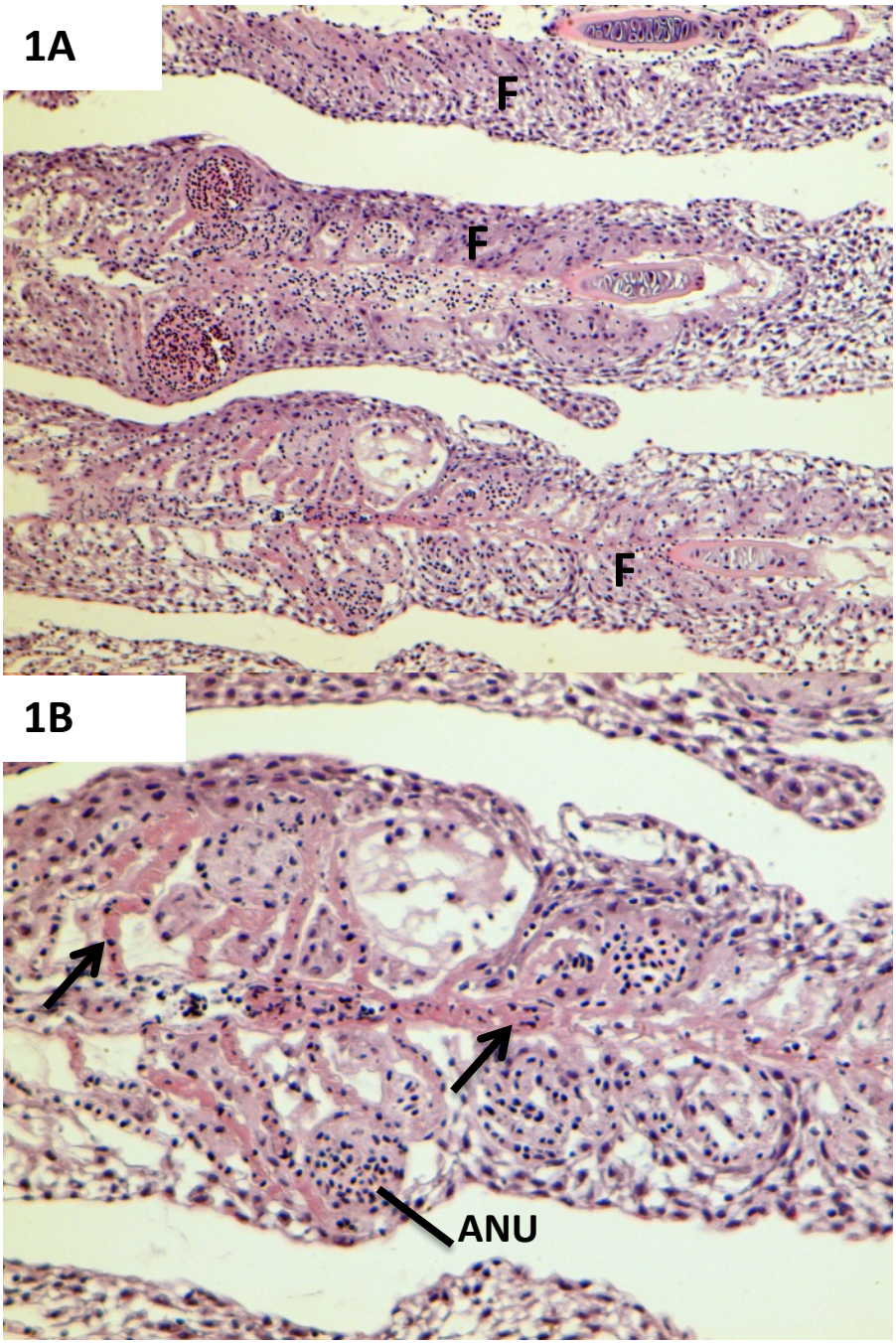

Figure 6-1. 1A: Severe Secondary Lamella Fusion (F) in 3 Gill Filaments at Low Magnification. 1B) Higher Magnification Showing Moderate Epithelial Cell Necrosis (arrows) and Aneurysm (ANU) in Delta Smelt Exposed to Water Collected from Site 3 – Cache Slough

Exposure 3

In livers, no single cell necrosis (SCN), macrophage aggregate (MA), sinusoidal congestion (SC) or inflammation (INF) were observed in any treatment. Glycogen depletion (GD) and lipidosis (LIP) were observed but scores were not different among treatments. In gills, there was no gill epithelial cell necrosis (GCN), secondary lamella edema, or inflammation (GINF) observed in

any treatment. However, chloride cell hyperplasia (CCH) was observed in all treatments. Several fish exposed to Site 3 -Cache/Lindsey Slough had more severe lesions (Figure 6-2), including one fish with severe gill aneurysm or telangiectasia (ANU; n=1/18) as well as three fish with mucus cell hyperplasia (MCH; n=3/18). One fish exposed to water from Site 4 - Sacramento River at Isleton had moderate gill aneurysm (n=1/19). In addition, 2 fish were intersex in control treatment.

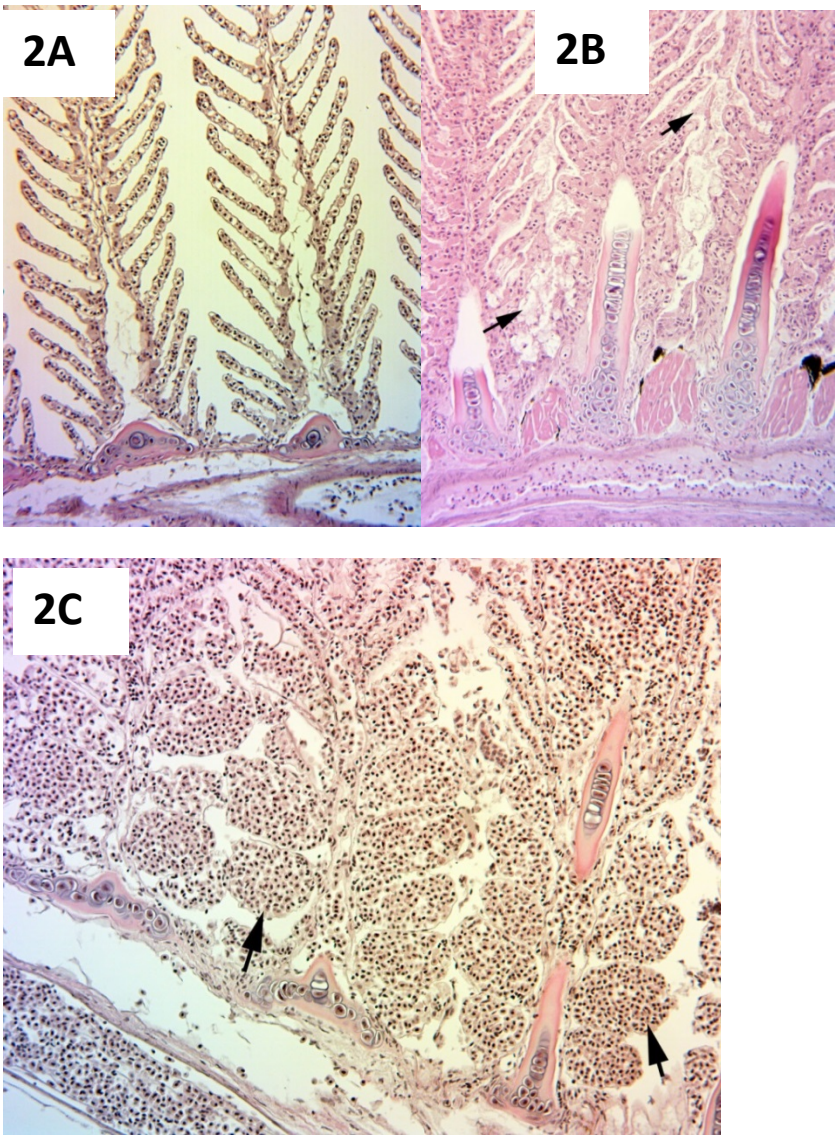

Figure 6-2. A) Typical Regular Thin Gill Lamellae Structure of Delta Smelt Exposed to FCCL Water for 96 hr. B) Severe Mucus Cell Hyperplasia in Gill Exposed to Site 3 - Cache Slough Water for 96 hr. Arrows Point to Mucus Discharges Filling the Space of Primary and Secondary Lamellae. C) Severe Gill Aneurysm or Telangiectasia (arrows) in Delta Smelt Exposed to Water from Site 3 - Cache Slough

Exposure 4

There were no significant lesions observed in livers and gills of any treatment.

Exposure 5

In livers, no single cell necrosis (SCN) or macrophage aggregate (MA) were observed in any treatment. Moderate glycogen depletion (GD) and lipidosis (LIP) were observed among all treatments but severe lipidosis was more prevalent in Site 1 – Montezuma Slough and Site 5 – Sacramento River at Decker Island. One fish in Site 3 – Cache/Lindsey Slough had severe inflammation (INF) and one fish in Site 5 – Sacramento River at Decker Island had severe lipidosis and moderate sinusoidal congestion (SC) (Fig 3). Except for one fish in Site 5 – Sacramento River at Decker Island which had moderate epithelial cell hyperplasia, no significant lesions were observed in gill of any treatments.

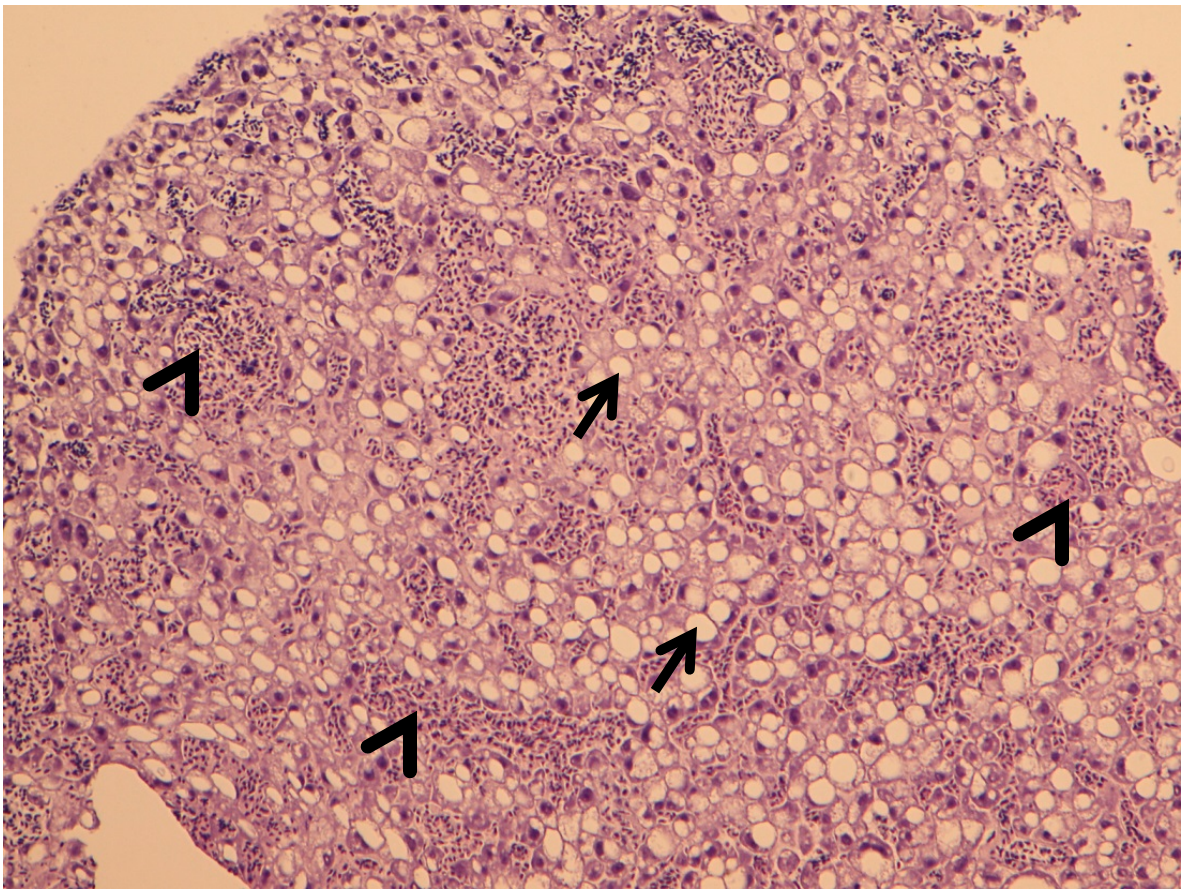

Figure 6-3. Severe Fatty Vacuolation (arrows) and Moderate Sinusoidal Congestion (arrowheads) in Delta Smelt Exposed to Water Collected from Site 5 – Sacramento River at Decker Island

Cytochrome P450s

Cytochrome P450 induction was immunohistochemically demonstrated in hepatocytes of Delta Smelt. No P450 inductions were observed in any treatment in Exposure Experiment 1 and 5. In Exposure 2, 3, and 4, moderate to weak staining was evident in hepatocytes among all treatments. Enhanced P450 staining (rank 3) were observed in Site 1 – Montezuma Slough (N=1) Site 2 – Toe Drain (N=1), and Site 3 – Cache Slough (N=1) of Exposure 3 and Site 1 – Montezuma Slough (N=1) and Site 2 – Toe Drain (N=1)2 – Toe Drain (N-1) of Exposure 4 (Fig 4). Among all exposures, higher prevalence of fish with moderate to enhanced P450 staining

were observed in Site 1 – Montezuma Slough and Site 2 – Toe Drain of Exposure 4, indicate potential for contaminant exposure.

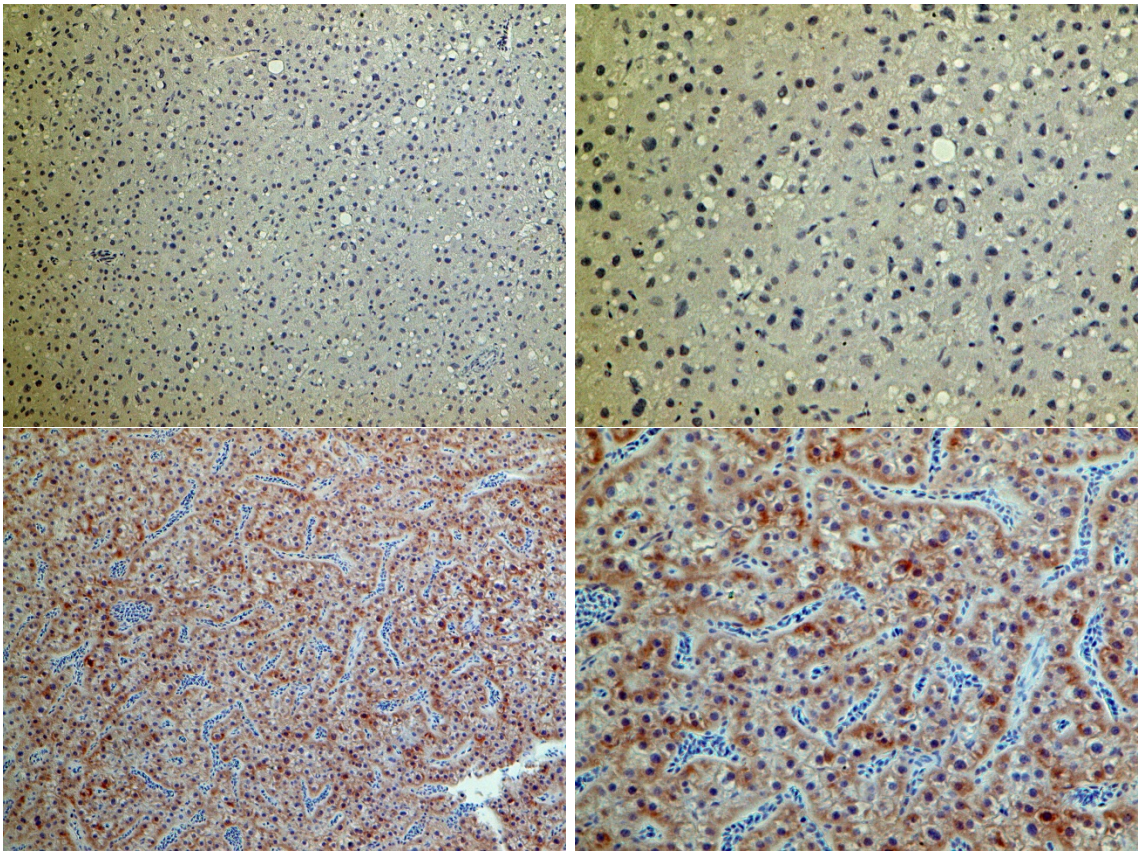

Figure 6-4. Top Micrograph Shows Negative CYP450 Reaction in Control Delta Smelt at Low and High Magnification. Bottom Micrograph Shows Enhanced CYP450 (brownish color) in Fish Exposed to Water Collected from Site 2 – Toe Drain in Exposure 4 initiated on November 24, 2017

Biomarkers of Contaminant Stress and Exposure

Acetylcholinesterase Activity in Brain

Significant effect ($P<0.05$) of sex on acetylcholinesterase activity was not found for Exposures 1, 2, 3, and 4, so the analysis was done by pooling data from females and males together for these exposures. Significant differences among groups were found only for Exposure 2 (ANOVA, $F_{[6, 21]} = 4.2857$, $P = 0.0057$). Significant differences found among groups in Exposure 2 (Table 6-2) were due to a higher activity at Site 3 – Cache Slough compared to the control and Site 4 – Sacramento River at Decker Island. However, no group had the acetylcholinesterase activity significantly lower than the control group. Biologically relevant changes in brain acetylcholinesterase are due to a decreased activity that affects motor control which was not observed in any exposure. Moreover, high salinity did not have any significant influence on the acetylcholinesterase activity, as it showed a similar level when compared to the control. On the other hand, for Exposure 5 there was a significant effect of sex ($P= 0.0031$) on acetylcholinesterase activity. A one-way ANOVA with post-hoc Tukey test was run for males

(ANOVA, $F_{[7, 12]} = 0.5102$, $P = 0.8104$) and females (ANOVA, $F_{[7, 10]} = 1.6199$, $P = 0.2358$) separately, which did not show significant differences among groups.

Table 6-2. Brain Activity of Acetylcholinesterase for Exposure 2 Initiated on October 27, 2017

Treatment	Acetylcholinesterase activity (µmol/min/mg prot)
Site 3 – Cache Slough	25.29 ± 5.45 a
Site 5 – Toe Drain	24.86 ± 1.66 a,b
Site 2 – Sacramento River at Isleton	22.01 ± 1.81 a,b,c
High Salinity Control	19.88 ± 2.50 a,b,c
Site 1 – Montezuma Slough	19.69 ± 1.66 a,b,c
Control	18.69 ± 2.05 b,c
Site 4 – Sacramento River at Decker Island	18.48 ± 1.60 c

Enzymatic antioxidants

The enzymatic activity of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione S-transferase was measured in the liver.

Exposures 1 and 2

Significant differences among sites were not found for any enzymatic antioxidant for Exposures 1 and 2 ($P<0.05$).

Exposure 3

There was a significant effect of sex on glutathione reductase and glutathione S-transferase activities for Exposure 3. Significant differences among groups for glutathione reductase (ANOVA, $F_{[7, 18]} = 2.5773$, $P = 0.0500$, Table 6-3) and glutathione s-transferase (ANOVA, $F_{[7, 20]} = 3.0821$, $P = 0.0226$, Table 6-4) were found for females only.

Table 6-3. Glutathione Reductase Activity in Females for Exposure 3 Initiated on November 10, 2017

	Glutathione reductase (mU/min/mg prot)	
Treatment	**Females**	**Males**
Site 6 - Grizzly Bay	0.41 ± 0.06 a	0.46 ± 0.07
Site 5 - Sacramento River at Decker Island	0.35 ± 0.08 a,b	0.55 ± 0.08
Site 4 - Sacramento River at Isleton	0.35 ± 0.02 a,b	0.41 ± 0.08
Site 1 - Montezuma Slough	0.32 ± 0.03 a,b	0.42± 0.03
Control	0.32 ± 0.01 a,b	0.40 ± 0.01
High Salinity Control	0.27 ± 0.03 a,b	0.51 ± 0.03
Site 3 - Cache Slough	0.31 ± 0.03 a,b	0.33 ± 0.02
Site 2 - Toe Drain	0.23 ± 0.04 b	0.40 ± 0.04

Table 6-4. Glutathione S-Transferase Activity in Females for Exposure 3 Initiated on November 10, 2017

Treatment	Glutathione S-transferase (µmol/min/mg prot)	
	Females	**Males**
Site 6 - Grizzly Bay	5.15 ± 0.55 a	3.39 ± 0.49
High Salinity Control	4.17 ± 0.17 a,b	3.50 ± 0.45
Site 4 - Sacramento River at Isleton	3.69 ± 0.29 a,b	3.38 ± 1.03
Site 1 - Montezuma Slough	3.65 ± 0.71 a,b	2.67 ± 0.59
Site 3 - Cache Slough	3.58 ± 0.38 a,b	2.35 ± 0.24
Site 5 - Sacramento River at Decker Island	3.05 ± 0.13 a,b	3.48 ± 0.43
Site 2 - Toe Drain	2.69 ± 0.09 b	2.89 ± 0.28
Control	2.61 ± 0.46 b	2.08 ± 0.18

Exposure 4

Significant differences among sites against the control were found for Catalase (ANOVA, $F_{[7, 23]} = 3.8330$, $P = 0.0067$, Table 6-5) and Glutathione S-transferase (ANOVA, $F_{[7, 23]} = 3.6946$, $P = 0.0081$, Table 6-6), as well as in females for superoxide dismutase for Exposure 4.

Table 6-5. Catalase Activity for Exposure 4 Initiated on November 24, 2017

Treatment	Catalase activity (U/min/mg prot)
Site 4 - Sacramento River at Isleton	707.1 ± 64.4 a
Site 2 - Toe Drain	608.6 ± 232.5 a,b
Site 5 - Sacramento River at Decker Island	599.1 ± 25.9 a,b
Site 6 - Grizzly Bay	585.9 ± 168.4 a,b
Site 3 - Cache slough	546.7 ± 133.5 a,b
Control	439.4 ± 50.7 b
Site 1 - Montezuma Slough	419.1 ± 29.4 b
High Salinity Control	417.2 ± 79.9 b

Table 6-6. Glutathione S-Transferase Activity for Exposure 4 Initiated on November 24, 2017

Treatment	GST Activity (µmol/min/mg prot)
Site 4 - Sacramento River at Isleton	5.7 ± 0.8 a
Site 2 - Toe Drain	5.5 ± 1.9 a,b
Site 6 -Grizzly Bay	5.4 ± 2.0 a,b
Site 5 - Sacramento River at Decker Island	5.1 ± 0.4 a,b
Site 3 - Cache Slough	4.4 ± 0.6 a,b
Site 1 - Montezuma Slough	3.7 ± 0.4 a,b
High Salinity Control	3.5 ± 1.1 a,b
Control	3.0 ± 0.6 b

There was a significant effect of sex on superoxide dismutase activity for Exposure 4. A one-way ANOVA with a post-hoc Tukey test was run for each sex. Significant differences among groups were found for females (ANOVA, $F_{[7, 14]} = 2.7766$, $P = 0.0493$, Table 6-7) but not in males (ANOVA, $F_{[7, 15]} = 1.1850$, $P = 0.3677$).

Table 6-7. Superoxide Dismutase Activity in Females for Exposure 4 Initiated on November 24, 2017

Treatment	SOD Activity (U/mg prot)	
	Females	Males
Site 4 - Sacramento River at Isleton	1130.8 ± 48.2 a	643.3 ± 244.6
Site 6 - Grizzly Bay	859 ± 472.6 a,b	289.4 ± 115.9
High Salinity Control	698.2 ± 182.1 a,b	267.4 ± 160.1
Site 2 - Toe Drain	687.1 ± 387.8 a,b	1007.2 ± 900.4
Site 5 - Sacramento River at Decker Island	677.1 ± 126.5 a,b	368.3 ± 54.3
Site 1 - Montezuma Slough	578.6 ± 157.9 a,b	364.7 ± 270.7
Control	365.6 ± 297.8 b	329.9 ± 87.5
Site 3 - Cache Slough	352.2 ± 153.6 a,b	268.9 ± 25.3

For Exposure 4 the highest activity of catalase and glutathione S-transferase, as well as of superoxide dismutase in females was found in Delta Smelt exposed to water from Site 4 - Sacramento River at Isleton.

Exposure 5

Significant differences among sites were found for Glutathione peroxidase (ANOVA, $F_{[7, 23]} = 3.7512$, $P = 0.0075$, Table 6-8), Glutathione reductase (ANOVA, $F_{[7, 23]} = 3.4963$, $P = 0.0106$, Table 6-9), and Glutathione S-transferase (ANOVA, $F_{[7, 23]} = 4.7477$, $P = 0.0020$, Table 6-10) for Exposure 5 but without significant differences against the control. However, it is noteworthy to mention that the highest activity for these three enzymatic antioxidants was found at Site 4 - Sacramento River at Isleton. Moreover, glutathione reductase activity at Site 6 - Grizzly Bay was significantly lower compared to control.

Table 6-8. Glutathione Peroxidase Activity for Exposure 5 Initiated on December 8, 2017

Treatment	GPx (mU/mg prot)
Site 4 - Sacramento River at Isleton	8.41 ± 1.68 a
Site 5 - Sacramento River at Decker Island	8.32 ± 0.72 a
Control	6.49 ± 2.5 a,b
Site 6 - Grizzly Bay	6.21 ± 1.08 a,b
Site 2 - Toe Drain	5.89 ± 1.03 a,b
Site 1 - Montezuma Slough	5.52 ± 0.98 a,b
Site 3 - Cache Slough	5.49 ± 0.21 a,b
High Salinity Control	5.12 ± 1.26 b

Table 6-9. Glutathione Reductase Activity for Exposure 5 Initiated on December 8, 2017

Treatment	GR Activity (mU/mg prot)
Site 4 – Sacramento River at Isleton	0.20 ± 0.03 a
Site 2 - Toe Drain	0.19 ± 0.04 a
Control	0.19 ± 0.05 a
Site 5 - Sacramento River at Decker Island	0.19 ± 0.02 a
Site 3 - Cache Slough	0.18 ± 0.03 a,b

Treatment	GR Activity (mU/mg prot)
High Salinity	0.16 ± 0.03 a,b
Site 1 - Montezuma Slough	0.15 ± 0.01 a,b
Site 6 - Grizzly Bay	0.10 ± 0.02 b

Table 6-10. Glutathione S-Transferase Activity for Exposure 5 Initiated on December 8, 2017

Treatment	GST Activity (µmol/min/mg prot)
Site 4 - Sacramento River at Isleton	3.67 ± 0.4 a
Site 5 - Sacramento River at Decker Island	3.33 ± 0.42 a,b
Site 6 - Grizzly Bay	3.12 ± 0.11 a,b,c
Control	3.10 ± 1.26 a,b,c
Site 2 - Toe Drain	2.55 ± 0.33 a,b,c
High Salinity Control	2.46 ± 0.31 a,b,c
Site 1 - Montezuma Slough	2.19 ± 0.13 b,c
Site 3 - Cache Slough	2.15 ± 0.263 c

Discussion

We did not observe acute toxicity in Delta Smelt exposed to water from the six sampling locations in each of five sub-strata within the full study area. There were no statistically significant reductions in acute toxicity and fish condition observed in any of the exposure treatments. Thus, outflow water did not have any negative acute effects to Delta Smelt.

Analytical chemistry results indicate that contaminants were present in Delta outflow water during this project period in concentrations unlikely to cause acute effects. Noteworthy detections included Fipronil, Fipronil-sulfone, Fipronil-desulfinyl, and Fipronil-sulfide, which were present at all sites in all exposures, at concentrations below ng/L. Likewise, Barium (both total and dissolved fractions) was detected in all sites in all exposures in the µg/L range. Chlorpyrifos was detected in the Sacramento River at Isleton site in Exposure 3, and in the Toe Drain, Cache Slough and Sacramento River at Decker Island sites in Exposure 4, however concentrations ranged from 0.11-0.12 ng/L. Iron was detected in the Grizzly Bay site in Exposure 3 at 82 µg/L, and at 92 µg/L in the Sacramento River at Isleton site in Exposure 4. These concentrations are orders of magnitude below USEPA chronic aquatic life benchmarks for the chemicals in question. The chronic aquatic life benchmarks for Fipronil and its transformation products range from 0.59 (Fipronil-desulfinyl) to 2.2 (Fipronil) µg/L for fish, and the chronic aquatic life benchmark for Chlorpyrifos is 0.57 µg/L. Aside from one detection of the herbicide Azoxystrobin, non-target suspect analyses did not find any compounds above the limit of quantification. Although the compounds detected during this study period were at low concentrations and by themselves not enough to cause acute adverse effects, we cannot rule out the possibility of potentially additive and/or synergistic mixture effects which could be responsible for the sub-lethal effects observed in this study.

Enzymatic and histopathologic biomarkers analyzed did not reveal significant changes when compared to the control and high salinity group. This suggest that Delta Smelt can tolerate 96hr short term exposure to salinity in the range of 2-6 ppt. We found two males with intersex testis.

Intersex is a chronic effect and occurs when fish are exposed to endocrine disrupting chemicals that disrupts the endocrine-hormonal systems. Therefore, it is unlikely the intersex in male is a result of a 96hr exposure. Although the incidence is low, this suggests that fish at the FCCL may have prior exposure to endocrine disrupting chemicals, as their culture water is sourced from ambient sources. Therefore, we did not perform estradiol (E2) analysis for this study. In addition, liver glycogen depletion, lipidosis, and gill chloride cell hyperplasia are consistent with FCCL environmental culture conditions and are likely a result from compensatory mechanisms from chronic stress (e.g., over-crowding). The gill lesions observed, such as mucus cell hyperplasia, secondary lamellar fusion, and lamellar aneurysms, were mostly severe in the Cache Slough site, and could indicate metals, pesticide, and mixed contaminants exposure in this region (Hammock et al. 2015). Mild to moderate mucus secretion is considered a protective response to contaminant exposure, but severe mucus secretion and aneurysm can impede respiration resulting in fish death due to hypoxia (Matey et al 2010).

Significantly higher activities of glutathione S-transferase and glutathione reductase were observed in females exposed to water from the Grizzly Bay site in Exposure 3. On the other hand, the highest activities of catalase, glutathione S-transferase, and superoxide dismutase for Exposure 4, and of glutathione peroxidase, glutathione reductase, and glutathione S-transferase for Exposure 5 were recorded for fish exposed to water from the Sacramento River at Isleton site. Glutathione is the main non enzymatic antioxidant and it scavenges reactive oxygen species, which can be generated by pollutants and changes in water quality and some environmental conditions. The higher activities of superoxide dismutase, catalase, and glutathione peroxidase indicate a higher production of reactive oxygen species. Results from enzymatic antioxidant assays indicate that fish exposed to water from the Grizzly Bay site during Exposure 3 and the Sacramento River at Isleton site from Exposures 4 and 5 were being exposed to a higher load of unspecified organic compounds and were producing more reactive oxygen species. However, these changes are in general mild and do not indicate severe responses against contaminants.

Taken together, data for the contaminant exposure study indicate that fish were potentially exposed to a higher load of organic contaminants during Exposures 3 and 4, which were initiated in the month of November 2017. However, it must be highlighted that most of the changes observed were mild and were not associated with biologically relevant changes in tissue morphology. Fish exposed to water collected from different sites of the Delta had higher expression of CYP1A and glutathione S-transferase (enzymes involved in phase I and II of biotransformation, respectively) compared to the control group for Exposures 3 and 4. Enhanced CYP1A was observed for the Montezuma Slough, Toe Drain, and Cache Slough sites in Exposure 3, and Montezuma Slough and Toe Drain sites for Exposure 4. In contrast, the activity of glutathione S-transferase was higher at all site locations compared to the control for Exposures 3 and 4, but significant changes were only observed at the Grizzly Bay site in Exposure 3 and at the Sacramento River at Isleton site in Exposure 4. These discrepancies in CYP1A and glutathione S-transferase activities among sites for Exposures 3 and 4 may be caused by induction of these enzymes by different sets of organic contaminants.

Results from this study indicate that the water quality of the augmented Delta outflow in 2017 had little negative health effects on Delta Smelt. As 2017 was a wet year, the good water quality observed during this study period may be due to dilution or depletion of contaminants in the Delta outflow water. However, contaminants are still present, albeit in low concentrations, as

indicated by the results of our histopathological analyses, enzymatic endpoints, and analytical chemistry results. In conjunction with our 2017 histopathology study on wild Delta Smelt as part of the larger Directed Outflow Project funded by USBR, absolute lesion scores in wild Delta Smelt were lower during this project period than in previous years, although histologic analyses continue to suggest contaminant effects on Delta Smelt in Cache Slough. Given the results of both studies, it would appear that in general augmented Delta outflow is beneficial to Delta Smelt health and condition.

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Supplemental Data: Tables

Table 1. Summary of Water Quality Measurements

Toxicity Test Date	Site Location	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Ammonia-nitrogen (mg/L)	pH	SC (µS/cm)
Exposure 1 10/13/2017	Lindsey Slough	56	80	0.11	7.81	148
	Cache Sl. @ mouth of Steamboat Sl.	48	34	0.22	7.58	116
	Montezuma Slough	352	60	0.14	7.38	2785
	Grizzly Bay	876	68	0.14	7.41	7443
Exposure 2 10/27/2017	Montezuma Slough	360	82	0.25	7.40	2765
	Toe Drain	60	74	0.37	7.95	304
	Cache Slough	52	44	0.20	7.78	167
	Sacramento River at Isleton	48	50	0.46	7.51	132
	Sacramento River at Decker Island	64	50	0.19	7.46	162
Exposure 3 11/10/2017	Montezuma Slough	400	58	0.15	7.40	3312
	Toe Drain	56	62	0.05	8.05	284
	Cache Slough	20	62	0.12	7.72	178
	Sacramento River at Isleton	48	58	0.52	7.60	142
	Sacramento River Decker Island	48	50	0.20	7.45	138
	Grizzly Bay	1160	70	0.13	7.32	7544
Exposure 4 11/24/2017	Montezuma Slough	556	66	0.11	7.32	3542
	Toe Drain	116	72	0.05	8.09	380
	Cache Slough	96	70	0.12	8.04	163
	Sacramento River at Isleton	60	62	0.21	7.91	125
	Sacramento River at Decker Island	84	64	0.12	7.74	147
	Grizzly Bay	1132	64	0.09	7.40	7344
Exposure 5 12/8/2017	Montezuma Slough	596	82	0.18	7.87	3590
	Toe Drain	116	104	0.01	8.18	194
	Cache Slough	60	86	0.13	8.00	203
	Sacramento River at Isleton	64	68	0.23	8.20	162
	Sacramento River at Decker Island	80	70	0.18	7.96	268
	Grizzly Bay	1112	80	0.04	7.67	11113

Table 2. Summary of Targeted Analytical Chemistry Results (ng/L) for Exposure 1, Initiated October 13, 2017

Compound	Site 1 Lindsey Slough	Site 2 Cache Slough	Site 3 Grizzly Bay	Site 4 Montezuma Slough
Novaluron	ND	ND	ND	ND
Chlorothalonil	ND	ND	ND	ND
Fipronil-desulfinyl	0.08	0.09	0.10	0.09
Chlorpyrifos	ND	ND	ND	ND
Fipronil-sulfide	0.05	0.06	0.05	0.05
Bioallethrin	ND	ND	ND	ND

Compound	Site 1 Lindsey Slough	Site 2 Cache Slough	Site 3 Grizzly Bay	Site 4 Montezuma Slough
Fipronil	0.19	0.15	0.13	0.14
Prallethrin	ND	ND	ND	ND
Tetramethrin	0.00	0.00	0.00	0.00
Fipronil-desulfinyl amide	ND	ND	ND	0.06
Fipronil-sulfone	0.15	0.12	<LOQ	0.11
Fipronil amide	ND	ND	ND	ND
Bifenthrin	ND	ND	ND	ND
Phenothrin	ND	ND	ND	ND
Cyhalothrin	ND	ND	ND	ND
Cyphenothrin	ND	ND	ND	ND
Permethrin	ND	ND	ND	ND
Cypermethrin	ND	ND	ND	ND
Esfenvalerate	ND	ND	ND	ND
Deltamethrin	ND	ND	ND	ND

Table 3. Summary of Targeted Analytical Chemistry Results (ng/L) for Exposure 2 Initiated October 27, 2017

Compound	Site 1 Montezuma Slough	Site 2 Sac River Isleton	Site 3 Cache Slough	Site 4 Sac River at Decker	Site 5 Toe Drain
Novaluron	ND	ND	ND	ND	ND
Chlorothalonil	ND	ND	ND	ND	ND
Fipronil-desulfinyl	0.09	0.07	0.10	0.11	0.10
Chlorpyrifos	ND	ND	0.23	ND	0.11
Fipronil-sulfide	0.06	0.05	0.06	0.06	0.05
Bioallethrin	ND	ND	ND	ND	ND
Fipronil	0.18	0.23	0.23	0.24	0.14
Prallethrin	ND	ND	ND	ND	ND
Tetramethrin	0.00	0.00	0.00	0.00	0.00
Fipronil-desulfinyl amide	0.05	ND	ND	ND	ND
Fipronil-sulfone	0.13	0.18	0.13	0.17	0.10
Fipronil amide	ND	ND	ND	ND	ND
Bifenthrin	ND	ND	ND	ND	ND
Phenothrin	ND	ND	ND	ND	ND
Cyhalothrin	ND	ND	ND	ND	ND
Cyphenothrin	ND	ND	ND	ND	ND
Permethrin	ND	ND	ND	ND	ND
Cypermethrin	ND	ND	ND	ND	ND
Esfenvalerate	ND	ND	ND	ND	ND
Deltamethrin	ND	ND	ND	ND	ND

Table 4. Summary of Targeted Analytical Chemistry Results (ng/L) for Exposure 3 Initiated November 10, 2017

Compound	Site 1 Montezuma Slough	Site 2 Toe Drain	Site 3 Cache Slough	Site 4 Sac River at Isleton	Site 5 Sac River at Decker	Site 6 Grizzly Bay
Novaluron	ND	ND	ND	ND	ND	ND
Chlorothalonil	ND	ND	ND	ND	ND	ND
Fipronil-desulfinyl	0.10	0.11	0.10	0.07	0.09	0.10
Chlorpyrifos	ND	ND	ND	0.11	ND	ND
Fipronil-sulfide	0.06	0.05	0.06	0.05	0.05	0.05
Bioallethrin	ND	ND	ND	ND	ND	ND
Fipronil	0.16	0.12	0.23	0.31	0.23	0.15
Prallethrin	ND	ND	ND	ND	ND	ND
Tetramethrin	0.00	0.00	0.00	0.00	0.00	0.00
Fipronil-desulfinyl amide	ND	ND	ND	ND	ND	ND
Fipronil-sulfone	0.17	0.11	0.14	0.16	0.15	0.10
Fipronil amide	ND	ND	ND	ND	ND	ND
Bifenthrin	ND	ND	ND	ND	ND	ND
Phenothrin	ND	ND	ND	ND	ND	ND
Cyhalothrin	ND	ND	ND	ND	ND	ND
Cyphenothrin	ND	ND	ND	ND	ND	ND
Permethrin	ND	ND	ND	ND	ND	ND
Cypermethrin	ND	ND	ND	ND	ND	ND
Esfenvalerate	ND	ND	ND	ND	ND	ND
Deltamethrin	ND	ND	ND	ND	ND	ND

Table 5. Summary of Targeted Analytical Chemistry Results (ng/L) for Exposure 4 Initiated November 24, 2017

Compound	Site 1 Montezuma Slough	Site 2 Toe Drain	Site 3 Cache Slough	Site 4 Sac River at Isleton	Site 5 Sac Rover at Decker	Site 6 Grizzly Bay
Novaluron	ND	ND	ND	ND	ND	ND
Chlorothalonil	ND	ND	ND	ND	ND	ND
Fipronil-desulfinyl	0.12	0.12	0.13	0.09	0.14	0.12
Chlorpyrifos	ND	0.11	0.11	ND	0.12	ND
Fipronil-sulfide	0.06	0.06	0.06	0.06	0.07	0.06
Bioallethrin	ND	ND	ND	ND	ND	ND
Fipronil	0.29	0.17	0.41	0.49	0.64	0.20
Prallethrin	ND	ND	ND	ND	ND	ND
Tetramethrin	0.00	0.00	0.00	0.00	0.00	0.00
Fipronil-desulfinyl amide	ND	ND	ND	ND	0.10	ND
Fipronil-sulfone	0.16	0.12	0.26	0.28	0.40	0.13
Fipronil amide	ND	ND	ND	<LOQ	<LOQ	ND
Bifenthrin	ND	ND	ND	ND	ND	ND
Phenothrin	ND	ND	ND	ND	ND	ND
Cyhalothrin	ND	ND	ND	ND	ND	ND
Cyphenothrin	ND	ND	ND	ND	ND	ND
Permethrin	ND	ND	ND	ND	ND	ND

Compound	Site 1 Montezuma Slough	Site 2 Toe Drain	Site 3 Cache Slough	Site 4 Sac River at Isleton	Site 5 Sac Rover at Decker	Site 6 Grizzly Bay
Cypermethrin	ND	ND	ND	ND	ND	ND
Esfenvalerate	ND	ND	ND	ND	ND	ND
Deltamethrin	ND	ND	ND	ND	ND	ND

Table 6. Summary of Targeted Analytical Chemistry Results (ng/L) for Exposure 5 Initiated December 8, 2017

Compound	Site 1 Montezuma Slough	Site 2 Toe Drain	Site 3 Cache Slough	Site 4 Sac River at Isleton	Site 5 Sac River at Decker	Site 6 Grizzly Bay
Novaluron	ND	ND	ND	ND	ND	ND
Chlorothalonil	ND	ND	ND	ND	ND	ND
Fipronil-desulfinyl	0.11	0.11	0.10	0.07	0.09	0.12
Chlorpyrifos	ND	ND	ND	ND	ND	ND
Fipronil-sulfide	0.06	0.06	0.07	0.06	0.06	0.06
Bioallethrin	ND	ND	ND	ND	ND	ND
Fipronil	0.32	0.16	0.25	0.28	0.23	0.21
Prallethrin	ND	ND	ND	ND	ND	ND
Tetramethrin	0.00	0.00	0.00	0.00	0.00	0.00
Fipronil-desulfinyl amide	0.06	ND	ND	ND	ND	0.06
Fipronil-sulfone	0.22	0.16	0.26	0.23	0.24	0.14
Fipronil amide	ND	ND	ND	ND	ND	ND
Bifenthrin	ND	ND	ND	ND	ND	ND
Phenothrin	ND	ND	ND	ND	ND	ND
Cyhalothrin	ND	ND	ND	ND	ND	ND
Cyphenothrin	ND	ND	ND	ND	ND	ND
Permethrin	ND	ND	ND	ND	ND	ND
Cypermethrin	ND	ND	ND	ND	ND	ND
Esfenvalerate	ND	ND	ND	ND	ND	ND
Deltamethrin	ND	ND	ND	ND	ND	ND

Table 7. Summary of Trace Metals Analysis, for Total and Dissolved Fractions

Toxicity Test Date	Site	Total Metals (ppm)			Dissolved Metals (ppm)			
		Ba	Fe	Mn	Ba	Fe	Mn	Ni
Exposure 1 10/13/2017	Lindsey Slough	0.021			0.023			
	Cache Sl. @ Steamboat Sl.	0.018			0.018			
	Montezuma Slough	0.042			0.034			
	Grizzly Bay	0.034	0.082	0.011	0.034			
Exposure 2 10/27/2017	Montezuma Slough	0.033		0.014	0.033		0.012	
	Toe Drain	0.024			0.025			
	Cache Slough	0.021			0.023			
	Sacramento River at Isleton	0.016			0.018			
	Sacramento River at Decker Island	0.018			0.02			
Exposure 3 11/10/2017	Montezuma Slough	0.038			0.038			
	Toe Drain	0.024			0.024			

Toxicity Test Date	Site	Total Metals (ppm)			Dissolved Metals (ppm)			
		Ba	Fe	Mn	Ba	Fe	Mn	Ni
	Cache Slough	0.024			0.024			
	Sacramento River at Isleton	0.021			0.021			
	Sacramento River at Decker Island	0.020			0.022			
	Grizzly Bay	0.042	0.082	0.011	0.041			
Exposure 4 11/24/2017	Montezuma Slough	0.040	0.084	0.058	0.041		0.040	
	Toe Drain	0.027			0.027			
	Cache Slough	0.027			0.028			
	Sacramento River at Isleton	0.024	0.092		0.027	0.013		
	Sacramento River at Decker Island	0.024	0.012		0.025			
	Grizzly Bay	0.038			0.039			
Exposure 5 12/8/2017	Montezuma Slough	0.040			0.041			
	Toe Drain	0.035	0.310	0.013	0.031			
	Cache Slough	0.030	0.072		0.030			0.010
	Sacramento River at Isleton	0.025	0.100		0.025			
	Sacramento River at Decker Island	0.027	0.086		0.028			
	Grizzly Bay	0.040		0.022	0.040		0.011	

Notes: Ba: Barium, Fe: Iron, Mn: Manganese, Ni: Nickel.

Table 8. Histopathology Scores for Individual Fish in Exposure 1 Initiated on October 13, 2017

	Liver						Gill									
Fish	GD	LIP	SCN	INF	MA	SC	ANU	GCN	CCH	MCH	ECH	SLE	Parasite	GINF	Fusion	P450
C-1	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
C-2	2	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0
C-3	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
C-4	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
C-5	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C-6	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0
C-7	0	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0
C-8	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S1-1	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0
S1-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1-3	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S1-4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1-5	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S1-6	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1-7	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S1-8	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S2-1	3	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0
S2-2	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2-3	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S2-4	1	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S2-5	2	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S2-6	0	1	0	0	0	0	0	0	2	0	2	0	0	0	0	0
S2-7	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S2-8	0	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S3-1	1	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S3-2	1	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S3-3	2	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S3-4	2	3	0	0	0	0	0	0	2	0	0	0	1	1	0	0
S3-5	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S3-6	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S3-7	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
S3-8	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Fish	Liver						Gill									
	GD	LIP	SCN	INF	MA	SC	ANU	GCN	CCH	MCH	ECH	SLE	Parasite	GINF	Fusion	P450
S4-1	3	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S4-2	1	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S4-3	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S4-4	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0
S4-5	1	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S4-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S4-7	3	2	0	0	0	0	2	0	2	0	0	0	0	0	0	0
S4-8	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
HS-1	3	1	0	0	0	0	1	0	2	0	0	0	1	0	0	0
HS-2	0	2	0	0	0	0	0	1	2	0	0	0	1	0	0	0
HS-3	2	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
HS-4	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-5	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-6	2	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0

(GD) Glycogen Depletion, (LIP) Lipidosis, (SCN) Single Cell Necrosis, (INF) Liver Inflammation, (MA) Macrophage Aggregate, (SC) Sinusoidal Congestion, (ANU) Aneurysm, (GCN) Epithelial Cell Necrosis, (CCH) IONOCYTE Hyperplasia, (MCH) Mucus Cell Hyperplasia, (ECH) Telangiectasia, (SLE) Secondary Lamella Edema, (GINF) Gill Inflammation, C – Control, Site 1 – Lindsey Slough, Site 2 – Cache Slough, Site 3 – Grizzly Bay, Site 4 – Montezuma Slough, HS – High Salinity Control

Table 9. Histopathology Scores for Individual Fish in Exposure 2 Initiated on October 27, 2017

	Liver						Gill									
Fish	GD	LIP	SCN	INF	MA	SC	ANU	GCN	CCH	MCH	ECH	SLE	Parasite	GINF	Fusion	P450
C-1	3	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0
C-2	2	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0
C-3	1	3	0	0	0	0	0	0	2	0	1	0	0	0	0	0
C-4	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0
C-5	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C-7	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	1
C-8	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-3	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-6	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-7	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-8	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S1-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S1-2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1-3	0	2	0	0	0	0	2	0	0	0	0	0	0	0	0	1
S1-4	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S1-5	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S1-6	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S1-7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1-8	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S2-1	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S2-2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S2-3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2-4	2	1	0	0	0	0	1	0	2	0	0	0	0	0	0	0
S2-5	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2-7	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2-8	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Fish	Liver						Gill									P450
	GD	LIP	SCN	INF	MA	SC	ANU	GCN	CCH	MCH	ECH	SLE	Parasite	GINF	Fusion	
S3-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S3-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S3-3	3	2	0	0	0	0	1	2	0	3	1	0	0	0	3	0
S3-4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S3-5	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S3-6	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S3-7	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S3-8	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S4-1	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S4-2	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S4-3	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
S4-4	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S4-5	2	3	0	0	0	0	1	0	0	0	0	0	0	0	0	0
S4-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S4-7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S4-8	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S5-1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S5-2	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	2
S5-3	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S5-4	2	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0
S5-5	1	2	0	0	0	0	1	0	2	0	0	0	0	0	0	0
S5-6	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S5-7	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S5-8	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1

(GD) Glycogen Depletion, (LIP) Lipidosis, (SCN) Single Cell Necrosis, (INF) Liver Inflammation, (MA) Macrophage Aggregate, (SC) Sinusoidal Congestion, (ANU) Aneurysm, (GCN) Epithelial Cell Necrosis, (CCH) Ionocyte Hyperplasia, (Mch) Mucus Cell Hyperplasia, (ECH) Telangiectasia, (SLE) Secondary Lamella Edema, (GINF) Gill Inflammation. C – Control, HS – High Salinity Control, Site 1 – Montezuma Slough, Site 2 – Sacramento River at Isleton, Site 3 – Cache Slough, Site 4 – Sacramento River at Decker Island, Site 5 – Toe Drain

Table 10. Histopathology Scores for Individual Fish in Exposure 3 Initiated on November 10, 2017

	Liver						Gill									
Fish	GD	LIP	SCN	INF	MA	SC	ANU	GCN	CCH	MCH	ECH	SLE	Parasite	GINF	Fusion	P450
C-1	1	0	0	0	0	0	0	0	2	0	1	0	0	0	0	1
C-2	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1
C-3	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1
C-4	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	1
C-5	2	1	0	0	0	0	0	0	2	0	0	0	0	0	0	1
C-6	1	1	0	0	0	0	0	0	3	0	1	1	0	0	0	0
C-7	1	0	0	0	0	0	0	0	3	0	1	0	0	0	0	1
C-8	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
C-9	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	1
C-10	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
C-11	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
C-12	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
C-13	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
C-14	2	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
C-15	1	3	0	0	0	0	0	0	2	0	0	0	0	0	0	1
C-16	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0
C-17	1	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0
C-18	0	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0
C-19	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1
C-20	2	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0
HS-1	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
HS-2	2	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
HS-3	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
HS-4	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	2
HS-5	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
HS-6	0	3	0	0	0	0	1	0	2	0	0	0	0	0	0	0
HS-7	0	3	0	0	0	0	1	0	2	0	1	0	0	0	0	0
HS-8	0	2	0	0	0	0	0	0	2	0	0	1	0	0	0	0
HS-9	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
HS-10	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	1
HS-11	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2
HS-12	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1

	Liver						Gill									
Fish	**GD**	**LIP**	**SCN**	**INF**	**MA**	**SC**	**ANU**	**GCN**	**CCH**	**MCH**	**ECH**	**SLE**	**Parasite**	**GINF**	**Fusion**	**P450**
HS-13	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
HS-14	2	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0
HS-15	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
HS-16	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
HS-17	2	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1
S1-1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	3
S1-2	2	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S1-3	2	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S1-4	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	1
S1-5	2	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S1-6	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S1-7	3	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S1-8	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S1-9	0	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S1-10	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S1-11	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S1-12	1	2	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S1-13	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S1-14	1	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S1-15	1	2	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S1-16	2	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S1-17	2	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S1-18	2	3	0	0	0	0	1	0	1	0	0	0	0	0	0	0
S1-19	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S1-20	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S2-1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S2-2	2	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S2-3	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S2-4	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	2
S2-5	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S2-6	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	2
S2-7	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S2-8	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S2-9	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	3

	Liver						Gill									
Fish	GD	LIP	SCN	INF	MA	SC	ANU	GCN	CCH	MCH	ECH	SLE	Parasite	GINF	Fusion	P450
S2-10	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S2-11	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S2-12	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0
S2-13	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2
S2-14	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2
S2-15	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	2
S2-16	0	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S2-17	0	3	0	0	0	0	0	0	2	0	0	0	0	0	0	1
S2-18	1	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S2-19	0	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S2-20	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S3-1	1	3	0	0	0	0	0	0	2	3	1	0	0	0	0	0
S3-2	3	0	0	0	0	0	0	0	2	3	1	0	0	0	0	1
S3-3	0	1	0	0	0	0	0	0	2	3	2	0	0	0	0	0
S3-4	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2
S3-5	1	3	0	0	0	0	0	0	1	0	0	0	0	0	0	3
S3-6	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	2
S3-7	2	0	0	0	0	0	1	0	2	0	1	0	0	0	0	2
S3-8	1	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S3-9	1	3	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S3-10	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S3-11	1	3	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S3-12	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S3-13	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2
S3-14	1	2	0	0	0	0	3	0	1	0	0	0	0	0	0	1
S3-15	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S3-16	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S3-17	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S3-18	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S3-19	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S4-1	2	2	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S4-2	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S4-3	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S4-4	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

	Liver						Gill									
Fish	GD	LIP	SCN	INF	MA	SC	ANU	GCN	CCH	MCH	ECH	SLE	Parasite	GINF	Fusion	P450
S4-5	2	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S4-6	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S4-7	1	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S4-8	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S4-9	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	1
S4-10	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S4-11	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S4-12	0	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S4-13	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S4-14	0	3	0	0	0	0	0	0	2	0	1	0	0	0	0	0
S4-15	1	2	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S4-16	2	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S4-17	1	1	0	0	0	0	1	0	2	0	0	0	0	0	0	0
S4-18	2	2	0	0	0	0	2	0	1	0	0	0	0	0	0	0
S4-19	2	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S5-1	1	0	0	0	0	0	0	0	2	1	0	0	0	0	0	1
S5-2	1	2	0	0	0	0	1	0	2	1	0	0	0	0	0	1
S5-3	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0
S5-4	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S5-5	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1
S5-6	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S5-7	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
S5-8	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1
S5-9	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S5-10	1	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S5-11	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S5-12	1	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S5-13	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S5-14	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S5-15	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S5-16	1	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S6-1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S6-2	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1
S6-3	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

	Liver						Gill									
Fish	GD	LIP	SCN	INF	MA	SC	ANU	GCN	CCH	MCH	ECH	SLE	Parasite	GINF	Fusion	P450
S6-4	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2
S6-5	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S6-6	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S6-7	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S6-8	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S6-9	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S6-10	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
S6-11	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S6-12	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	2
S6-13	3	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1
S6-14	2	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S6-15	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S6-16	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S6-17	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1

(GD) Glycogen Depletion, (LIP) Lipidosis, (SCN) Single Cell Necrosis, (INF) Liver Inflammation, (MA) Macrophage Aggregate, (SC) Sinusoidal Congestion, (ANU) Aneurysm, (GCN) Epithelial Cell Necrosis, (CCH) Ionocyte Hyperplasia, (MCH) Mucus Cell Hyperplasia, (ECH) Telangiectasia, (SLE) Secondary Lamella Edema, (GINF) Gill Inflammation. C – Control, HS – High Salinity Control, Site 1 – Montezuma Slough, Site 2 – Toe Drain, Site 3 – Cache Slough, Site 4 – Sacramento River at Isleton, Site 5 – Sacramento River at Decker Island, Site 6 – Grizzly Bay

Table 11. Histopathology Scores for Individual Fish in Exposure 4 Initiated on November 24, 2017

	Liver						Gill									
Fish	**GD**	**LIP**	**SCN**	**INF**	**MA**	**SC**	**ANU**	**GCN**	**CCH**	**MCH**	**ECH**	**SLE**	**Parasite**	**GINF**	**Fusion**	**P450**
C-1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C-2	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C-3	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	1
C-4	3	1	0	0	0	0	0	0	2	0	0	0	0	0	0	1
C-5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C-6	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
C-7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
C-8	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2
HS-1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-3	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
HS-5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-6	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
HS-7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1-1	3	3	0	0	0	0	1	0	0	0	0	0	0	0	0	2
S1-2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
S1-3	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S1-4	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	2
S1-5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
S1-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
S1-7	0	3	0	0	0	0	1	0	0	0	0	0	0	0	0	2
S1-8	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2-1	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S2-2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
S2-3	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S2-4	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S2-5	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S2-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2-7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
S2-8	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	Liver						Gill									
Fish	GD	LIP	SCN	INF	MA	SC	ANU	GCN	CCH	MCH	ECH	SLE	Parasite	GINF	Fusion	P450
S3-1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S3-2	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
S3-3	1	1	0	0	0	3	0	0	0	0	0	0	0	0	0	1
S3-4	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S3-5	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S3-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S3-7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S3-8	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S4-1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S4-2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S4-3	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S4-4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S4-5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S4-6	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
S4-7	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	NA
S4-8	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	NA
S5-1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S5-2	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S5-3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S5-4	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
S5-5	0	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S5-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S5-7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S5-8	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S6-1	0	1	0	0	0	0	1	0	2	0	0	0	0	0	0	1
S6-2	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S6-3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S6-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S6-5	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
S6-6	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	Liver						Gill									
Fish	**GD**	**LIP**	**SCN**	**INF**	**MA**	**SC**	**ANU**	**GCN**	**CCH**	**MCH**	**ECH**	**SLE**	**Parasite**	**GINF**	**Fusion**	**P450**
S6-7	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S6-8	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(GD) Glycogen Depletion, (LIP) Lipidosis, (SCN) Single Cell Necrosis, (INF) Liver Inflammation, (MA) Macrophage Aggregate, (SC) Sinusoidal Congestion, (ANU) Aneurysm, (GCN) Epithelial Cell Necrosis, (CCH) IONOCYTE Hyperplasia, (MCH) Mucus Cell Hyperplasia, (ECH) Telangiectasia, (SLE) Secondary Lamella Edema, (GINF) Gill Inflammation. C- Control, HS – High Salinity Control, Site 1 – Montezuma Slough, Site 2 – Toe Drain, Site 3 – Cache Slough, Site 4 – Sacramento River at Isleton, Site 5 – Sacramento River at Decker Island, Site 6 – Grizzly Bay

Table 12. Histopathology Scores for Individual Fish in Exposure 5 Initiated on December 8, 2017

	Liver						Gill									
Fish	GD	LIP	SCN	INF	MA	SC	ANU	GCN	CCH	MCH	ECH	SLE	Parasite	GINF	Fusion	P450
C-1	1	2	0	0	0	0	0	0	2	0	0	0	0	0	0	1
C-2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C-4	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C-5	0	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0
C-6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C-7	2	1	0	0	0	0	0	0	2	1	0	0	0	0	0	0
C-8	3	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0
HS-1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-5	0	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0
HS-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-7	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HS-8	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1-1	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1-2	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1-3	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1-5	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1-6	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S1-7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1-8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2-1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2-2	3	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0
S2-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2-4	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2-5	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2-6	0	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S2-7	2	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0
S2-8	0	2	0	0	0	0	0	0	2	0	1	0	0	0	0	0

	Liver						Gill									
Fish	**GD**	**LIP**	**SCN**	**INF**	**MA**	**SC**	**ANU**	**GCN**	**CCH**	**MCH**	**ECH**	**SLE**	**Parasite**	**GINF**	**Fusion**	**P450**
S3-1	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0
S3-2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S3-3	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S3-4	3	0	0	3	0	0	0	0	2	0	0	0	0	0	0	0
S3-5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S3-6	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S3-7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S3-8	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0
S4-1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S4-2	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S4-3	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
S4-4	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S4-5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S4-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S4-7	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S4-8	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S5-1	0	3	0	0	0	2	0	0	2	0	0	0	0	0	0	0
S5-2	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
S5-3	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S5-4	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S5-5	0	1	0	0	0	0	1	0	3	0	2	0	0	0	0	0
S5-6	0	3	0	0	0	0	0	0	3	0	1	0	0	0	0	0
S5-7	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S5-8	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S6-1	1	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0
S6-2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S6-3	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S6-4	1	2	0	0	0	0	1	0	2	0	0	0	0	0	0	0
S6-5	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
S6-6	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	Liver						Gill									
Fish	**GD**	**LIP**	**SCN**	**INF**	**MA**	**SC**	**ANU**	**GCN**	**CCH**	**MCH**	**ECH**	**SLE**	**Parasite**	**GINF**	**Fusion**	**P450**
S6-7	0	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0
S6-8	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(GD) glycogen depletion, (LIP) lipidosis, (SCN) single cell necrosis, (INF) liver inflammation, (MA) macrophage aggregate, (SC) sinusoidal congestion, (ANU) aneurysm, (GCN) epithelial cell necrosis, (CCH) Ionocyte hyperplasia, (MCH) mucus cell hyperplasia, (ECH) telangiectasia, (SLE) secondary lamella edema, (GINF) gill inflammation. C- Control, HS – High Salinity Control, Site 1 – Montezuma Slough, Site 2 – Toe Drain, Site 3 – Cache Slough, Site 4 – Sacramento River at Isleton, Site 5 – Sacramento River at Decker Island, Site 6 – Grizzly Bay.

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Chapter 7: Tidal Wetlands Associated with Foraging Success of Delta Smelt

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Tidal wetlands associated with foraging success of Delta Smelt:

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Abstract

Delta Smelt (*Hypomesus transpacificus*), an annual fish endemic to the San Francisco Estuary (SFE), is imperiled. One recovery strategy is to restore tidal wetlands, thereby increasing productivity and prey abundance. However, the link between tidal wetlands and foraging of Delta Smelt is not yet established. Using GIS, we quantified the area of tidal wetlands (km^2) within a 2 km radius around sampling stations from which 1380 Delta Smelt were collected over four years (2011-15). We quantified stomach fullness, a metric of foraging success, for each fish and regressed it against tidal wetland area, turbidity, water temperature, and other factors known to influence foraging success of Delta Smelt. Stomach fullness increased with both increasing tidal wetland area and increasing water temperature and was reduced at turbidities >80 NTU. Model estimates show that stomach fullness increased 2-fold from the minimum (0 km^2) to the maximum (4.89 km^2) tidal wetland area. Of this increase, 60% was due to increased predation on larval fish, while 40% was due to increased predation on zooplankton. Delta Smelt collected from areas with the highest tidal wetland area were 6 times more likely to have a larval fish in their guts than those collected from areas with the lowest. Thus, tidal wetland appears to confer substantial benefits to the foraging success of Delta Smelt, mainly via increased predation on larval fish.

Introduction

An apparent pattern in aquatic ecosystems is that the combination of stable substrate and sunlight elevates productivity, and this pattern holds across gradients of both salinity and current. In freshwater lakes for example, the shallow areas close to shore tend to be more productive than the surface waters off-shore (i.e., littoral versus limnetic zones; Kalff 2002, Vadeboncoeur et al. 2011,Vander Zanden et al. 2011). Similarly, the sunlit bottoms of streams provide habitat for benthic algae and plants, while the primary producer community in the water column is relatively depauperate (Allan and Castillo 2007). Shallow, tidally influenced areas within estuaries, called tidal wetlands or tidal marshes, are extremely productive (Shaffer and Sullivan 1988, Beck et al. 2001). Tidal wetlands are therefore potentially important sources of productivity for nearby pelagic ecosystems, where they may provide both foraging habitat and subsidies of primary and secondary production to the surrounding channels (i.e., the Outwelling Hypothesis; Odum and de la Cruz 1967, Dame et al. 1986).

California's San Francisco Estuary (hereafter SFE) is formed by the confluence of the Sacramento and San Joaquin rivers and the San Francisco Bay, and is a relatively unproductive estuary (i.e., <100 g C m^{-2} yr^{-1}; Cloern et al. 2014; Wilkerson and Dugdale 2016). Although approximately 97% of the tidal wetland in the estuary was drained during the 19th and early 20th centuries (Whipple et al 2012), loss of tidal wetland habitat is not a proximate cause of its oligotrophy. The estuary was productive as recently as the early 1980s, well after the bulk of the tidal wetland was drained (Jassby and Powel 1994). Instead, several other factors are hypothesized to suppress productivity, including grazing by invasive clams (Alpine and Cloern 1992, Jassby et al. 2002), low residence times and loss of phytoplankton due to fresh water export from the south Sacramento-San Joaquin Delta (i.e., upstream and south of the SFE; Jassby and Powell 1994), and possibly changes in nitrogen concentration or form (e.g., Glibert et al. 2011, Parker et al. 2012, Wilkerson and Dugdale 2016), although nutrients are generally considered replete (Jassby et al. 2002, Cloern and Jassby 2012).

There is increasing evidence that the low productivity of the SFE is contributing to the declining abundance of several fish species, including the Delta Smelt (*Hypomesus transpacificus*; Feyrer et al. 2003, Sommer et al. 2007, Miller et al. 2012, Hammock et al. 2015). Delta Smelt is listed on the state and federal endangered species acts and is endemic to the SFE (Bennett 2005). It is pelagic, migratory, and annual, spawning mainly in freshwater in the spring (Bennett 2005, Sommer et al. 2011). One current recovery strategy is to restore tidal wetland habitat, in part to increase the food supply for Delta Smelt (USFWS 2008, California Natural Resource Agency 2017). Tidal wetlands support both detrital and autochthanous food web pathways via high rates of primary production (e.g., macrophytes, phytoplankton; Conway-Cranos et al. 2015). In consequence, tidal wetlands are rich in zooplankton, larval fish, and benthic invertebrates (Shaffer and Sullivan 1988, Beck et al. 2001, Visintainer et al. 2006, Howe et al. 2014), prey of Delta Smelt (Slater and Baxter 2014, Hammock et al. 2017). Many tidal wetland restoration projects are planned in the SFE, and several are underway or completed (USFWS 2008). A second recovery strategy is to release additional water from reservoirs, pushing the salinity field seaward and increasing the area of the low salinity zone (salinity ranging from 0.5-6)—a relatively productive salinity range occupied by Delta Smelt (Kimmerer et al. 1998, Feyrer et al. 2011, California Natural Resource Agency 2017). This strategy may also increase habitat quality because seaward areas are less channelized and have more remnants of tidal wetland (e.g.,

Grizzly Bay, Suisun Marsh; Matern et al. 2002, Feyrer et al. 2011, Hammock et al. 2015). Given the general importance of shallow water habitat to the productivity of aquatic ecosystems, these strategies appear sound. However, there is currently no direct evidence linking tidal wetland to increased foraging success of Delta Smelt (Hobbs et al. 2017).

Here, we examine whether the amount of surrounding tidal wetland correlates with foraging success of Delta Smelt, while accounting for covariables and examining underlying mechanisms. Delta Smelt are associated with higher turbidities in the wild (Feyrer et al. 2007, Grimaldo et al. 2009), potentially because it improves their foraging success while limiting predation (Feyrer et al. 2007, Bennett and Burau 2015, Hasenbein et al. 2016, Kimmerer and Slaughter 2016). In laboratory experiments, foraging success decreased linearly with increasing turbidity for juvenile Delta Smelt (Hasenbein et al. 2013), and at both high and low turbidities for larval Delta Smelt (optimal foraging success occurred between ~12 or 25 and 80 NTU, Hasenbein et al. 2016). Temperature is a well-known driver of foraging in other ectotherms (Brown et al. 2004), but is less well studied for Delta Smelt. We suggest that there are two mechanisms by which tidal wetland could directly improve the foraging success of Delta Smelt. Wetlands may export zooplankton into open water habitat (Odum and de la Cruz 1967, Dame et al. 1986) or Delta Smelt may forage within or along the edge of tidal wetland before returning to the open water where they were collected (Herbold et al. 2014). We consider the likelihood of these mechanisms for Delta Smelt, and whether area of adjacent tidal wetland—nursery habitat for many fishes (Baltz et al. 1993, Beck et al. 2001, Grimaldo et al. 2004, 2017)—increases the probability of observing larval fish in the guts of Delta Smelt.

Materials and Methods

Fish Collection, Dissection, and Diet

Juvenile, sub-adult, and adult Delta Smelt were sampled with trawls conducted by California Department of Fish and Wildlife (CDFW) Interagency Ecological Program surveys in bays and channels in the SFE (Bennett 2005, Merz et al. 2011, Hammock et al. 2015, Damon et al. 2016). Delta Smelt were flash-frozen in dewars of liquid nitrogen on CDFW boats and then measured for a variety of growth, health, reproduction, and condition endpoints at UC Davis (e.g., Hammock et al. 2015 and 2017, Teh et al. 2016, Kurobe et al. 2016). Juveniles were collected in summer during the Summer Townet survey (40 stations sampled twice per month, June-Aug), sub-adults in fall during the Fall Midwater Trawl survey (122 stations sampled monthly, Sept-Dec), and adults in winter and spring during the Spring Kodiak Trawl survey (40 stations sampled monthly, Jan-May). At each station, temperature (°C), turbidity (Nephelometric Turbidity Units; NTU), and specific conductance (µS cm^{-1}) were measured. This study focuses on Delta Smelt that were collected over a four-year period, from Aug 23, 2011 through Aug 12, 2015 from 55 stations (Figure 7-1, Table S1, n = 1380). During summer and fall surveys, a zooplankton tow (160 µm mesh size) accompanied the fish trawl at all 40 Summer Townet stations and 32 of the 122 Fall Midwater Trawl stations.

The flash-frozen Delta Smelt were kept immersed in liquid nitrogen until individuals were weighed, measured for fork length, and dissected as each fish thawed (5-10 min per fish; Teh et al. 2016). Following excision, the digestive tract was preserved in 70% ethanol, and sent to the CDFW Diet Study Lab for stomach fullness and content analysis. At CDFW, stomach contents

were weighed, identified, and enumerated, with lengths measured for larger prey items (i.e., amphipods, mysids and fish). The wet weight of prey was determined by either multiplying the count of each prey type by a wet weight estimate, or from calculations based on length-weight equations for larger zooplankton (Slater and Baxter 2014). Stomach fullness was calculated as the weight of the gut contents divided by the weight of the Delta Smelt, multiplied by 100. Detailed diet analysis methods are available in Slater and Baxter (2014), and dissection methods and flash-freezing justification are available in Teh et al. (2016).

Determining Tidal Wetland Area

To obtain a metric that reflects the foraging access of Delta Smelt to tidal wetland, we quantified the combined areas of tidal emergent wetlands, tidal flats, tidal pannes, and muted tidal emergent wetlands within circles around each of the 55 sampling stations with positive Delta Smelt catch using ArcGIS (ESRI, Redlands, CA; Figure 7-1, Table S1). We based the radius of these circles (i.e., ArcGIS buffers) on our estimate of the area within which Delta Smelt, which feed during daylight, potentially foraged before collection (Hammock et al. 2015, 2017). Potential foraging area was based on the mean time Delta Smelt had to forage during the day up until collection (4 h; n = 1380), movement speed of Delta Smelt (0.72 km hr^{-1} in slack water, Swanson et al. 1998), and trawl length, which is strongly influenced by tidal strength (median distance traveled over land by Fall Midwater Trawl and Summer Townet was 0.32 km). Therefore, an average Delta Smelt collected during a typical trawl could have foraged up to 3.3 km from the sampling station coordinates, although this requires that the boat trawled in the opposite direction that the fish swam at 0.72 km hr^{-1} from sunrise until collection, and that the fish was collected at the end of the trawl.

Given the multiple uncertainties in precise collection point and foraging range, and the circuitous routes taken by pelagic fish (e.g., Marsac and Cayré 1998, Dagorn et al. 2000), we quantified tidal wetland area within both a 1 and a 2 km radius around each station (i.e., buffers). While our estimate above of 3.3 km suggests that Delta Smelt collected from a station could conceivably have been foraging beyond this range, larger buffers would have overlapped one-another considerably (Fig. 1). Preliminary results were quite similar between the two radii, so we chose to use 2 km buffers. We note that obstructions and channel networks may prevent access to all areas within this buffer in some cases, but a standard circular buffer allowed us to apply the same metric across all stations, and nearby wetland area is a first approximation of wetland availability.

We quantified areas of tidal wetlands using data compiled from three sources: The Bay Area Aquatic Resources Inventory (BAARI, http://www.sfei.org/baari#sthash.palEXR8x.dpbs), a data set compiled by Wetlands and Water Resources, Inc. from three sources: the Delta Plan, the Cache Slough Conservation Assessment, and the Bay Delta Conservation Plan (http://baydeltaconservationplan.com/Home.aspx; Jeff Schlueter *personal communication*), and the CDFW VegCAMP survey of Suisun Marsh plants from 2009 (https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=48108&inline).

The data were updated where necessary to reflect known changes to wetland areas caused by levee breaches. We used the “Intersect” tool in ArcGIS to calculate the overlap between the tidal wetlands in the data sets listed above and the buffers around the sampling stations. We then

calculated the area of the intersection features in km^2. Wetland areas around each station ranged from 0.0 to 4.89 km^2.

To provide a variable with which to test whether simply open-water area, rather than wetland area, might be driving foraging success, we quantified the area of open water habitat around the stations using the same method and data sets described above for wetland area (i.e., all wetted area except tidal wetlands). The calculated tidal wetland area and open water area values were visually checked against the map to ensure the areas were reasonable (2 km buffers shown in Fig. 1).

Data Analysis

We used model comparison to identify predictors of stomach fullness for Delta Smelt (n = 1380). An arcsin square-root transformation was applied to the proportional stomach fullness data to improve normality (examined using quantile-quantile plots), and used as the response variable in thirteen Gaussian models (Table 7-1). The main goal was to determine whether tidal wetland area predicted stomach fullness, while accounting for potential confounding and masking variables (McElreath 2016). We built models of increasing complexity, beginning with an intercept model (Model 1, Table 7-1) and adding potential cofactors known or hypothesized to be important to Delta Smelt foraging success. Pairs of models were built that were identical except that they either included (Models 3, 5, 6, 7, 8, 10, and 13) or omitted (Models 1, 2, 4, 9, 11, and 12) tidal wetland area.

Following Hammock et al. (2017), time of collection (binned into 6:00-8:00, 8:00-10:00, 10:00-12:00, 12:00-14:00, and 14:00-16:00), season (summer, fall, and winter/spring), and salinity (<0.55 and >0.55) were included in Models 2-13 (Table 7-1). Salinity was included because there is twice the tidal wetland area in brackish habitat in the SFE (see Results), and stomach fullness of Delta Smelt is higher in brackish habitat most of the year (Hammock et al. 2017). Models 3-13 included an interaction of known importance between salinity (<0.55 and >0.55) and season (summer, fall, winter/spring; Hammock et al. 2017). Turbidity was included because it is known to affect Delta Smelt foraging success (Feyrer et al. 2007, Bennett and Burau 2015, Hasenbein et al. 2016, Kimmerer and Slaughter 2016, and Hasenbein et al. 2013), and could conceivably increase with tidal wetland area if export of particulate matter from the wetland affects turbidity in adjacent channels or bays (Shaffer and Sullivan 1988). To distinguish between reduced foraging at both high and low turbidity, reduced foraging at only high turbidity, or a continuous response to turbidity, turbidity was left as a continuous variable (Model 6), divided into three bins (<12, 12-80, and >80 NTU; Model 7), and divided into two bins (< and >80 NTU; Model 8). The model with two turbidity bins (Model 8) outperformed Models 6 and 7, as well as an identical model without a turbidity variable (Model 5), so turbidity (< and >80 NTU) was included in Models 9-13 (Table 7-1). Relatively shallow tidal wetland habitat may be more strongly influenced by air temperature than channels, potentially influencing water temperature and the metabolic demand of Delta Smelt at nearby sampling stations (Brown et al. 2004), so temperature was included in Models 10-13 (Table 7-1). To test whether Delta Smelt foraging success was driven by simply the availability of aquatic habitat, we replaced tidal wetland area with open water area in the top-ranked of models 1-11 (Model 12). Finally, water year type varied from wet (2011) to critically dry (2014, 2015) during the study period (http://cdec.water.ca.gov/reportapp/javareports?name=wsihist). Therefore, a variable for year-

class of Delta Smelt was included in the top-ranked of models 1-12 to account and test for potential differences in foraging success due to water year type.

All models were fit in R using the 'lm' command (R Core Team). The models were compared using Akaike Information Criterion corrected for small sample size (AIC_c; Burnham and Anderson 2002, McElreath 2016), and ANCOVAs were used to determine significance of variables. The 'visreg' R package was used to plot the partial residuals to show the influence of each variable on stomach fullness (Breheny and Burchett 2013). The top-ranked model was used to make predictions across the ranges of tidal wetland area, turbidity, and temperature to calculate the effect sizes of each variable on stomach fullness (additional details in Supplemental material, *Effect sizes*).

Some of the Delta Smelt had extremely high stomach fullness due to the presence of larval fish in their guts (Figs. 2 and 3). To determine to what extent our results were driven by these outliers, we excluded all 69 Delta Smelt with fish in their guts and reanalyzed the dataset with models 1-11 described above (n = 1311). This also allowed us to determine the extent to which the benefit of tidal wetland area was due to predation on larval fish versus zooplankton.

If Delta Smelt feed directly on zooplankton in the pelagic zone, and do not forage within or along tidal wetland, replacing tidal wetland area with zooplankton density should improve the foraging models. Zooplankton density is a direct measure of food availability where the fish was collected, whereas tidal wetland area is a proxy for food availability (either via export or direct foraging). Thus, if tidal wetland area is a better predictor of stomach fullness than zooplankton density, tidal wetland likely confers foraging benefits beyond simple zooplankton export, perhaps because Delta Smelt utilize tidal wetland—or the edges of tidal wetland—for foraging (Herbold et al. 2014). We note that wetlands can also export nutrients, detritus, and phytoplankton to stimulate the open water food web (e.g., Lehman et al. 2010), but we do not address this less direct mechanism here.

To differentiate between the two mechanisms (tidal wetland export versus foraging within/along tidal wetland), we excluded all Delta Smelt without an associated zooplankton sample (all Spring Kodiak Trawl fish and Fall Midwater Trawl fish from stations where zooplankton were not sampled), and 5 Fall Midwater Trawl Delta Smelt that had larval fish in their guts, leaving 434 Delta Smelt. To obtain an estimate of local food availability using the associated zooplankton samples we summed across all species of Cladocera and Copepoda, two major prey items of Delta Smelt (Nobriga 2002, Slater and Baxter 2014, Hammock et al. 2017, Table S2). While this is a very rough metric of food availability (not all zooplankton are of equivalent food quality for Delta Smelt; Nobriga 2002), the variable nevertheless correlated positively with stomach fullness (Hammock et al. 2017). As with the previous models, the response variable was 'proportion stomach fullness' that was arcsin square-root transformed. The models for this comparison included an intercept model (Model 1), the top-ranked model from the previous analysis (~tidal wetland area + time of day + season + salinity + salinity × season + turbidity + temperature; Model 2), the same model except that tidal wetland area was replaced with zooplankton density (Model 3), a model that included both zooplankton density and tidal wetland area (Model 4), and a model that included a zooplankton density by temperature interaction (Model 5; Table 7-2). This final model was included because metabolic theory predicts that temperature should increase feeding rate and therefore stomach fullness, if food is available and the critical thermal

optima is not exceeded (Brown et al. 2004), but decrease stomach fullness at low food availability due to increased metabolic demand (Vinagre et al. 2007).

Next, we used model comparison to determine whether the incidence of larval fish in the guts of Delta Smelt increases with tidal wetland area, since tidal wetland acts as nursery habitat for fishes (Baltz et al. 1993, Beck et al. 2001, Grimaldo et al. 2004, 2017). Because the response variable was a proportion (i.e., the proportion of Delta Smelt at each station with fish in their guts) with an uneven distribution of fish among stations (Table S1), we fit beta-binomial models to the data (McElreath 2016). Two models were built: an intercept model and a model with tidal wetland area as a linear predictor. The models were fit using mle2 from the bbmle package in R (Bolker 2010). More complex models were not included because the dataset was far smaller than above (n = 69) and mostly included Delta Smelt collected during winter/spring (92.8%).

Finally, stations were divided between 'fresh' and 'brackish' based on weighted average salinity (as above, 0.55 was the boundary). Mean proportion of tidal wetland area within the 2 km buffer was calculated for both categories and compared with a generalized linear model with a beta distribution, since the data were non-normal (Cribari-Neto et al. 2009).

Results

The 1380 Delta Smelt ranged in fork length from 23 to 90 mm (mean: 58.7 mm) and in body weight from 0.09 to 6.69 g (mean: 1.70 g). Tidal wetland area, higher temperatures, and turbidities below 80 NTU were strongly associated with increased stomach fullness of Delta Smelt (Table 7-1, Figure 7-2 and 7-3). The top-ranked model included a parameter for tidal wetland area (ANCOVA, $F_{1,1370} = 57.75$, $P < 0.0001$, Fig. 2), temperature (ANCOVA, $F_{1, 1370} = 33.68$, $P < 0.0001$; Fig. 3A), and turbidity (<80 and >80 NTU; ANCOVA, $F_{1, 1370} = 43.55$, $P < 0.0001$; Fig. 3B). It also included other variables that previous work has shown to be important (Hammock et al. 2017). Time of day was significant (ANCOVA, $F_{1,1370} = 92.90$, $P < 0.0001$), with stomach fullness increasing during the day (Hammock et al. 2017). Salinity (fresh vs brackish; ANCOVA, $F_{1,1370} = 13.42$, $P = 0.0002$), season (summer, fall, winter/spring; ANCOVA, $F_{2,1370} = 1.52$, $P = 0.220$), and a salinity by season (summer, fall, winter/spring) interaction (ANCOVA, $F_{1, 1370} = 31.11$, $P < 0.0001$) were included in the top-ranked model (Hammock et al. 2017). During summer, stomach fullness was higher in freshwater, but stomach fullness was higher in brackish habitat during fall and spring/winter (further explanation of this interaction in Hammock et al. 2017). Parameter estimates and their 95% confidence intervals are in Table S3. In all cases, including tidal wetland area as a predictor substantially improved the AIC_c score of the model (Table 7-1). That is, Model 3 outperformed Model 2, Model 5 outperformed Model 4, Model 8 outperformed Model 9, and Model 10 outperformed Model 11, all by substantial margins (Table 7-1). In addition, Model 10 outperformed Model 12, indicating that stomach fullness increases with tidal wetland area, not simply availability of open water habitat. Model 13, which included a variable for year-class, received some AIC_c weight (0.26, Table 7-1). However, year-class was not a significant predictor of stomach fullness (ANCOVA, $F_{4, 1366} = 0.7313$, $P = 0.570$).

Based on predictions from the top-ranked model (Table 7-1), increasing tidal wetland area from the minimum of 0.0 km^2 to the maximum of 4.89 km^2 increased stomach fullness by 2.0-fold,

from 0.28 to 0.55%. For turbidity, predicted stomach fullness was 0.32% at <80 NTU and 0.09% at >80 NTU, a 3.7-fold difference. Increasing temperature from 7.4 to 25.5°C increased predicted stomach fullness 2.7-fold, from 0.21 to 0.56%.

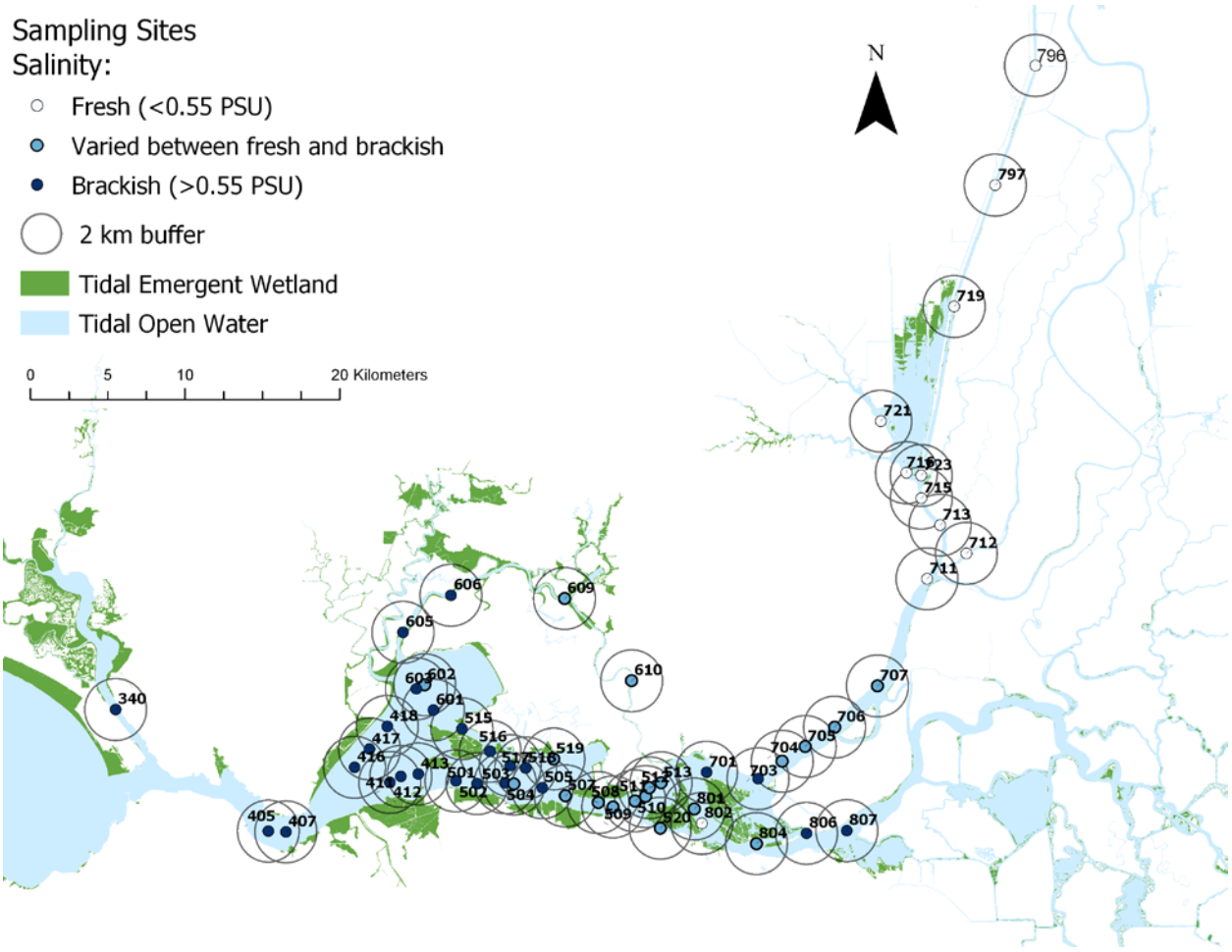
When the 69 Delta Smelt with fish in their guts were excluded from the analysis (n = 1311), the top-ranked model remained the same, with a ΔAIC_c value 4.3 units better than the next best model and an AIC_c weight proportion of 0.90 (Table S4). However, while tidal wetland area was still included in the best model, and it was still significant (ANCOVA, $F_{1, 1301} = 16.65$, P < 0.0001), its influence on stomach fullness was diminished. Predicted stomach fullness was 0.28% at zero tidal wetland area and 0.40% at 4.89 km^2 tidal wetland area, a 1.4-fold difference. Thus, of the approximate doubling of stomach fullness as tidal wetland area increased from the minimum to the maximum for the top-ranked model fit to the full dataset, 60% can be attributed to increased predation rates on larval fish, and 40% to increased predation rates on zooplankton. Parameter estimates and 95% confidence intervals are in Table S5.

Mean stomach fullness for the 1311 Delta Smelt without fish in their stomachs was 0.39%, while for the 69 Delta Smelt with fish in their stomachs it was 1.41%, a 3.7-fold difference. Of the organisms found in Delta Smelt guts, 78.8% were invertebrates, 19.2% were larval fish, and 1.9% were unidentified by weight (Table S2). Of the 464 larval fish found in Delta Smelt stomachs, 52% were Pacific Herring, 8% were Prickly Sculpin, 1% were Longfin Smelt, 1% were *Tridentiger* spp., and 38% were unidentified.

Comparing models fitted to the Delta Smelt dataset that included zooplankton tows (n = 434), tidal wetland area was a better predictor of foraging success than zooplankton abundance (Model 2 vs 3; Table 7-2). Overall, the models that included tidal wetland area received an AIC_c weight proportion of 0.998 (Table 7-2). The ANCOVA results for the top-ranked model were: tidal wetland area ($F_{1, 426} = 7.14$, P = 0.008), time of day ($F_{1, 426} = 22.81$, P < 0.0001), season ($F_{1, 426} = 0.51$, P = 0.477), salinity ($F_{1, 426} = 2.41$, P = 0.122), turbidity ($F_{1, 426} = 16.03$, P < 0.0001), and the season by salinity interaction ($F_{1, 426} = 36.07$, P < 0.0001). Parameter estimates and 95% confidence intervals are in Table S6.

The proportion of Delta Smelt with fish in their guts increased substantially with increasing tidal wetland area. The top-ranked beta-binomial model included tidal wetland area, and received an AIC_c weight proportion of 0.925 (Table 7-3). The tidal wetland area parameter was positive and significant (parameter = 0.42, 95% CI: 0.17, 0.67, P = 0.0012). Based on model estimates, increasing tidal wetland area from the minimum to the maximum increased the probability of observing fish in the gut of a Delta Smelt by 6.4-fold, from 3.3 to 21.2%.

Tidal wetland area was lower in fresh water than in brackish water (beta regression of proportion of wetland area with the 2 km buffer, $z = -6.61$, P <0.001). Mean tidal wetland area in fresh water was 0.69 km^2 and in brackish water it was 1.44 km^2, a 2.1-fold difference.



Notes: The circles show the 2 km ArcGIS buffers used to quantify tidal wetland area (km^2) around the 55 stations in our analysis. Salinity bins are based on salinities during which the 1380 Delta Smelt were collected. Sites that were <0.55 when every Delta Smelt was collected are designated 'fresh', brackish is analogous but >0.55, and 'varied' means that the site varied between fresh and brackish depending on time of year and amount of freshwater flow.

Figure 7-1. Tidal Wetland Areas and CDFW Sampling Stations in the San Francisco Estuary Positive for Delta Smelt Catch (Aug 2011- Aug 2015)

Table 7-1. Comparison of Stomach Fullness Models Fit to the Full Dataset (n = 1380)

Model #	Model	ΔAIC_c	df	AIC_c wt
10	~Tw + Hr + Seas + Sal + Sal × Seas + Turb2 + Temp	0	11	0.74
13	~Tw + Hr + Seas + Sal + Sal × Seas + Turb2 + Temp + Yc	2.1	15	0.26
12	~Ow + Hr + Seas + Sal + Sal × Seas + Turb2 + Temp	21.4	11	<0.001
11	~Hr + Seas + Sal + Sal × Seas + Turb2 + Temp	23.9	10	<0.001
8	~Tw + Hr + Seas + Sal + Sal × Seas + Turb2	27.5	10	<0.001
7	~Tw + Hr + Seas + Sal + Sal × Seas + Turb3	29.5	11	<0.001
6	~Tw + Hr + Seas + Sal + Sal × Seas + Turb	43.6	10	<0.001
9	~Hr + Seas + Sal + Sal × Seas + Turb2	64.1	9	<0.001
5	~Tw + Hr + Seas + Sal + Sal × Seas	70.4	9	<0.001
4	~Hr + Seas + Sal + Sal × Seas	115.8	8	<0.001
3	~Tw + Hr + Seas + Sal	125.7	7	<0.001

Model #	Model	ΔAIC_c	df	AIC_c wt
2	~Hr + Seas + Sal	165.2	6	<0.001
1	~Intercept only	260.5	2	<0.001

Notes: 'Tw' is Tidal Wetland Area (km^2), 'Hr' is Time of Day Divided into Two Hour Blocks (treated as a continuous variable), 'Seas' is Season (summer, fall, winter/spring), 'Sal' is Salinity (fresh [<0.55] or Brackish [>0.55]), 'Yc' is Delta Smelt Year-Class, 'Turb2' is Turbidity Divided into Two Bins (<80 and >80 NTU), 'Turb3' is Turbidity Divided Among Three Bins (<12, >12 and <80, and >80 NTU), 'Turb' is Turbidity as a Continuous Variable, 'Temp' is Water Temperature in °C, and 'Ow' is Open Water Area (km^2)

ΔAIC_c difference between model of interest and top-ranked model in Akaike Information Criterion Units corrected for small sample size, *df* degrees of freedom, *AIC_c wt* Akaike weight

Table 7-2. Comparison of Stomach Fullness Models Fit to Dataset with Associated Zooplankton Abundance Data (n = 434)

Model #	Model	ΔAICc	df	AICc wt
2	~Tw + Hr + Seas + Sal + Sal × Seas + Turb2 + Temp	0.0	9	0.6111
4	~Tw + Hr + Seas + Sal + Sal × Seas + Turb2 + Temp + Z	2.0	10	0.2296
5	~Tw + Hr + Seas + Sal + Sal × Seas + Turb2 + Temp + Z + Temp × Z	2.7	11	0.1572
3	~Hr + Seas + Sal + Sal x Seas + Turb2 + Temp + Z	11.4	9	0.0021
1	~Intercept only	83.4	2	<0.001

Note: 'Tw' is Tidal Wetland Area (km^2), 'Hr' is Time of Day Divided into Two Hour Blocks (a continuous variable), 'Seas' is Season (summer, fall, winter/spring), 'Sal' is Salinity (fresh [<0.55] or brackish [>0.55]), 'Turb2' is Turbidity Divided into Two Bins (<80 and >80 NTU), 'Temp' is Water Temperature in °C, and 'Z' is Zooplankton Abundance

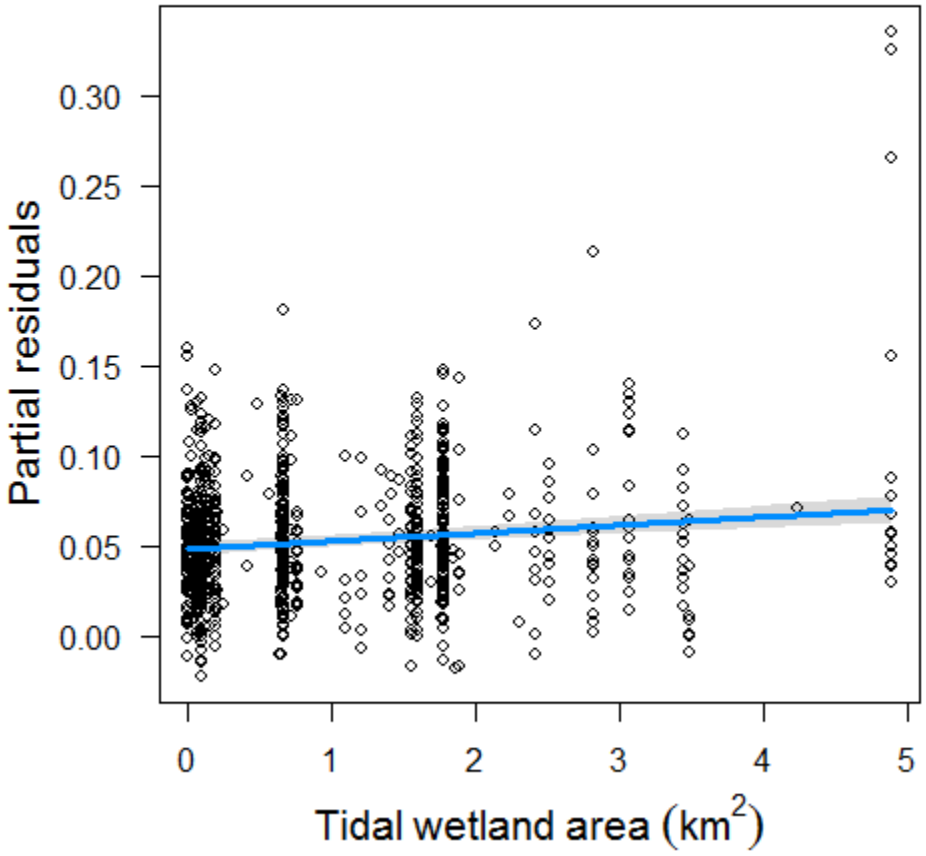
ΔAIC_c difference between model of interest and top-ranked model in Akaike Information Criterion Units corrected for small sample size, *df* degrees of freedom, *AIC_c wt* Akaike weight

Table 7-3. Comparison of Beta-Binomial Models in Which the Proportion of Delta Smelt at Each Station with Fish in their Guts was the Response Variable (n = 1380)

Model #	Model	ΔAIC_c	df	AIC_c wt
2	~Tw	0.0	3	0.925
1	~Intercept only	5.0	2	0.075

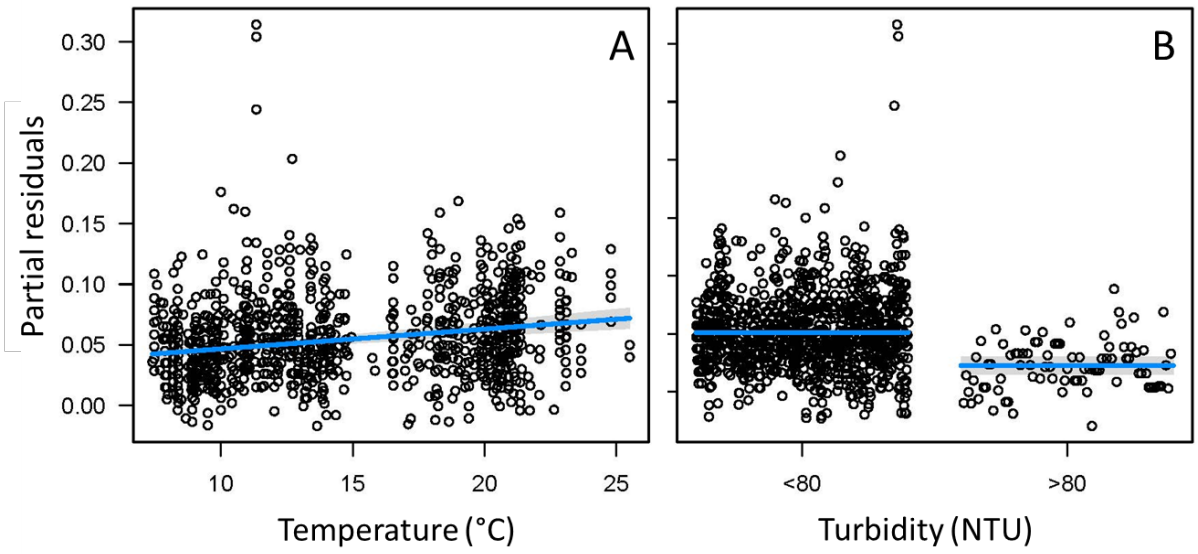
Notes: 'Tw' is Tidal Wetland Area (km^2).

ΔAIC_c difference between model of interest and top-ranked model in Akaike Information Criterion Units corrected for small sample size, *df* degrees of freedom, *AIC_c wt* Akaike weight



Note: The y-Axis is on the 'Arcsin (square-root)' of 'proportion stomach fullness' scale. The grey shading represents the 95% confidence Interval.

Figure 7-2. Partial Residuals of the Top-Ranked Model in Table 7-1, Plotted Against Tidal Wetland Area (km^2)



Note: The y-axis is on the 'arcsin(square-root)' of 'proportion stomach fullness' scale, and is identical in panels A and B. The grey shading represents the 95% confidence interval.

Figure 7-3. Partial Residuals of the Top-Ranked Model in Table 7-1, Plotted Against Water Temperature (°C; panel A) and Turbidity (NTU; panel B)

Discussion

Over a four-year period, wild Delta Smelt collected from stations with proximity to greater tidal wetland area exhibited increased stomach fullness. As with any observational study, this result could be misleading if a co-variable is in fact responsible for the relationship. However, we somewhat mitigated this possibility by including potential confounders in the models (e.g., salinity, turbidity, and temperature; McElreath 2016), and tidal wetland area remained an important predictor of stomach fullness. Moreover, tidal wetlands are productive habitat (Shaffer and Sullivan 1988, Beck et al. 2001), and are well known to act as nurseries for larval fish (e.g., Baltz et al. 1993, Grimaldo et al. 2004), to which 60% of the influence of tidal wetland area on stomach fullness was attributed. Therefore, we suggest that tidal wetlands, or more specifically the productivity of tidal wetlands, improved the foraging success of Delta Smelt collected nearby through increased access to prey.

The influence of tidal wetland area on Delta Smelt foraging success is consistent with previous work on other fishes. Allen et al. (1994) found that Mummichogs exiting tidal marsh had fuller stomachs than when they entered. Gulf Killifish had greater foraging success on the surface of brackish marsh than in subtidal areas (Rozas and LaSalle 1990). In southern California, California Killifish collected on tidal marsh had 6-times more food in their guts than individuals restricted to tidal creeks within the marsh (West and Zedler 2000). Our study is a less direct test of the influence of tidal wetland on fish foraging because it did not compare Delta Smelt collected from inside and outside of tidal wetland. Instead, we used GIS to quantify tidal wetland

area in the vicinity of Delta Smelt sampling stations, and regressed that area against stomach fullness. This methodology assumes that Delta Smelt have equal access to all tidal wetland within the buffers and no access to tidal wetland outside the buffers, and these assumptions are unlikely to be fully met. Routes to areas of tidal wetland may be circuitous or impossible in some cases, and riverine or tidal currents could make certain areas of tidal wetland relatively inaccessible. Despite these assumptions, tidal wetland area was an important predictor of Delta Smelt foraging success.

We compared two possible mechanisms for how tidal wetland area improved the foraging success of Delta Smelt. One possibility is that tidal wetlands export phytoplankton, detritus, and zooplankton to bays and channels, increasing prey availability directly and indirectly. This potential mechanism was first proposed by Odum and de la Cruz (1967) and is known as the Outwelling Hypothesis (Dame et al. 1986). Two local studies indicate that tidal wetlands are net exporters of organic material, though it is highly variable temporally (Lehman et al. 2010, Lucas et al. 2006). However, a third SFE study found a tidal marsh to be a sink for the mysid *Neomysis kadiakensis* (Dean et al. 2005), so support in the SFE for the Outwelling Hypothesis is mixed. In any case, Herbold et al. (2014) suggests that given the relatively small volume of water in tidal wetlands compared to channels, the flux of phytoplankton and zooplankton to the pelagic foodweb is likely inconsequential. Herbold et al. (2014) argues instead that tidal wetland improves prey availability for fish by providing rich foraging habitat within or along the edges of wetlands. Indeed, the edges of tidal wetland habitat, either around the outside of wetlands or along tidal creeks within wetlands, are considered to be particularly important to fish foraging success (Gewant and Bollens 2012). Rich foraging is perhaps why Baltz et al. (1993) found that larvae and juvenile fishes in estuarine wetlands in Louisiana were concentrated within 0 - 1.25 m of the edge of wetlands. In the SFE, Grimaldo et al. (2004) observed densities of larval fish that were over three times higher in marsh edge habitat than in adjacent river channels. Given that the ‘tidal wetland area’ model outperformed the ‘zooplankton density’ model, our results suggest that there is a foraging benefit provided by tidal wetland that is unrelated to purely zooplankton export (Model 2 versus 3, Table 7-2). The most abundant larval fish taxon in Delta Smelt guts was Pacific Herring, which occurred in high densities in brackish tidal marsh during recent SFE surveys (L. Grimaldo *personal communication*). Thus, our results are more consistent with the hypothesis that tidal wetlands provide foraging habitat than substantial export of prey, although both mechanisms may occur. However, this result should be considered preliminary, given that the zooplankton variable is likely a crude measure of Delta Smelt food availability.

This potential mechanism—that Delta Smelt forage within or along tidal wetlands—could explain why Delta Smelt appear to be far more efficient predators in brackish than in freshwater habitat (Fig. 3D, Hammock et al. 2017). If Delta Smelt forage along the periphery of tidal wetlands, zooplankton tows in channels near tidal wetlands may underestimate prey availability. Because tidal wetland is more prevalent in brackish habitat in the SFE (see Results), relative prey availability may be underestimated by zooplankton tows compared to those in fresh water. Delta Smelt may also be able to take advantage of other resources in wetlands that are not available in channels, such as increased access to epibenthic and epiphytic chironomids and amphipods (Whitley and Bollens 2014, Howe et al. 2014), although demersal prey are of relatively limited use compared to pelagic prey like copepods (Table S2). While our study provides only indirect evidence that Delta Smelt use tidal wetlands as foraging habitat, other studies provide stronger evidence that Delta Smelt use relatively shallow habitat. Sommer et al. (2004), Sommer and

Mejia (2013), and Mahardja et al. (2015) show that Delta Smelt inhabit tidal sloughs in the Yolo Bypass floodplain, and Aasen (1999) found that densities of Delta Smelt were higher in shallow water habitat in Sherman Lake and Honker Bay than in channels. But whatever the mechanism, our study indicates that tidal wetlands improve the foraging success of Delta Smelt.

The relationship between stomach fullness and temperature is consistent with metabolic theory and physiological work on cultured Delta Smelt. Depending on the temperature of acclimation, the critical thermal maximum (CT_{max}) of Delta Smelt is 27-29 °C (Komoroske et al. 2014). In our study, stomach fullness increased linearly with increasing temperature, up to a maximum of 25.5 °C (Fig. 3A). Thus, Delta Smelt behaved as expected, continuing to increase food consumption as temperature approached their CT_{max} (e.g., Fonds et al. 1992). Because stomach fullness increased with temperature, the increase in feeding rate must have outpaced the increase in gastric evacuation rate, which also increases with temperature in fishes (e.g., Persson 1981, Booth 1990, Handeland et al. 2008). However, as temperature increases toward the CT_{max} of ectotherms, metabolic demand increases (Brown et al. 2004). With energy shifting from growth to metabolism, it is possible for fish to eat more at higher temperature but grow more slowly (e.g., Handeland et al. 2008). Delta Smelt in the 2013/14 year-class, collected during an extreme drought in CA, had significantly higher stomach fullness than the previous two year classes, both of which were substantially cooler (Hammock et al. 2017). However, the elevated stomach fullness in 2013/14 did not lead to improved fitness, as sexually mature females from 2013/14 were smaller and less fecund than those of the previous two year-classes (B. Hammock, *unpublished results*). Thus, the positive influence of temperature on stomach fullness does not indicate that high temperature improves conditions for Delta Smelt (Fig. 3A).

The influence of turbidity on stomach fullness was also consistent with previous research. Turbidity has been depressed in the SFE for decades (Feyrer et al. 2007), which may be problematic for Delta Smelt because its occurrence is associated with turbid water (Feyrer et al. 2007, Grimaldo et al. 2009). For Delta Smelt, clear water is thought to increase predation pressure, decrease prey availability as zooplankton exhibit predator avoidance behaviors, and decrease foraging success by, counterintuitively, reducing visual acuity (Feyrer et al. 2007, Bennett and Burau 2015, Hasenbein et al. 2016, Kimmerer and Slaughter 2016). For example, to promote feeding of larval Delta Smelt in aquaculture, an alga is added to rearing systems to bring turbidity up to 9 NTU (Lindberg et al. 2013). In concurrence, Hasenbein et al. (2016) found a parabolic response of prey consumption of larval Delta Smelt to turbidity, with optimal foraging success between ~25 and 80 NTU and relatively low cortisol levels (cortisol is a stress hormone; Hasenbein et al. 2016). While we also observed reduced foraging success above 80 NTU, we found no reduction in stomach fullness below 12 NTU (n = 152). In fact, mean stomach fullness was higher below 12 NTU than it was from 12-80 NTU (0.51 and 0.44%, respectively), although not significantly so (Table 7-1). However, the fish in our study were more mature than those used by Hasenbein et al. (2016), and it is larval Delta Smelt that require turbid water to feed successfully (Lindberg et al. 2013). Juvenile Delta Smelt show a more linear decrease in foraging efficiency with turbidity (Hasenbein et al. 2013), similar to our results.

In summary, stomach fullness of Delta Smelt increased with increasing tidal wetland area, increasing temperature, and at turbidities below 80 NTU. We detected no difference in foraging success between moderate and low turbidity in juvenile through adult Delta Smelt. Our results appear inconsistent with the Outwelling Hypothesis, because tidal wetland area was a better

predictor of foraging success than zooplankton density (Table 7-2). Our results are more consistent with the hypothesis that Delta Smelt forage within or along tidal wetlands, although detections of Delta Smelt in tidal wetlands and their peripheries is needed to support this conclusion. Overall, our results support two recommendations from the Delta Smelt Resiliency Strategy meant to benefit Delta Smelt: (1) restoration of tidal wetlands, and (2) outflow actions that maximize the amount of tidal wetland area within the low salinity zone (California Natural Resource Agency 2017, USFWS 2008).

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Chapter 8: Confirming Species Identification of 2017 Wild Delta Smelt and the Feasibility of Formalin-Fixed Delta Smelt for Genetic Identification

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Abstract

Monitoring abundance of Delta Smelt (*Hypomesus transpacificus*) is critical for understanding the population dynamics of the species. Federal and state agencies have been morphologically identifying Delta Smelt sampled during their routine surveys and reporting their abundances. While morphological identification is practical, the method has limitations. For example, the method requires intact specimens, and distinguishing Wakasagi and Delta Smelt, especially at early life stages, can be challenging. To ensure accurate identifications, researchers can use genetic test(s) for identifying damaged specimens and for confirming morphological identifications. In Task 1, we performed species identification based on sequences of the cytochrome b gene for 151 fish that were morphologically identified as Delta Smelt in the field (2017 EDSM, SKT, and STN surveys). Our genetic test results confirmed that all 151 fish were Delta Smelt. In addition, we explored the feasibility of using genetic sequencing to identify Delta Smelt fixed in formalin. Formalin is commonly used for preserving biological samples for morpho-histological analyses but is not ideal for genetic tests because long term storage in formalin can cause DNA fragmentation. In Task 2, we performed species identification by sequencing the cytochrome b gene for Delta Smelt preserved in 10% phosphate-buffered formalin for up to 11 years to test the feasibility of the method. We have successfully amplified a portion of the cytochrome b gene and obtained DNA sequences from these archived samples. The process requires extra steps to remove formalin, however it is feasible to extract genomic DNA from formalin-fixed fish tissue for genetic tests.

Task 1. Confirming Species Identification of 2017 Wild Delta Smelt

Introduction

Monitoring abundance of Delta Smelt (*Hypomesus transpacificus*) is critical for understanding population dynamics of the species in the Bay-Delta. The California Department of Fish and Wildlife (CDFW) and United States Fish and Wildlife Service (USFWS) have been morphologically identifying Delta Smelt and reporting their abundances in their routine surveys (CDFW 2019). Morphological identification is practical; however, the method requires skilled taxonomists and intact specimens. If key morphological features are lost or identifications are done in the field (i.e., without a microscope), taxonomic identification can be difficult. In addition, distinguishing Wakasagi and Delta Smelt, especially at early life stages, can be challenging since both Wakasagi and Delta Smelt belong to the same genus, *Hypomesus*, and the fish species share morphological features (Benjamin et al. 2018).

We received sub-adult and adult stages of fish collected by the CDFW and USFWS for confirming species identification (n=151). Those fish were morphologically identified as Delta Smelt and flash-frozen in the field and brought to the Aquatic Health Program, University of California, Davis. Given the extremely low abundance of Delta Smelt, misidentification of even a few fish could result in large differences in the abundance index, which is routinely reported by the CDFW (CDFW 2019). In addition, we want to be certain that health and condition data reported as from Delta Smelt actually come from that species. Therefore, in Task 1 we sequenced the cytochrome b gene in the 151 fish identified as Delta Smelt our lab received in 2017 from the field (including both USFWS and CDFW surveys).

Materials and Methods

Genomic DNA extraction, PCR, and sequencing for wild fish captured by CDFW under the Enhanced Delta Smelt Program-2017 and the SKT and STN -2017

Wild caught fish morphologically identified as Delta Smelt by USFWS and CDFW crews were flash-frozen in liquid nitrogen immediately after capture and brought back to the Aquatic Health Program, University of California, Davis (Teh et al. 2016). A total of 151 fish collected from the 2017 surveys (EDSM, SKT, and STN) were subjected to species identification by genetic test (Table 8-1). While dissecting fish, a small portion of pectoral fin was clipped from the fish and preserved in ethanol (100%). Genomic DNA was extracted using QIAGEN kits (QIAamp DNA extraction kit). A set of primers, 064_Hypomesus_F1 and 066_Hypomesus_R1, targeting a conserved region of Delta Smelt and Wakasagi cytochrome b gene was used (Table 8-2, Supplemental Data 1). Conventional PCR was carried out using the reagents and cycling conditions shown in Tables 8-3 and 8-4. The amplified DNA bands at the expected size (approximately 270 bp) were submitted to the UC Davis Sequencing Facility for direct sequencing reactions using the same custom primers described above.

Table 8-1. List of Wild Fish Subjected to Species Identification by Genetic Test (sequencing cytochrome b gene)

Survey	Fish IDs
EDSM	E1 through 60, E151 through 177
SKT	A1 through 15, A21 through 39
STN	7339, 7341 through 7361, 7409, 7610 through 7615, 7750

Note: The fish were collected by the 2017 EDSM, SKT, and STN surveys and flash-frozen in liquid nitrogen.

Table 8-2. PCR Primers Used for Amplifying the Cytochrome b Gene

Primers	Primer sequences (5' to 3')
064_Hypomesus_F1	ACTACAAGAACCCTAATGG
066_Hypomesus_R1	GATGCTCCGTTAGCGTGCATG

Table 8-3. PCR Cocktail for Amplifying the Cytochrome b Gene from Flash-Frozen Samples

Reagents	Volume (µL per reaction)
10X Buffer*	5.0
dNTP (10 mM)	1.0
MgCl2 (50 mM)	1.5
H2O	33.3
Taq (Platium Taq Polymerase)	0.2
Primer (Fw, 10 µM)	2.0
Primer (Rv, 10 µM)	2.0
Template DNA	5.0
Total	50 µL per reaction

* 10X Buffer is supplied in the Platinum Taq Polymerase Kit (ThermoFisher Sci, Cat. Number: 10966034)

Table 8-4. PCR Cycling Conditions for Amplifying the Cytochrome b Gene from Flash-Frozen Field Fish

Temperatures	Incubation Times	Steps	
95° C	5 min.	Initial denaturing	
95° C	30 sec.	Denaturing	30 cycles
55° C	30 sec.	Annealing	
72° C	1 min.	Extension	
72° C	5 min.	Final extension	

Results and Discussion

The identification of all the fish collected by 2017 EDSM, SKT, and STN surveys were confirmed to be Delta Smelt by sequencing of the cytochrome b gene. A majority of fish (115 of the 151) had identical sequences to the reference cyto-b gene sequence of *Hypomesus transpacificus* in the NCBI database. The rest of the fish (36 of the 151) had 99% similarity to the *H. transpacificus* cyto-b gene. Thus, all of the 151 fish were correctly identified in the field as Delta Smelt.

Task 2. Feasibility of Formalin Fixed Delta Smelt for Genetic Tests

Introduction

Preserving fish in fields can be a challenging task when researchers want to maximize outcomes from same individual fish using various endpoints such as morphometric analyses, otolith measurements, genetic tests, and histology. There are a couple of critical criteria for field sampling; firstly, preserving fish in fields has to be simple and fast enough to process fish samples immediately after collection to avoid compromising quality of samples. Secondly, sampling method shouldn't rely on use of sophisticated equipment. Equipment is very limited in fields, especially on research vessels. A simple task such as measuring accurate body weight can be challenging as analytical scales require flat and stable surface without any movement. Finally, researchers need to consider compatibility of preservative solutions and analyses. For example, otolith cannot be stored in formalin while ethanol cannot be used for histology. These compatibility issues related to preservative solutions can make field sampling complicated. To address these issues, our laboratory developed the 'flash-freezing' method using liquid nitrogen for subadult and adult stages of Delta Smelt (Teh et al. 2016). In the field, crews from the California Fish and Wildlife (CDFW) wrap individual fish with unique identification tag using aluminum foil and freeze down in liquid nitrogen for preservation. Later in a laboratory, researchers dissect fish and preserve tissues in the proper preservative solutions for each analysis. This cryopreservation method enables researchers to obtain data from (1) morphometric analyses (body weight, fork length), (2) histology (gills, gonads, livers), (3) enzymatic assays, (4) otolith measurements, and (5) disease analyses (Teh et al. 2016). However, this cryopreservation method is not ideal for small specimens such as larval stage of Delta Smelt since researchers need to sort fish from other environmental debris first. Currently field sampling crews are using formalin for preserving larval fish. Formalin preserved fish samples are ideal for morphometric analyses (e.g., measuring fork/total length) and histology, however it is unclear whether researchers can use such fish samples for genetic tests since formalin causes fragmentation on genomic DNA and formation of protein-DNA cross-links (Campos and Gilbert 2012). Although there are papers reporting genomic DNA extraction from 10% phosphate-buffered formalin fixed and preserved tissues (Yuan et al. 2014), our research group did not have any data, particularly on Delta Smelt. Studying larval Delta Smelt fitness is critically important since larval fish are susceptible to various stressors, such as starvation. Survival during early life stage can directly affect their abundances at later stages. In Task 2 we tested our ability to conduct species identifications on fish preserved in 10% phosphate-buffered formalin. We included Delta Smelt specimens preserved in formalin for up to 11 years to test whether genetic test can be performed using archived samples.

Materials and Methods

Genomic DNA extraction, PCR, and sequencing for formalin fixed and preserved Delta Smelt tissue samples

Delta Smelt samples, preserved in 10% phosphate buffered formalin for 11 years or 1 year, were used for genomic DNA extraction (Table 8-5; see Table 8-6 for the contents of 10% phosphate-buffered formalin). The caudal fin was clipped for use in this experiment. We extracted genomic DNA using QIAGEN kit (QIAamp DNA FFPE Tissue kit, Cat No./ID: 56404). Since residual

formalin can inhibit Proteinase K (Page 9 in the instruction, "Starting materials" in the Qiagen instructions), the fin clip samples were dehydrated prior to the genomic DNA extraction as follows: 1) washing in 70% ethanol for 30 minutes twice, 2) washing in 80% ethanol for 30 minutes twice, 3) washing in 90% ethanol for 30 minutes twice, 4) washing in 100% ethanol for 30 minutes twice.

We skipped the first 8 steps in the protocol (Procedure 1-8, Page 12) because these steps are procedures for removing paraffin and dehydration. The genomic DNA was eluted in 100 µL of ATE buffer. The genomic DNA concentrations were measured by Nanodrop (Table 8-5).

PCR was performed using a pair of custom designed primers developed in our laboratory (64_Hypomesus_F1 and 66_Hypomesus_F2, Table 2, Supplemental Data 2). The PCR cocktail and PCR cycling conditions are shown in Tables 8-7 and 8-8. We doubled the recommended amount of Taq polymerase (0.4 µl per reaction) to maximize the amplification efficiency (Table 8-7). This is because genomic DNA extracted from formalin preserved tissues is severely degraded and fragmented. For the same reason, the PCR was performed for 45 cycles (Table 8-8). In the reaction, we included two positive controls (samples fixed and preserved in 70% ethanol) and one negative control (reaction cocktail without genomic DNA). PCR amplified DNA fragments were submitted to UC Davis DNA Sequencing Facility for direct sequencing reactions using the custom primers (http://dnaseq.ucdavis.edu/).

Table 8-5. Delta Smelt Samples Used for Species Identification by Genetic Test

Sample ID	Description	Preserved in Formalin for	Tissue Wet Weight (mg) Used for Extraction	gDNA Concentration
1	Sex Maturation 2006-07, Date 02/14/2007 DELTA SMELT, #3 Cultured_ Wild_X_ Study AC Lindberg, FCCL, Byron Group W1W 10% Buffered Formalin	11 years	1.3 mg	10.4 ng/µL
2	Sex Maturation 2006-07, Date 02/14/2007 DELTA SMELT, #10 Cultured_ Wild_X_ Study AC Lindberg, FCCL, Byron Group W1W 10% Buffered Formalin	11 years	2.6 mg	14.3 ng/µL
3	Sex Maturation 2006-07, Date 02/14/2007 DELTA SMELT, #1 Cultured_ Wild_X_ Study AC Lindberg, FCCL, Byron Group W1W 10% Buffered Formalin	11 years	1.9 mg	23.7 ng/µL
4	263 dpn 05-AT 1/30/2017 10% Formalin (Alejandro's sample)	1 year	1.4 mg	8.1 ng/µL
5	231 dpn 3 AT 1/30/2017 10% formalin (Alejandro's sample)	1 year	2.2 mg	11.9 ng/µL
6	DS-171 Dph 10% formalin	1 year	1.1 mg	10.0 ng/µL

Sample ID	Description	Preserved in Formalin for	Tissue Wet Weight (mg) Used for Extraction	gDNA Concentration
	AT-00 3-14-17 (Alejandro's sample)			

Notes: These fish samples were fixed and preserved in 10% phosphate-buffered formalin.

Table 8-6. Recipe for Preparing 10% Phosphate-Buffered Formalin

Ingredients	Volume/Weight
Distilled water	3600 mL
37% formalin	400 mL
Na_2HPO_4	26 g
$NaH_2PO_4 \cdot H_2O$	16 g

Table 8-7. PCR Cocktail for Amplifying the Cytochrome b Gene from 10% Phosphate Buffered Formalin Fixed Samples

Reagents	Volume (µL per reaction)
10X Buffer*	5.0
dNTP (10 mM)	1.0
$MgCl_2$ (50 mM)	1.5
H_2O	33.1
Taq (Platium Taq Polymerase)	0.4
Primer (Fw, 10 µM)	2.0
Primer (Rv, 10 µM)	2.0
Template DNA	5.0
Total	50 µL per reaction

* 10X Buffer is supplied in the Platinum Taq Polymerase Kit (ThermoFisher Sci, Cat. Number: 10966034)

Table 8-8. PCR Cycling Conditions for Amplifying the Cytochrome b Gene from 10% Phosphate-Buffered Formalin Fixed and Preserved Samples

Temperatures	Incubation Times	Steps	
95° C	5 min.	Initial denaturing	
95° C	30 sec.	Denaturing	45 cycles
55° C	30 sec.	Annealing	
72° C	1 min.	Extension	
72° C	5 min.	Final extension	

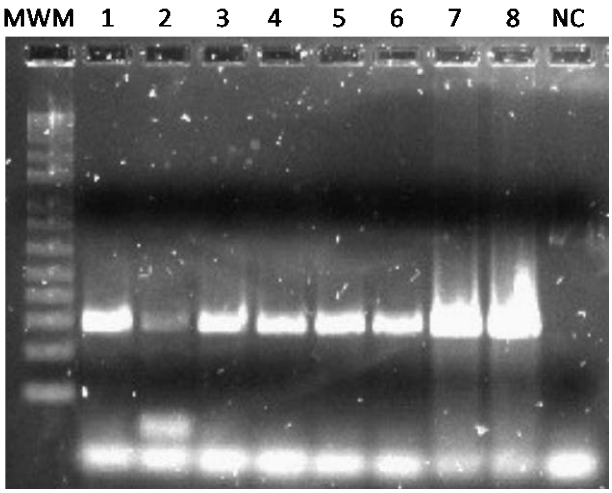
Results and Discussion

DNA bands at the expected size were amplified from the 10% phosphate-buffered formalin fixed samples as well as positive controls (Fig. 1). No band was observed in the negative control. The BLASTN search results demonstrated that the PCR amplified DNA fragments encoded the Delta Smelt cytochrome b gene (Table 8-9). The DNA sequences in FASTA format are available in Supplemental Data 2.

We were able to amplify a portion of the cytochrome b gene from archived samples that were preserved for over 10 years in 10% phosphate-buffered formalin. As expected, the band intensity from the formalin-fixed samples was not as strong as the ones from ethanol preserved samples (Fig. 1). Although the instructions of the genomic DNA extraction kit indicate that DNA fragmentation becomes more severe as tissues are preserved in formalin for longer periods, we did not observe significant differences between 11 years and 1 year preserved tissue samples in the PCR and sequencing results (Fig. 1, Supplemental Data 2).

The 10% borax-buffered formalin is commonly used for fixing various types of tissue samples (Tucker and Chester 1984), however, the solution is not ideal for long term storage. The 10% borax-buffered formalin can become acidic over time due to the loss of buffering capacity of sodium borate, resulting in damage to specimens. Therefore, an alternative buffering method is recommended. One such alternative is 10% phosphate buffered-formalin, as used in this study. It is one of the standard solutions for fixing and storing various types of specimens for morpho-histological analyses (Tucker and Chester 1984). Tissue samples preserved in 10% phosphate-buffered formalin are very stable and pH remains between 7-8 for at least five years (Able 1983). In addition, there are commercially available kits for extracting genomic DNA from 10% phosphate-buffered formalin fixed tissues, which allows researchers to perform genetic tests including species identification by sequencing marker genes (as demonstrated here).

The results from this study indicate that specimens preserved and archived in 10% phosphate-buffered formalin can be used for genetic tests, which potentially provide us opportunities to expand research for better understanding health or population structure of Delta Smelt. For example, larval stage of Delta Smelt preserved in 10% phosphate-buffered formalin can be used for morphometric analysis to assess fitness of fish, followed by histological analysis to evaluate energy storage in liver, and genetic test for confirming species identification (Takács et al. 2016, Teh et al. 2016). In addition, we can use archived wild Delta Smelt samples collected from pre-Pelagic Organism Decline period if there are any specimens available in universities or state agencies. Such fish specimens could be used for genetic analyses and may provide key information regarding population structure of Delta Smelt or presence of hybrids once Delta Smelt were abundant (Benjamin et al. 2018). Although additional steps are required for processing formalin preserved tissues and quality of gDNA extracted from formalin preserved fish was not as good as frozen fish, it is still feasible to amplify short DNA fragments by PCR for genetic analyses. Thus, we can maximize outcomes by fixing and preserving fish tissues with 10% phosphate-buffered formalin.



Notes: (From left) Molecular weight marker (MWM); 1-3: formalin preserved samples (11 years); 4-6: formalin preserved samples (1 year); 7-8: positive control (ethanol preserved tissue, A1 EDSM 12/2017); NC: negative control (no genomic DNA).

Figure 8-1. PCR Results for Delta Smelt Cytochrome b Gene

Table 8-9. BLASTN Sequence Similarity Search Results

Sample	Primer	Description	Query coverage	Identity	Subject Accession No.
SampleNo1	Primer 64	Hypomesus transpacificus cytochrome b gene, partial cds; mitochondrial	100%	100%	HQ667171.1
SampleNo1	Primer 66	Hypomesus transpacificus isolate Msax_912 cytochrome b (cytb) gene, partial cds; mitochondrial	100%	100%	KF013217.1
SampleNo2	Primer 64	Not available*			
SampleNo2	Primer 66	Not available			
SampleNo3	Primer 64	Hypomesus transpacificus cytochrome b gene, partial cds; mitochondrial	100%	100%	HQ667171.1
SampleNo3	Primer 66	Hypomesus transpacificus isolate Msax_912 cytochrome b (cytb) gene, partial cds; mitochondrial	100%	100%	KF013217.1
SampleNo4	Primer 64	Hypomesus transpacificus cytochrome b gene, partial cds; mitochondrial	100%	100%	HQ667171.1
SampleNo4	Primer 66	Hypomesus transpacificus cytochrome b gene, partial cds; mitochondrial	99%	100%	HQ667171.1
SampleNo5	Primer 64	Hypomesus transpacificus cytochrome b gene, partial cds; mitochondrial	100%	100%	HQ667171.1
SampleNo5	Primer 66	Hypomesus transpacificus cytochrome b gene, partial cds; mitochondrial	100%	100%	HQ667171.1

Sample	Primer	Description	Query coverage	Identity	Subject Accession No.
SampleNo6	Primer 64	Hypomesus transpacificus cytochrome b gene, partial cds; mitochondrial	100%	100%	HQ667171.1
SampleNo6	Primer 66	Hypomesus transpacificus isolate Msax_912 cytochrome b (cytb) gene, partial cds; mitochondrial	100%	100%	KF013217.1
SampleNo7	Primer 64	Hypomesus transpacificus cytochrome b gene, partial cds; mitochondrial	100%	99%	HQ667171.1
SampleNo7	Primer 66	Hypomesus transpacificus isolate Msax_912 cytochrome b (cytb) gene, partial cds; mitochondrial	100%	99%	KF013217.1
SampleNo8	Primer 64	Hypomesus transpacificus cytochrome b gene, partial cds; mitochondrial	100%	100%	HQ667171.1
SampleNo8	Primer 66	Hypomesus transpacificus isolate Msax_912 cytochrome b (cytb) gene, partial cds; mitochondrial	100%	100%	KF013217.1

* The PCR product was not submitted to the sequencing facility due to the amplification of non-specific band (Fig. 2)

Acknowledgments

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Supplemental Data:1. Primers for Delta Smelt Mitochondrial Cytochrome b Gene

```
                      (5′ => 3′)
064_Hypomesus_F1      ACTACAAGAACCCTAATGG
065_Hypomesus_F2      TGGCCAACCTTCGGAAAACC
066_Hypomesus_R1      GATGCTCCGTTAGCGTGCATG
067_Hypomesus_R2      TAAAGACCTCGGCCAATATG

HQ667171.1: Delta Smelt
HQ667170.1: Wakasagi

                                  ACTACAAGAACCCTAATGG 064_Hypomesus_F1
                               065_Hypomesus_F2   TGGCCAACCTTCGGAAAACC
HQ667171.1      AAAAACCATCGTTGTTAATTCAACTACAAGAACCCTAATGGCCAACCTTCGGAAAACCCA
HQ667170.1      AAAAACCATCGTTGTCAATTCAACTACAAGAACCCTAATGGCCAACCTTCGGAAAACCCA
                ********************************************************

HQ667171.1      TCCCCTCCTGAAAATTACCAACGACGCTCTTGTTGATCTGCCTGCACCCTCCAATATTTC
HQ667170.1      CCCCCTCCTAAAAATTACCAATGACGCCCTAGTTGATTTACCTGCACCCTCCAATATTTC
                 ******** *********** ***** ** ****** * ********************

HQ667171.1      TATCTGATGAAACTTTGGCTCCCTCCTTGGACTATGTCTTATTATTCAAATCCTCACAGG
HQ667170.1      AATCTGATGAAACTTTGGATCCCTTCTTGGGCTGTGTCTTATTATCCAAATCCTTACGGG
                 ***************** ***** ***** ** *********** ******** ** **

HQ667171.1      CCTATTCCTAGCCATGCACTACACTGCCGAGACTGCTACAGCATTTTCTTCTGTAGTACA
HQ667170.1      CCTCTTTTTGGCTATGCACTATACTGCTGAGACTGCTACCGCTTTTTCCTCTGTTGTTCA
                *** **  * ** ******** ***** *********** ** ***** ***** ** **

                                 066_Hypomesus_R1   CATGCACGCTAACGGAGCATC
HQ667171.1      CTTATGCCGGGACGTTAATTACGGGTGACTAATCCGGAACATGCACGCTAACGGAGCATC
HQ667170.1      CCTCTGCCGAGACGTTAATTACGGCTGACTAATCCGTAACATGCACGCTAACGGAGCATC
                * * ***** ************** *********** ***********************

                       067_Hypomesus_R2  CATATTGGCCGAGGTCTTTA
HQ667171.1      TTTCTTCTTTATTTGTATTTATCTTCATATTGGCCGAGGTCTTTACTACGGCTCCTTCCT
HQ667170.1      TTTCTTCTTTATTTGCATTTACCTTCATATTGGCCGAGGTCTTTATTACGGCTCATTCCT
                *************** ***** ********************** ******** *****

HQ667171.1      TTATAAGGAAACCTGAAACATCGGCGTAGTCCTTCTCCTTTTGGTTATAATGACTGCCTT
HQ667170.1      GTACAAGGAAACTTGAAACATCGGTGTGGTTCTACTACTTTTAGTCATAATGACCGCTTT
                 ** ******** *********** ** ** ** ** ***** ** ******** ** **

HQ667171.1      TGTTGGCTATGTTCTTCCCTGAGGACAAATATCATTCTGAGGG
HQ667170.1      TGTGGGCTATGTTCTTCCCTGAGGACAAATATCATTCTGAGGG
                *** ************************************

>HQ667171.1 Hypomesus transpacificus cytochrome b gene, partial cds; mitochondrial
AAAAACCATCGTTGTTAATTCAACTACAAGAACCCTAATGGCCAACCTTCGGAAAACCCATCCCCTCCTG
AAAATTACCAACGACGCTCTTGTTGATCTGCCTGCACCCTCCAATATTTCTATCTGATGAAACTTTGGCT
CCCTCCTTGGACTATGTCTTATTATTCAAATCCTCACAGGCCTATTCCTAGCCATGCACTACACTGCCGA
GACTGCTACAGCATTTTCTTCTGTAGTACACTTATGCCGGGACGTTAATTACGGGTGACTAATCCGGAAC
ATGCACGCTAACGGAGCATCTTTCTTCTTTATTTGTATTTATCTTCATATTGGCCGAGGTCTTTACTACG
GCTCCTTCCTTTATAAGGAAACCTGAAACATCGGCGTAGTCCTTCTCCTTTTGGTTATAATGACTGCCTT
TGTTGGCTATGTTCTTCCCTGAGGACAAATATCATTCTGAGGG

>HQ667170.1 Hypomesus nipponensis cytochrome b gene, partial cds; mitochondrial
AAAAACCATCGTTGTCAATTCAACTACAAGAACCCTAATGGCCAACCTTCGGAAAACCCACCCCCTCCTA
AAAATTACCAATGACGCCCTAGTTGATTTACCTGCACCCTCCAATATTTCAATCTGATGAAACTTTGGAT
CCCTTCTTGGGCTGTGTCTTATTATCCAAATCCTTACGGGCCTCTTTTTGGCTATGCACTATACTGCTGA
GACTGCTACCGCTTTTTCCTCTGTTGTTCACCTCTGCCGAGACGTTAATTACGGCTGACTAATCCGTAAC
ATGCACGCTAACGGAGCATCTTTCTTCTTTATTTGCATTTACCTTCATATTGGCCGAGGTCTTTATTACG
GCTCATTCCTGTACAAGGAAACTTGAAACATCGGTGTGGTTCTACTACTTTTAGTCATAATGACCGCTTT
TGTGGGCTATGTTCTTCCCTGAGGACAAATATCATTCTGAGGG
```

Supplemental Data: 2. Sequencing Results for Delta Smelt Cytochrome b Gene Amplified from 10% Phosphate-Buffered Formalin

>SampleNo1_64
ACGACGCTCTTGTTGATCTGCCTGCACCCTCCAATATTTCTATCTGATGAAACTTTGGCTCCCTCCTTGGACTATGTCTTATTATTCAAATCCTCACAGGCCT
ATTCCTAGCCATGCACTACACTGCCGAGACTGCTACAGCATTTTCTTCTGTAGTACACTTATGCCGGGACGTTAATTACGGGTGACTAATCCGGAACATGCAC
GCT

>SampleNo1_66
TAAGTGTACTACAGAAGAAAATGCTGTAGCAGTCTCGGCAGTGTAGTGCATGGCTAGGAATAGGCCTGTGAGGATTTGAATAATAAGACATAGTCCAAGGAGG
GAGCCAAAGTTTCATCAGATAGAAATATTGGAGGGTGCAGGCAGATCAACAAGAGCGTCGTTGGTAATTTTCAGGAGGGGATGGGTTTTCCGAAGGTTGGCCA
TTAGGGTTCTTGTAGT

>SampleNo3_64
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TATTCCTAGCCATGCACTACACTGCCGAGACTGCTACAGCATTTTCTTCTGTAGTACACTTATGCCGGGACGTTAATTACGGGTGACTAATCCGGAACATGCA
CGCTAACGGAG

>SampleNo3_66
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AAAGTTTCATCAGATAGAAATATTGGAGGGTGCAGGCAGATCAACAAGAGCGTCGTTGGTAATTTTCAGGAGGGGATGGGTTTTCCGAAGGTTGGCCATTAGG
GTTCTTGTA

>SampleNo4_64
GACGCTCTTGTTGATCTGCCTGCACCCTCCAATATTTCTATCTGATGAAACTTTGGCTCCCTCCTTGGACTATGTCTTATTATTCAAATCCTCACAGGCCTAT
TCCTAGCCATGCACTACACTGCCGAGACTGCTACAGCATTTTCTTCTGTAGTACACTTATGCCGGGACGTTAATTACGGGTGACTAATCCGGAACATGCACGC
TAACGGAGCA

>SampleNo4_66
TCCGGCATAAGTGTACTACAGAAGAAAATGCTGTAGCAGTCTCGGCAGTGTAGTGCATGGCTAGGAATAGGCCTGTGAGGATTTGAATAATAAGACATAGTCC
AAGGAGGGAGCCAAAGTTTCATCAGATAGAAATATTGGAGGGTGCAGGCAGATCAACAAGAGCGTCGTTGGTAATTTTCAGGAGGGGATGGGTTTTCCGAAGG
TTGGCCATTAGGGTTCTTGTAGT

>SampleNo5_64
ACGCTCTTGTTGATCTGCCTGCACCCTCCAATATTTCTATCTGATGAAACTTTGGCTCCCTCCTTGGACTATGTCTTATTATTCAAATCCTCACAGGCCTATT
CCTAGCCATGCACTACACTGCCGAGACTGCTACAGCATTTTCTTCTGTAGTACACTTATGCCGGGACGTTAATTACGGGTGACTAATCCGGAACATGCACGCT
AACGGAGCAT

>SampleNo5_66
CCGGCATAAGTGTACTACAGAAGAAAATGCTGTAGCAGTCTCGGCAGTGTAGTGCATGGCTAGGAATAGGCCTGTGAGGATTTGAATAATAAGACATAGTCCA
AGGAGGGAGCCAAAGTTTCATCAGATAGAAATATTGGAGGGTGCAGGCAGATCAACAAGAGCGTCGTTGGTAATTTTCAGGAGGGGATGGGTTTTCCGAAGGT
TGGCCATTAGGGTTCTTGTAG

>SampleNo6_64
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CTAACGGAGC

>SampleNo6_66
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GGAGCCAAAGTTTCATCAGATAGAAATATTGGAGGGTGCAGGCAGATCAACAAGAGCGTCGTTGGTAATTTTCAGGAGGGGATGGGTTTTCCGAAGGTTGGCC
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>SampleNo7_64
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CTAACGGAGCAT

>SampleNo7_66
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GAGGGAGCCAAAGTTTCATCAGATGGAAATATTGGAGGGTGCAGGCAGATCAACAAGAGCGTCGTTGGTAATTTTCAGGAGGGGATGGGTTTTCCGAAGGTTG
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>SampleNo8_64
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ATTCCTAGCCATGCACTACACTGCCGAGACTGCTACAGCATTTTCTTCTGTAGTACACTTATGCCGGGACGTTAATTACGGGTGACTAATCCGGAACATGCAC
GCTAA

>SampleNo8_66
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AATATTGGAGGGTGCAGGCAGATCAACAAGAGCGTCGTTGGTAATTTTCAGGAGGGGATGGGTTTTCCGAAGGTTGGCCATTAGGGTTCTTGTAG

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Chapter 9: Effect of Isohaline (X_2) and Region on Delta Smelt Habitat, Prey and Distribution During the Summer and Fall: Insights into Managed Flow Actions in a Highly Modified Estuary

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Abstract

Decline of the endangered Delta Smelt has continued and the population remains at an all-time low. Outflow-related management actions to benefit Delta Smelt are currently in place or proposed. However, uncertainty remains as to how these actions may affect habitat factors for Delta Smelt. The foundation for the actions and their associated hypotheses/predictions are that summer and fall habitat conditions are improved for juvenile Delta Smelt when the low salinity zone (LSZ; 0.5-6 ppt) moves seaward, particularly, when overlapping the Suisun Bay-Marsh area of the Sacramento-San Joaquin Delta (Delta). The latter is more likely during wet years and the last notable increase in the Delta Smelt population occurred in the wet year of 2011. We used survey data from paired fish and habitat sampling to evaluate several predictions. We focused on years 2011 through 2017 as this period began and ended with a wet year and encompassed the majority of the available paired fish and zooplankton survey data.

In line with predictions and similar to 2011, Suisun Bay and Marsh turbidity was elevated in summer and fall of 2017 and water temperature lower in summer of 2017 compared to most other regions. While in 2017 Delta Smelt prey density/biomass in general showed an increase for Suisun Bay and Marsh when compared to non-wet years, analyses and observational data did not show an increase in prey in these areas during the fall. Delta Smelt prey density/biomass were comparable but generally not greater in Suisun Bay and Marsh during the summer of 2017 compared to other regions. Prey decreased between summer to fall in both Suisun Bay and Marsh in 2017, and both regions had less zooplankton biomass than freshwater regions in the northern part of the Delta. Our results for 2017 do not support the prediction that when the LSZ overlaps with Suisun Bay/Marsh in the fall period Delta Smelt prey will be greater in this area

than other regions and/or that prey will increase during the Fall X_2 Action period. In 2011, catch density of Delta Smelt was greater in the LSZ during the summer and fall when compared to the freshwater regions of the northern Delta. During most other years and seasons, including summer and fall of 2017, catch density of Delta Smelt was greatest in the freshwater regions of the northern Delta. Results for the summer/fall of 2017 and the summer of 2011, do not fully support the prediction that when compared to other regions Delta Smelt catch density will be higher in Suisun Bay and Marsh when the LSZ overlaps this area. However, Delta Smelt were often present in the Suisun Bay and Marsh area during the study period and periodically greater than or comparable in catch density when compared to other regions.

The continued decline in overall population abundance of Delta Smelt during the study period was largely believed to be due to sub-optimal habitat conditions caused during the mostly drought years of 2012-2016. Although 2017 was one of the wettest years on record, the Delta Smelt population did not show a corresponding increase as in 2011 during the fall or the following spring of 2018 for adults. While it is clear wet years and outflow-related actions can produce a variety of conditions beneficial to Delta Smelt during the rearing and other life-cycle periods, other habitat variables such as water temperature and competition from other species (especially invasive clam species) may offset any potential benefit of increased outflow for Delta Smelt. Continued research on factors limiting Delta Smelt throughout its ontogeny and associated management options to reduce their impact is needed.

Introduction

Delta Smelt (*Hypomesus transpacificus*) is small slender-bodied, mostly annual fish of the Osmeridae family (smelts) endemic to the Sacramento-San Joaquin Delta (Delta) and connecting San Francisco Estuary (SFE), the largest estuary on the Pacific Coast (Knowles and Cayan 2002). Rapid decline of the once abundant Delta Smelt led to its listing as threatened in 1993 and endangered in 2009 under federal and state endangered species acts, respectively (USFWS 1993; CFGC 2009). Since its listings, the Delta Smelt population abundance has continued to decline and remains at an all-time low (Hobbs 2017). The species has been, and will likely continue to be, at the heart of the debate over competing uses of Delta water and is perhaps the most important fish species in the Delta with respect to water management and policy (Moyle et al. 2018). This relates well beyond a regional issue given the Delta is the most important source of freshwater in the state of California, a state which recently became the fifth-largest economy in the world.

The historical record demonstrates the SFE-Delta is now managed at a salinity level that is much greater than would have occurred under pre-1900 conditions (CCWD 2010). Operational strategies for salinity management reduces salinity intrusion somewhat, but salinity still exceeds pre-1900 levels (CCWD 2010). In addition, seasonal and inter-annual variation in salinity of the SFE-Delta has been attenuated, with peak flows following the winter wet season being reduced and low flows during the fall pre-wet season being increased. These changes are largely the result of manipulation of freshwater flows into the SFE-Delta (CCWD 2010). Outflows through the SFE-Delta impact the position of the low salinity zone (LSZ; 0.5-6 ppt) and the distance in kilometers from the Golden Gate Bridge to where the tidally averaged bottom salinity is 2 ppt (denoted by X_2 [Jassby et al. 1995) in the SFE-Delta.

Rearing habitat within estuarine ecosystems is shaped by freshwater flow and its intersection with other dynamic (e.g., water temperature, salinity) and stationary habitat features (Peterson 2003). Variability in freshwater flow therefore influences habitat quantity and quality for many estuarine biota, especially in estuaries where freshwater flow varies substantially seasonally from year to year (Whitfield 1999; Kimmerer et al. 2009). Understanding where and how rearing habitat and its ecological function varies with flow can be particularly important for identifying successful recruitment of estuarine biota (Ramos et al. 2009). This is especially true for organisms that spawn and rear in shallow habitats and are sensitive to changes in salinity and water temperature (Greenwood 2007). Thus, an understanding of rearing habitats occupied by estuarine fishes is foundational for understanding potential mechanisms that link habitat to survival, growth, and ultimately recruitment to later life stages.

In the SFE-Delta, research on estuarine biota and its response to freshwater flow has been focused on the LSZ. This area is considered important rearing habitat for many fishes and invertebrates (Stevens and Miller 1983; Jassby et al. 1995; Kimmerer 2002; Feyrer et al. 2007; Nobriga et al. 2008; Kimmerer et al. 2009). The Delta is also important because it is where two large water export facilities are located, diverting up to 4.5 km^3 of fresh water annually to other regions (mostly southern) of the state. These water diversions can directly affect SFE-Delta biota through entrainment mortality (Kimmerer 2008; Grimaldo et al. 2009) but can also have impacts on biota through degradation of water quality and habitat availability (Kimmerer 2002; Feyrer et al. 2007; Kimmerer et al. 2009; Feyrer et al. 2011,).

Prior research portrayed Delta Smelt as a semi-anadromous species that migrated to and reared in the LSZ, with adults moving upstream to spawn in freshwater (Dege and Brown 2004; Bennett 2005). Recent analyses have found Delta Smelt residing in freshwater habitats of the north Delta (Sacramento Deepwater Ship Channel [SDWSC] and Cache Slough complex) year-round (Sommer et al. 2011; Sommer and Mejia 2013). Using isotopic analysis of otoliths from over a thousand Delta Smelt, Bush (2017) found Delta Smelt exhibits partial migration through three different life history phenotypes, which include freshwater residents, brackish water residents, and a migratory phenotype hatching in fresh water then moving to brackish water as immature fish (juvenile through sub-adult stage). The relative abundance of each life history phenotype varied inter-annually with the migratory phenotype most common in every year but not always dominant.

The positive relationship of freshwater flows to populations of various SFE-Delta fish species and their habitat has been clearly demonstrated for some taxa (Stevens and Miller 1983; Kimmerer 2002; Feyrer et al. 2006; Perry et al. 2018). Previous work shows that Delta Smelt physical habitat increases during the fall when outflow, indexed as X_2, is located seaward of the freshwater region of the Sacramento-San Joaquin Delta (Feyrer et al. 2007; Feyrer et al. 2011; Bever et al. 2016). However, several authors found either no relationship, or a weak relationship, between Delta Smelt and freshwater outflow/X_2 (Stevens and Miller 1983; Moyle et al. 1992; Jassby et al. 1995; Kimmerer 2002; Bennett 2005; Mac Nally et al. 2010; Thomson et al. 2010; Miller et al. 2012) and fall X_2 has not been found to be an important predictor of Delta Smelt population growth rate (Thomson et al. 2010; Maunder and Deriso 2011). This has led to uncertainty among fisheries and water resources managers. Regardless, outflow-related management actions to benefit Delta Smelt are currently in place or proposed (USFWS 2008; CNRA 2016; Frantzich et al. 2018; Schultz et al. 2018). Action 4 (Fall X_2 Action) of the Delta

Smelt Biological Opinion (USFWS 2008) currently requires Delta outflow be maintained at an average X_2 no greater than 74 km for September and October following wet years and 81km following above normal years. While uncertainty remains as to how these actions may affect certain habitat factors for Delta Smelt, the prevailing hypothesis is that summer and fall habitat conditions are improved for juvenile Delta Smelt when X_2 moves seaward (USBR 2012; Brown et al. 2014), especially when X_2 overlaps the Suisun Bay-Marsh area of the Delta. It is predicted that lower water temperatures, harmful algal constituents, nonnative competitor and predator abundance, and increased habitat area, turbidity and prey density/biomass will facilitate an increase in Delta Smelt catch density, health, growth, and survival within Suisun Bay and Marsh and at levels greater than other regions. As part of the multi-agency Directed Outflow Project (DOP), in this paper we aim to evaluate several of these predictions (water temperature, turbidity, prey, catch density) using data from surveys conducted during 2009-2018.

Methods

Study Area and General Sampling Design

The study area ranged from San Pablo Bay and the lower Napa River, upstream into the connecting Delta to Stockton on the San Joaquin River, Hood on the Sacramento River, and the SDWSC (Figure 9-1). The study area includes the North Delta Arc (Moyle et al. 2016) an area consistently occupied by a large portion of the Delta Smelt population.

We used concurrently measured fish and habitat data taken during daylight hours from both state (2011-2018) and federal (2017-18) monitoring surveys. Surveys conducted by the California Department of Fish and Wildlife (CDFW) included the Summer Townet (STN) and Fall Midwater Trawl (FMWT). Survey data from U.S. Fish and Wildlife Service (USFWS) and U.S. Bureau of Reclamation (USBR) included Enhanced Delta Smelt Monitoring (EDSM) which was paired through much of the study area with a DOP-specific habitat survey. Surveys conducted by CDFW were taken at fixed long-term monitoring stations whereas survey data from USFWS-DOP surveys were conducted using a generalized random-tessellation stratified sampling method (Stevens and Olsen 2004; Starcevich et al. 2016).

Water Quality and Physical Habitat

Flow. – Estimates of daily net freshwater outflow (cfs) of the Delta past Chipps Island were obtained from the California Department of Water Resources (DWR) DAYFLOW website (https://water.ca.gov/Programs/Environmental-Services/Compliance-Monitoring-And-Assessment/Dayflow-Data).

Water quality. – For all surveys water quality was taken immediately upon arrival at each sampling station. Measurements reported for this paper include temperature (± 0.01°C), salinity (calculated from conductivity and temperature; ± 0.1 ppt), turbidity (± 0.3 NTU), and chlorophyll a (± 0.01 mg/L).

Lower Trophic Level Resources

We generated zooplankton data as a count of organisms per cubic meter of water. All zooplankton data was paired (same location and time) with a Delta Smelt survey. Oblique trawls were used in CDFW surveys to sample prey using a Clarke-Bumpus (CB) net to target meso-zooplankton (adult and juvenile copepods, and cladocerans. The CB net frame consisted of an

acrylic cylinder 12.5 cm in outer diameter (interior mouth diameter of 12.0 cm, due to new acrylic frame being thicker than previous metal frame) by 19.0 cm long with a General Oceanics model 2030 flow meter bracketed inside. The net was made of 0.160-mm mesh nylon cloth (No. 10 mesh), with an inner mouth diameter of 13 cm, and a length of 73 cm (84.75 cm including the canvas mouth and end). It tapered to 50 mm at the cod-end where a polyethylene bottle screened with 0.140-mm mesh wire cloth collected the organisms. One tow of about 10 minutes in length was used to collect the CB samples. The STN CB net was mounted on a tubular steel frame, with the CB net mounted directly above the STN fish net. The FMWT conducted an additional tow with a mysid sled of tubular steel with both a CB and Mysid net attached. Samples were preserved in 10% formalin with Rose Bengal dye to aid in separating organisms from detritus and algae. Samples were transferred to the CDFW laboratory (Stockton, CA USA) for processing.

For DOP surveys, zooplankton were sampled using a 50-cm diameter bongo net frame. One of the bongo cylinders was outfitted with 0.500-mm mesh for macro-zooplankton, the other cylinder was outfitted with 0.150-mm mesh for meso-zooplankton. A General Oceanics Model 2030 flow meter was used to determine the volume of water filtered through the nets. Concurrent fixed-depth tows of seven minutes were used at each site just under the surface of the water column and in the lower half of the water column. For example, if the channel was 10 m deep, the bottom tow occurred below 5 m deep for the entire length of the tow. For deep channel tows, the bridle of the bongo net was weighted with a chain or downrigger ball (~2.2-3.2 kilograms). This sank the net to sample in a fixed position within the bottom half of the water column. Measures of the warp length and angle of the tow rope from the stern tow bar were used to target desired sampling depths using trigonometry. In addition, HOBO pressure loggers (Onset Hobo™ U20-L02) were attached to the bongo net frame to verify and continuously record sampling depth and temperature of each tow. Upon retrieval, the net was systematically washed down towards the cod end. The cod end was emptied into sample jars with 10% formalin.

Zooplankton sample processing followed methods detailed in the Interagency Ecological Program's Zooplankton Study (https://www.wildlife.ca.gov/Conservation/Delta/Zooplankton-Study), although in 2017 10 aliquot counts were used instead of 20 aliquot counts due to the dense nature of the DOP samples. The different zooplankton sampling methods (CDFW and DOP) were compared in a separate study. No significant differences were found with regard to catch density or composition (ANOSIM statistic R = -0.01521, $P = 0.622$ with 999 permutations). Confirming the results of the ANOSIM, there was no clear distinction in non-metric multi-dimensional scaling (NMDS) space between the oblique and surface/bottom samples. Analysis of paired zooplankton counts obtained from the two methods revealed significant differences (Wilcoxon test: $P < 0.05$) for only 2 of 14 zooplankton categories (e.g., *Sinocalanus doerri* and other prey). Data from the CDFW and DOP were therefore pooled for periods of overlap. More detail on the results of the analyses are provided in the supplemental material.

Delta Smelt

Trawling methods to sample Delta Smelt differed somewhat between CDFW and USFWS, and details on methods can be found in Von Geldern (1972) and Chadwick (1964), and at https://www.fws.gov/lodi/juvenile_fish_monitoring_program/jfmp_index.htm, respectively.

Delta Smelt were preserved in liquid nitrogen on survey boats using methods in Teh et al. (2016) and transferred to University of California at Davis (UCD).

Analyses

We examined prey availability for nine regions within and among years, including the 2017 Fall X_2 Action period. Prey density (individuals/m^3) and prey biomass density ($\mu g/m^3$) were used in the analyses and reporting (rounding up to the nearest whole number to discretize), and for this paper are synonymous with abundance and biomass, respectively. Response variables included total prey (the sum of all zooplankton) and various key prey species and groups (adult *Pseudodiaptomus forbesi*, juvenile *Pseudodiaptomus* spp., *Limnoithona* spp., *Sinocalanus doerrii*, *Acartiella sinensis* and *Tortanus* spp.)

To evaluate the environmental conditions and water quality covariates impact on the prey density and biomass of these same response variables in Suisun Bay during the 2017 Fall X_2 Action period, the data was subset seasonally and the mean and variance of the response variables were examined within each subset for equality. A Poisson generalized linear model was used to study the relationship between the response variables and environmental and water quality covariates.

For comparisons of prey density and biomass across regions during the summer and fall of 2017, response variables were evaluated for equality of variance using a Levene test for equality of variances. All response variables failed the Levene Test ($p<0.05$) and thus a non-parametric Kruskal-Wallis rank-sum test followed by a Dunn multiple comparison post-hoc test was used to compare the equality of the median response variables across the nine regions.

A one-way Analysis of Variance (ANOVA) was used to compare temperature and turbidity across years (2010-2017) for Suisun Bay and all regions combined during the summer and fall. A one-way ANOVA was used to compare water temperature (°C), turbidity (NTU) and salinity (ppt) across regions during the summer and fall of 2017. Any water quality data used in comparisons and analyses was matched with the survey that was related to the response variable of interest.

Results

Outflow

During fall (September and October) of 2017, X_2 averaged 75 km (Figure 9-2). Correspondingly and outflow increased per the Fall X_2 Action (Figure 9-3). A similar pattern was observed for 2011, another year when the Fall X_2 Action was implemented. For all other fall periods in years post 2010, X_2 was located above 81km; the highest X_2 observed during period was in 2014 (~88-90 km).

Relationship Between Prey Density/Biomass, X_2, and Fall X_2 Action

In 2017, total prey density and prey biomass density appeared to trend upward in response to increased *X2* during the fall period (Figure 9-4). Similarly, a positive trend in late fall was evident in prey density and prey biomass density in response to a turbidity gradient (Figure 9-5) but declined in response to rising salinity gradients (Figure 9-6). In 2017 in Suisun Bay, total prey and most prey groups analyzed, did not show a significant increase in prey density and prey biomass density during the Fall X_2 Action period when compared to preceding summer

conditions (Table 9-1). There was some evidence that *Limnoithona* spp. prey density, but not biomass, increased during the Fall X_2 Action period when compared to preceding summer conditions (Table 9-1). In Suisun Bay and Marsh, adult *P. forbesi* biomass, and prey density and biomass in general, were greater during wet years than non-wet years in the summer and fall periods but markedly decreased from summer to fall (Figure 9-7 and 9-8). More landward regions had no decrease, to less of an overall decrease, in adult *P. forbesi* biomass and abundance from summer to fall during wet years and between wet years and non-wet years (i.e., no-action years) (Figure 9-7).

Regional Comparisons of Delta Smelt Prey Density/Biomass

Summer Period. – Among wet years (2011 and 2017), prey density and biomass were greatest in the SDWSC followed by Cache Slough in 2011, and Cache Slough followed by SDWSC in 2017 (Figure 9-8). The Southern Delta largely dominated summer prey density and biomass in the non-wet years, while Suisun Bay and Marsh had some of the lowest levels of summer prey density and biomass among regions during non-wet years in all seasons (Figure 9-8). The pattern was similar in the fall and late fall, with prey density and biomass generally greatest in either Cache Slough, SDWSC and Southern Delta. Suisun Bay and Marsh had some of the lowest levels of fall key prey abundance and biomass among regions during both wet and non-wet years (Figure 9-8).

Summer 2017. – In summer of 2017 mean total prey density was highest in the Lower San Joaquin region followed by the Lower Sacramento and lowest in the Western region (Table 9-2A). Although a significant difference was found among regions with respect to total prey density ($X^2 = 64.4$, df = 7, $p < 0.0001$), only the Western region significantly differed from the other regions (Table 9-2A). A significant difference ($X^2 = 66.5$, df = 7, $p < 0.0001$) was also found in mean total prey biomass among regions (Table 9-2B). Prey biomass was highest in Cache Slough and the SDWSC and significantly higher than Susuin Bay. Prey biomass was lowest in the Western region and significantly lower than all other regions (Table 9-2B).

In summer of 2017 there was a significant difference ($X^2 = 74.21$, df = 7, $p < 0.0001$) in mean adult *P. forbesi* density among regions (Table 9-2A). Mean adult *P. forbesi* density was greatest in Cache Slough compared to other regions, however, the difference was found to be significant only when compared to the Susuin Bay and Western regions (Table 9-2A). There was also a significant difference ($X^2 = 74.2$, df = 7, $p < 0.0001$) in mean adult *P. forbesi* biomass among regions (Table 9-2B). Biomass was highest in Cache Slough and Susuin Marsh, with the former significantly higher than Susuin Bay. Similar to total prey biomass, prey biomass for *P. forbesi* was lowest in the Western region and significantly lower than all other regions (Table 9-2B).

In summer of 2017 there was a significant difference ($X^2 = 75.4$, df = 7, $p < 0.0001$) in mean juvenile *Pseudodiaptomus* spp. density among regions (Table 9-2A). Mean juvenile *Pseudodiaptomus* spp. density was highest in Cache Slough followed by the SDWSC, with the former significantly higher than both Suisun Bay and Suisun Marsh and the latter significantly higher than Suisun Bay. Mean juvenile Pseudodiaptomus spp. density and was significantly lower in the Western than all other regions (Table 9-2A). There was a significant difference ($X^2 = 75.4$, df = 7, $p < 0.0001$) in mean juvenile *Pseudodiaptomus* spp. biomass among regions (Table 9-2B). Biomass was highest in Cache Slough and SDWSC with the former significantly

higher than Susuin Bay. Prey biomass for juvenile *Pseudodiaptomus* spp. was lowest in the Western region and significantly lower than all other regions (Table 9-2B).

In summer of 2017 there was a significant difference (X^2 = 189.5, df = 7, p < 0.0001) in mean *Limnoithona* spp. density among regions (Table 9-2A). Mean *Limnoithona* spp. density was highest in Suisun Bay, which was significantly higher than all other regions outside of Susuin Marsh (Table 9-2A). There was also significant difference (X^2 = 233.4, df = 7, p < 0.0001) in mean *Limnoithona* spp. biomass among regions, with biomass significantly higher in the three more seaward regions than all others (Table 9-2B).

In summer of 2017 there was a significant difference (X^2 = 70.7, df = 7, p < 0.0001) in mean *Sinocalanus doerri* density among regions (Table 9-2A). Mean *Sinocalanus doerri* density was highest in SDWSC, and significantly higher than all other regions other than Susuin Bay and Cache Slough (Table 9-2A). Similar to density, there was a significant difference (X^2 = 71.4, df = 7, p < 0.0001) in mean *Sinocalanus doerri* biomass among regions (Table 9-2B), with biomass highest in the SDWSC. *Sinocalanus doerri* biomass was significantly greater in the SDWSC than Suisun Bay, and lowest in the Western region which was significantly lower than all other regions (Table 9-2B).

Fall 2017. – In fall of 2017 mean total prey density was significantly different among regions (X^2 = 64.4, df = 7, p < 0.0001) being highest in the SDWSC followed by the Lower Sacramento, and lowest in the Upper Sacramento region (Table 9-3A). Total prey biomass was significantly different among regions (X^2 = 89.2, df = 7, p < 0.0001) being highest in the SDWSC followed by the Southern Delta, and lowest in the Upper Sacramento region (Table 9-3B).

In fall of 2017 there was a significant difference (X^2 = 128.2, df = 8, p < 0.0001) in mean adult *P. forbesi* density among regions (Table 9-3A). Mean adult *P. forbesi* density was greatest in Cache Slough, followed closely by the SDWSC and Lower Sacramento regions, and lowest in the Upper Sacramento region. Adult *P. forbesi* density in Suisun Bay was significantly lower than most of the more landward regions (Table 9-3B). The pattern for biomass was similar, with a significant difference (X^2 = 128.2, df = 8, p < 0.0001) in mean adult *P. forbesi* density among regions (Table 9-3B), biomass greatest in Cache Slough followed closely by the SDWSC and Lower Sacramento regions, and lowest in the Upper Sacramento region. As with density, biomass of adult *P. forbesi* in Suisun Bay was significantly lower than most of the more landward regions (Table 9-3B).

In fall of 2017 there was a significant difference (X^2 = 163.3, df = 8, p < 0.0001) in mean juvenile *Pseudodiaptomus* spp. density among regions (Table 9-3A). Mean juvenile *Pseudodiaptomus* spp. density was highest in the Southern Delta region, with the three most seaward regions (i.e., Western, Suisun Bay and Suisun Marsh) being significantly lower than more landward regions (Table 9-3A). A similar pattern was apparent for biomass, with a significant difference (X^2 = 161.3, df = 8, p < 0.0001) in mean juvenile *Pseudodiaptomus* spp. among regions (Table 9-3B), with the three most seaward regions (i.e., Western, Suisun Bay and Suisun Marsh) being significantly lower than most of the landward regions (Table 9-3B).

In fall of 2017 there was a significant difference (X^2 = 124.7, df = 8, p < 0.0001) in mean *Limnoithona* spp. density among regions (Table 9-3A). Mean *Limnoithona* spp. density was

highest in the SDWSC followed closely by Susuin Marsh (Table 3A). There was also a significant difference (X^2 = 103.9, df = 8, p < 0.0001) in mean *Limnoithona* spp. biomass among regions, with biomass highest in in the SDWSC and Susuin Marsh (Table 9-3B).

In fall of 2017 there was a significant difference (X^2 = 139.1, df = 8, p < 0.0001) in mean *Sinocalanus doerri* density among regions, with biomass highest in the SDWSC and significantly greater than the three most seaward regions (i.e., Western, Suisun Bay and Suisun Marsh) (Table 9-3A). The same pattern was noted for mean biomass of *Sinocalanus doerri* significantly different among regions (X^2 = 133.2, df = 8, p < 0.0001), highest in the SDWSC and significantly greater than the three most seaward regions (Table 9-3B).

Water Quality

Overall water temperatures during the summer exceeded 20°C in all regions, except for the Western region (Figure 9-9). Summer water temperatures were higher in landward regions, especially in the Southern Delta and SDWSC, and often approached or exceeded 23°C. Summer water temperatures in Suisun Bay and Suisun Marsh were generally lower than 23°C. Fall water temperatures were varied with landward regions largely similar to or lower than Suisun bay and Suisun Marsh (Figure 9-9). Overall mean water temperatures were significantly different across years for the summer (one-way ANOVA = 68.8; df = 7; $P < 0.001$) and were significantly greater during summer for the last four years of the period analyzed (2014-2017) than the first four years (2010-2013) (Table 9-4). A similar pattern was noted within Suisun Bay, as mean water temperatures significantly differed across years for the summer (one-way ANOVA = 60.23; df = 7; $P < 0.001$) and were significantly greater for the last three years of the period analyzed (2015-2017) than the first four years (2010-2013) (Table 9-4). Fall mean temperatures both overall and for Suisun Bay were significantly different across years (one-way ANOVA = 55.8; df = 7; $P < 0.001$ and one-way ANOVA = 24.27; df = 7; $P < 0.001$, respectively), differences varied across years during the study period with no clear trend across years or between wet and non-wet years (Table 9-4).

In 2017 mean summer temperature was significantly different (X^2 = 266.5; df = 8; $P < 0.001$) among regions (Table 9-5). Mean summer temperature was highest in the SDWSC and lowest in the Western region, with the ship channel significantly greater in summer temperature than the Suisun regions and lower in the Western region than all others (Table 9-5). In 2017 mean fall temperature was also significantly different (X^2 = 53.3; df = 8; $P < 0.001$) among regions (Table 9-6). Mean fall temperature was highest in the SDWSC and lowest in the Upper Sacramento region. stern region, with significant differences varied across regions (Table 9-6).

Among years and seasons turbidity was often greater in Susuin Bay and Suisun Marsh compared to the more landward regions (Figure 9-10). Overall mean turbidity was significantly different across years for the summer (one-way ANOVA = 24.12; df = 7; $P < 0.001$) and fall (one-way ANOVA = 32.36; df = 7; $P < 0.001$) periods, with no clear trend across years or between wet and non-wet years (Table 9-4). A similar pattern was noted within Suisun Bay for the summer period (one-way ANOVA = 21.14, df = 7; $P < 0.001$). However, in the fall (one-way ANOVA = 37.96; df = 7; $P < 0.001$) turbidity in Susiun Bay was significantly greater for 2011 than all other years, with all other years being similar to each other (Table 9-4).

In 2017 mean turbidity was significantly different across regions (X^2 = 298.2; df = 8; P < 0.001) during the summer with the three most seaward regions (Western, Suisun Bay and Suisun Marsh) significantly greater in turbidity than all other regions (Table 9-5). In 2017 results for mean turbidity in the fall were similar to those in the summer, with a significant difference among regions (X^2 = 173.8; df = 8; P < 0.001) and the highest turbidity for the three most seaward regions (Western, Suisun Bay and Suisun Marsh) although the differences were not always found to be significant (Table 9-6).

In wet years (2011 and 2017) salinities were in the suitable range (0-6 ppt) for Delta Smelt occupation (Sommer et al. 2007) during the summer and fall at all locations except for the Western region (Figure 9-11). Suisun Bay and Suisun Marsh often exceeded 6 ppt salinity in non-wet years (Figure 9-11). There was a significant difference in salinity among regions for both the summer (X^2 = 378.7; df = 8; P < 0.001) and fall (X^2 = 363.9; df = 8; P < 0.001) periods (Table 9-5 and 9-6). A similar pattern was evident across seasons, with salinity in the three most seaward regions (i.e., Western, Suisun Bay and Suisun Marsh) significantly greater than all other regions (Table 9-5 and 9-6).

Overall, median chlorophyll-*a* was low across all regions during fall of 2017; the highest observed values occurred in Suisun Marsh and SDWSC (Figure 9-12). A more detailed description of chlorophyll-*a* trends is in development for another study of the DOP.

Delta Smelt

Mean sampling station catch density of Delta Smelt in the summer of 2017 was greatest in the SDWSC and Cache Slough, followed by Suisun Marsh (Figure 9-13). A somewhat similar pattern occurred in summer 2011 with Cache Slough having the greatest catch density, followed by Suisun Marsh and the SDWSC (Figure 9-13). During the study period, Suisun Bay or Marsh were greater in catch density than all other regions only in fall of 2009 (Suisun Marsh and the SDWSC) and 2011 (Suisun Bay), and late fall of 2015 (Suisun Marsh) and 2017 (Suisun Marsh). While the freshwater areas of the Cache Slough Complex and SDWSC largely dominated catch density in summer of 2017, abundance dropped precipitously in late July. Because Delta Smelt catch density dropped to such low levels (Figure 9-14), statistical modelling of their distribution as a function of prey and habitat was not pursued in this paper.

Overall mean catch density of Delta Smelt was higher in freshwater areas (<0.5 ppt) than in the LSZ (0.5-6 ppt) for the summer, and highest in the LSZ in the fall (Figure 9-15a). Freshwater areas (<0.5 ppt) were highest in catch density in the summer for wet years and non-wet years, with the non-wet years having the same pattern in catch density across the salinity range but with a lower density level (Figure 9-15b). Catch density was higher in wet years in the LSZ for the fall and the freshwater areas for the late fall. Catch density was highest in the freshwater areas for 2017 and highest in the LSZ for 2011 during the summer (Figure 9-15c).

Discussion

Outflow

In general, our study period began and ended with wet years (2011 and 2017), with mostly drought years between. Overall summer Delta outflow was greater in 2011 than 2017 although both followed a similar pattern peaking in June and quickly receding by August. During fall of

2011 and 2017, the Fall X_2 Action was implemented, and outflow was increased to shift X_2 towards 75 km.

Lower Trophic Food Web

Zooplankton. – While food in general showed an increase in 2017 for Suisun Bay and Marsh when compared to non-wet years, analyses and observational data did not show an increase in food in these areas during the 2017 Fall X_2 Action. Zooplankton biomass was greatest in Cache Slough followed by the SDWSC in the summer of 2017. Zooplankton decreased between summer to fall in both Suisun Bay and Marsh, and both regions had less zooplankton biomass than regions in the more landward part of the Delta (Cache Slough, SDWSC and Lower Sacramento River). The latter does not support the prediction that when the LSZ overlaps with Suisun Bay/Marsh in the fall period food will be greater in this area than other regions. This pattern largely holds up when looking across years and seasons with the northern part of the Delta having a greater abundance of food in general.

The 2017 results presented here are also consistent with recent work by Kimmerer et al. (2018) showing that *P. forbesi* abundance and biomass are depressed in Suisun Bay during the fall. Overall, adult *P. forbesi* abundance and biomass was highest in Cache Slough during fall of 2017, also consistent with findings of Kimmerer et al. (2018). Juvenile *Pseudodiaptomus* spp. abundance was low in Suisun Bay and Suisun Marsh compared to other regions, which is not surprising given recent findings by Kimmerer et al. (2018) showing that *P. forbesi* nauplii and juveniles experience high mortality in Suisun Bay from clam grazing. In fact, Kimmerer et al. (2019) indicate that without subsidies of *P. forbesi* (all life stages) into Suisun Bay during the summer and fall, *P. forbesi* abundance would be near zero. Until a box-model analysis is performed (currently underway by the DOP), it is unknown if the Fall X_2 Action during 2017 yielded higher subsidies of *P. forbesi* to Suisun Bay. Nonetheless, data presented for *P. forbesi* and other key Delta Smelt zooplankton prey (*Limnoithona* spp., *S. doerri*) suggest prey was not enhanced in Suisun Bay during the 2017 Fall X_2 Action, which may be due to high mortality caused by clam grazing.

In general, *P. forbesi* abundance and biomass were lower in Suisun Bay compared to most other regions across years (2011-2017), which is not surprising given the potential of clam grazing to cause high mortality of nauplii and juvenile life stages. Similar to Kimmerer et al. (2018), the data presented here shows that zooplankton abundance increases with X_2 where clam grazing is lower.

Water Quality

Salinity, water temperature and turbidity are all key water quality variables that shape Delta Smelt habitat (Feyrer et al. 2007; Nobriga et al. 2008). In wet years (2011 and 2017) salinities were in the suitable range (0-6 ppt) for Delta Smelt (Sommer et al. 2007) during the summer and fall at all locations except for the Western region, although Suisun Bay and Suisun Marsh often exceeded 6 ppt salinity in non-wet years. While Delta Smelt have a fairly broad salinity (Komoroske et al. 2016) found Delta Smelt body condition was reduced at high salinities and acclimating to salinities outside the LSZ could impose energetic costs that constrain the species ability to exploit these habitats.

It has been shown that larval Delta Smelt will not feed in clear water (Baskerville-Bridges et al. 2004), and that turbidity may provide the visual contrast needed for Delta Smelt to see their prey. In addition, turbidity may provide Delta Smelt cover from predators. Overall, turbidity was elevated in Suisun Bay and Suisun Marsh during summer and fall of 2017 compared to most other regions. This supports the prediction that when the LSZ overlaps with Suisun Bay/Marsh, turbidity as an aspect of Delta Smelt habitat, is greater in this area than other regions. Recent modeling work by Bever et al. (2018) demonstrate wind is an important driver of turbidity in Suisun Bay during fall and winter. Wind speed was not examined but it is possible that wind was higher in this region than others.

Overall mean water temperatures were significantly greater during summer for the last four years of the study period analyzed (2014-2017) than the first four years (2010-2013) with a similar pattern noted within Suisun Bay. At the juvenile to sub-adult life stage Delta Smelt start to experience stress at about 22-23 °C (Komoroske et al. 2014; Jefferies et al. 2016). Water temperature was lower in Suisun Bay and Suisun Marsh during the summer of 2017 compared to several landward regions and was generally lower than the thermal stress level (~ 22-23 °C). Summer water temperatures in landward regions, especially in the Southern Delta and SDWSC, often approached or exceeded 23°C. Suisun Bay and Suisun Marsh were generally similar in water temperature during the fall period compared to other regions and most fall temperatures were below the aforementioned thermal stress level. Although results are mixed, the summer data roughly support the prediction that when the LSZ overlaps with Suisun Bay/Marsh, water temperature as an aspect of Delta Smelt habitat, is lower in this area than other regions. Similar to turbidity, water temperature is driven by larger atmospheric effects rather than purely flow (Wagner et al. 2011), especially during the summer and fall months when Delta outflow is reduced.

Overall, chlorophyll *a* was low in all regions during the fall of 2017, especially in Suisun Bay, which it not surprising given clam grazing limits buildup of phytoplankton biomass in the low salinity zone of the estuary (Kimmerer and Thompson 2014). Chlorophyll *a* was highest in the SDWSC and Suisun Marsh during fall of 2017. Kimmerer and Thompson (2014) suggest that upstream subsidies of phytoplankton into the low salinity zone can be quickly offset by clam grazing effects. More in-depth chlorophyll-*a* analyses are presented in another DOP-related study.

Delta Smelt

The last notable increase in the Delta Smelt population occurred in the wet year of 2011. The continued decline in overall population abundance of Delta Smelt during the study period was largely believed to be due to sub-optimal habitat conditions caused during the mostly drought years of 2012-2016 (Moyle et al. 2016). Although 2017 was one of the wettest years on record, the Delta Smelt population did not show a corresponding increase as in 2011 during the fall or the following spring (2018). Catch numbers from surveys remained at record lows during the latter part of our study period. The wet year prior to 2011 was 2006. Baxter et al. (2015) mention not finding a notable increase in juvenile and adult abundance in 2006 and emphasized the need for favorable habitat conditions throughout the year, especially during the larval period.

Preliminary evidence suggests that water temperature, especially in the more landward regions of the study area, approached or passed levels (≥ 22-23 C) where physiological stress has been

shown to occur (Komoroske et al. 2015) and likely led to the complete disappearance in late July of the relatively high catch density of Delta Smelt in the Cache Slough Complex and SDWSC in the summer of 2017. This may have led to mortality or egress from these areas due to cumulative thermal stress. However, overall low catch rates in the late summer/early fall made it difficult to assess if the Delta Smelt population shifted its distribution, with a significant change in relative distribution and catch density not occurring until later in the fall in the Lower Sacramento region.

The results presented here for the summer/fall of 2017 and the summer of 2011, do not fully support our prediction that when compared to other regions in our study area, Delta Smelt catch density will be higher in Suisun Bay and Marsh when the LSZ overlaps this area. However, Delta Smelt were often present in the Suisun Bay and Marsh area during the study period and periodically greater than or comparable in catch density when compared to other regions.

Although the pattern varied across years and season, Delta Smelt in our study period were captured at relatively higher and similar catch densities in the freshwater areas (<0.5 ppt) compared to the LSZ. This pattern is not in line with previous research suggesting abundance of rearing Delta Smelt should be greater in the LSZ than other areas (Moyle et al. 1992; Dege and Brown 2004; Bennett 2005; Kimmerer et al. 2013; Sommer and Mejia 2013). Our data mostly correspond to the distribution of Delta Smelt in relation to salinity presented in Komoroske et al. (2014). Our data are also somewhat in line with Bush (2017) who found the species exhibits a variation in life history phenotypes (migratory, freshwater resident, and brackish water resident), with the latter being relatively uncommon. Thus, not all Delta Smelt should be expected to occupy the LSZ, especially if habitat and food are suitable in upstream habitats. While summer outflows and Fall X_2 Action created some suitable habitat conditions for Delta Smelt to occupy Suisun Bay and Marsh during the summer (salinity, turbidity, temperature, food), and fall (salinity, turbidity), it appears other factors (e.g., food supply, predation, physical habitat features) were important in shaping why Delta Smelt were mostly located upstream of the LSZ during 2017.

While catch density of Delta Smelt showed significant variation within and across regions, Delta Smelt were noticeably absent from the Upper Sacramento and Southern Delta regions. The former is a now channelized stretch of the river with relatively less shallow and low-velocity habitats. The Southern Delta is known to have a high presence of non-native fishes, is closer to the major water export operations, and has relatively higher water temperatures and lower turbidity than most other regions. Delta Smelt were present in the Western region during the summer in both wet years and one dry year but were not captured in this region in the fall or late fall of any year. This is likely due to the reduction in suitable habitat from higher water salinities in this region as X_2 moves landward during the summer and fall.

Management Implications

Wet years and outflow-related actions can produce a variety of conditions beneficial to Delta Smelt during the rearing and other life-cycle periods, however, other habitat variables such as water temperature and competition from other species may offset any potential benefit of increased outflow for Delta Smelt. Managed flow actions should consider how shifting

low-salinity habitat seaward affects key mechanistic responses beyond just potential habitat occupied by Delta Smelt. For example, recent research suggests that increased outflow can help subsidize phytoplankton and meso-zooplankton from freshwater habitats to the LSZ but the effects of clam grazing and predatory pressures appears to rapidly offset subsidized production (Gould and Kimmerer 2010; Greene et al. 2011; Kimmerer and Thompson 2014; Slaughter et al. 2016; Kimmerer et al. 2018, Kimmerer et al. 2019). In addition, outflow appears to have little effect on water temperature (Wagner et al. 2011) and turbidity (Bever et al. 2018) during the summer and fall, both key water quality variables that shape Delta Smelt habitat (Feyrer et al. 2007; Nobriga et al. 2008). Mechanistic models, such as the box-model recently applied to examine zooplankton subsidies to the LSZ (Kimmerer et al. 2019) can be informative for understanding expected responses for different flow actions.

Managed flow actions should also consider the effects of previous conditions to understand how managed flow action affect Delta Smelt distribution/abundance and food web actions. For example, this study did not observe a change in the distribution of Delta Smelt into Suisun Bay during the fall of 2017. However, prior to the fall, Delta Smelt were abundant in Suisun Bay during early summer before water temperatures elevated to levels known to be stressful to Delta Smelt. For zooplankton, previous summer abundance was accounted for when examining fall abundance patterns, allowing us to at least partially discriminate effects due to the flow action during fall of 2017. In doing so, we show that in 2017, *P. forbesi* abundance in Suisun Bay was actually lower compared to other regions and years. In short, to assess the effects of a managed flow action in a complex estuarine ecosystem, it is important to consider the conditions the biological response variables experience prior to the action itself.

The potential for future flow actions to have the desired ecological effect may increase the more its design is related to the natural seasonal hydrograph the system's native biota evolved with (Propst and Gido 2004; Kiernan at al. 2012). Northern and Central California receives most of its rain and snowfall during the winter, with little to no rainfall in summer-fall period. Historically, flows were therefore highest in winter following large storm events and in late winter/early spring following snow melt, with the lowest flows occurring in the pre-wet season fall. However, the SFE-Delta is now a highly modified system with competing water demands and various operational and flood control directives which create substantial challenges to implementing and evaluating environmental-based flow actions. In addition, it is known that restoration efforts seeking to mimic natural flow regimes in modified river systems will not always yield successful ecological outcomes unless such flows trigger functional processes (Yarnell et al. 2015). Yarnell et al. (2015) proposes a 'functional flows' approach rather than attempting to mimic a natural hydrograph, to identify and restore aspects of the flow regime that support key ecosystem functions and drive geomorphological and ecological processes.

Continued research on factors limiting Delta Smelt throughout its ontogeny and associated management options to reduce their impact is needed. Recent research (Hammock et al. 2019a) suggests the 22-fold decrease in Delta chlorophyll-*a* from 1969–2014, and the cascading effects on zooplankton and pelagic fishes, occurred largely due to combined impacts of *P. amurensis* invasion and increased water diversions. Kimmerer (2002) did not find a link between flow and upper trophic food web responses, in part, because fish and food supplies can move and exhibit extreme temporal variability. The question of whether modifications to water diversion

operations would provide lower trophic subsidies to the LSZ, and if such subsidies could offset clam grazing effects, could be explored in future empirical and modeling efforts.

Alternative summer and fall flow and non-flow-related actions may prove efficacious in efforts to benefit the Delta Smelt population. Several alternative actions can be found in the Delta Smelt Resiliency Strategy (CNRA 2016). Actions geared toward increasing available food for Delta Smelt, such as the North Delta Food Web Adaptive Management Project (e.g., Yolo Bypass) and Roaring River Distribution System Food Production should consider if any increases in food may also benefit other competitor and predator species. The Suisun Marsh Salinity Control Gate Project began its pilot year in 2018 and intends to bring fresher water to the Suisun Marsh area in hopes of attracting Delta Smelt to this relatively intact habitat. Habitat restoration actions, such as efforts to increase tidal marsh habitat may also be options to consider. Hammock et al. (2019b) found tidal wetland habitat appears to confer substantial benefits to the foraging success of Delta Smelt. Little is known about Delta Smelt spawning habitat in the wild and/or if spawning habitat is currently limiting the population. Further insight may guide restoration activities that have the best potential to benefit the Delta Smelt population.

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Tables

Table 9-1. Poisson Generalized Linear Model Results Showing Whether there was a Change in Delta Smelt Prey Density (individuals/m3) and Prey Biomass Density (µg/m3) of Total Prey and Four Prey Species from Summer to Fall of 2017 in Suisun Bay

	Density (individuals/m3)					Biomass Density (µg/m3)				
Response	**Term**	**Model Estimate**	**Standard Error**	**Z Statistic**	**P-value**	**Term**	**Model Estimate**	**Standard Error**	**Z Statistic**	**P-value**
Total Prey	Intercept	33,962.72	10,206.72	3.33	0.001	Intercept	16,624.30	43,809.80	0.38	0.71
	Summer Mean	48.88	90.54	0.54	0.59	Summer Mean	1,842.80	3,897.10	0.47	0.64
	Temperature	*-8,173.02*	3,433.42	-2.38	0.02	Temperature	-8,366.30	8,091.20	-1.03	0.30
	NTU	*-2,019.59*	535.41	-3.77	0.0003	NTU	-1,199.50	1,463.50	-0.82	0.42
	Salinity	*-408.10*	724.46	-0.56	0.57	Salinity	-240.60	1,542.40	-0.16	0.88
Pseudodiaptomus forbesi Adults	Intercept	9,787.61	7,875.30	1.24	0.22	Intercept	26,101.33	21,094.94	1.24	0.22
	Summer Mean	-14.63	773.22	-0.02	0.98	Summer Mean	-6.67	2,075.41	0.00	1.00
	Temperature	-2,855.10	1,564.14	-1.83	0.07	Temperature	-7,725.09	4,223.43	-1.83	0.07
	NTU	52.56	284.30	0.18	0.85	NTU	155.47	764.45	0.20	0.84
	Salinity	-562.79	305.63	-1.84	0.07	Salinity	-1,472.98	825.51	-1.78	0.08
Pseudodiaptomus spp. Juveniles	Intercept	25.15	4.71	5.34	9.51^{-8}	Intercept	25.36	4.74	5.35	8.95^{-08}
	Summer Mean	-0.55	0.31	-1.74	0.08	Summer Mean	-0.53	0.31	-1.70	0.09
	Temperature	-3.57	1.21	-2.95	0.003	Temperature	-3.62	1.23	-2.95	0.003
	NTU	-0.47	0.22	-2.13	0.03	NTU	-0.46	0.22	-2.05	0.04
	Salinity	-2.08	0.23	-8.85	8.42^{-19}	Salinity	-2.07	0.24	-8.70	3.39^{-18}
Limnoithona spp.	Intercept	1618.94	341.27	4.74	0.00	Intercept	211.91	73.16	2.90	0.005
	Summer Mean	7.57	3.77	2.01	**0.05**	Summer Mean	-0.81	6.13	-0.13	0.90
	Temperature	-478.28	114.73	-4.17	0.00	Temperature	-65.55	20.70	-3.17	0.002
	NTU	-13.56	17.93	-0.76	0.45	NTU	6.14	3.40	1.81	0.07
	Salinity	-46.59	24.19	-1.93	0.06	Salinity	-10.97	3.94	-2.78	0.007

Response	Density (individuals/m3)					Biomass Density (µg/m3)				
	Term	Model Estimate	Standard Error	Z Statistic	P-value	Term	Model Estimate	Standard Error	Z Statistic	P-value
Sinocalanus doerri	Intercept	2,467.24	1,752.83	1.41	0.16	Intercept	-11.41	7.55	-1.51	0.13
	Summer Mean	-1.24	120.60	-0.01	0.99	Summer Mean	-2.58	0.86	-3.01	**0.003**
	Temperature	-773.22	509.69	-1.52	0.11	Temperature	6.86	2.40	2.86	0.004
	NTU	-13.91	91.45	-0.15	0.88	NTU	3.09	1.46	2.12	0.03
	Salinity	-30.05	101.12	-0.30	0.77	Salinity	-0.25	0.25	-0.99	0.32

Notes: Significant changes ($P < 0.05$) in prey are bolded. Intercept line is for response variables at average summer mean temperature, turbidity (NTU) and salinity.

Table 9-2. Comparisons of Delta Smelt Prey (A.) Density (individuals/m3) and (B.) Biomass (µg/m3) Across Regions in Summer of 2017

Prey	Region	Mean	Standard Error	Lower CI	Upper CI	Sample Size	Group			
A.										
Total Prey Kruskal-Wallis $X^2 = 64.4$, df = 7, $p < 0.0001$	Western	1020	281	459	1581	71		b		
	Suisun Bay	9891	1267	7385	12398	131	a			
	Suisun Marsh	7053	1700	3571	10534	29	a			
	Southern Delta	12143	1408	9350	14936	102	a			
	Lower San Joaquin	17060	2667	11765	22355	97	a			
	Lower Sacramento	14675	3297	8091	21260	66	a			
	Cache Slough	8890	3042	2668	15111	30	a	b		
	Sacramento Shipping Channel	12034	2292	7436	16633	53	a			
Pseudodiaptomus forbesi Adult	Western	157	59	38	275	71			c	
	Suisun Bay	1275	179	920	1630	131		b		
	Suisun Marsh	3794	914	1923	5665	29	a	b		
	Southern Delta	1788	175	1441	2135	102	a	b		
Kruskal-Wallis $X^2 = 74.21$, df = 7, $p < 0.0001$	Lower San Joaquin	1212	138	938	1486	97	a	b		
	Lower Sacramento	2399	411	1578	3220	66	a	b		
	Cache Slough	5779	1525	2661	8897	30	a			
	Sacramento Shipping Channel	3154	871	1407	4901	53	a	b		

Tables

Prey	Region	Mean	Standard Error	Lower CI	Upper CI	Sample Size	Group			
Pseudodiaptomus spp. Juveniles	Western	599	200	200	999	71				d
	Suisun Bay	4843	774	3311	6374	131			c	
	Suisun Marsh	3224	820	1544	4904	29		b	c	
	Southern Delta	4534	467	3607	5461	102	a	b	c	
Kruskal-Wallis X^2 = 75.4, df = 7, p < 0.0001	Lower San Joaquin	4894	380	4141	5647	97	a	b		
	Lower Sacramento	6337	1027	4286	8388	66	a	b	c	
	Cache Slough	13876	2074	9635	18117	30	a			
	Sacramento Shipping Channel	8569	1389	5781	11357	53	a	b		
Limnoithona spp. Kruskal-Wallis X^2 = 189.5, df = 7, p < 0.0001	Western	273	84	106	441	71		b		d
	Suisun Bay	1318	330	665	1972	131			c	
	Suisun Marsh	63	15	31	94	29			c	d
	Southern Delta	0	0	0	0	102	a			
	Lower San Joaquin	41	27	-13	95	97	a			
	Lower Sacramento	6	2	3	10	66	a	b		
	Cache Slough	0	0	0	1	30	a			
	Sacramento Shipping Channel	8	3	1	14	53	a	b		
Sinocalanus doerri Kruskal-Wallis X^2 = 70.7, df = 7, p < 0.0001	Western	62	27	9	115	71			c	
	Suisun Bay	198	27	145	251	131	a			
	Suisun Marsh	305	89	122	487	29	a	b		
	Southern Delta	50	6	38	62	102	a			
	Lower San Joaquin	267	54	160	374	97	a			
	Lower Sacramento	296	72	153	440	66	a			
	Cache Slough	72	17	38	107	30	a	b	c	
	Sacramento Shipping Channel	1328	224	879	1778	53		b		
B.										
Total Prey Kruskal-Wallis X^2 = 66.5, df = 7, p < 0.0001	Western	4314	837	2596	6032	28			c	
	Suisun Bay	13191	1570	10076	16305	102		b		
	Suisun Marsh	16645	3822	8740	24551	24	a	b		
	Southern Delta	13103	1002	11107	15098	78	a	b		
	Lower San Joaquin	12789	1024	10740	14838	60	a	b		
	Lower Sacramento	17681	2900	11847	23514	48	a	b		
	Cache Slough	34201	10250	11642	56761	12	a			

Prey	Region	Mean	Standard Error	Lower CI	Upper CI	Sample Size	Group			
	Sacramento Shipping Channel	27546	4853	17684	37409	35	a			
Pseudodiaptomus forbesi Adult Kruskal-Wallis X^2 = 74.2, df = 7, p < 0.0001	Western	417	252	-100	934	28			c	
	Suisun Bay	3393	541	2320	4467	102		b		
	Suisun Marsh	10099	2673	4570	15629	24	a	b		
	Southern Delta	4758	532	3699	5818	78	a	b		
	Lower San Joaquin	3225	467	2290	4160	60	a	b		
	Lower Sacramento	6384	1283	3803	8966	48	a	b		
	Cache Slough	15383	6417	1259	29507	12	a			
	Sacramento Shipping Channel	8396	2852	2600	14192	35	a	b		
Pseudodiaptomus spp. Juveniles Kruskal-Wallis X^2 = 75.4, df = 7, p < 0.0001	Western	750	399	-69	1570	28				d
	Suisun Bay	6063	1099	3883	8242	102			c	
	Suisun Marsh	4036	1129	1701	6371	24		b	c	
	Southern Delta	5676	669	4344	7008	78	a	b	c	
	Lower San Joaquin	6127	604	4918	7336	60	a	b		
	Lower Sacramento	7934	1508	4901	10967	48	a	b	c	
	Cache Slough	17373	4105	8337	26408	12	a			
	Sacramento Shipping Channel	10728	2141	6378	15078	35	a	b		
Limnoithona spp. Kruskal-Wallis X^2 = 233.4, df = 7, p < 0.0001	Western	111	30	50	173	28		b		
	Suisun Bay	226	55	115	336	102		b		
	Suisun Marsh	10	2	6	15	24		b		
	Southern Delta	0	0	0	0	78	a			
	Lower San Joaquin	9	6	-3	21	60	a			
	Lower Sacramento	1	0	1	2	48	a			
	Cache Slough	0	0	0	0	12	a			
	Sacramento Shipping Channel	2	1	0	3	35	a			
Sinocalanus doerri Kruskal-Wallis X^2 = 71.4, df = 7, p < 0.0001	Western	121	83	-49	290	28			c	
	Suisun Bay	428	62	304	552	102	a			
	Suisun Marsh	896	298	280	1513	24	a	b		

Tables

Prey	Region	Mean	Standard Error	Lower CI	Upper CI	Sample Size	Group			
	Southern Delta	136	19	98	174	78	a			
	Lower San Joaquin	624	163	298	951	60	a			
	Lower Sacramento	923	278	363	1482	48	a			
	Cache Slough	234	88	40	427	12	a	b	c	
	Sacramento Shipping Channel	3472	736	1976	4968	35		b		

Notes: Group column shows results from Dunn's Kruskal-Wallis Multiple Comparison post-hoc test, with significant differences ($P \leq 0.05$) represented by regions not sharing a letter. Group membership is comparable within, not across, a prey item.

Table 9-3. Comparisons of Delta Smelt Prey (A.) Density (individuals/m^3) and (B.) Biomass (µg/m^3) Across Regions in Fall of 2017

Prey	Region	Mean	Standard Error	Lower CI	Upper CI	Sample Size	Group				
A.											
Total Prey (X^2 = 56.9, df = 8, p-value = 1.9^{-9})	Western	1045	402	229	1861	35					e
	Suisun Bay	2734	361	2019	3449	110	a	b	c	d	
	Suisun Marsh	4367	1072	2171	6562	29	a	b	c	d	e
	Southern Delta	5161	1867	1379	8943	38			c	d	e
	Lower San Joaquin	4350	808	2733	5968	57	a		c	d	e
	Lower Sacramento	9379	3477	2418	16340	59	a	b			
	Cache Slough	8836	1735	5315	12358	36	a	b	c		
	Sacramento Shipping Channel	14574	3315	7874	21275	41		b			
	Upper Sacramento	500	149	196	804	31				d	e
Pseudodiaptomus forbesi Adult (X^2= 128.2, df = 8, p-value < 2.2^{-16})	Western	27	5	16	37	35			c	d	
	Suisun Bay	332	124	86	578	110			c		
	Suisun Marsh	338	153	25	651	29		b	c	d	
	Southern Delta	634	98	435	832	38	a	b	c		
	Lower San Joaquin	2095	186	1722	2468	57	a				
	Lower Sacramento	2355	516	1321	3389	59	a				
	Cache Slough	2858	504	1834	3881	36	a				
	Sacramento Shipping Channel	2490	652	1173	3807	41	a	b			

Prey	Region	Mean	Standard Error	Lower CI	Upper CI	Sample Size	Group				
	Upper Sacramento	11	4	2	19	31				d	
Pseudodiaptomus spp. Juveniles (X^2= 163.3, df = 8, p-value < 2.2^{-16})	Western	170	26	118	222	35		b	c	d	
	Suisun Bay	332	78	177	487	110			c		
	Suisun Marsh	418	151	109	726	29			c	d	
	Southern Delta	9403	1428	6510	12296	38	a				
	Lower San Joaquin	5419	452	4514	6323	57	a				
	Lower Sacramento	3652	1268	1114	6190	59	a				
	Cache Slough	4345	458	3416	5274	36	a				
	Sacramento Shipping Channel	2661	352	1950	3371	41	a	b			
	Upper Sacramento	15	5	5	26	31				d	
Limnoithona spp. (X^2= 124.7, df = 8, p-value < 2.2^{-16})	Western	26	13	1	51	35	a			d	
	Suisun Bay	73	12	48	97	110			c		
	Suisun Marsh	200	77	43	356	29		b	c		
	Southern Delta	0	0	0	0	38				d	
	Lower San Joaquin	5	2	2	8	57	a			d	
	Lower Sacramento	15	4	7	22	59	a	b			
	Cache Slough	110	45	19	201	36	a	b	c		
	Sacramento Shipping Channel	207	49	108	307	41			c		
	Upper Sacramento	9	9	-9	27	31	a			d	
Sinocalanus doerri (X^2= 139.1, df = 8, p-value < 2.2^{-16})	Western	0	0	0	0	35			c		
	Suisun Bay	46	40	-32	125	110			c		
	Suisun Marsh	1	1	-1	3	29			c		
	Southern Delta	30	5	20	39	38	a	b			
	Lower San Joaquin	22	9	5	39	57	a				
	Lower Sacramento	8	2	4	12	59	a		c		
	Cache Slough	170	64	40	299	36	a	b			
	Sacramento Shipping Channel	1353	273	802	1905	41		b			
	Upper Sacramento	3	1	1	5	31	a		c		

Tables

Prey	Region	Mean	Standard Error	Lower CI	Upper CI	Sample Size	Group				
B.											
Total Prey (X^2 = 89.2, df = 8, p-value = 6.8^{-16})	Western	5086	1890	925	9246	12		b	c	d	
	Suisun Bay	5013	721	3580	6447	83			c		
	Suisun Marsh	6065	1205	3566	8563	23		b	c		
	Southern Delta	16031	4179	6833	25228	12	a	b	c		
	Lower San Joaquin	13473	1380	10624	16321	25	a				
	Lower Sacramento	11996	3535	4881	19111	47	a	b	c		
	Cache Slough	15315	2234	10654	19976	21	a				
	Sacramento Shipping Channel	24958	6276	12102	37813	29	a	b			
	Upper Sacramento	1922	1183	-562	4407	19				d	
Pseudodiaptomus forbesi Adult (X^2= 126.8, df = 8, p-value < 2.2^{-16})	Western	69	23	18	120	12			c	d	
	Suisun Bay	893	383	131	1655	83			c		
	Suisun Marsh	900	457	-48	1847	23		b	c	d	
	Southern Delta	1686	464	665	2707	12	a	b	c		
	Lower San Joaquin	5576	749	4029	7122	25	a				
	Lower Sacramento	6268	1540	3168	9368	47	a				
	Cache Slough	7606	1757	3941	11272	21	a				
	Sacramento Shipping Channel	6212	2049	2014	10409	29	a	b			
	Upper Sacramento	19	11	-5	42	19				d	
Pseudodiaptomus spp. Juveniles (X^2= 161.3, df = 8, p-value < 2.2^{-16})	Western	213	55	93	333	12		b	c	d	
	Suisun Bay	420	113	195	646	83			c		
	Suisun Marsh	523	212	84	961	23			c	d	
	Southern Delta	11773	3181	4771	18774	12	a				
	Lower San Joaquin	6784	854	5022	8546	25	a				
	Lower Sacramento	4572	1778	992	8152	47	a				
	Cache Slough	5440	750	3875	7005	21	a				
	Sacramento Shipping Channel	3409	535	2313	4505	29	a	b			
	Upper Sacramento	15	7	-1	30	19				d	

Prey	Region	Mean	Standard Error	Lower CI	Upper CI	Sample Size	Group				
Limnoithona spp. (X^2= 103.9, df = 8, p-value < 2.2^{-16})	Western	13	7	-2	28	12	a	b		d	
	Suisun Bay	10	2	6	14	83		b			
	Suisun Marsh	26	13	0	52	23		b			
	Southern Delta	0	0	0	0	12			c		
	Lower San Joaquin	1	0	1	2	25	a		c	d	
	Lower Sacramento	2	0	1	3	47	a		c	d	
	Cache Slough	24	10	4	44	21	a	b			
	Sacramento Shipping Channel	33	8	16	49	29		b			
	Upper Sacramento	2	2	-2	6	19			c	d	
Sinocalanus doerri (X^2= 133.2, df = 8, p-value < 2.2^{-16})	Western	0	0	0	0	12			c		
	Suisun Bay	155	152	-148	457	83			c		
	Suisun Marsh	3	3	-3	9	23			c		
	Southern Delta	71	22	23	118	12	a	b			
	Lower San Joaquin	67	44	-24	158	25	a				
	Lower Sacramento	19	6	7	31	47	a		c		
	Cache Slough	458	243	-50	965	21	a	b			
	Sacramento Shipping Channel	3534	975	1538	5530	29		b			
	Upper Sacramento	9	4	0	17	19	a		c		

Notes: Group column shows results from Dunn's Kruskal-Wallis Multiple Comparison post-hoc test, with significant differences ($P \leq 0.05$) represented by regions not sharing a letter. Group membership is comparable within, not across, a prey item.

Tables

Table 9-4. Analysis of Variance Output Comparing Water Temperature and Turbidity Across Years for Suisun Bay (A) and for All Regions Combined (B)

	Year	Mean	LCL	UCL	Group		Year	Mean	LCL	UCL	Group
A											
Summer Turbidity	2010	41.8	36.9	46.8	cd	Summer Temperature	2010	19.9	19.7	20.1	a
	2011	31.7	26.8	36.6	ab		2011	20.5	20.3	20.7	b
	2012	25.1	20.2	29.9	a		2012	20.8	20.6	20.9	bc
	2013	44.2	39.4	49.0	cd		2013	20.8	20.7	21.0	cd
	2014	38.9	33.9	43.9	bc		2014	21.1	21.0	21.3	de
	2015	27.0	22.1	31.9	a		2015	21.4	21.2	21.6	e
	2016	47.6	42.7	52.4	d		2016	21.2	21.0	21.4	e
	2017	38.7	33.9	43.5	bc		2017	21.4	21.2	21.6	e
Fall Turbidity	2010	17.8	9.8	25.8	a	Fall Temperature	2010	18.9	18.2	19.6	a
	2011	54.3	49.0	59.6	b		2011	19.3	18.8	19.7	ab
	2012	21.7	16.4	27.0	a		2012	19.0	18.5	19.5	a
	2013	23.5	18.3	28.7	a		2013	20.3	19.8	20.8	cd
	2014	21.9	16.6	27.3	a		2014	20.9	20.4	21.4	d
	2015	19.4	13.8	25.0	a		2015	20.9	20.4	21.4	d
	2016	17.7	12.3	23.0	a		2016	18.7	18.3	19.2	a
	2017	23.7	20.0	27.3	a		2017	19.8	19.5	20.1	bc
B											
Summer Turbidity	2010	25.4	22.9	27.9	c	Summer Temperature	2010	21.0	20.8	21.2	a
	2011	22.2	19.8	24.5	bc		2011	21.1	21.0	21.3	a
	2012	17.4	15.1	19.8	a		2012	21.5	21.3	21.7	b
	2013	25.7	23.4	28.0	c		2013	21.7	21.5	21.9	b
	2014	20.6	18.3	23.0	ab		2014	22.4	22.2	22.6	d
	2015	18.8	16.4	21.1	ab		2015	22.4	22.3	22.6	d
	2016	29.7	27.4	32.0	d		2016	22.2	22.0	22.4	cd
	2017	25.3	23.8	26.8	c		2017	22.0	21.9	22.2	c
Fall Turbidity	2010	18.0	10.9	25.0	ab	Fall Temperature	2010	19.0	18.1	19.9	a
	2011	32.5	29.5	35.5	c		2011	19.6	19.2	19.9	a
	2012	15.6	12.6	18.5	ab		2012	19.7	19.3	20.0	a
	2013	18.1	15.2	21.1	b		2013	20.4	20.1	20.8	b
	2014	15.3	12.3	18.3	ab		2014	21.5	21.1	21.8	c
	2015	14.4	11.3	17.4	ab		2015	21.7	21.3	22.1	c
	2016	13.2	10.2	16.2	a		2016	19.4	19.0	19.7	a
	2017	15.7	14.4	17.1	a		2017	19.5	19.1	19.6	bc

Notes: Group column shows results from Bonferroni Multiple Comparison Procedure, with significant differences ($P \leq 0.05$) represented by regions not sharing a letter. Group membership is comparable within, not across, seasons or water quality variables. Wet years are shaded.

Table 9-5. Comparisons of Water Temperature, Turbidity and Salinity Across Regions in the Summer of 2017

	Region	Mean	Standard Error	Lower CI	Upper CI	Count	Group			
Temperature (°C) X^2 = 266.5, df = 8, $P < 2.2^{-16}$	Western	19.9	0.2	19.6	20.2	71				d
	Suisun Bay	21.3	0.1	21.2	21.5	131	a			
	Suisun Marsh	21.6	0.2	21.2	21.9	29	a			
	Southern Delta	23.2	0.2	22.8	23.5	102		b	c	
	Lower San Joaquin	23.0	0.1	22.7	23.2	97		b	c	
	Lower Sacramento	21.7	0.2	21.2	22.1	66	a			
	Cache Slough	21.4	0.8	19.9	23.0	30	a	b		
	Sacramento Shipping Channel	23.8	0.3	23.2	24.3	53			c	
	Upper Sacramento	22.3	0.1	22.0	22.6	18	a	b	c	
Turbidity (NTU) X^2 = 298.2, df = 8, $P < 2.2^{-16}$	Western	49.2	4.3	40.6	57.8	71			c	
	Suisun Bay	34.1	1.8	30.6	37.6	131			c	
	Suisun Marsh	59.4	4.5	50.1	68.7	29			c	
	Southern Delta	9.9	0.4	9.2	10.6	102		b		
	Lower San Joaquin	13.2	0.6	12.0	14.4	97	a	b		
	Lower Sacramento	17.5	1.1	15.4	19.7	66	a			
	Cache Slough	14.3	1.7	10.7	17.8	30	a	b		
	Sacramento Shipping Channel	21.4	2.0	17.4	25.5	53	a			
	Upper Sacramento	12.2	1.2	9.7	14.6	18	a	b		
Salinity (ppt) X^2 = 378.7, df = 8, $P < 2.2^{-16}$	Western	14.2	0.5	13.2	15.3	71				d
	Suisun Bay	2.89	0.23	2.43	3.34	131			c	
	Suisun Marsh	2.41	0.31	1.77	3.04	29			c	d
	Southern Delta	0.06	0.00	0.06	0.07	102	a			
	Lower San Joaquin	0.17	0.03	0.12	0.23	97	a			
	Lower Sacramento	0.29	0.06	0.16	0.41	66	a			
	Cache Slough	0.11	0.02	0.07	0.15	30	a	b		
	Sacramento Shipping Channel	0.29	0.02	0.25	0.33	53		b		
	Upper Sacramento	0.10	0.03	0.04	0.15	18	a	b		

Notes: Group column shows results from Dunn's Kruskal-Wallis Multiple Comparison post-hoc test, with significant differences ($P \leq 0.05$) represented by regions not sharing a letter. Group membership is comparable within, not across, temperature (°C), turbidity (NTU), and salinity (ppt).

Table 9-6. Comparisons of Water Temperature, Turbidity and Salinity Across Regions in the Fall of 2017

	Region	Mean	Standard Error	Lower CI	Upper CI	Count	Group			
Temperature (°C) X^2 = 53.3, df = 8, $P = 9.6^{-9}$	Western	19.4	0.51	18.3	20.4	35	a	b		
	Suisun Bay	19.7	0.21	19.3	20.1	110				d
	Suisun Marsh	19.8	0.46	18.8	20.7	29	a		c	d
	Southern Delta	18.7	0.35	18.0	19.4	38	a		c	d
	Lower San Joaquin	19.6	0.34	18.9	20.3	57	a		c	d
	Lower Sacramento	18.7	0.28	18.1	19.2	59	a		c	
	Cache Slough	18.3	0.42	17.5	19.2	36	a	b		
	Sacramento Shipping Channel	20.3	0.43	19.4	21.1	41			c	d
	Upper Sacramento	17.1	0.31	16.5	17.8	31		b		d
Turbidity (NTU) X^2 = 173.8, f = 8, $P < 2.2^{-16}$	Western	20.4	2.00	16.4	24.5	35		b	c	
	Suisun Bay	23.0	1.78	19.5	26.6	110			c	
	Suisun Marsh	28.7	2.87	22.8	34.6	29			c	
	Southern Delta	5.9	0.61	4.7	7.1	38	a	b		
	Lower San Joaquin	10.6	0.68	9.3	12.0	57	a	b		
	Lower Sacramento	8.7	1.18	6.3	11.0	59	a			
	Cache Slough	7.3	1.48	4.2	10.3	36	a			
	Sacramento Shipping Channel	15.2	1.69	11.8	18.6	41		b	c	
	Upper Sacramento	5.2	0.70	3.8	6.6	31	a			
Salinity (ppt) X^2 = 363.9, df = 8, $< 2.2^{-16}$	Western	15.85	0.68	14.47	17.24	35				d
	Suisun Bay	4.71	0.24	4.24	5.18	110				d
	Suisun Marsh	4.94	0.44	4.04	5.84	29				d
	Southern Delta	0.09	0.01	0.07	0.10	38	a	b	c	
	Lower San Joaquin	0.22	0.04	0.13	0.30	57	a		c	
	Lower Sacramento	0.16	0.03	0.09	0.23	59	a	b		
	Cache Slough	0.09	0.01	0.07	0.11	36	a	b	c	
	Sacramento Shipping Channel	0.27	0.01	0.24	0.30	41			c	
	Upper Sacramento	0.05	0.00	0.05	0.05	31	b			

Notes: Group column shows results from Dunn's Kruskal-Wallis Multiple Comparison post-hoc test, with significant differences ($P \leq 0.05$) represented by regions not sharing a letter. Group membership is comparable within, not across, temperature (°C), turbidity (NTU), and salinity (ppt).

Figures

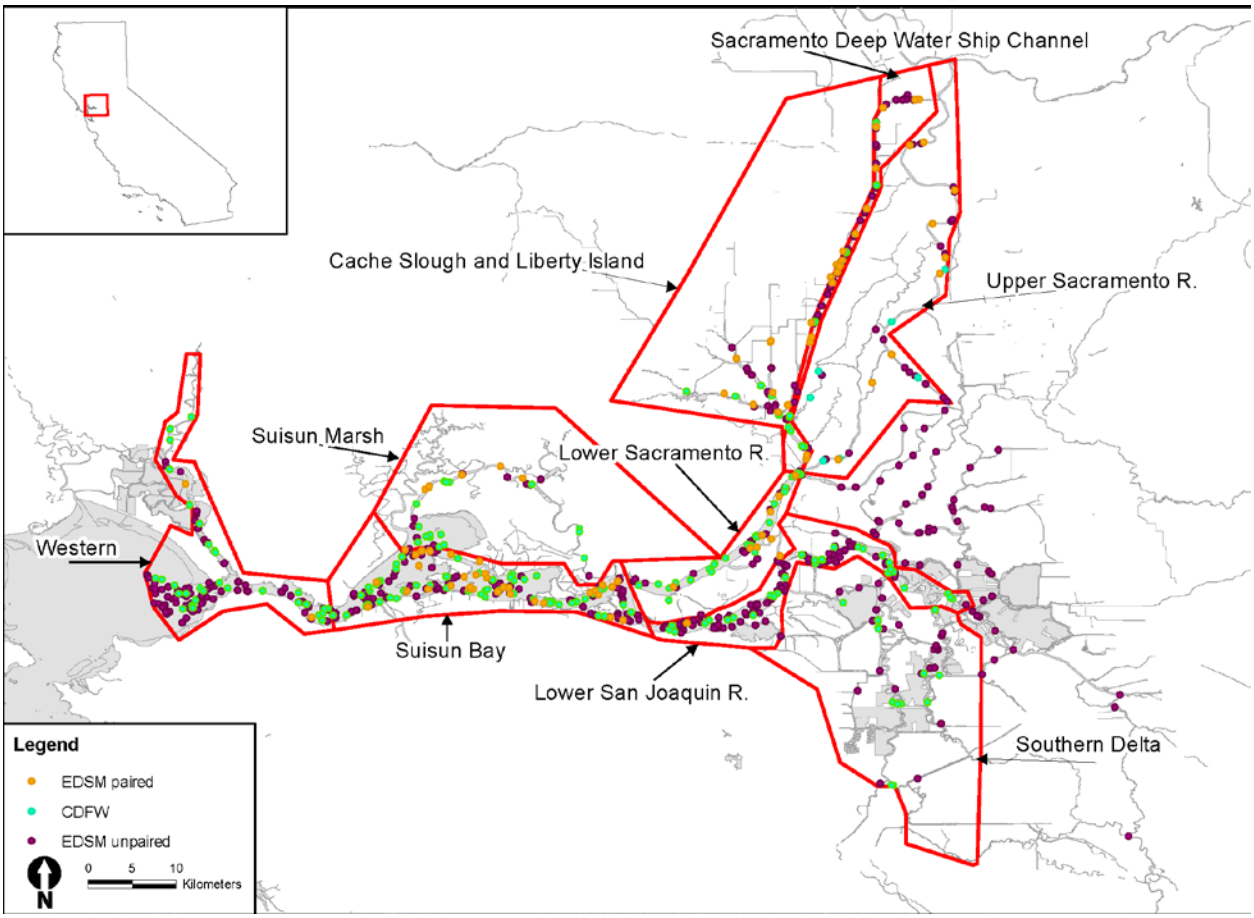
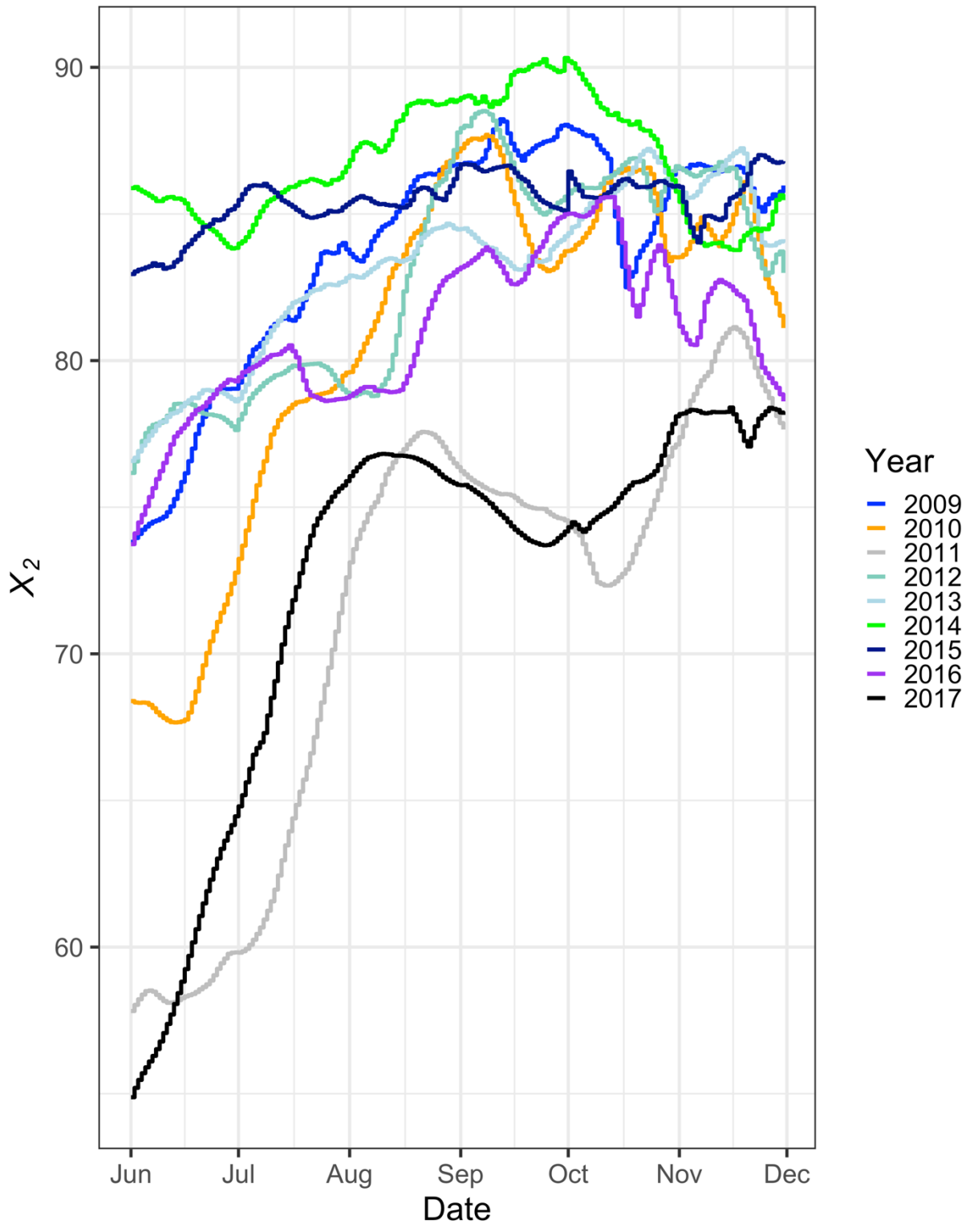
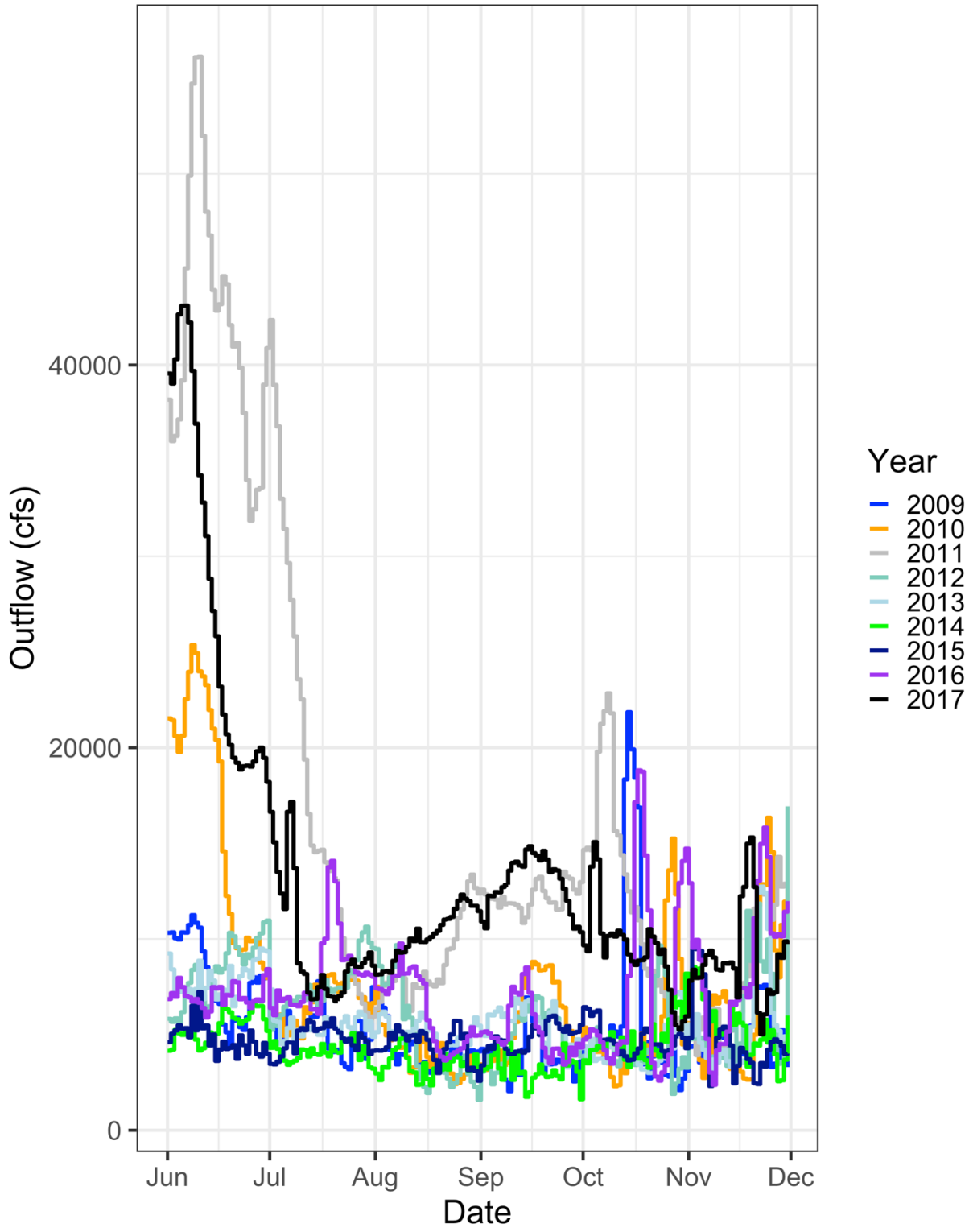


Figure 9-1. Map of the Study Area Depicting Polygons of the Regional Strata and Sampling Stations Used in Analyses



Note: The wet years are in grayscale and non-wet years are depicted in colors.

Figure 9-2. Profiles of X_2 from June to December from 2009 to 2017



Notes: Wet years are depicted in grayscale and other years are represented by colors.

Figure 9-3. Profiles of Freshwater Outflow (cfs) from 2009 to 2017

Figures

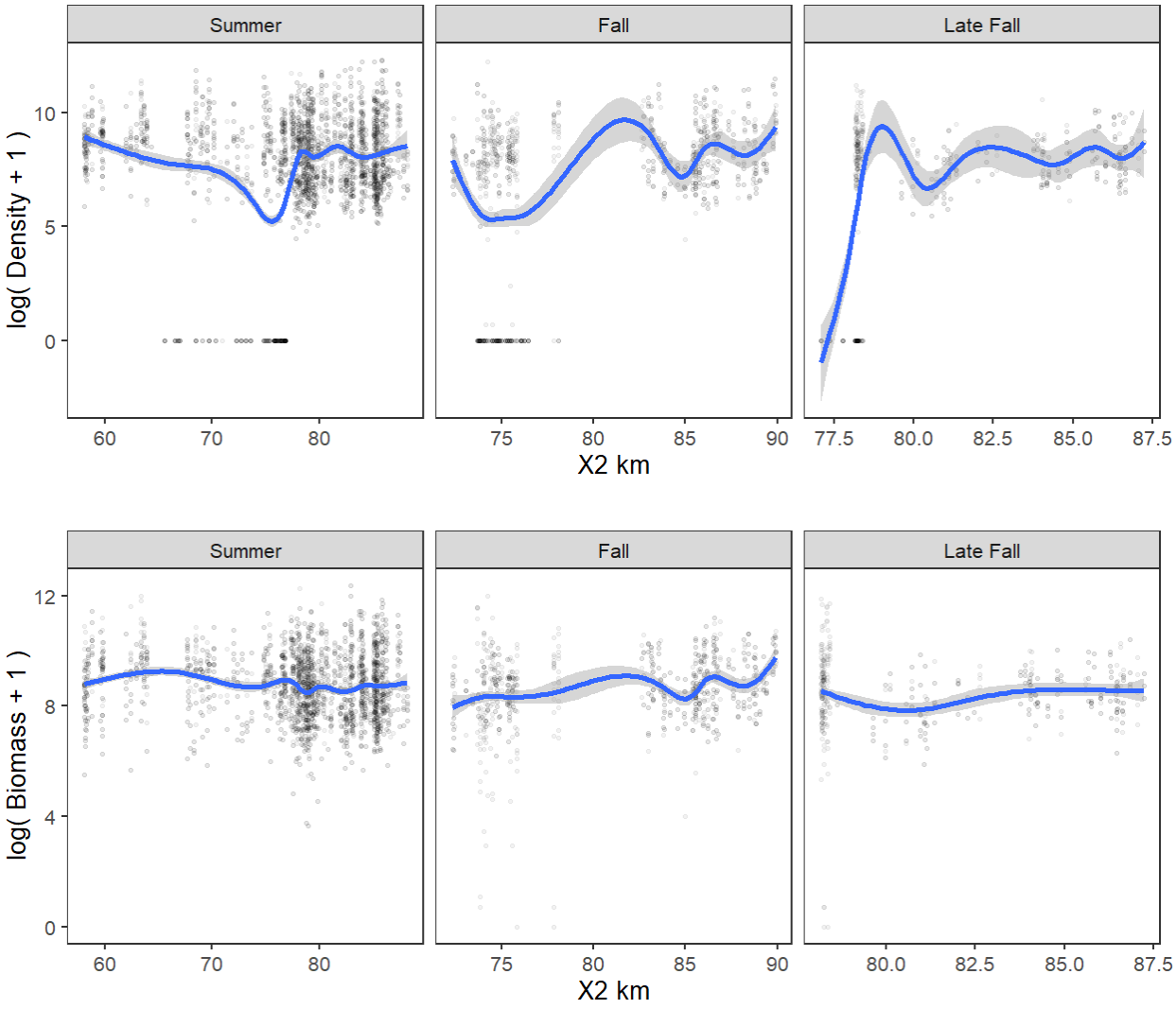


Figure 9-4. Total Prey Density (individuals/m^3; top) and Prey Biomass Density (µg/m^3; bottom) Along the X2 (isohaline gradient) of the San Francisco Estuary in the Summer, Fall and Late Fall from 2010 to 2017

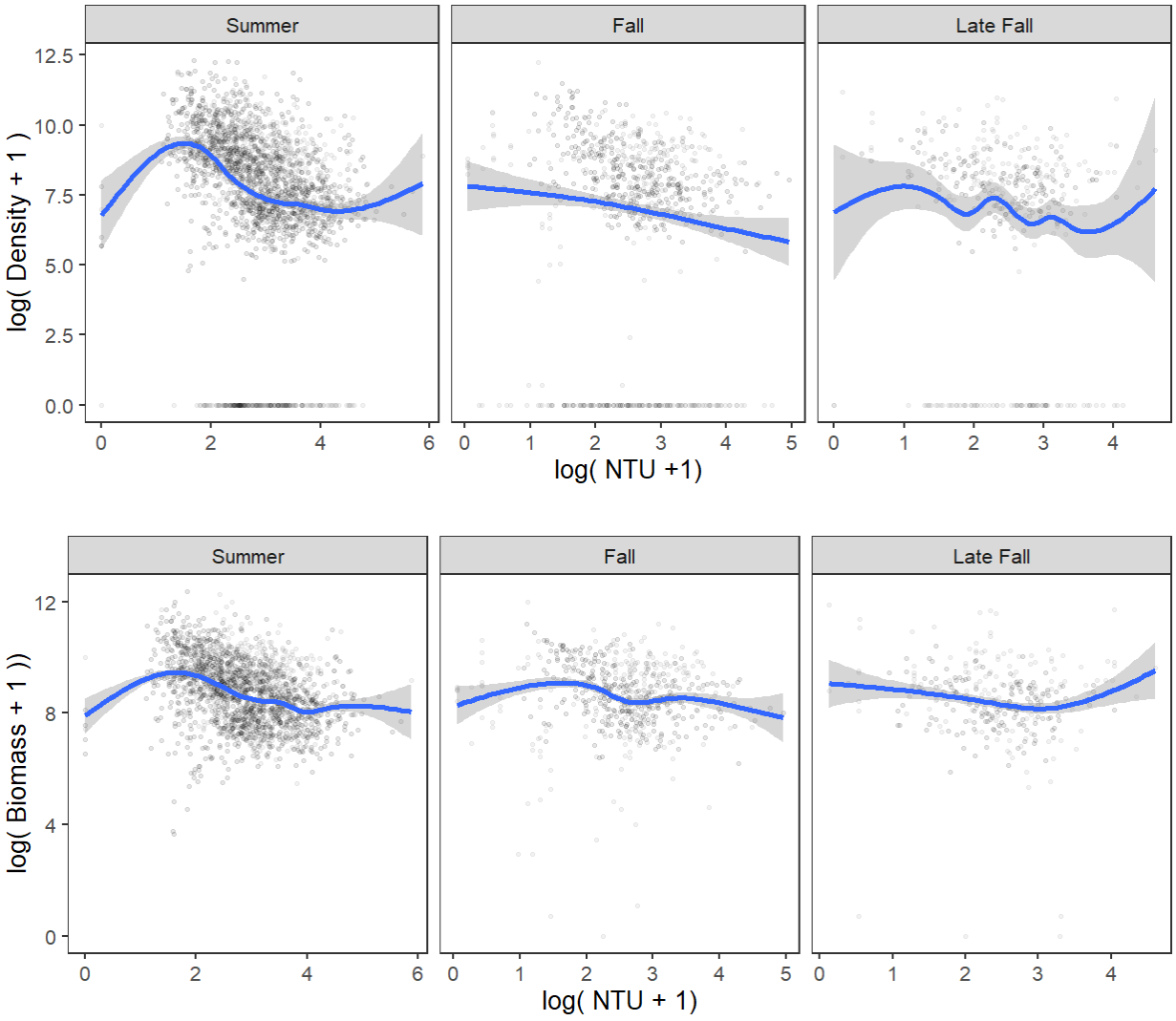


Figure 9-5. Total Prey Density (individuals/m^3; top) and Prey Biomass Density (µg/m^3; bottom) Along a Turbidity (NTU) Gradient of the San Francisco Estuary in the Summer, Fall and Late Fall from 2010 to 2017

Figures

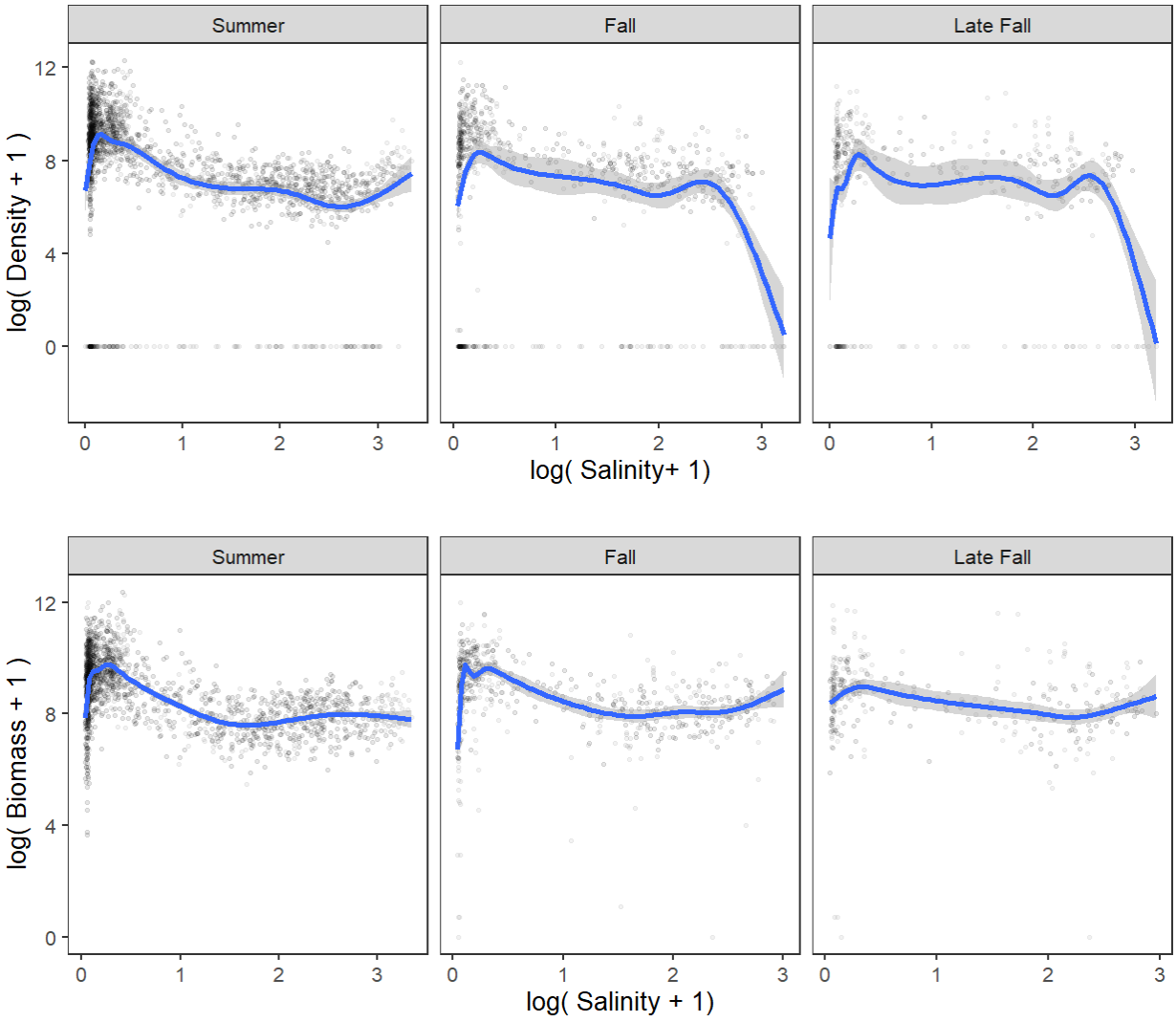
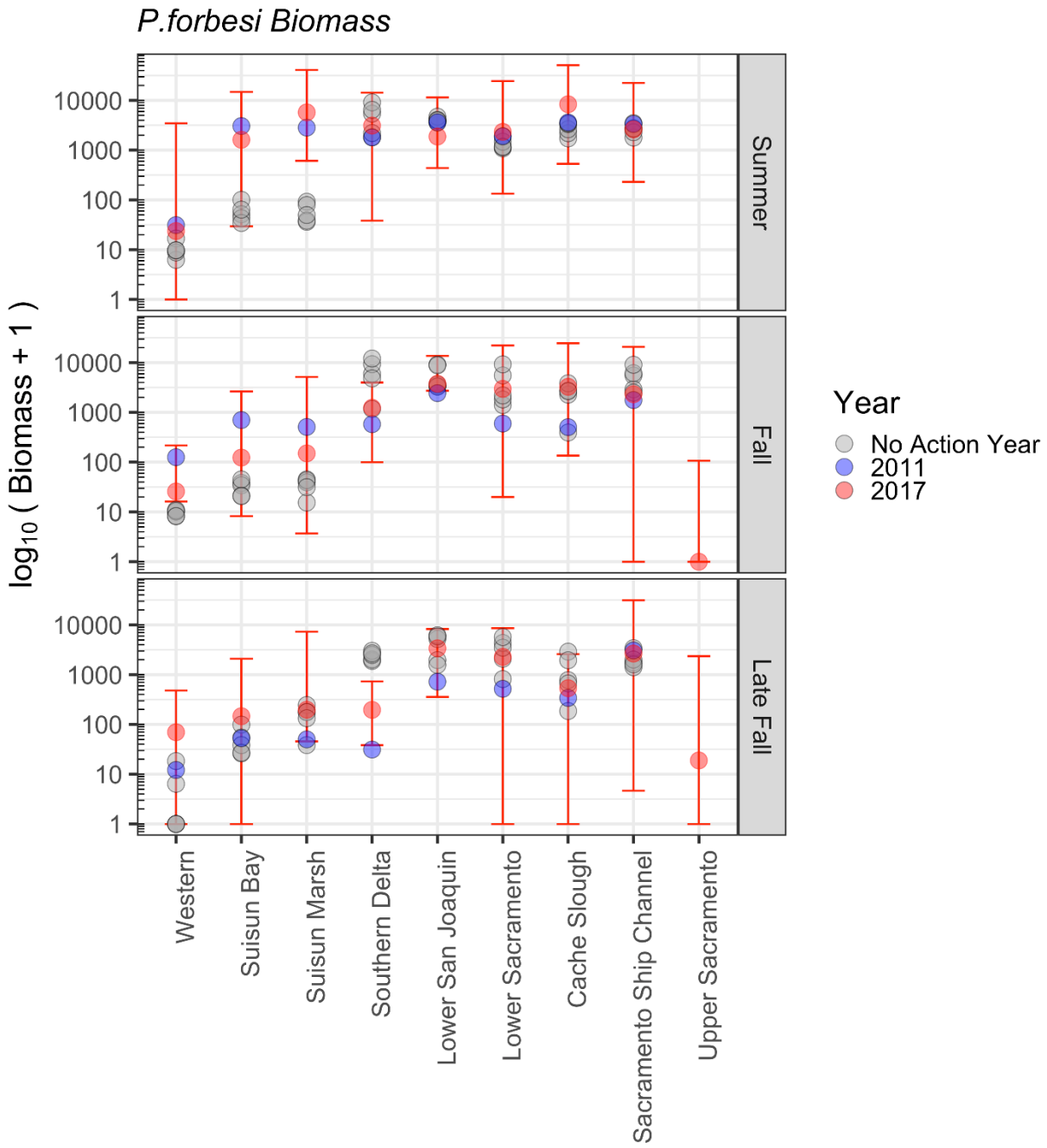


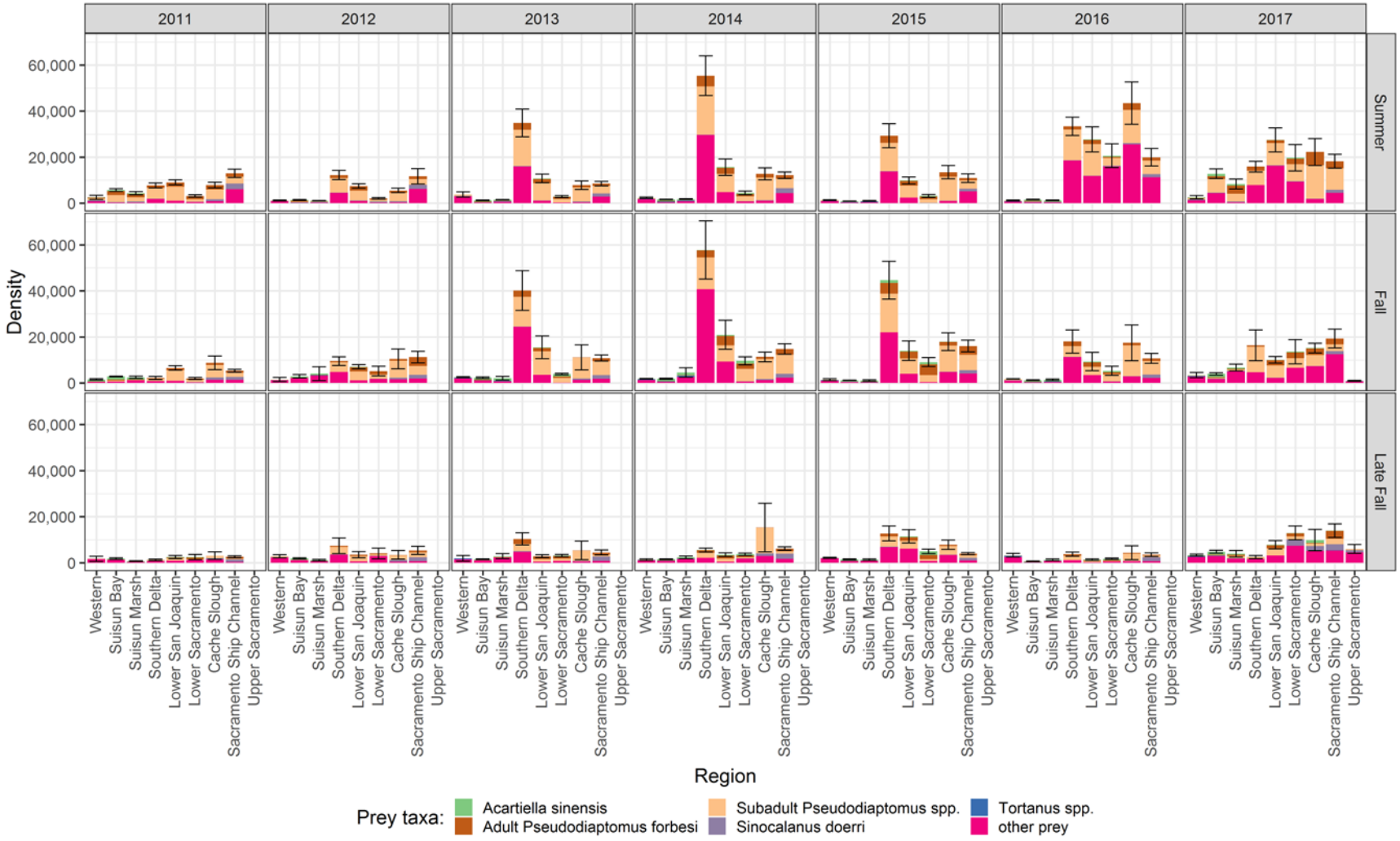
Figure 9-6. Total Prey Density (individuals/m^3; top) and Prey Biomass Density (µg/m^3; bottom) Along Salinity Gradient of the San Francisco Estuary in the Summer, Fall and Late Fall from 2010 to 2017

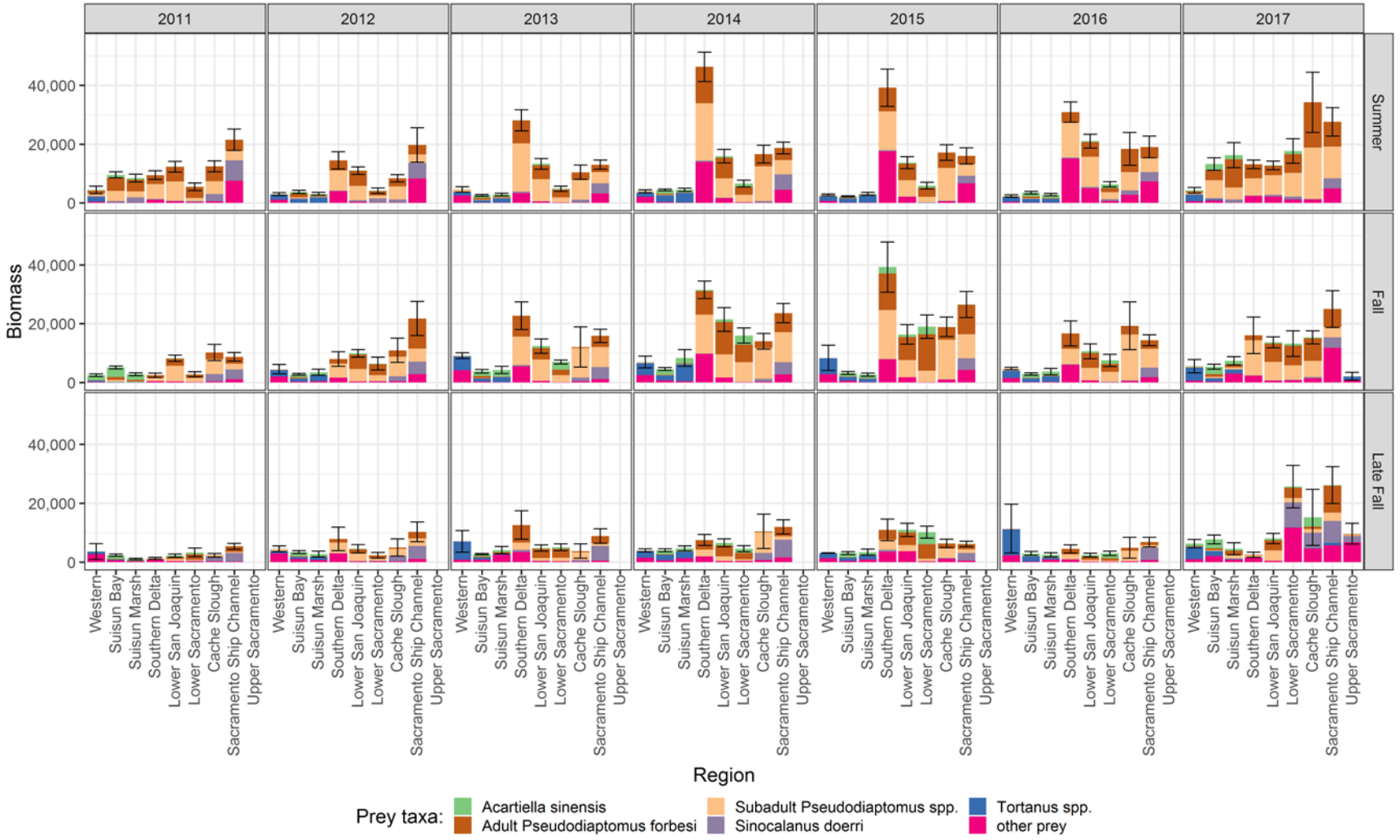


Notes: The blue points represent 2011 values and the red points represent 2017. The vertical red lines represent the 5th and 95th percentiles. Regions are ordered roughly from seaward (to the left) to landward (to the right).

Figure 9-7. Median Values (points) are Shown for the Logarithms of Adult *Pseudodiaptomus forbesi* Biomass Density (µg/m^3)

Figures

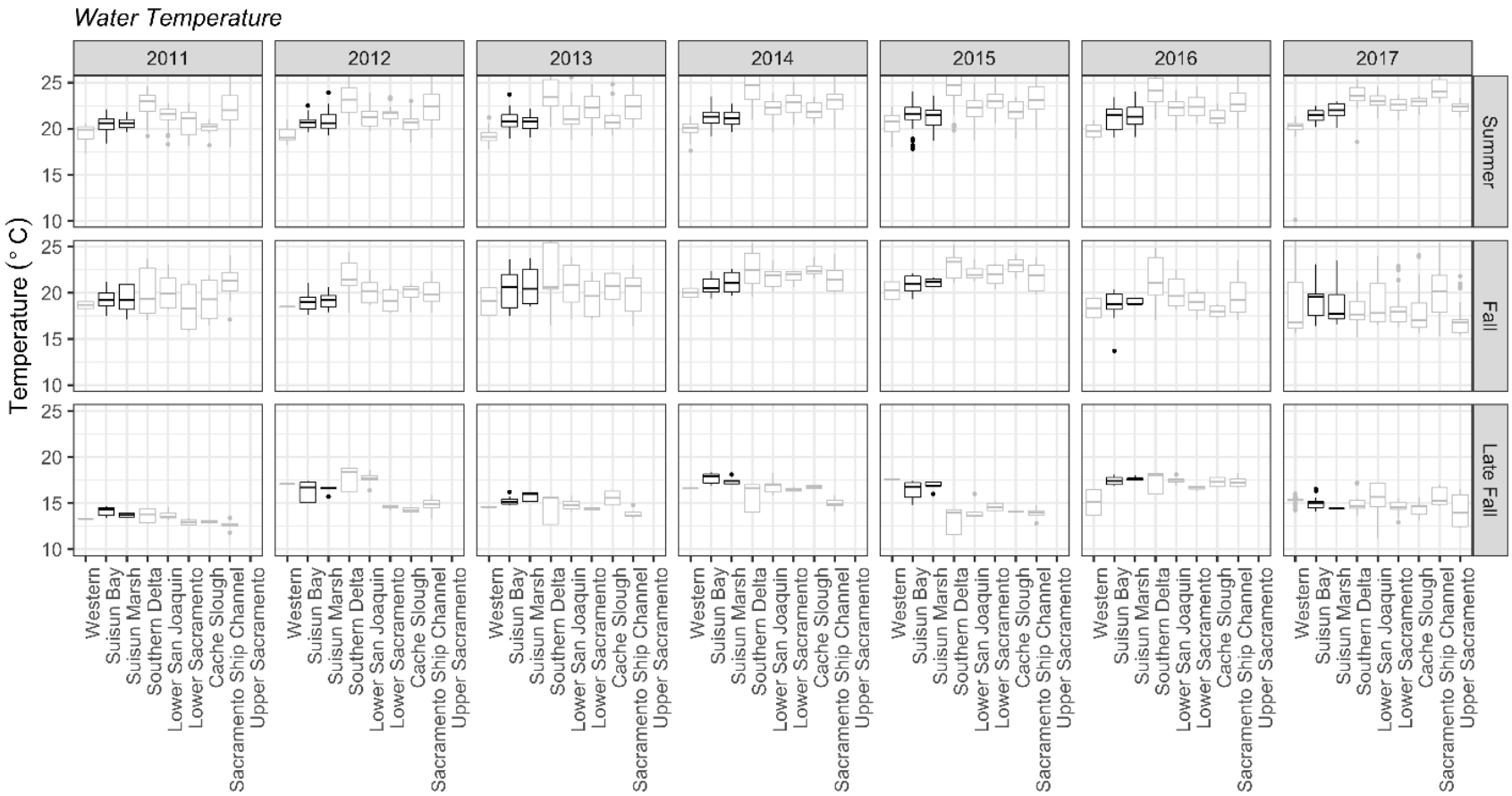

2011
2012
2013
2014
2015
2016
2017
Summer
Fall
Late Fall
Density
60,000
40,000
20,000
0
Western
Suisun Bay
Suisun Marsh
Southern Delta
Lower San Joaquin
Lower Sacramento
Cache Slough
Sacramento Ship Channel
Upper Sacramento
Region
Prey taxa:
Acartiella sinensis
Adult Pseudodiaptomus forbesi
Subadult Pseudodiaptomus spp.
Sinocalanus doerri
Tortanus spp.
other prey



Notes: (top) and Biomass Density (µg/m^3) (bottom) of Five of the Most Prominent Delta Smelt Prey Groups/Species Per Region from 2011 to 2017.

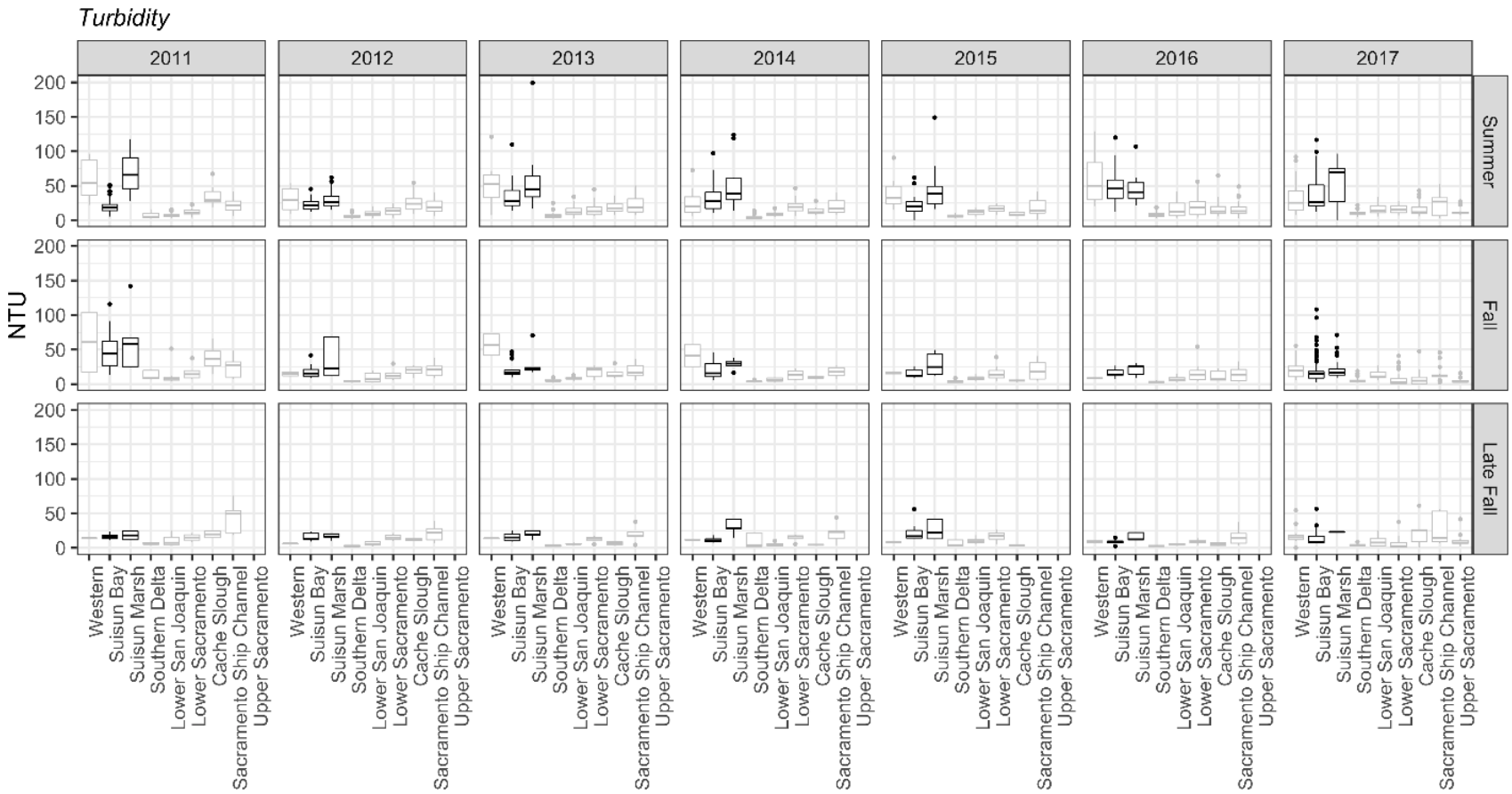
Figure 9-8. Summer, Fall, and late-Fall Mean Catch Density (individuals/m^3)

Figures



Notes: CDFW Surveys for 2011-2016 and EDSM and DOP Surveys for 2017.

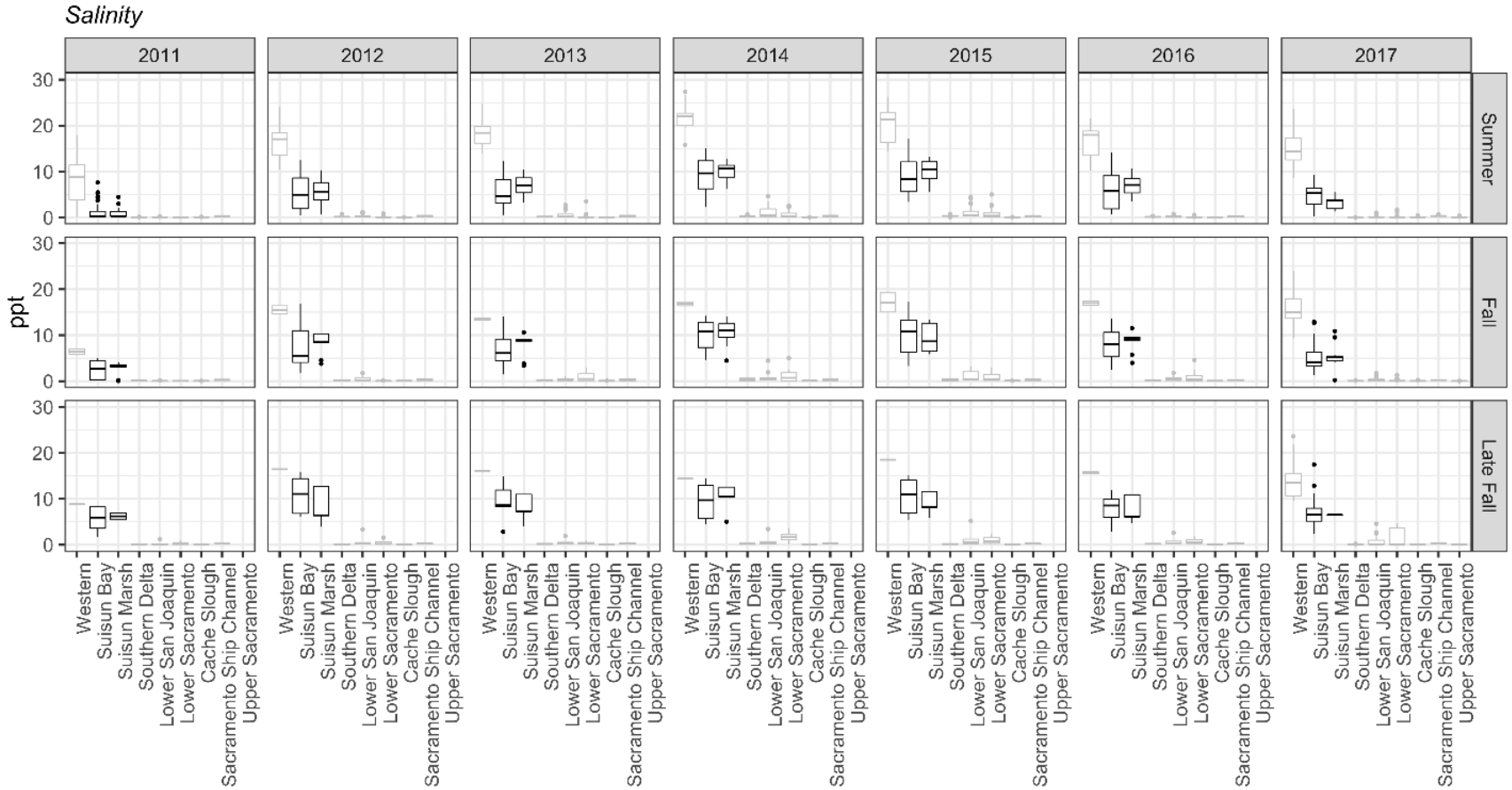
Figure 9-9. Variation in Water Temperature Across Years (2011-2017) for Summer, Fall and Late-Fall Periods



Notes: CDFW Surveys for 2011-2016 and EDSM and DOP Surveys for 2017.

Figure 9-10. Variation in Water Turbidity (NTU) Across Years (2011-2017) for Summer, Fall and Late-Fall Periods

Figures



Notes: CDFW Surveys for 2011-2016 and EDSM and DOP Surveys for 2017.

Figure 9-11. Variation in Water Salinity (ppt) Across Years (2011-2017) for Summer, Fall and Late-Fall Periods

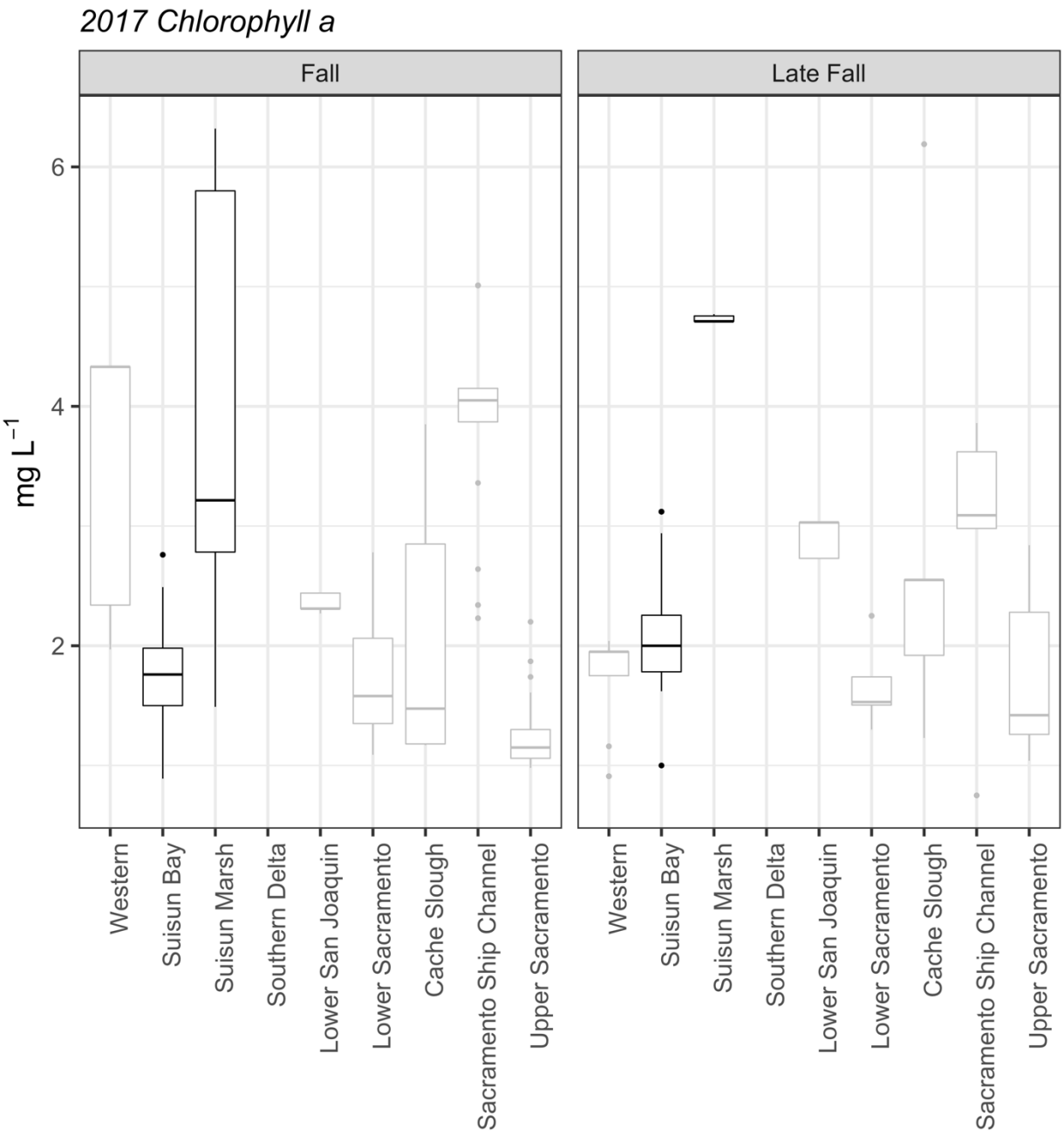
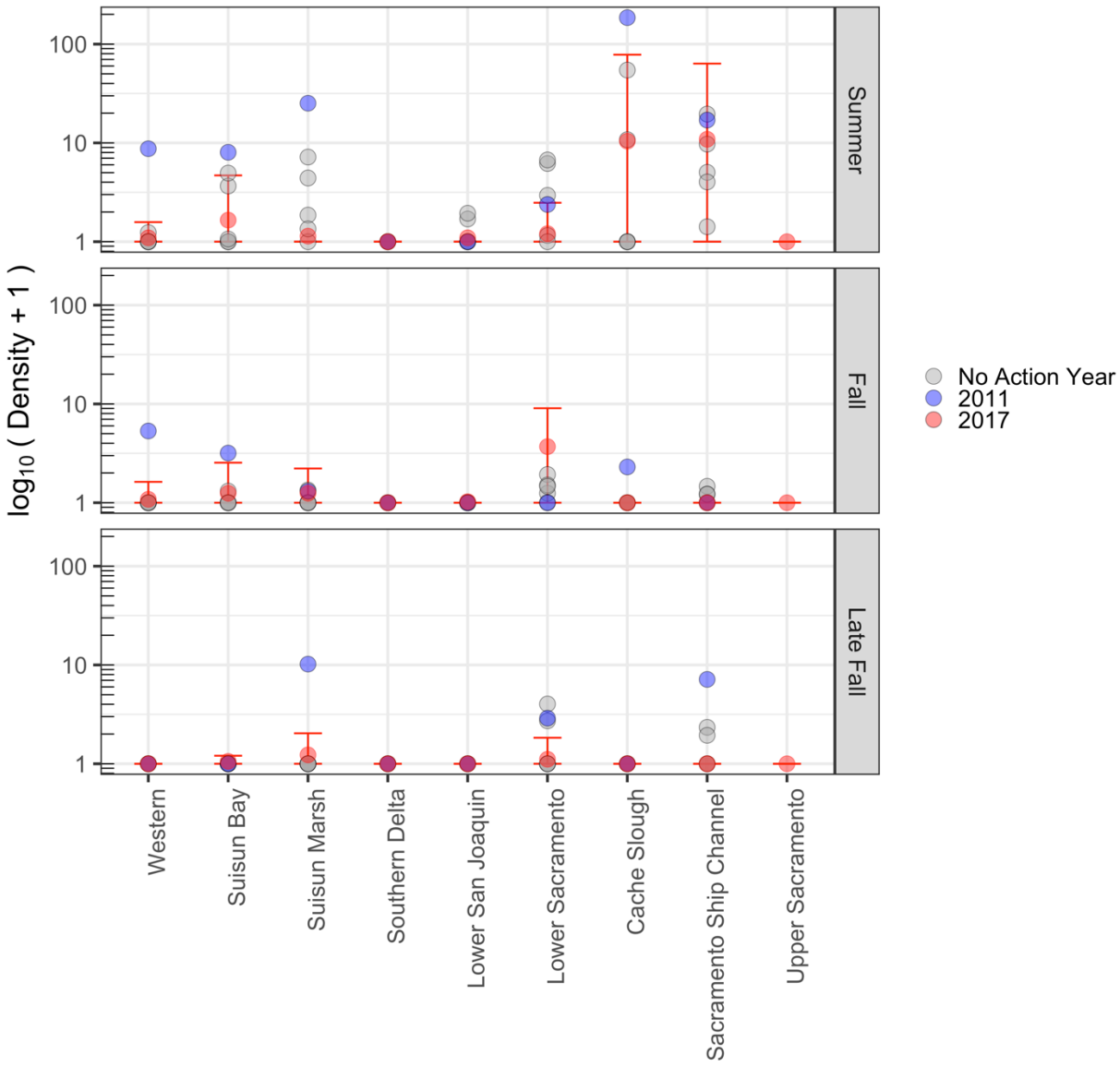


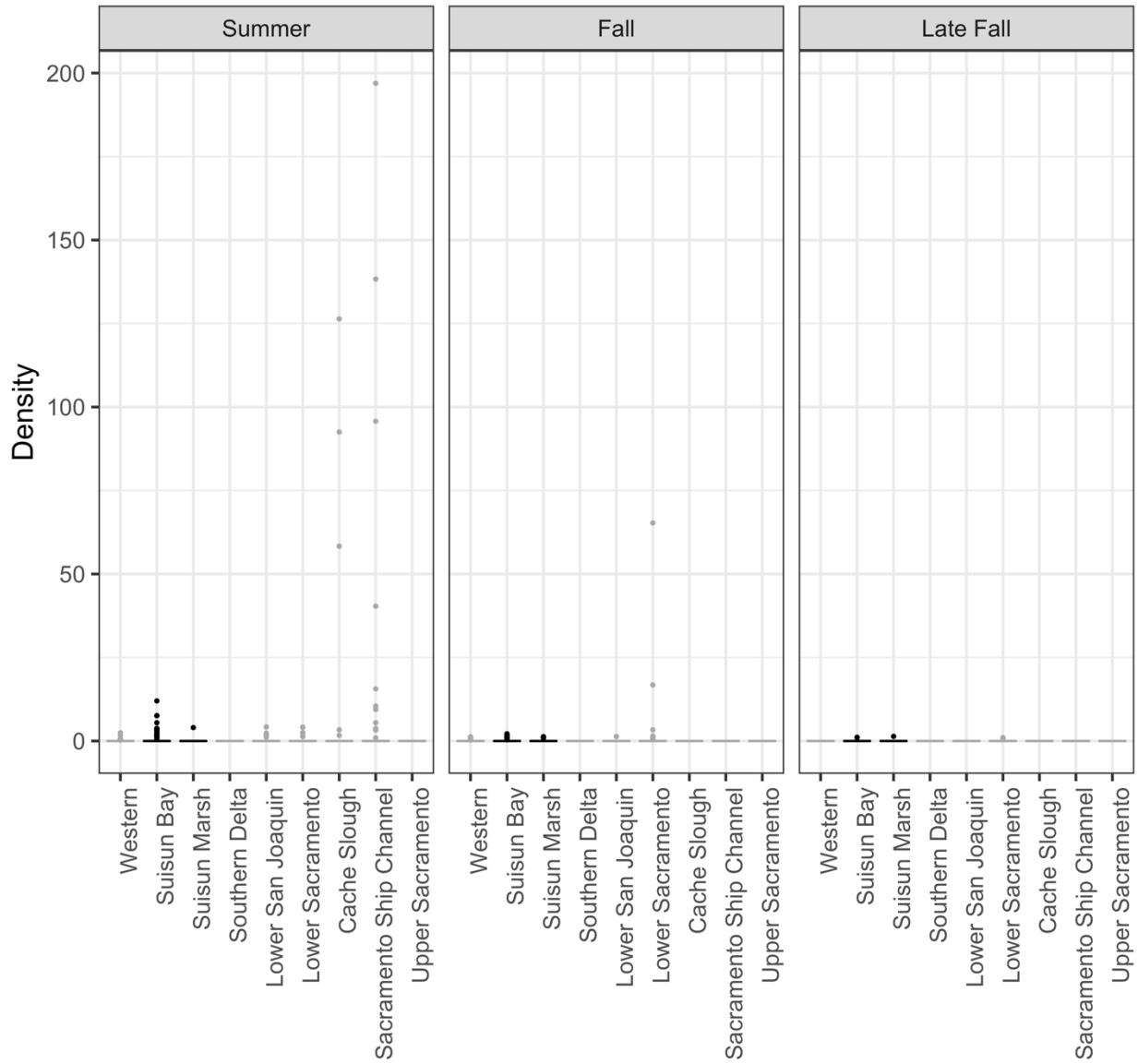
Figure 9-12. Patterns in Chlorophyll *a* During 2017 are Shown for the DOP Data, with Suisun Bay and Suisun Marsh in Bold



Notes: The blue points represent 2011 values and the red points represent 2017. Regions are ordered roughly from seaward (to the left) to landward (to the right).

Figure 9-13. Mean Values (points) are Shown for the Logarithms of Delta Smelt Catch Density (individuals/10,000 m^3) Across Years and Regions

Catch Density of Delta Smelt in 2017



Log10 of Delta Smelt Catch Density in 2017

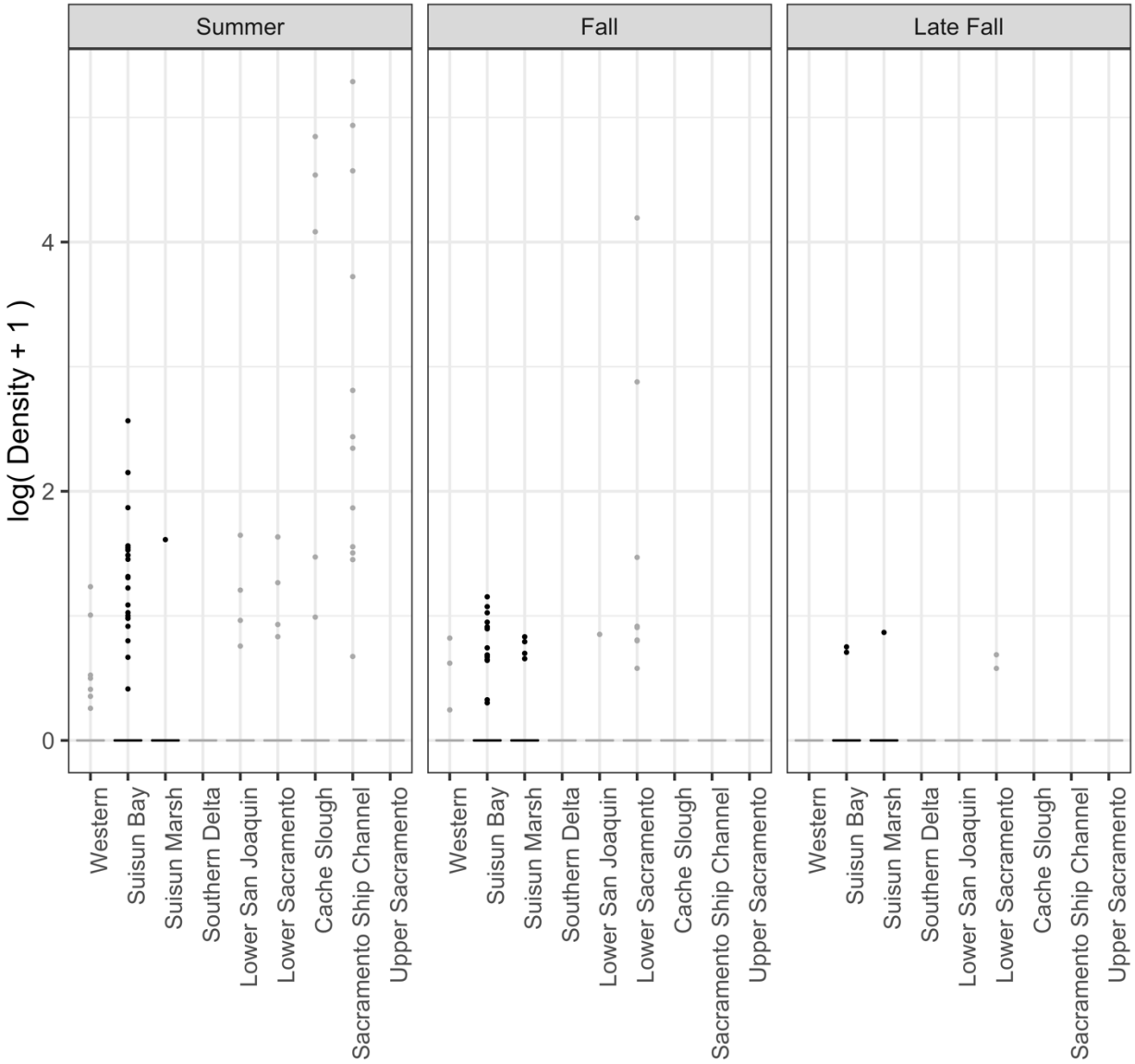
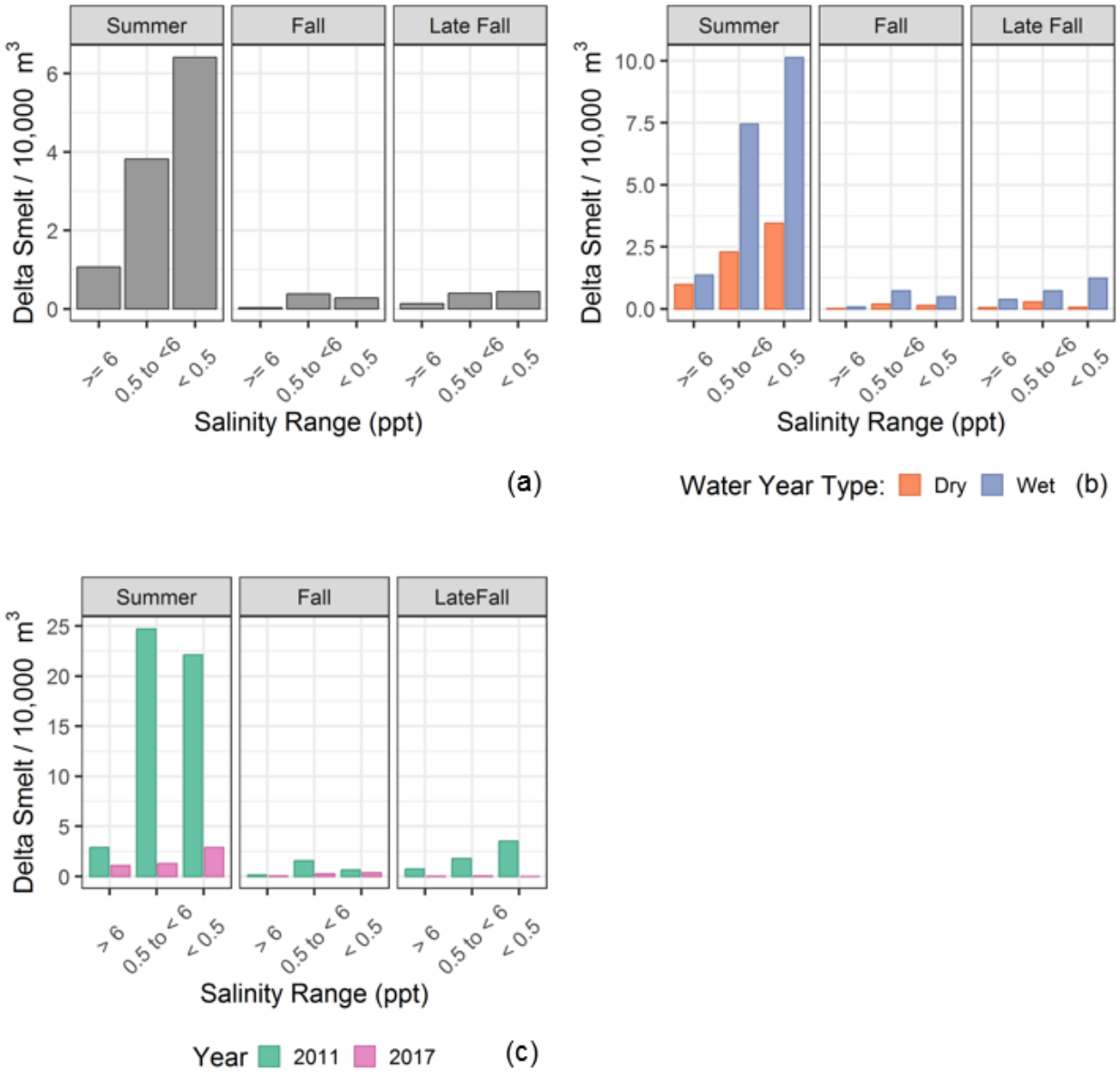


Figure 9-14. Patterns in Delta Smelt Density (individuals/10,000 m^3) During 2017 are Shown in Untransformed Space (top panels) and in Log Space (bottom panels) for the Combined DOP and CDFW Data



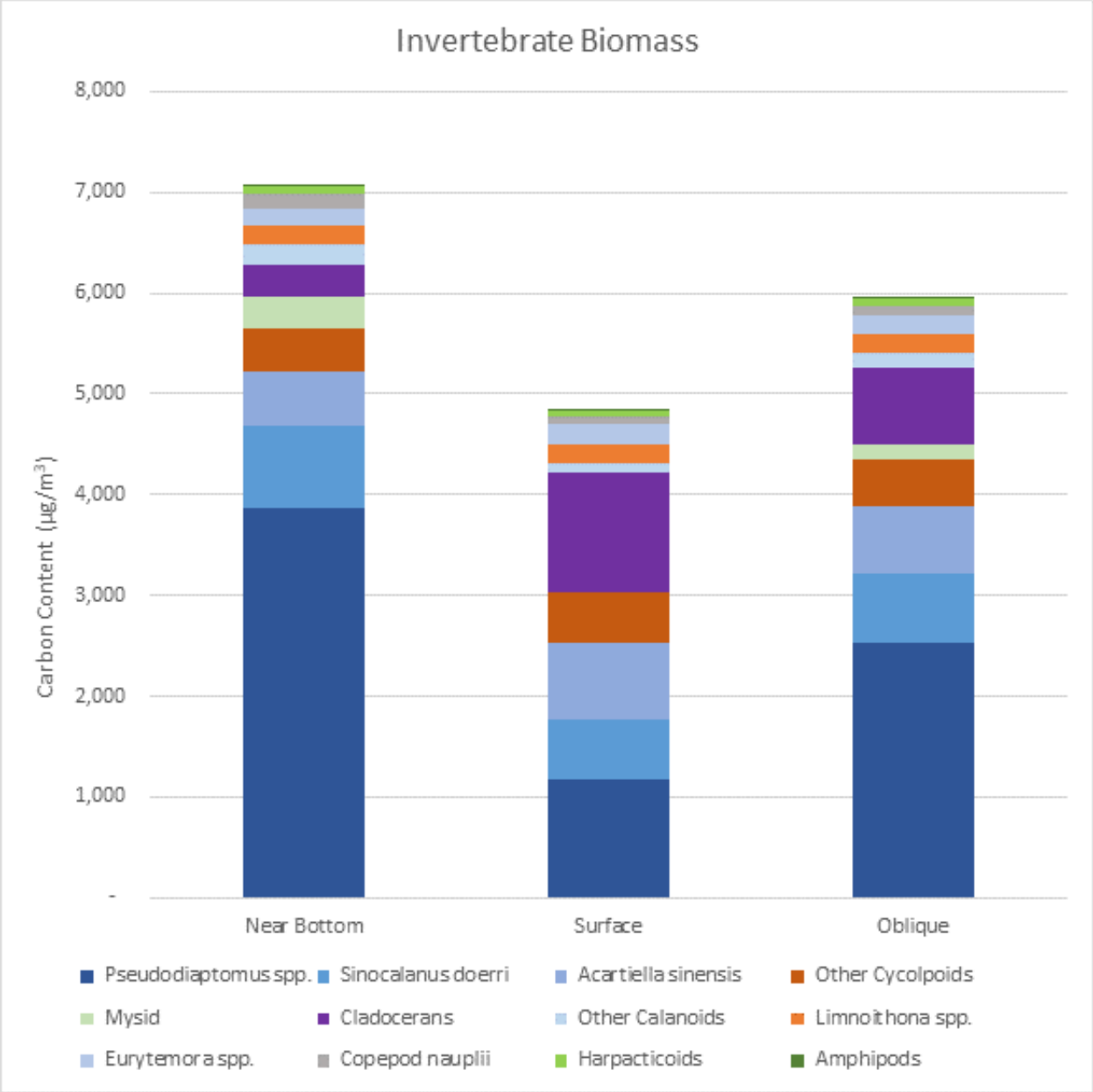
Notes: Combined (a), Wet and Non-Wet Years (b), and between Wet Years 2011 and 2017, Using CDFW and USFWS Survey Data.

Figure 9-15. Patterns in Seasonal Delta Smelt Mean Catch Density (individuals/10,000 m3) by Salinity Level for All Years (2011-2017)

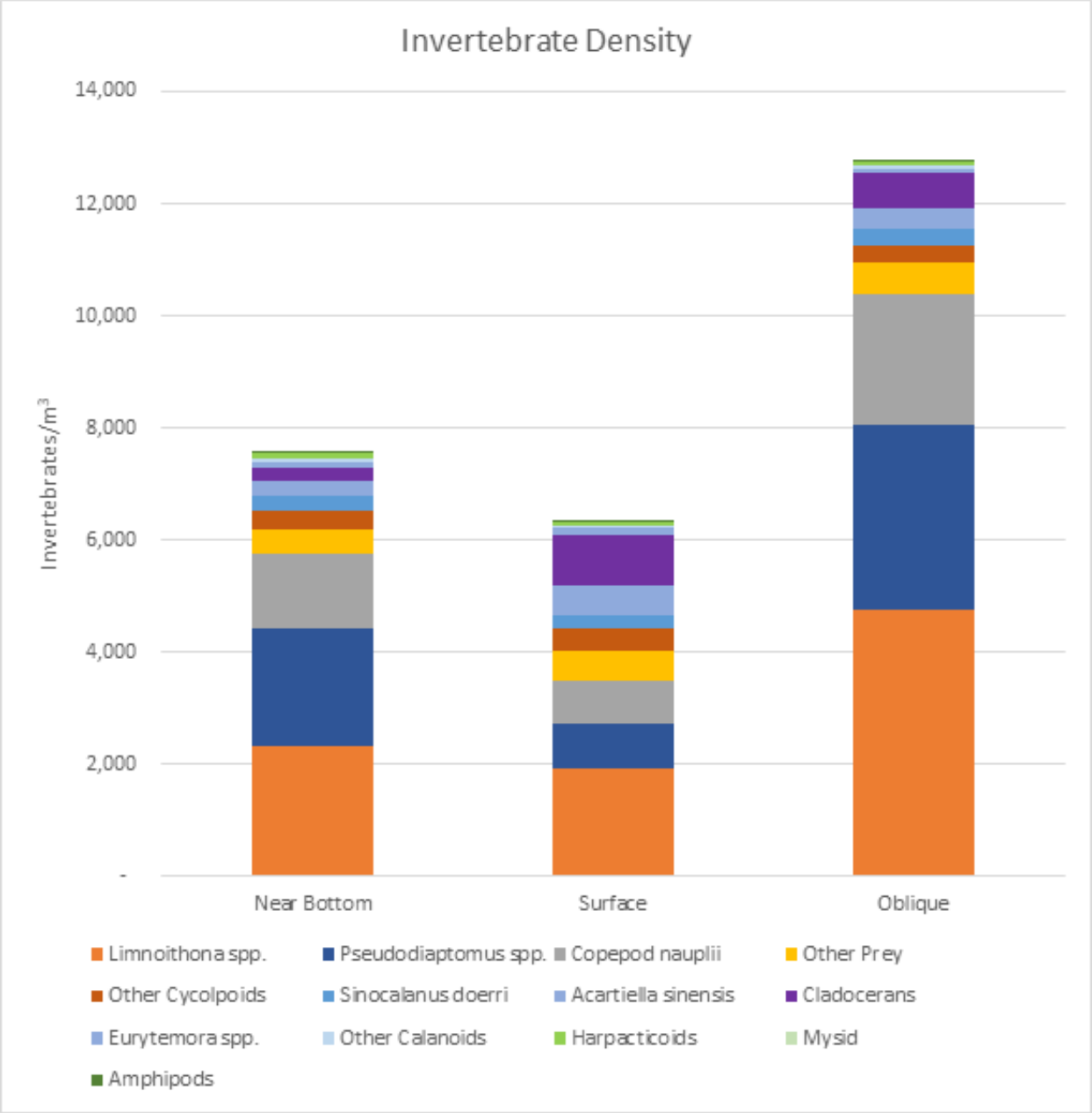
Supplemental Material

Supplemental Table 1. Comparisons of zooplankton catch densities between two tow methods (surface/bottom versus oblique) for all sampling dates both were taken. The surface/bottom zooplankton catch density consists of the combination of surface and bottom tows. Column 2: Ratio of abundances for oblique to surface/bottom tows (± 1 Standard Deviation); Column 3: mean catch density with standard deviation; Column 4: number of sampling locations with positive abundances recorded from both tow methods; Column 5: Pearson's correlation coefficients between catch densities log10(x + 1) from both tow methods at all 31 sampling locations (note that all correlations are positive); Column 6: *P*-value resulting from the Wilcoxon Signed Rank test on raw catch density values recorded from both tow methods. A bold *P*-value (> 0.05) indicates there is no significant difference in the series recorded by the two tow methods (i.e. the median difference of the distributions is close to zero).

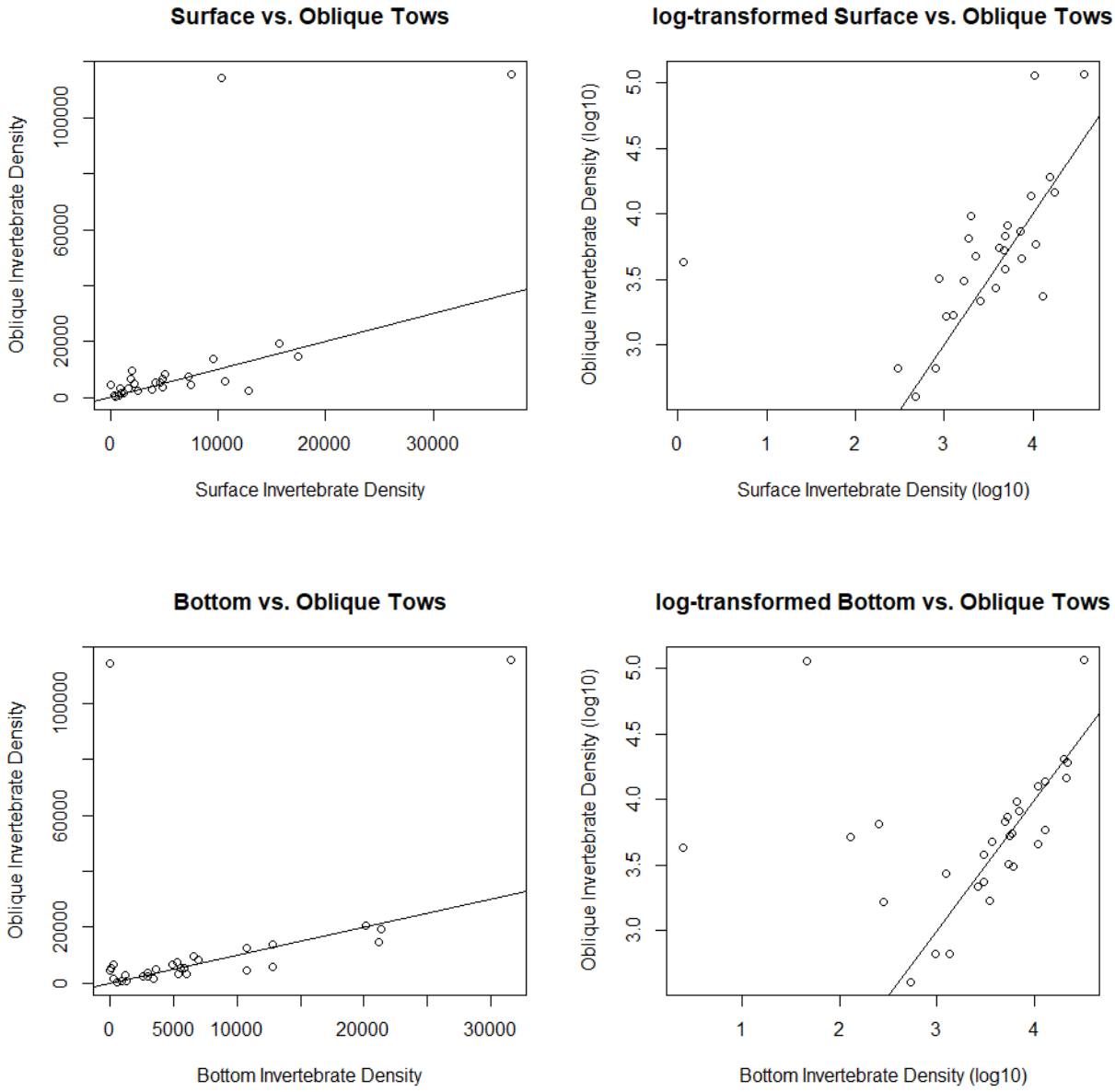
Taxa	Ratio (oblique / surface and bottom) of catch densities	Mean catch density (standard deviation)	Number of points	Correlation coefficient across all 31 stations	Wilcoxon Test *P*-Value
Acartiella sinensis	1.2	44.6 (161.6)	14	0.63 (0.016)	**0.58**
Amphipods	0.6	1.2 (1.4)	16	0.48 (0.06)	**0.18**
Cladocerans	1.0	3.5 (8.8)	20	0.79 (<0.001)	**0.78**
Copepod nauplii	0.9	2.2 (4.4)	26	0.32 (0.11)	**0.71**
Eurytemora spp.	1.3	81.7 (216.8)	8	0.85 (0.01)	**0.84**
Harpacticoids	1.2	11 (34.2)	25	0.91 (<0.001)	**0.25**
Limnoithona spp.	1.2	340.5 (1693.8)	25	0.80 (<0.001)	**0.40**
Mysid	0.8	1 (0.9)	14	0.97 (<0.001)	**0.86**
Other Calanoids	1.0	5.3 (12.2)	10	0.90 (<0.001)	**0.85**
Other Cycolpoids	1.0	2.9 (5.5)	27	0.34 (0.09)	**0.59**
Other Prey	1.7	4.9 (10.1)	27	0.65 (<0.001)	0.03
Pseudodiaptomus spp.	1.2	3 (5.8)	28	0.29 (0.13)	**0.47**
Sinocalanus doerri	1.2	1.4 (1.1)	11	0.99 (<0.001)	0.03
Tortanus spp.	1.6	15.2 (36.5)	7	0.66 (0.11)	**0.47**



Supplemental Figure 1. Comparison of Zooplankton Catch Biomass Density (µg/m3) between CDFW (Oblique) and DOP (Surface/Bottom) Sampling Methods for Zooplankton Taxa/Groups Contributing to at Least 1% of the Total Zooplankton Abundance at Each Sampling Location

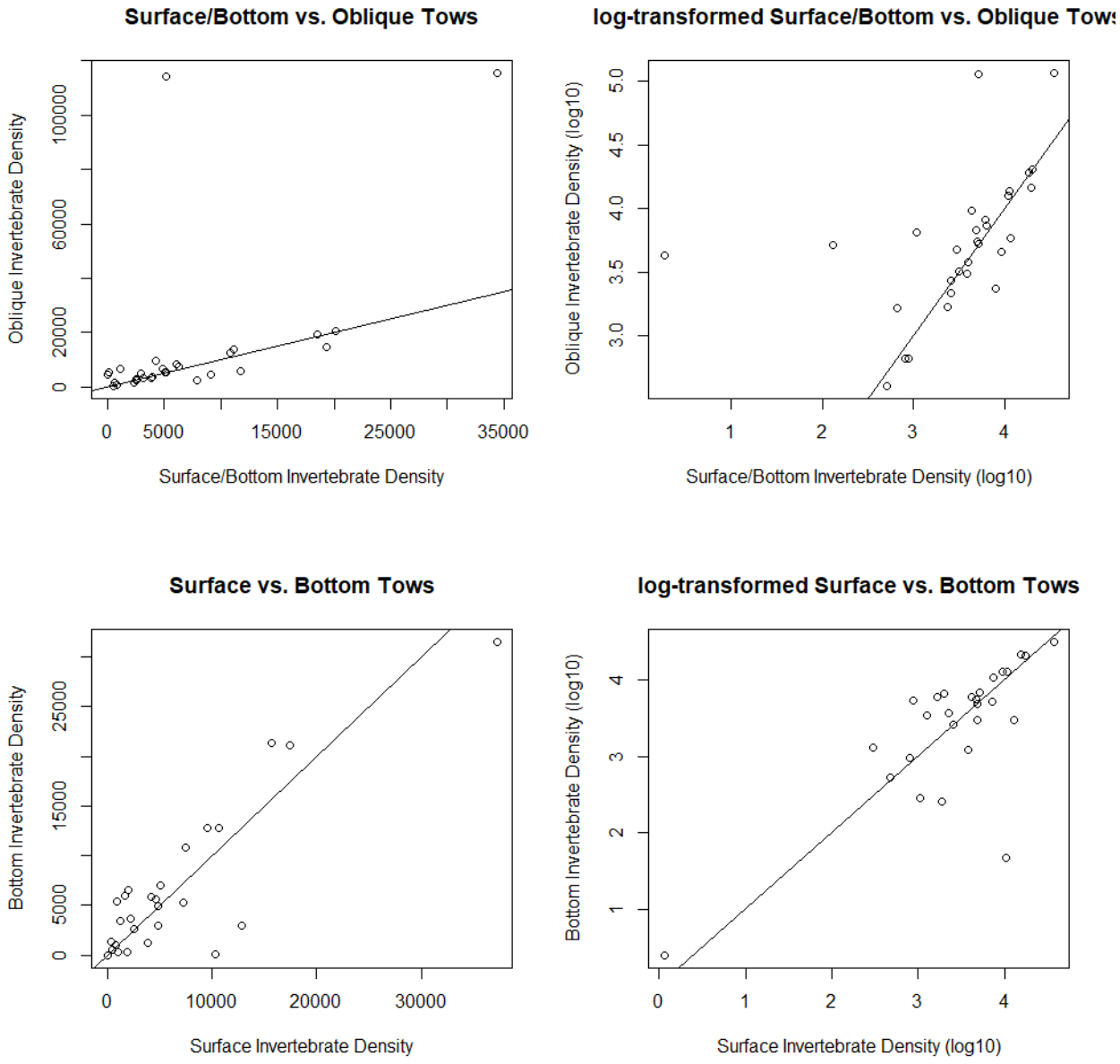


Supplemental Figure 2. Comparison of Zooplankton Catch Density (invertebrates/m3) between CDFW (Oblique) and DOP (Surface/Bottom) Sampling Methods for Zooplankton Taxa/Groups Contributing to at Least 1% of the Total Zooplankton Abundance at Each Sampling Location

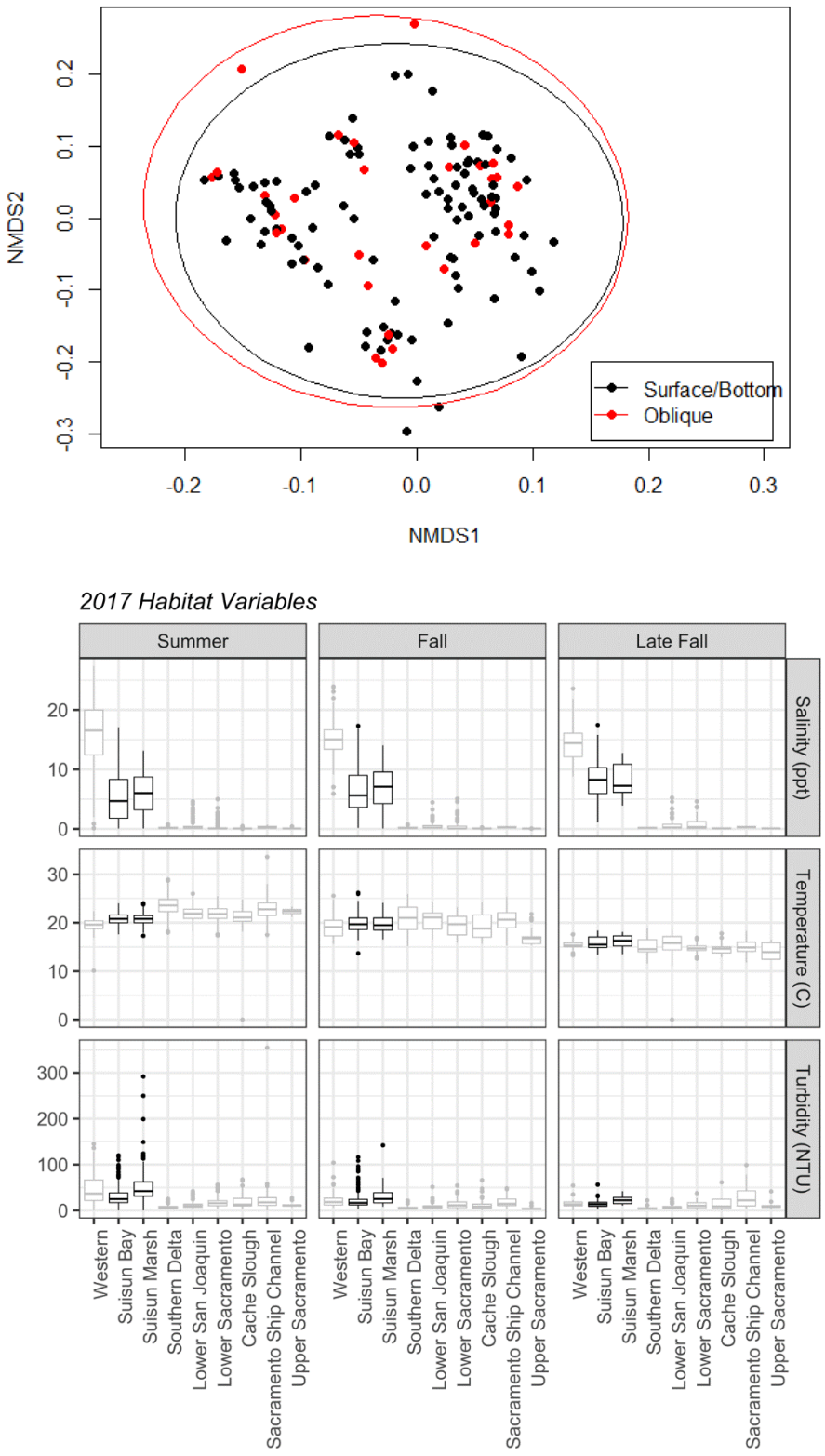


Supplemental Figure 3. Plots Comparing Zooplankton Catch Density (invertebrates/m3) between CDFW (Oblique) and DOP (Surface/Bottom) Sampling Methods

Figures

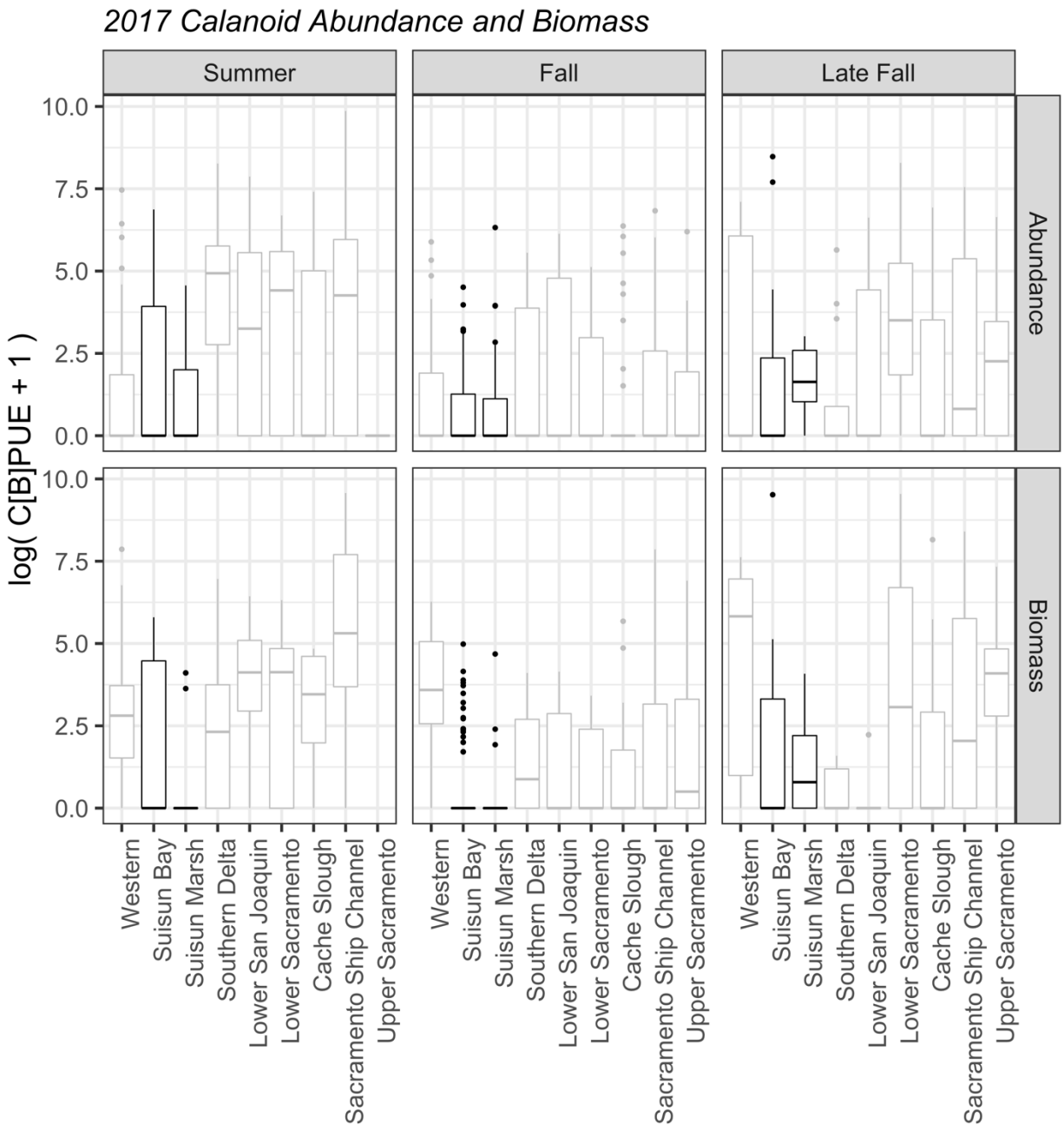


Supplemental Figure 4. Non-Metric Multi-Dimensional Scaling (NMDS) Scatter Plot Comparing Zooplankton Catch Density (invertebrates/m3) between CDFW (oblique) and DOP (surface/bottom) Sampling Methods

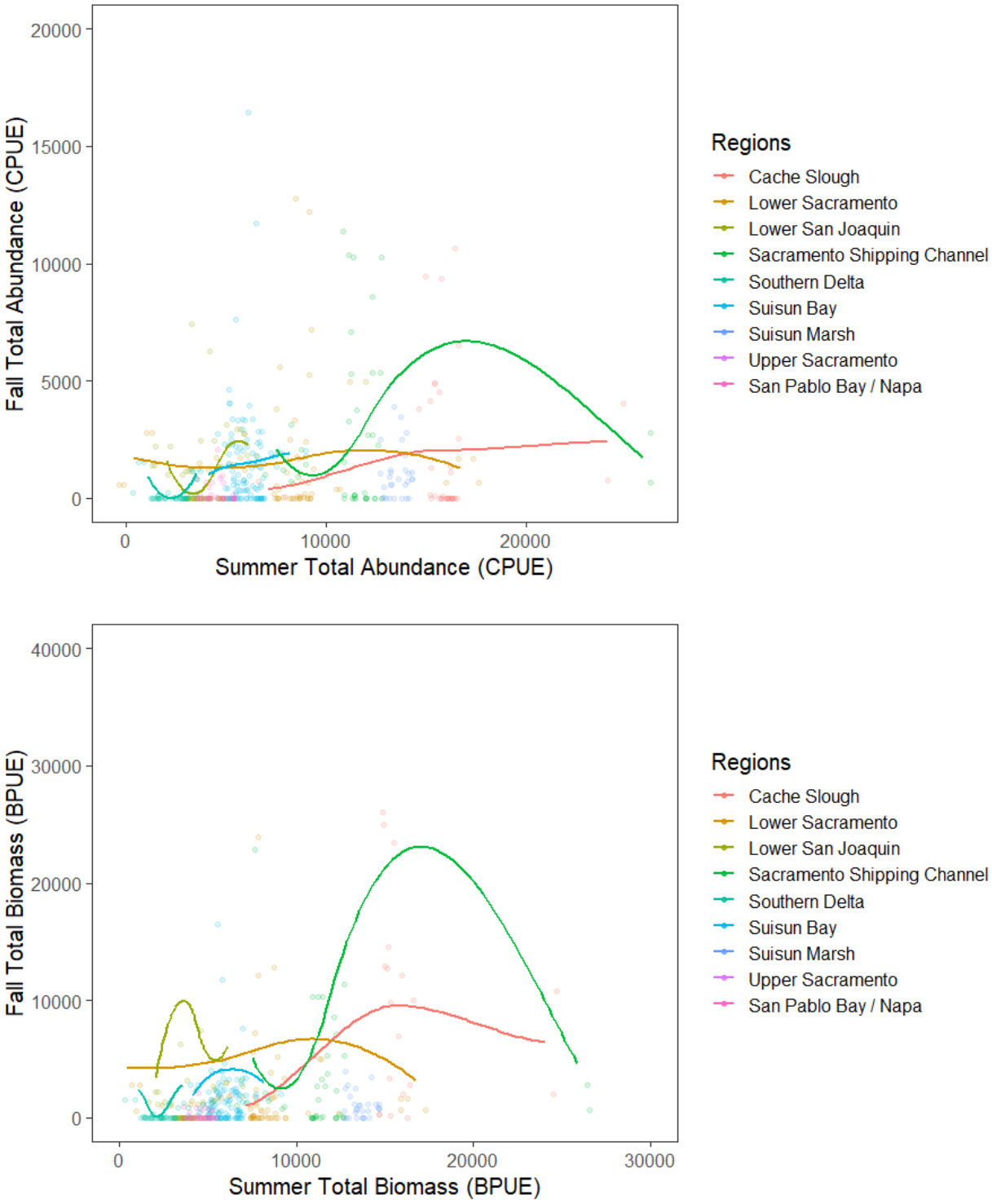


Notes: The months in each season, as well as the downstream order and colors of the strata are as per Figure 6.

Supplemental Figure 5. Patterns in Salinity, Temperature and Turbidity During 2017 are Shown for the Combined DOP and CDFW Data



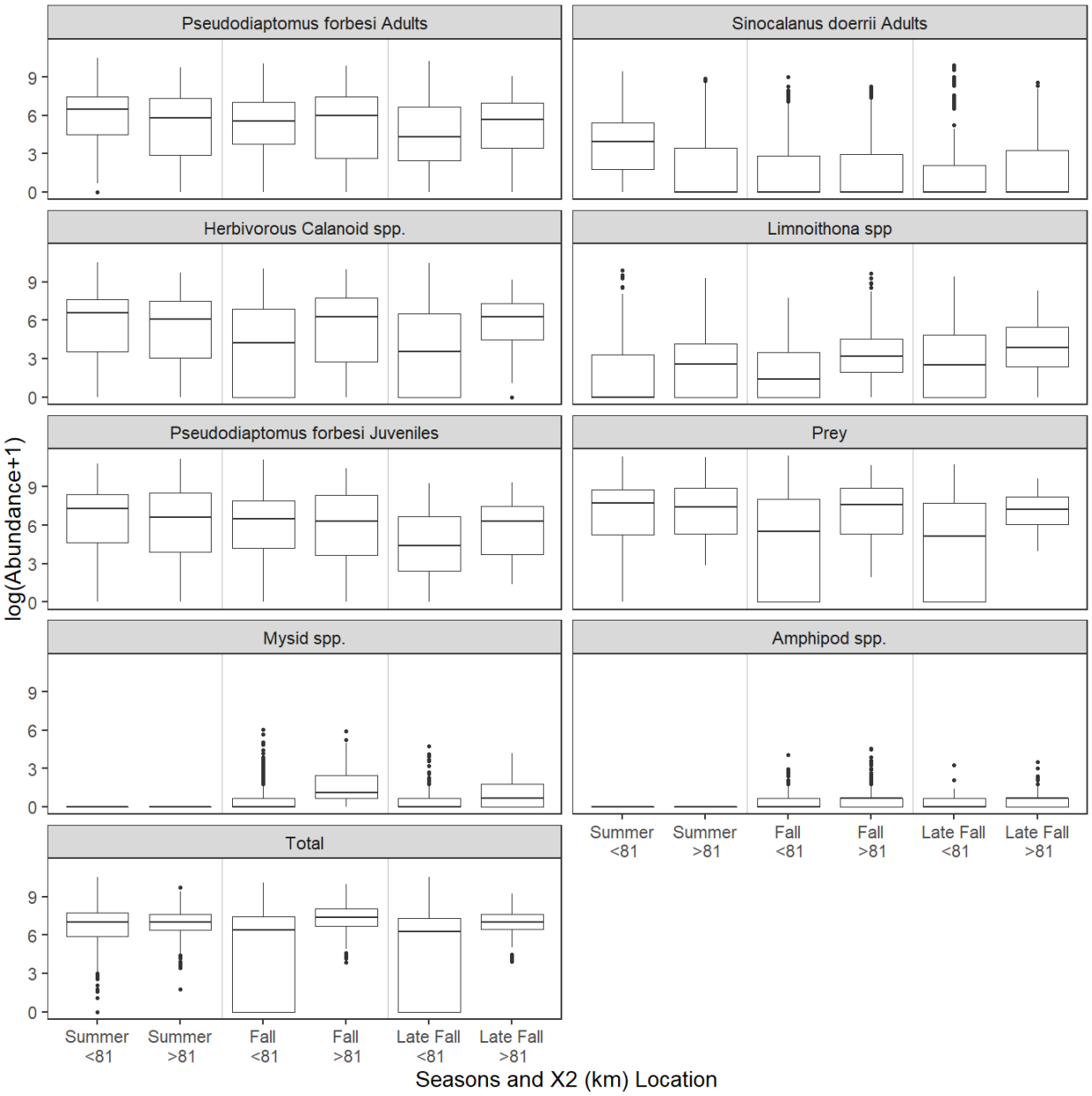
Supplemental Figure 6. Patterns in Calanoid Abundance and Biomass During 2017 are Shown for the Combined DOP and CDFW Data

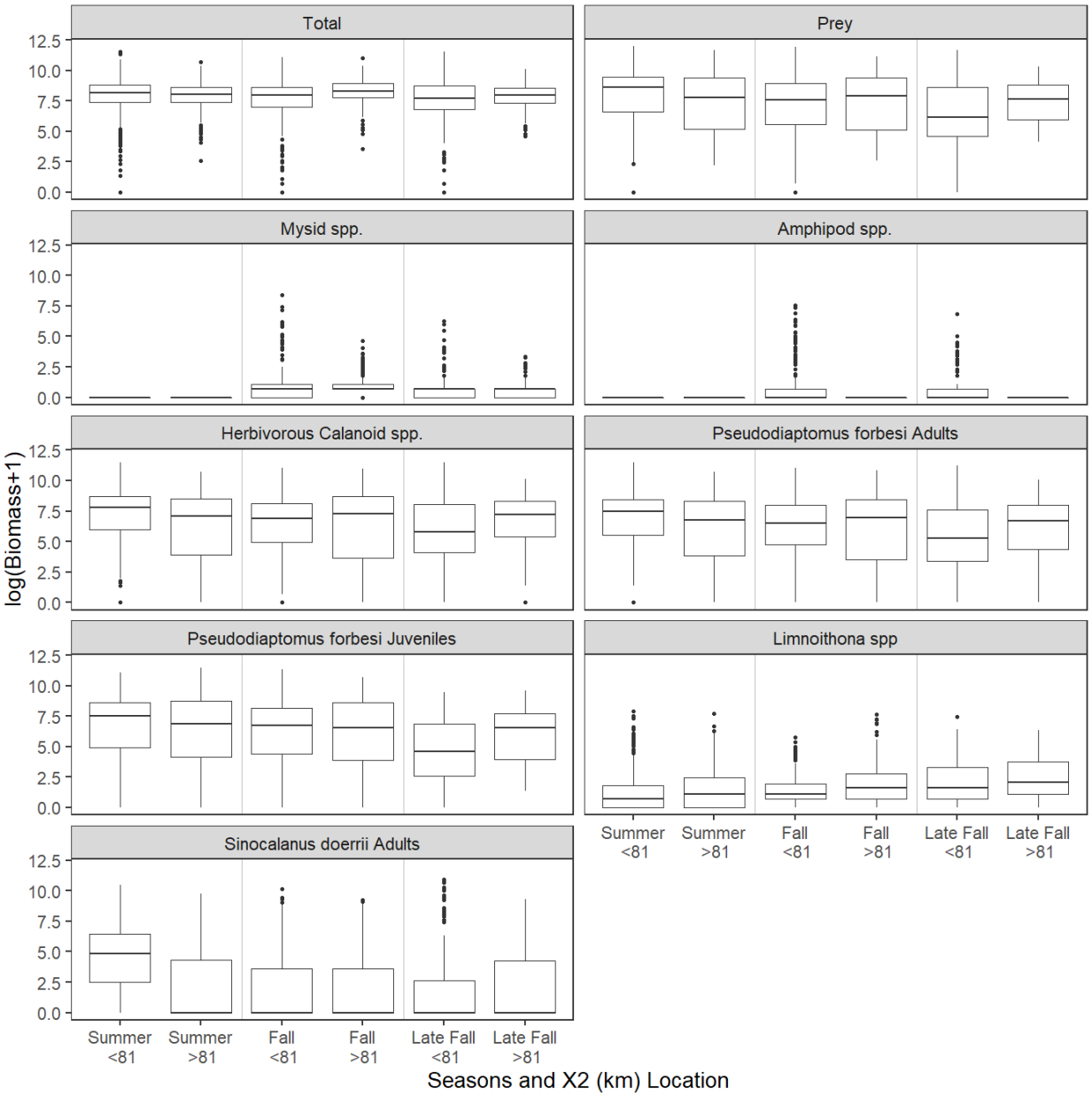


Notes: Not all regions are shown with lines if sample size was too small to adequately model a trend line. One outlier was removed so trend lines for other regions could be shown: 90692 prey abundance and 146016 prey biomass were both in the Lower Sacramento strata. Points are jittered to improve visibility.

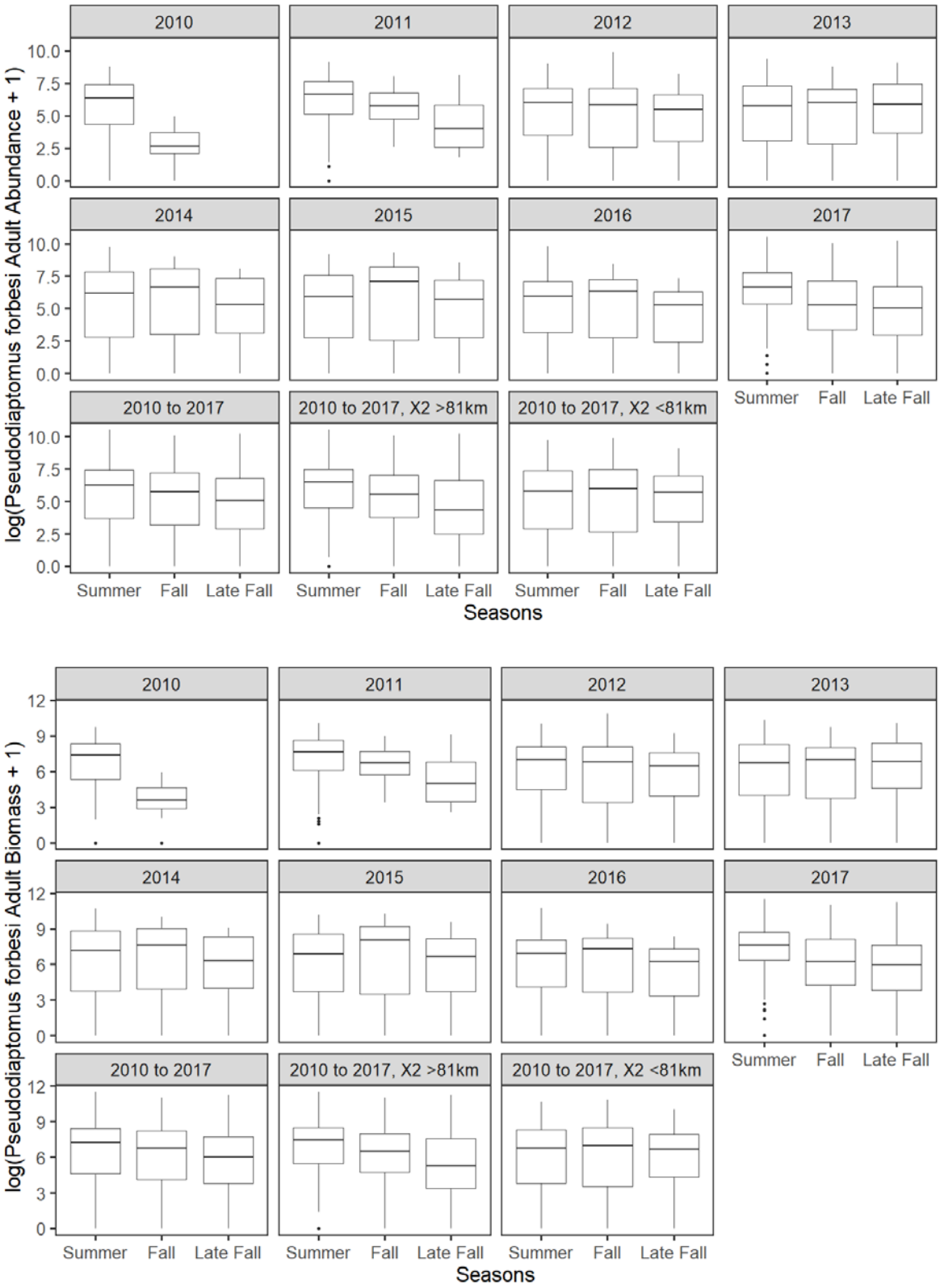
Supplemental Figure 7. Total Abundance (top) and Biomass (bottom) in the Fall of 2017 Predicted by Summer Biomass in 2017

Figures

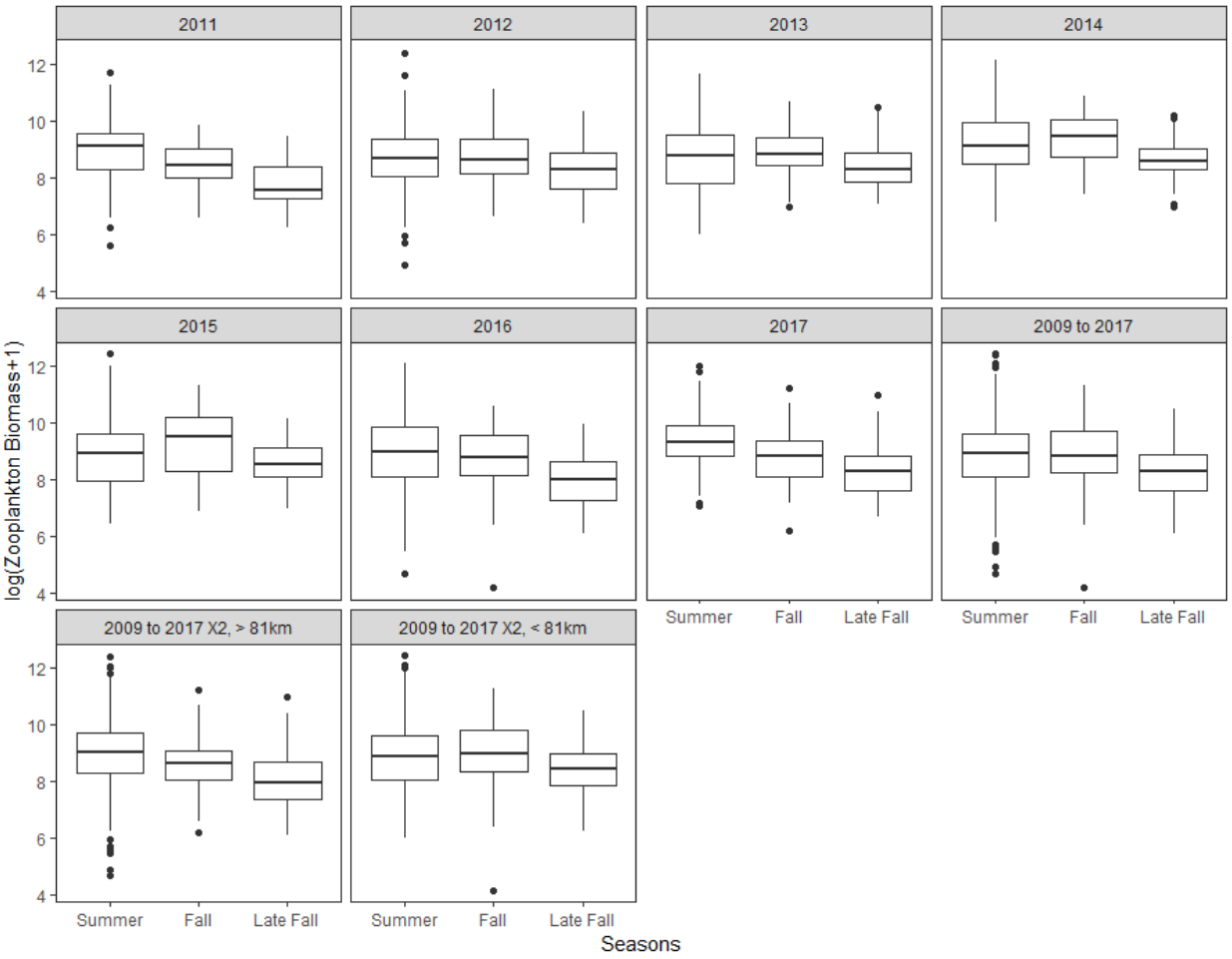

Pseudodiaptomus forbesi Adults
Sinocalanus doerrii Adults
Herbivorous Calanoid spp.
Limnoithona spp
Pseudodiaptomus forbesi Juveniles
Prey
Mysid spp.
Amphipod spp.
Total
log(Abundance+1)
0
3
6
9
Summer <81
Summer >81
Fall <81
Fall >81
Late Fall <81
Late Fall >81
Seasons and X2 (km) Location



Supplemental Figure 8. Boxplots of Years from 2010-2017 Total Prey Abundance (top) Biomass (bottom) by Season for Years in which X2 was Positioned Seaward (<81 km) and Years in Which X2 was Positioned Inland (>81 km)

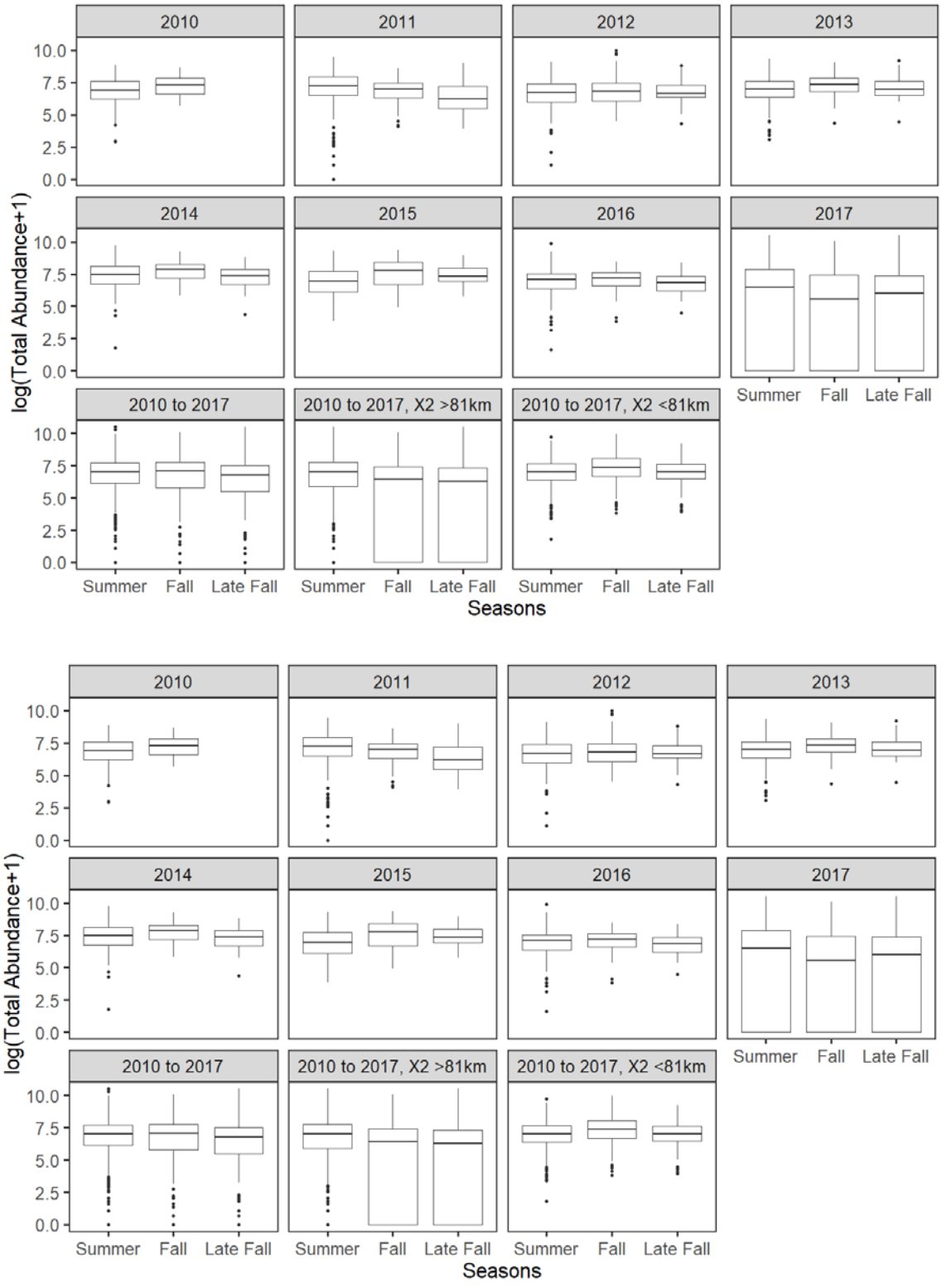


Supplemental Figure 9. Boxplots of *Pseudodiaptomus forbesi* Abundance (top) and Biomass (bottom) by Year and Season

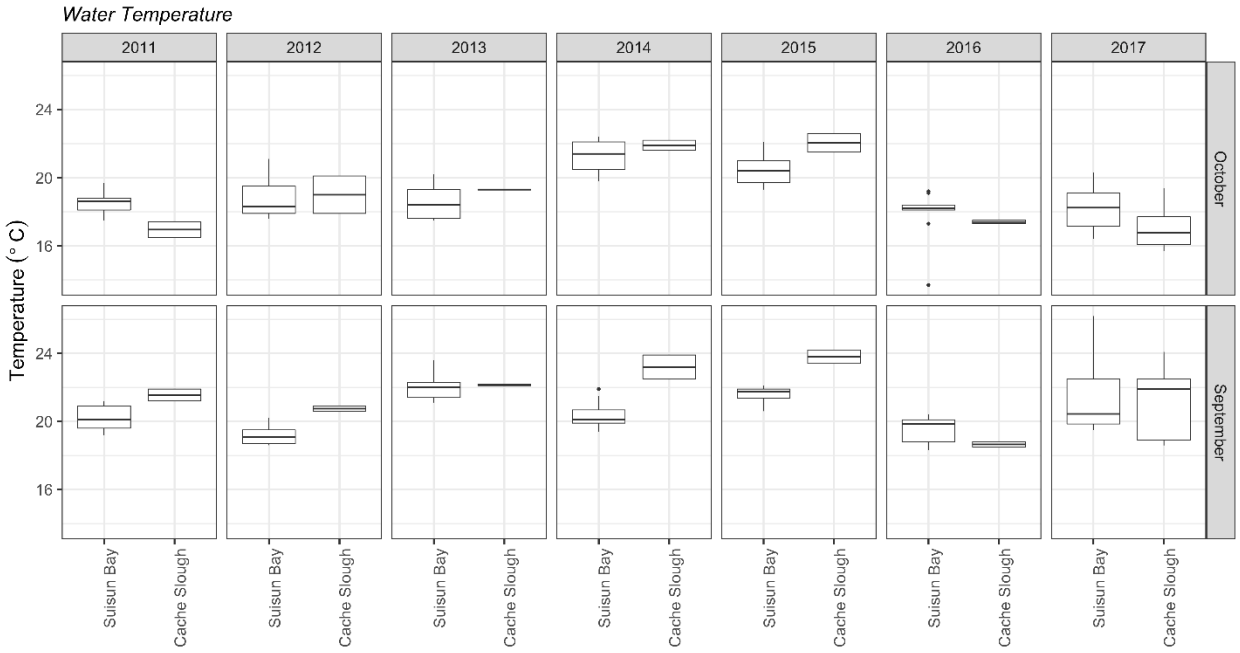


Supplemental Figure 10. Boxplots of all Zooplankton by Season

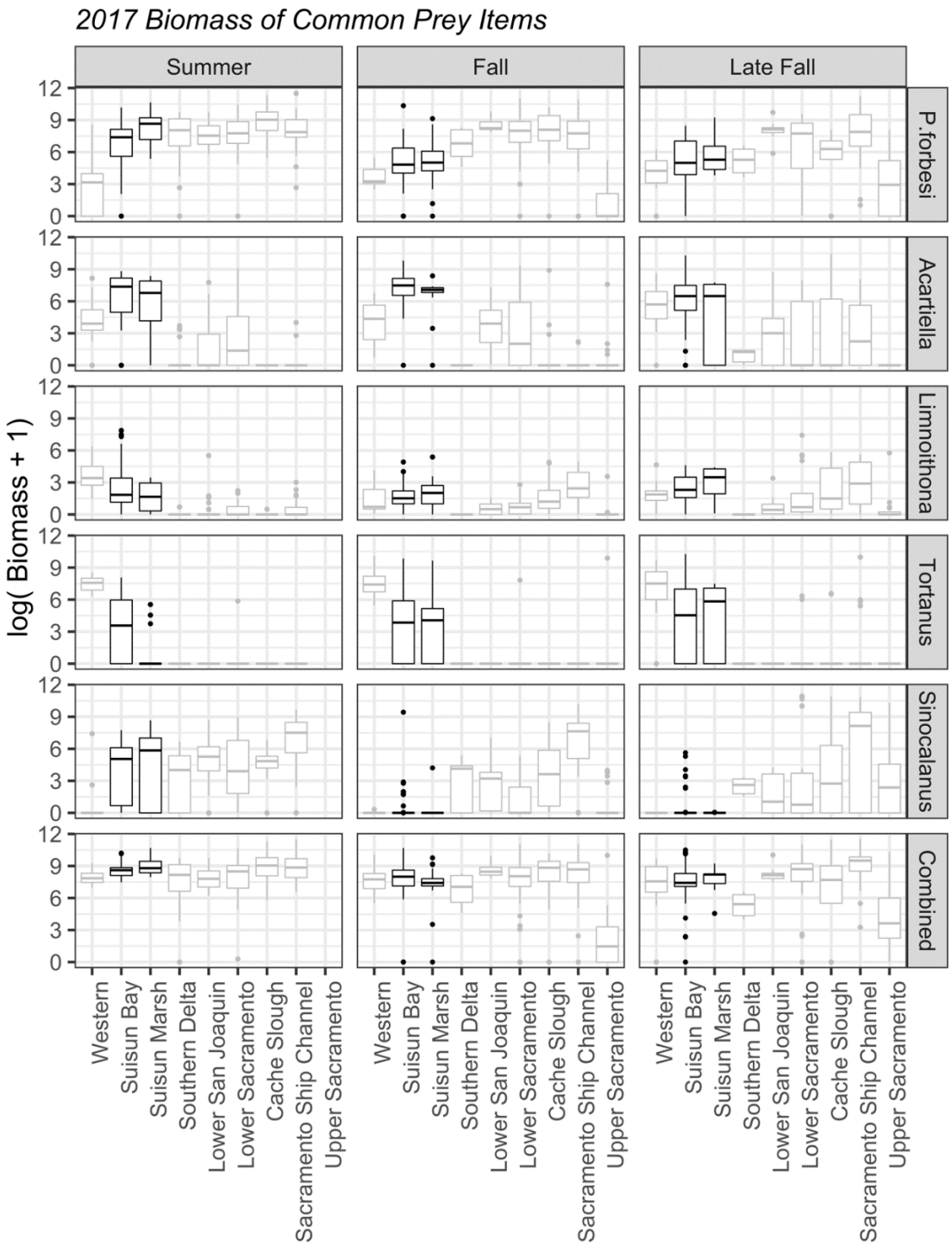
Figures



Supplemental Figure 11. Boxplots of Total Lower Trophic Abundance (top) and Biomass (bottom) by Season

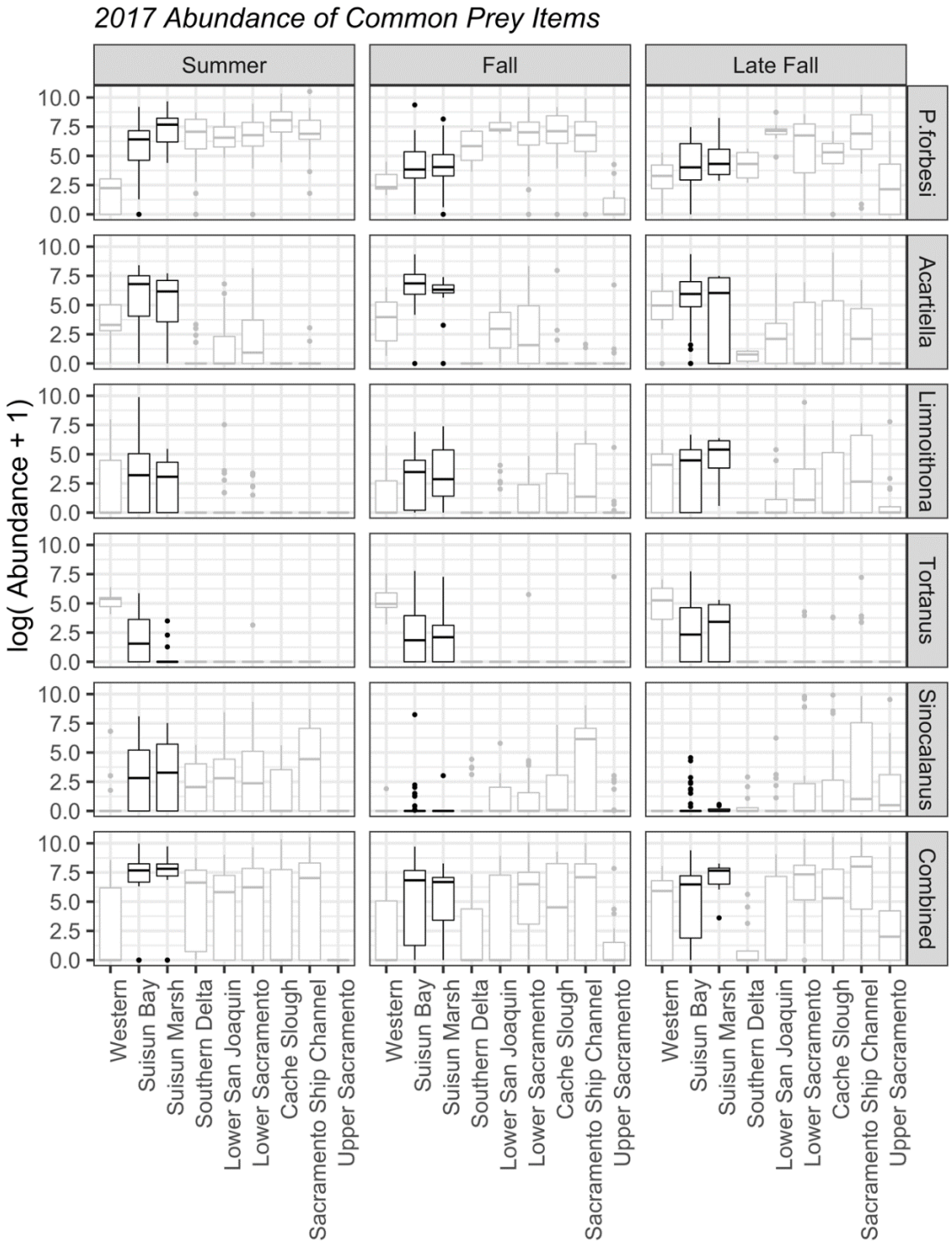


Supplemental Figure 12. Changes in Median Water Temperatures between September and October between Suisun Bay and Cache Slough



Notes: The box-plots represent variability across the combined DOP and CDFW sets of tows. Suisun Bay and Suisun Marsh regions are plotted in black. All other regions are plotted in gray. The regions are ordered from upstream to downstream (left to the right). The seasons are as follows: Summer (Jun – Aug); Fall (Sep – Oct); and Late Fall (Nov).

Supplemental Figure 13. Patterns in 2017 Biomass Density (µg/m^3) for Five Common Prey Items, Combined In Log-Space



Notes: The box-plots represent variability across the combined DOP and CDFW sets of tows. Suisun Bay and Suisun Marsh regions are plotted in black. All other regions are plotted in gray. The regions are ordered from upstream to downstream (left to the right). The seasons are as follows: Summer (Jun – Aug); Fall (Sep – Oct); and Late Fall (Nov).

Supplemental Figure 14. Patterns in 2017 Catch Density (count/m^3) for Five Common Prey Items, Combined In Log-Space

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