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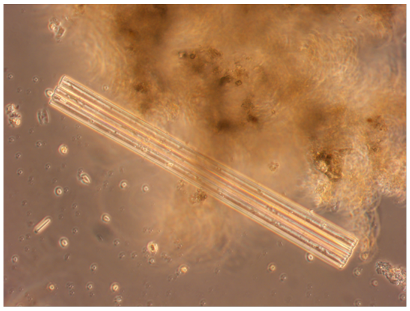
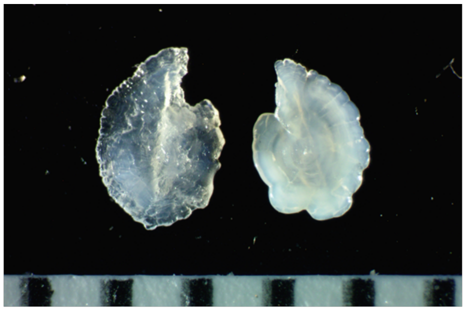
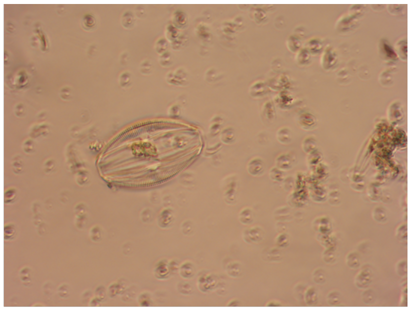
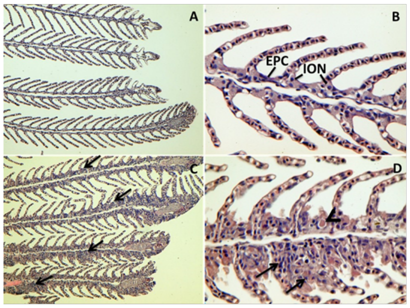
Directed Outflow Project Technical Report 5

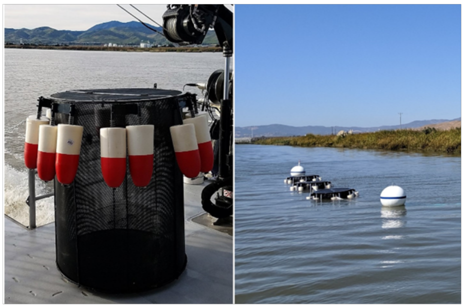
Directed Outflow Project, California
California Great Basin Region

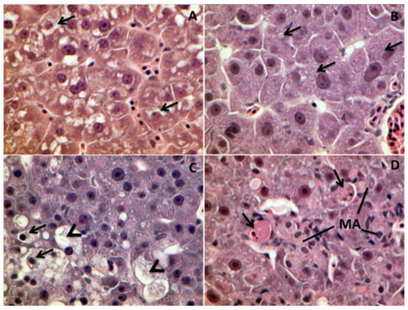












Cover Photos: top left: two pennate diatoms Ulnaria sp. (synonym Synedra sp.) preserved in lugols at 400X magnification under phase contrast microscopy; top right: single pennate diatom Amphora sp. preserved in lugols at 400X magnification under phase contrast microscopy, diatom photos by Julia Taylor; Fish and Aquatic Sciences, ICF 980 9th St Suite 1200, Sacramento, CA 95814; top middle: A pair of asymmetrical Delta Smelt otoliths exhibiting 100% vaterite (abnormal, left) and 100% aragonite (0% vaterite, normal, right) dissected from a cultured Delta Smelt collected in field surveys, photos by the Otolith Geochemistry and Fish Ecology Laboratory, University of California, Davis, Davis CA, 95616, USA; bottom left: gills of Delta Smelt at 100X and 400X; bottom right: livers of Delta Smelt H&E 600X, histology photos by Swee Teh, University of California, Davis, Davis, California, 95616, USA; bottom middle: Delta Smelt enclosure design used in 2019 deployments and three enclosure replicates in Suisun Marsh. Photographs provided by the Department of Water Resources, Sacramento CA, 95691, USA.

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The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

Directed Outflow Project Technical Report 5

Directed Outflow Project, California
California Great Basin Region

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13. SUPPLEMENTARY NOTE

14. ABSTRACT The U.S. Bureau of Reclamation’s Directed Outflow Program (DOP), along with collaborating agencies and non-governmental groups, are continuing efforts to evaluate the hypothesized benefits of outflow/outflow alteration to habitats and species of the Sacramento-San Joaquin Delta and connecting upper estuary (Delta). A particular focus of the DOP is to improve ecological understanding of the critically endangered Delta Smelt (*Hypomesus transpacificus*), a small short-lived osmerid fish endemic to the Delta. The DOP technical report series (https://www.usbr.gov/mp/bdo/directed-outflow.html) aims to periodically showcase ongoing DOP-related research studies. Results from DOP-related studies are anticipated to assist decision-making processes and better inform general management actions to benefit Delta habitat conditions and species such as Delta Smelt.

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

The U.S. Bureau of Reclamation's (Reclamation) Directed Outflow Program (DOP), along with collaborating agencies and non-governmental groups, are continuing efforts to evaluate the hypothesized benefits of outflow/outflow alteration and improve ecological understanding of the critically endangered Delta Smelt (*Hypomesus transpacificus*), a small short-lived osmerid fish endemic to the Sacramento-San Joaquin Delta and connecting upper estuary (Delta). The DOP technical report series aims to periodically showcase ongoing DOP-related research studies. Each chapter within this report is intended for eventual submittal to a peer-reviewed scientific journal, thus formatting may vary 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 following provides additional background information.

In 2008, the U.S. Fish and Wildlife Service (USFWS) issued a Biological Opinion (2008 BiOp; USFWS 2008) on Central Valley Project/State Water Project operations that concluded aspects of those operations jeopardize the continued existence of Delta Smelt and adversely modify the species' critical habitat. Action 4 (Fall X2 Action) of the 2008 BiOp required Delta outflow be maintained at an average X2 (average position of the 2 parts per thousand (ppt) isohaline from Golden Gate) no greater than 74 kilometers (km) for September and October following wet years and 81 km following above normal years (water-year type [wet, above normal, below normal, dry, critical] is based on measured unimpaired runoff;. In 2011, Reclamation produced a fall outflow adaptive management plan based on the science underlying the Fall X2 Action and outlining how adaptive management might proceed (Reclamation 2012).

In spring 2016, USFWS requested augmentation of summer outflow from the Sacramento River to benefit the habitat and declining population of Delta Smelt, although the action never occurred. Slightly thereafter the Delta Smelt Resiliency Strategy (DSRS) was finalized in July 2016 (CNRA 2016). The DSRS 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). These actions included augmentation of Delta outflow to push the low salinity zone (0.5-6 ppt) seaward and routing of water through Yolo Bypass Toe Drain to promote food production, to benefit Delta Smelt.

In winter of 2016/2017 Reclamation formed the DOP to assist in evaluating outflow-related hypotheses and predictions (Table 1) using targeted paired biological and physical monitoring. The over-arching hypothesis is summer and fall habitat conditions are improved for juvenile Delta Smelt when X2 moves seaward (Reclamation 2012; Brown et al. 2014), especially when X2 overlaps the Suisun Bay-Marsh area of the Delta. Predictions are largely based on conceptual models within Baxter et al. (2015) (figures 48 and 49 in particular) and predictions in Brown et al. (2014).

In August of 2016, Reclamation and California Department of Water Resources (DWR) jointly requested a Reinitiation of Consultation on the Coordinated Long-Term Operation of the Central Valley Project (CVP) and State Water Project (SWP). The USFWS accepted the request shortly after and stated therein: "…new information is demonstrating the increasingly imperiled state of the delta smelt and its designated critical habitat, and that emerging science shows the importance of outflows to all life stages of delta smelt and to maintaining the primary constituent elements of designated critical habitat." The new Biological Opinion on operations was finalized in October of 2019 (2019 BiOp; USFWS 2019). The Delta Smelt Summer-Fall Habitat Action (SFHA) and additional measures in Table 2-1 in the 2019 BiOp outlines multiple outflow-related actions geared toward benefitting Delta Smelt habitat and ultimately its population. Such actions include the following:

> *Fall X2: Modify water operations to maintain X2 at 80 km in above normal and wet water years in September and October. Maintain low salinity habitat in Suisun Marsh and Grizzly Bay when water*

> *temperatures are suitable. Manage the low salinity zone to overlap with turbid water and available food supplies. Establish contiguous low salinity habitat from Cache Slough Complex to the Suisun Marsh.*
>
> *Suisun Marsh Salinity Control Gate: The freshening of Montezuma Slough through gate operations could provide additional low salinity habitat for Delta Smelt to forage, spawn and rear.*
>
> *Suisun Marsh and Roaring River Distribution System Food Subsidies Study: Add fish food to Suisun Marsh through coordinating managed wetland flood and drain operations in Suisun Marsh, Roaring River Distribution System food production, and reoperation of the Suisun Marsh Salinity Control Gates.*
>
> *North Delta Food Subsidies/Colusa Basin Drain Study: Augment flow in the Yolo Bypass in July and/or September by closing Knights Landing Outfall Gates and routing water from Colusa Basin into Yolo Bypass to promote fish food production.*
>
> *Sacramento River Deepwater Ship Channel Food Study: Repair or replace the West Sacramento lock system to hydraulically reconnect the ship channel with the mainstem of the Sacramento River. The ship channel has the potential to flush food production into the north Delta for Delta Smelt.*

While much has been learned regarding the impacts of environmental conditions on Delta Smelt habitat, some uncertainty remains as to how outflow-related actions, such as those listed above, may affect certain habitat factors and the species' response. We anticipate results from DOP-related studies will assist decision-making processes regarding the SFHA and better inform general management actions to benefit the wild Delta Smelt population, including augmentation of the population through supplementation using cultured fish.

As Delta Smelt populations declined and wild Delta Smelt catch reached all-time lows in 2021 the ability of DOP studies to focus on wild fish to evaluate these actions became more limited. However, in 2022 Reclamation, DWR, California Department of Fish and Wildlife, and USFWS began to release Delta Smelt under a short-term program called Experimental Release. The goal of this program was to begin releasing Delta Smelt bred at the Fish Conservation and Culture Laboratory (FCCL) and test methods for release prior to the development of a full supplementation program. Over the initial first 3 years 200 days post hatch fish would be released between December and February with the intent for them to spawn in the months after February. As support for Experimental Release all fish recaptured from these releases would be processed following DOP protocols. The data collected from these fish would be considered used when appropriate to inform the key hypothesizes of the DOP as well as for informing the success of Experimental Release.

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Table 1. Qualitative Predictions Regarding the Effect of X2 (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 X2 Action Periods

Dynamic Abiotic Habitat Components	X2 in/near Suisun Region During Summer or Fall Compared to Other Regions and Within Suisun Region During Summer or Fall X2 Action Periods (in parentheses)	Chapters in the DOP Technical Report 5 with Related Data
Low Salinity Habitat Area	Higher (Increases)	1,4,5
Habitat Complexity	Higher (Increases)	--
Hydrodynamic Complexity	Higher (Increases)	2,6
Water Temperature	Lower (Decreases)	--
Turbidity	Higher (Increases)	1,2
Contaminants*	Lower (Decreases)	5
Dynamic Biotic Habitat Components		
Delta Smelt Prey Density and Biomass	Higher (Increases)	3,7
Phytoplankton Density and Biomass	Higher (Increases)	2
Harmful Algal Constituents/Cyanotoxins	Lower (Decreases)	5
Impact of Non-Native Competitors	Lower	3
Impact of Non-Native Predators	Lower	--
Delta Smelt Responses		
Occupancy/Residence	Greater (Increases)	1
Health	Greater (Increases)	4,5,6
Growth	Higher (Increases)	
Survival	Higher (Increases)	6
Prey Quality, Foraging Success	Better (Increases)	7
Fecundity	Higher	--
Population Range/Distribution	Broader, Less Constricted	1,4
Life History Diversity	Greater, More Even Spread	4

Contents

Page

Chapter 1. Evaluation of Delta Smelt Distribution Reveals Association With Other Small Pelagic Species: Implications for Competitive Dynamics and Proxy Monitoring 1-1
- Abstract 1-1
- Introduction 1-1
- Methods 1-4
  - Data Processing 1-5
  - Model Fitting 1-6
  - Model Validation 1-6
- Results 1-7
  - Delta Smelt Models 1-7
  - Delta Smelt and Wakasagi Presence/Absence 1-8
  - Small Pelagic Fish Presence/Absence 1-8
  - Abundance 1-9
  - Regional Dynamics 1-9
- Discussion 1-9
- Acknowledgements 1-13
- References 1-13
- Tables 1-21
- Figures 1-25

Chapter 2. Phytoplankton Community Structure and Nutrient Conditions in the Upper San Francisco Estuary During Managed Flow Action Periods 2-1
- Abstract 2-1
- Introduction 2-1
- Methods 2-4
  - Study Area and General Sampling Design Location and Sampling Period 2-4
  - Sampling Methods 2-4
  - Lab Analysis Methods 2-5
  - Statistical Analysis 2-5
- Results 2-6
  - Water Flow and Abiotic Water Quality Variables 2-6
  - Phytoplankton Biomass 2-6
  - Phytoplankton Diversity 2-7
  - Nutrient and Water Quality Data 2-7
  - Harmful Algal Blooms 2-7
- Discussion 2-8
  - Phytoplankton Biomass 2-8
  - Phytoplankton Diversity and Abundance 2-9
  - Dissolved Nutrients 2-10
  - Harmful Algal Blooms 2-11
- Conclusions and Management Implications 2-11
- Acknowledgements 2-12
- References 2-13

Tables 2-22
Figures 2-25
Supplemental Information 2-34
Chapter 3. Implementing Rapid Genetic Identification of Delta Smelt in a Non-Genetics Agency Laboratory to Support Species Monitoring 3-1
Abstract 3-1
Introduction 3-2
Materials and Methods 3-3
  Frozen SHERLOCK Reaction Stability Testing 3-3
  SHERLOCK Assay Validation on Mucus Samples from Frozen Specimens 3-4
  SHERLOCK Training and Assay Implementation at the Field Office 3-5
Results 3-5
  Stability of Pre-Frozen SHERLOCK Reaction Aliquots Over Time in -80°C Storage 3-5
  Validation Testing of Mucus Swabs from Frozen *Hypomesus* Specimens 3-5
  SHERLOCK Implementation in the Field Office: Winter 2023-2024 Genetic ID Results for *Hypomesus* Specimens Morphologically Identified as Delta Smelt 3-5
Discussion 3-6
Acknowledgments 3-7
References 3-8
Tables 3-10
Figures 3-15
Chapter 4. Otolith-Based Comparisons of Wild Versus Hatchery-Origin Delta Smelt 4-1
Abstract 4-1
Introduction 4-1
  Delta Smelt Status & Supplementation 4-1
  Otoliths in Fish and Fisheries 4-2
  Otoliths as Natural Tags 4-3
  Otolith Crystalline Structure 4-3
  Otolith-Somatic (O-S) Proportionality 4-3
  Approach & objective 4-4
Methods 4-4
  Fish Collection and Archival 4-4
  Otolith Crystalline Structure 4-4
  Otolith-Somatic Proportionality 4-5
  Otolith Age and Growth 4-5
  Otolith Geochemistry 4-6
Results 4-6
  Otolith Crystalline Structure 4-6
  Otolith-Somatic Proportionality 4-7
  Age and Growth 4-7
  Otolith Geochemistry 4-7
Discussion 4-8
  Summary of main findings 4-8
  Otolith Crystalline Structure 4-8
  Otolith-Somatic Proportionality 4-9
  Otolith Age & Growth 4-9
  Otolith Chemistry Classification & Clustering 4-10
  Conclusions 4-11

Acknowledgements ... 4-11
References ... 4-11
Tables ... 4-17
Figures ... 4-19
Supplementary Information ... 4-23
Chapter 5. Sub-lethal Responses of Delta Smelt to Contaminants Under Different Flow Conditions ... 5-1
Abstract ... 5-1
    Keywords ... 5-2
Introduction ... 5-2
Materials and Methods ... 5-4
    Sampling Design and Water Collections ... 5-4
    Water Years and Outflow Actions ... 5-4
    Chemical Analyses ... 5-4
    Delta Smelt Toxicity Testing ... 5-5
    Histopathology ... 5-6
Results ... 5-6
    Analytical Chemistry ... 5-6
    Toxicity Testing and Histopathology ... 5-7
Discussion ... 5-8
    Hypothesis 1: Site-Specific Responses to Contaminants ... 5-9
    Hypothesis 2: Water-Year Effects ... 5-10
    Hypothesis 3: Managed Flows and Contaminant Exposure ... 5-11
Conclusion ... 5-12
Acknowledgements ... 5-12
References ... 5-13
Tables ... 5-19
Figures ... 5-20
Supplemental Information ... 5-25
    Supplemental Tables ... 5-25
    Supplemental Figures ... 5-33
Chapter 6. Novel Field Enclosures Inform the Conservation of a Critically Endangered Fish ... 6-1
Abstract ... 6-1
Introduction ... 6-2
Methods ... 6-4
    Field Sites and Deployments ... 6-4
    Field Monitoring ... 6-6
    Post-deployment Fish Sampling and Health Metrics ... 6-7
    Statistical Analysis ... 6-9
Results ... 6-10
    Field Monitoring ... 6-10
    Survival and Growth ... 6-10
    Zooplankton and Diets ... 6-11
    Histopathology ... 6-11
    Temperature Tolerance ... 6-12
Discussion ... 6-13
    Survival and health ... 6-13

Food availability and consumption 6-15
Thermal Tolerance 6-16
Conclusions and Implications 6-18
Acknowledgements 6-18
Author Contributions 6-18
Conflict of Interests 6-19
Funding 6-19
Data Availability 6-19
References 6-19
Tables 6-27
Figures 6-33
Supplemental Information 6-42
Supplemental Tables 6-42
Supplemental Figures 6-45
Chapter 7. The Influence of Zooplankton Availability on Delta Smelt Condition and Foraging Across Habitat Contexts 7-1
Abstract 7-1
Introduction 7-2
Methods 7-3
Dataset 7-3
Response variables 7-4
Predictor Variables 7-4
Results 7-5
HSI Models 7-6
Gut Fullness Models 7-6
Discussion 7-6
Acknowledgments 7-9
References 7-9
Tables 7-14
Figures 7-16
Supplemental Information 7-23
Statistical Methods 7-23
Results 7-23

Tables

Table 1-1. Overview of Models 1-21
Table 1-2. Model variables with summary statistics 1-22
Table 1-3. Summary of Model Cross-Validation Statistics 1-24
Table 2-1. Mean and Standard Deviation in Parentheses of Water Quality Parameters by Region from September Through November 2-22
Table 2-2. Generalized Linear Model Summary for Action Year Status in Individual Terms and Interaction Terms 2-23
Table 2-3. R^2 and p Values for Correlation Between NMDS Configuration of Phytoplankton Taxonomic Groups and Environmental Parameters 2-24
Table 3-1. SHERLOCK RPA Primers, crRNA, and gBlock Sequences 3-10
Table 3-2. SHERLOCK Assay Reaction Components, Per RPA Pellet 3-11

Table 3-3. PCR Primers Targeting Cytochrome B Gene for Sanger Sequencing 3-11
Table 3-4. Validation Wakasagi Swab Specimens 3-11
Table 3-5. Validation Delta Smelt swab specimens 3-12
Table 3-6. Summary of SHERLOCK and Sanger DNA Data for Six *Hypomesus* Specimens Collected by The field Office in Late 2023-Early 2024. 3-13
Table 3-7. DNA Sequences for Five Smelt Specimens Collected by USFWS-LFWO in Winter 2023-2024 Sampling Season 3-14
Table 4-1. Total Number of Archived Delta Smelt Available for the Study by Year and Known Origin, Assuming Complete (100%) Fractional Tagging (fin clip and/or elastomer) of Cultured Individuals That Were Released Into the Wild 4-17
Table 4-2. Total Number of Delta Smelt Assigned to Each Vaterite Category by Year and Origin 4-17
Table 4-3. Results of a Logistic Regression Model Examining the Probability of a Delta Smelt Exhibiting Relatively High Vaterite (category II-IV) as the Interactive Effects of Size (fork length) and Origin 4-17
Table 4-4. Results of a Linear Model Examining Otolith-Somatic Proportionality of Hatchery and Wild Delta Smelt 4-18
Table 4-5. Results of the Generalized Additive Model (GAM) Examining Otolith Growth as the Interactive Effects of Fish Age and Origin 4-18
Table 4-6. Confusion Matrix for MDA Models Predicting Delta Smelt Origins Based on the First 100 µm or 400 µm of Each Fish's Otolith ^{87}Sr/^{86}Sr Profile 4-18
Table S4-1. Delta Smelt Included in the Study by Year and Survey 4-23
Table S4-2. Sample Sizes of Cultured and Wild Delta Smelt Included in Each Analysis 4-23
Table 5-1. Summary of Sampling Events and Exposure Test Initiation Dates Across the Study 5-19
Table S5-1. Summary of Conductivities (µS/cm) of Individual Site Locations. 5-25
Table S5-2. Summary of Contaminants Detected in the Control in 2018 and 2019 5-25
Table S5-3. Summary of Contaminants Detected Across the 2017 Study Period 5-26
Table S5-4. Summary of Contaminants Detected Across the 2018 Study Period 5-30
Table S5-5. Summary of Contaminants Detected Across the 2019 Study Period 5-31
Table 6-1. Deployment Dates, Age (days-post-hatch, DPH) of Fish when Put in Enclosures and Sampled, Number of Fish Deployed (n) and Estimated Percentage Survival of Delta Smelt for the Study 6-27
Table 6-2. Discrete Water Quality Measures Taken at Enclosure Checks During the Deployments 6-27
Table 6-3. Mixed Effect Model Results for the Effect of Season (at Rio Vista) and Site (within fall) on the Change in Condition (Delta K) and Change in Weight (Delta Weight) 6-28
Table 6-4. Permutational MANOVA Model Results Comparing Zooplankton Communities Across Seasons at Rio Vista, Across Site During the Fall (i.e. Rio Vista, Suisun Marsh, Yolo Bypass), and Comparing Zooplankton and Diet Composition Across Seasons 6-29
Table 6-5. Gill and Liver Condition Across Sites and Season 6-30
Table 6-6. Results of Linear Model of Delta Smelt Critical Thermal Maximum (CTmax) Versus Aclimation/Acclimatization Temperature and Location (field versus lab) 6-32
Table S6-1. Continuous Water Quality Stations Used in the Study for Year-Round Data 6-42
Table S6-2. Result Summary Growth Metrics of Delta Smelt in Enclosures 6-43

Table S6-3. Results from Pairwise Comparisons for DeltaK and DeltaWeight by Season (at Rio Vista) and Location (sites in fall) 6-44
Table 7-1. Effects of Zooplankton Biomass, Habitat, and Seasonality on HSI 7-14
Table 7-2. Effects of Zooplankton Biomass, Habitat, and Seasonality on Gut Fullness 7-15
Table S7-1. Effects of Salinity (brackish and freshwater) and Season (summer and fall) on Zooplankton Biomass, HSI, and Gut Fullness 7-24

Figures

Figure 1-1. Map of the Sacramento-San Joaquin Delta 1-25
Figure 1-2. Comparison of Mean CPUV (catch/10,000 m^3) for Individual Competitor Species and All Small Pelagic Fish Combined 1-26
Figure 1-3. Comparison of Mean CPUV (catch/10,000 m^3) by Sampling Stratum for Delta Smelt and Their Small Pelagic Competitors 1-27
Figure 1-4. Variable Importance Rankings for Selected Presence/Absence Models 1-28
Figure 1-5. Boxplots of Competitor Catch Density Relative to Binned Delta Smelt Catch 1-29
Figure 1-6. Conditional Effect of Important Covariates on A) Delta Smelt Presence, B) Combined Delta Smelt and Wakasagi Presence, and C-F) Small Pelagic Fish Presence 1-30
Figure 1-7. Conditional Effect of Competitor Density on A) Delta Smelt Detection probability, B) Delta Smelt or Wakasagi Detection Probability, and C) Delta Smelt/Wakasagi CPUV 1-30
Figure 1-8. Variable Importance Rankings for Selected CPUV Models 1-31
Figure 1-9. Conditional Effect of Important Covariates on A-C) Combined Delta Smelt and Wakasagi CPUV, and D-F) Small Pelagic Fish CPUV 1-32
Figure 1-10. Shapley Values Relative to Selected Covariate Values for Delta Smelt/Wakasagi Model 4 with Comparison of the Sacramento River Deep Water Ship Channel to other Sampled Regions 1-33
Figure 2-1. Map of the Regions and Locations Sampled in the Upper San Francisco Estuary and Average Position of X2 in Action and No-Action Years 2-25
Figure 2-2. Outflow and X2 from June Through January 2017 – 2021 2-26
Figure 2-3. Nutrient Concentrations Between Action (blue) and No-Action (orange) Years and Study Regions in the Upper SFE 2-27
Figure 2-4. Environmental Parameters Between Action (blue) and No-Action (orange) Years and Study Regions in the Upper SFE 2-28
Figure 2-5. Non-Metric Multidimensional Scaling (NMDS) Ordination of Phytoplankton Community Structure in the upper San Francisco Estuary for Action Year Status 2-29
Figure 2-6. Ratios of Dissolved Nutrients Between Action (blue) and No-Action (orange) Years and Regions of the Upper SFE, with Dissolved Inorganic Nitrogen to Phosphate (top) and Nitrate to Ammonium (bottom) 2-30
Figure 2-7. Chlorophyll Concentrations Between Action (action) and No-Action (no-action) Years in the Five Regions of the Upper SFE 2-31
Figure 2-8. Chlorophyll-a Generalized Linear Model with Action Year Status, Region, Temperature, Salinity, Ammonium, Phosphate, and X2 with 95% Confidence Intervals 2-32
Figure 2-9. Proportion of Sites with Colonial Microcystis spp. Observed in Each Region and Year 2-32

Figure 2-10. Mean Abundance of Each Phytoplankton Group by Year and Region as (A) Proportion of the Total Phytoplankton Abundance and (B) Scaled to Mean Chlorophyll-a to Highlight the Respective Contribution of Each Group to the Phytoplankton Biomass 2-33
Figure S2-1. NMDS Ordination of Phytoplankton Community Structure and Environmental Parameters by Region 2-34
Figure 3-1. Workflow for Rapid and Minimally Invasive Species Identification Using SHERLOCK 3-15
Figure 3-2. Stability of Pre-Frozen SHERLOCK Reaction Mixes Over Time at -80°C 3-16
Figure 3-3. SHERLOCK Validation Testing of Wakasagi and Delta Smelt Frozen Specimens 3-17
Figure 4-1. Crystalline Structure of Cultured and Wild Delta Smelt Collected in Field Surveys 4-19
Figure 4-2. Otolith-Somatic Proportionality for Wild (black) and Cultured (red) Delta Smelt 4-20
Figure 4-3. Otolith Increment Profiles of Wild and Cultured Delta Smelt 4-21
Figure 4-4. Core-to-Edge ^{87}Sr/^{86}Sr Profiles of Wild and Cultured Delta Smelt 4-22
Figure 5-1. Map of Study Area and Sampling Sites in the Sacramento-San Joaquin River Delta and San Francisco Estuary, Located in California, USA 5-20
Figure 5-2. Net Delta Outflow (m^3/sec) Encompassing the Three-Year Project Period, Flow Actions, and Exposures 5-21
Figure 5-3. Concentrations of Organic Compound Detections Shown for Each Exposure Test from the Six Sites for A) 2017, B) 2018, and C) 2019 5-22
Figure 5-4. Number of Detections of Organic Compounds Per Exposure in 2019 5-23
Figure 5-5. Summary of Gill Lesion Scores for A) 2017, B) 2018, C) 2019, and Liver Lesion Scores for D) 2017, E) 2018, and F) 2019 5-23
Figure 5-6. Summary of Gill Lesion Scores for A) 2017, B) 2018, C) 2019, and Liver Lesion Scores for D) 2017, E) 2018, and F) 2019, Across Toxicity Exposures 5-24
Figure S5-1. Summary of Delta Smelt Endpoints Compared Among Years: A) Survival, B) Gill Lesions, and C) Liver Lesions 5-33
Figure S5-2. A) Severe Secondary Lamella Fusion (F) in 3 Gill Filaments at Low Magnification; B) Higher Magnification Showing Moderate Epithelial Cell Necrosis (arrows) and Aneurysm (ANU) in Delta Smelt Exposed to Water Collected from Cache Slough in a Toxicity Exposure Initiated on October 27, 2017 5-33
Figure S5-3. Delta Smelt Exposed to Water from Cache Slough in a Toxicity Exposure Initiated on November 10, 2017 5-34
Figure S5-4. Mucous Cell Hyperplasia (black arrows), Epithelial Cell Necrosis (yellow arrows), Small Aneurysm (ANU), and Edema (SLE) in Secondary Lamella of Gill in Delta Smelt Exposed to Water Collected from the Sacramento River at Decker Island (200× magnification) in a Toxicity Exposure Initiated November 8, 2019 5-34
Figure 6-1. Smelt Enclosure Sites in the San Francisco Estuary of California 6-33
Figure 6-2. Delta Smelt Enclosure Design Used in 2019 Deployments (Photo on Left) and Three Enclosure Replicates in Suisun Marsh (Photo on Right) 6-34
Figure 6-3. Water Quality Parameters Measured Every 15 Minutes Across Seasons and Enclosure Locations in 2019 6-35
Figure 6-4. Modeled Estimate of Proportional Change (with 95% Confidence Intervals) of Delta Smelt Growth Metrics Across Season (A-B) and Sites (C-D) 6-36
Figure 6-5. Relative Abundance of All Zooplankton Caught at Each Site During Each Deployment Season and Mean Contribution of Observed Taxa to Delta Smelt Diets at Each Site at the End of Each Deployment 6-37

Figure 6-6. Mean Liver Lesion Score (A), Glycogen Depletion (B), and Gill Lesion Score (C) by Season at Each of Five Locations: the Fish Conservation and Culture Laboratory (FCCL), Rio Vista, the Sacramento Deep Water Shipping Channel (SRDWSC), Suisun Marsh, and Yolo Bypass 6-38
Figure 6-7. Livers of Delta Smelt H&E 600X 6-39
Figure 6-8. Gills of Delta Smelt 6-40
Figure 6-9. Critical Thermal Maximum (mean CTmax in °C ± SD) of Delta Smelt by Acclimation Temperature in the Field (blue) or Laboratory (red) 6-41
Figure S6-1. Percent Survival of Delta Smelt After Deployments Across Sites and Seasons 6-45
Figure S6-2. Length (cm), Weight (g), and Condition Factor (K) of Delta Smelt Before and After Enclosure Deployments Across Sites and Season 6-46
Figure S6-3. (A) Relative Abundance of all Zooplankton Caught at Each Site During Each Deployment Season; (B) Relative Abundance of Macrozooplankton Caught at Each Site During Each Deployment Season 6-47
Figure S6-4. Contribution of Different Taxa to Delta Smelt Diets at Each Location and Season 6-48
Figure S6-5. Critical Thermal Maxima (CTmax) of all Fish Measured in 2019 Including Cages in the Summer at Rio Vista 6-49
Figure 7-1. Distribution of the Abundance of Tidal Wetland Within a 2-km Radius of Each Station Included in our Study (n = 74) 7-16
Figure 7-2. Map of the Sacramento-San Joaquin Delta and San Francisco Estuary (the Estuary) 7-17
Figure 7-3. Habitat Categorizations for Each Station (n = 74) Included in our Study 7-18
Figure 7-4. HSI Shown as a Function of Zooplankton Biomass (BPUE), Salinity, and Season 7-19
Figure 7-5. HSI Shown as a Function of Zooplankton Biomass (BPUE), Season, and Wetland Abundance 7-19
Figure 7-6. Panels A and B Show HSI as a Function of Zooplankton Biomass (BPUE), Season, and Region 7-20
Figure 7-7. Gut Fullness Shown as a Function of Zooplankton Biomass (BPUE), Salinity, and Season 7-20
Figure 7-8. Gut Fullness Shown as a Function of Zooplankton Biomass (BPUE), Season, and Wetland Abundance 7-21
Figure 7-9. Gut Fullness Shown as a Function of Zooplankton Biomass (BPUE), Season, and Region 7-22
Figure S7-1. Season and Salinity Effects on Zooplankton Biomass (BPUE) 7-25
Figure S7-2. Season and Salinity Effects on HSI 7-25
Figure S7-3. Season and Salinity Effects on Gut Fullness 7-26
Figure S7-4. Temporal Trends in Biotic Variables from Our Dataset 7-26
Figure S7-5. HSI Shown as a Function of Zooplankton Biomass (BPUE), Season, and Wetland Abundance Modeled from a Dataset that Excluded the Minimum and Maximum Zooplankton Biomass, Both of Which Were Observed in the 'Medium' Wetland Category 7-27

Acronyms

°C	degrees Celsius
µg/L	micrograms per liter

μL	microliter
μm	micrometer
μmol/L	micromole per liter
AIC	Akaike Information Criteria
ANOVA	Analysis of Variance
AUC	area under the receiver operating curve
Bay-Delta	San Francisco Bay Estuary and Sacramento-San Joaquin Delta
BDL	Beldon's Landings
BIM	biological intercept model
BiOp	Biological Opinion
BLAST	Basic Local Alignment Search Tool
BPUE	Biomass per unit effort
BRT	boosted regression tree
$CaCO_3$	calcium carbonate
CDEC	California Data Exchange Center
CDFW	California Department of Fish and Wildlife
cfs	cubic feet per second
CHIP	Chipp's Island Survey
cm	centimeter
cm/s	centimeters per second
CPUV	catch per unit volume
Cq	quantification cycle
CSC	Cache Slough and Liberty Island
CTmax	critical thermal methodology
CVP	Central Valley Project
Delta	Sacramento-San Joaquin River Delta
DO	dissolved oxygen
DOC	dissolved organic carbon
DOP	Directed Outflow Program
dph	days post hatch
DSRS	Delta Smelt Resiliency Strategy
DWR	California Department of Water Resources
EDSM	Enhanced Delta Smelt Monitoring

EI	electron ionization
EMM	estimated marginal means
ESA	Endangered Species Act
Estuary	San Francisco Estuary
FCCL	Fish Conservation and Culture Laboratory
FMWT	Fall Midwater Trawl
FNU	Formazin Nephelometric Unit
g	gram
GAM	generalized additive model
GBM	gradient boosting machine
GC	Gas Chromatography
GC-QTOF-MS	Gas Chromatography coupled with Quadrupole Time-Of-Flight Mass Spectrometry
GLM	generalized linear model
GRTS	generalized random tessellated stratified
HAB	harmful algal bloom
Hg	Mercury
HNLC	High Nutrient Low Chlorophyll
HSC	high salinity control
HSI	hepatosomatic index
km	kilometer
L	liter
LA-MC-ICPMS	Laser Ablation Multi-Collector Inductively Coupled Plasma Mass Spectrometry
LC	Liquid Chromatography
LC-QTOF-MS	Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry
LFWO	Lodi Fish and Wildlife Office
LIS	Lisbon Weir
LSZ	Low Salinity Zone
m	meter
m^2	square meter
m^3/sec	cubic meters per second
MDA	mixture discriminant analysis

mg/L	milligrams per liter
mL	milliliter
mm	millimeter
NCI	negative chemical ionization
NDOI	Net Delta Outflow Index
NDSF	North Delta Food Subsidy
ng/L	nanogram per liter
NGO	non-governmental organization
NMDS	Non-metric Multidimensional Scaling
NTU	nephelometric turbidity unit
o-s	otolith-somatic
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PERMANOVA	permutational multivariate analysis of variance
ppt	parts per thousand
PSU	Practical Salinity Unit
qPCR	quantitative polymerase chain reaction
Reclamation	U.S. Bureau of Reclamation
RMSE	root mean square error
RPA	Recombinase Polymerase Amplification
RV	Rio Vista
RVB	Rio Vista Bridge
SFE	San Francisco Estuary
SFHA	Summer-Fall Habitat Action
SHERLOCK	Specific High-sensitivity Enzymatic Reporter Unlocking
SKT	Spring Kodiak Trawl
SM	Suisun Marsh
SNP	single nucleotide polymorphism
SRDWSC	Sacramento River Deep Water Ship Channel
SRWTP	Sacramento Regional Wastewater Treatment Plant
STN	Summer Townet
SWP	State Water Project
TL	total length

USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey
VIA	Visible Implant Alphanumeric
VIE	visual identification elastomer
YB	Yolo Bypass

Chapter 1. Evaluation of Delta Smelt Distribution Reveals Association With Other Small Pelagic Species: Implications for Competitive Dynamics and Proxy Monitoring

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Abstract

Detections of endangered or rare species can require enormous efforts and even then, positive detections may nevertheless be sparse, making development of habitat occupancy models or evaluation of management actions difficult. One possible solution to the challenge of low detection probability is to incorporate data from proxy species that share the same ecological niche. In the San Francisco Bay Estuary and Sacramento-San Joaquin Delta (Bay-Delta), the decline of the endemic Delta Smelt *Hypomesus transpacificus* has reached critical levels where detections are rare even with extensive survey efforts. With paired data from an intensive pelagic fish survey and a similarly robust lower-trophic monitoring study, we applied a tree-based machine learning algorithm, to evaluate habitat characteristics associated with detection of Delta Smelt. Preliminary analysis using only Delta Smelt data produced a model with low predictive ability, while models combining Delta Smelt with a closely related congener species Wakasagi smelt *Hypomesus nipponensis* or with a broader set of small pelagic fishes (Clupeidae, and Mississippi Silversides *Menidia audens*) were better supported. Therefore, we applied the same method to this suite of potential surrogate species. Unexpectedly, the catch per unit volume (CPUV) of the small pelagic fish community was the strongest explanatory variable for both Delta Smelt and Delta Smelt plus Wakasagi detections. Zooplankton food variables and salinity had the highest relative influence on small pelagic fish species CPUV. We conclude that Wakasagi and the small pelagic fish community are good proxies for Delta Smelt with caution. The relationship for abiotic water quality variables (e.g., salinity) matches what is known about Delta Smelt, while the prey species that had the highest relative influence (*Sinocalanus doerrii*) is not a commonly consumed Delta Smelt prey during the summer and fall, suggesting some differences in niches among the fish species. This study suggests that Wakasagi and the small pelagic fish community may provide important information for Delta Smelt habitat models.

Introduction

Efforts to establish habitat preferences for endangered and vulnerable species can be difficult given the rarity of detecting the species of interest, as much time and effort can be required for even a few detections (Jeliazkov et al. 2022, Belbachir et al. 2015, Thompson 2013, Shea and Mangel 2001). Even when large-scale efforts for sampling rare species are feasible, the resulting data can be challenging to analyze, as large numbers of absences can prevent models from correctly identifying suitable habitat (Cianfrani et al. 2010) or create models with large predictive uncertainties (Hamilton et al. 2015). This may lead to ineffective actions or even failed efforts to bolster populations. Notwithstanding these challenges, understanding relationships between endangered species and their preferred habitat is key to conservation efforts (Rushton et al. 2004).

Delta Smelt *Hypomesus transpacificus* in the northern Bay-Delta) have undergone a drastic decrease in abundance over the past several decades and inhabit a heavily altered and degraded environment. Delta Smelt is a small, semi-anadromous, planktivorous fish endemic to the Bay-Delta with an annual life cycle (Moyle et al. 2016). Delta Smelt spawn during the winter-spring season, larval fish hatch in the spring and the majority make their way to the low salinity zone (LSZ, 0.5-6 parts per thousand (ppt)) where they transition into juveniles from summer to fall before migrating upstream to freshwater regions to spawn. Various anthropogenic stressors have contributed to the decline of Delta Smelt including destruction of habitat, exposure to contaminants, decreased prey availability, introduced invasive predators, competitors and prey, changes in flow regimes due to water diversions, and increased temperatures during critical life stages (FLOAT-MAST 2020, Hamilton and Murphy 2018, Moyle et al. 2016). Numerous and substantial conservation and restoration efforts have been undertaken since Delta Smelt's listing as threatened under the Federal and California Endangered Species Acts in 1993 (The California listing was upgraded to endangered in 2009). Recently, large-scale management actions including flow augmentation during autumn and flow pulses during the summer were implemented to improve LSZ habitat for Delta Smelt (Frantzich et al. 2021, Sommer et al. 2020, USFWS 2019). However, Delta Smelt abundance has continued declining in recent years making detections of Delta Smelt even rarer and recovery efforts more urgent (Bacher 2022, USFWS 2022, CDFW 2022).

Understanding habitat preferences of Delta Smelt using the best available analytical tools and data therefore remains crucial to restoration efforts and management actions designed to increase and improve habitat (Hamilton and Murphy 2020), and many models have been produced to predict Delta Smelt habitat suitability or habitat usage. Previous efforts have identified various combinations of abiotic and biotic variables as important components of Delta Smelt habitat (Hamilton and Murphy 2018, Polanksy et al. 2018, Bever et al. 2016, Feyrer et al. 2011). Other studies of Delta Smelt have utilized occupancy models to better separate the potentially independent processes of habitat preference and the ability to detect a species, given its presence (Hendrix et al. 2022, Davis et al. 2022, Duarte and Peterson 2021, Peterson and Barajas 2018, Mahardja et al. 2017). While these modeling efforts have collectively advanced the theoretical understanding of historic Delta Smelt habitat preferences, verifying model results through additional sampling has become challenging, given that Delta Smelt are now rarely encountered in the long-term surveys on which many prior analyses were based. Even intensive, targeted Delta Smelt monitoring efforts have captured relatively few fish, and further insights are therefore likely to require novel analytical tools, new approaches for detecting rare species and/or use of surrogates such as cultured conspecifics or other, more common taxa with shared habitat preferences.

A relatively recent development in modeling habitat preferences has been the use of machine learning algorithms that, in theory, can overcome some of the challenges associated with multiple, potentially interacting variables, and non-linear relationships while relaxing some of the assumptions associated with classical modeling approaches (e.g., linear and generalized linear models). Tree-based algorithms are now commonly used in ecological studies (Stupariu et al. 2022, Tuia et al. 2022), and have been used previously to predict species distribution and identify key habitat associations over different spatial scales for other endangered and threatened species (e.g., Wang et al. 2015, Hopkins 2009). This family of machine-learning algorithms are all fundamentally based upon classification and decision trees, that employ various ensemble approaches to combine multiple, stochastically generated trees to improve the predictive performance and generalizability of the final ensemble. Additional advantages of using tree-based models are their ability to handle diverse types of predictor variables and accommodate missing data (Elith et al. 2008). When using tree-based models, the form of relationships between response and predictor, and the extent of interaction between covariates is dictated primarily by the data rather than making *a priori* assumptions as with generalized linear and additive models (Abeare 2009). Recent application of both tree-based machine learning and occupancy models to Delta Smelt have been met with mixed success. Grimaldo et al. (2021) used gradient boosting machines (GBM) (i.e., boosted regression trees (BRT)); to evaluate factors that could predict Delta Smelt entrainment at regional water projects. However, despite identifying a model capable of explaining a large proportion of the null deviance, the predictive performance of the model was poor, potentially due to the ongoing decline in species abundance and the resulting low number of salvaged fish.

The overall objective of our study was to characterize the relative importance of abiotic factors, zooplankton prey, and other potential small pelagic fish competitors on the presence and abundance of Delta Smelt and we hypothesized that salinity, turbidity and calanoid copepod prey, especially *Pseudodiaptomus forbesi* (Slater et al. 2019, Slater and Baxter 2014), would be among the strongest predictors. We sought to achieve this objective by utilizing a powerful and flexible, tree-based machine learning approach and by combining two unusually robust datasets; the first resulting from an intensive sampling effort targeting Delta Smelt and other small pelagic fishes in the Bay-Delta, and the second from a similarly intensive effort to sample the invertebrate prey-base of the Bay-Delta that was frequently paired in space and time with the fish sampling effort. The models resulting from preliminary analyses using Delta Smelt alone as the response variable had limited predictive ability, but did identify the presence of potential competitors, including Wakasagi Smelt *H. nipponensis*, American Shad *Alosa sapidissima*, Threadfin Shad *Dorosoma petenense* and Mississippi Silverside *Menidia audens* as the strongest predictor of Delta Smelt presence.

Given the continued decline of Delta Smelt in recent years, researchers are left with two suboptimal options for analyzing Delta Smelt-habitat relationships: rely on historical data that may have little bearing on current and future conditions, given the fundamental changes that have occurred in the Bay-Delta, or rely on more recent data where the low rate of detections challenge even sophisticated models. The use of indicator or proxy species may offer a third, complementary option to draw inferences about habitat preferences and quality. Studies suggest that other non-native competitors such as the closely related Wakasagi Smelt and Threadfin Shad may be suitable proxies for Delta Smelt, because they have overlapping ecological niches with Delta Smelt (Davis et al. 2022, Feyrer et al. 2003), are associated with Delta Smelt occupancy (Hendrix et al. 2022) and are caught in the same monitoring gear types (McKenzie and Mahardja 2021, Stompe et al. 2020).

Proxy species and community level analyses have been successfully used for modeling other endangered and low abundance species (Rodríguez et al. 2021, Silvano et al. 2017, Dunn et al. 2015). Dunn et al (2015) used two proxy bird species with similar macro-habitat requirements to the critically endangered Himalayan quail to model suitable survey areas. Rodríguez et al. (2021) used community-level models to understand vulnerable fish species responses to environmental stressors through shared species responses to the environment. Prior research has shown that when using proxy species, appropriate precautions need to be considered such as if the proxy species shares the same key ecological traits that make the endangered species population vulnerable to disturbance or if the appropriate habitat scale is being considered (Loman et al. 2021, Caro et al. 2005). With the precautions in mind, and based on the strong, linear, and positive relationship between the presence of Delta Smelt and other members of the small pelagic community, we proceeded to evaluate whether a proxy species approach involving the grouping of Delta Smelt with one or more of the other Bay-Delta small pelagic species could improve model performance and identification of important habitat features. In addition to potentially increasing power to evaluate Delta Smelt habitat associations, this approach may provide broader insight into the drivers of the Bay-Delta's pelagic forage fishes and serve as a potential modeling framework for other rare species that are now infrequently detected.

Methods

We focused our study on the upper portion of the Bay-Delta (Figure 4-1) where the U.S. Fish and Wildlife Service (USFWS) conducts routine monitoring of Delta Smelt through the Enhanced Delta Smelt Monitoring program (EDSM: USFWS 2022). Sampling locations were chosen using a generalized random tessellated stratified (GRTS) sampling design, which creates random sites that are spatially well distributed across a sampling region (Starcevich et al. 2016, Stevens Jr and Olsen 2004). Kodiak trawls were conducted from July through March for juvenile and adult Delta Smelt in different regions of the overall sample frame. Environmental parameters (temperature, turbidity, salinity, and chlorophyll-*a*) and lower trophic invertebrate samples (mesozooplankton and macrozooplankton) were collected alongside EDSM sampling from 2017 – 2021 as part of the Directed Outflow Project (DOP) Lower Trophic Study (Schultz and Kalmbach 2022). This paired sampling was conducted with a subset of the EDSM monitoring sites; pairing occurred for three different sites within a region each week. Each week a new set of sites were generated and sampled. The total number of sampling strata used by EDSM has varied over time and DOP does not sample all strata visited by EDSM; 19 regions were considered for this analysis with five regions typically sampled each week (Figure 1-1). While sampling was conducted year-round, this study focuses on the summer through late autumn period (July through November). Data were examined from 2017 through 2021. Only autumn season data were available for 2017-2018 from the DOP; summer and autumn data were available for 2019 – 2021.

Kodiak trawling was conducted following methods described by USFWS (2022). Between two to ten tows were completed at each site, with a minimum duration of two minutes and a maximum of ten minutes per tow. The Kodiak trawl net was towed in the first few meters of the water column against the water current. Mean net mouth size was estimated to be 12.54 square meters (m^2). The distance traveled during each tow was recorded using a calibrated mechanical flow meter, which was used to calculate CPUV. If a Delta Smelt was captured within the first two tows at a site, then the site was complete, otherwise tows were repeated until a Delta Smelt was caught or the maximum number of tows (typically four) were completed. A suite of water quality parameters was also collected during fish sampling, including temperature (degrees Celsius (°C)), dissolved oxygen (milligrams per liter (mg/L)), specific conductance (microsiemens/centimeter for freshwater and millisiemens/centimeter for saltwater), turbidity (nephelometric turbidity unit (NTU)), and Secchi depth (centimeters (cm)). Water quality parameter measurements were taken from the top meter of the water column, using a YSI PRO 2030 Meter. Turbidity values were obtained using Hach 2100Q turbidity meters.

Macrozooplankton and mesozooplankton sampling was conducted according to methods described in Schultz et al. (2019). One tow was conducted for each habitat type (shoal, channel surface, channel deep) present at a site. A shoal was defined as habitat that was roughly 3 meters deep or less. Channel habitat was greater than 3 meters deep; a surface tow was conducted in all channel and shoal habitat in the first meter of the water column. In channels greater than 6 meters deep, an additional tow was conducted in the bottom half to third of the water column. Not all habitat types were present at each site, so between one to three tows were conducted per site. In 2017, invertebrates were sampled with two, 50-cm-diameter Clarke-Bumpus nets attached to a bongo net frame; mesozooplankton were targeted with a 150-micron mesh size net and macrozooplankton were targeted with a 50-micron mesh size net. Beginning in 2018, the mesozooplankton net was switched to a 20-cm-diameter net. The tow duration was 8 minutes in 2017 but was shortened to 5 minutes beginning in 2018. Zooplankton samples were processed according to methods described in the Interagency Ecological Program's Environmental Monitoring Program (Kayfetz et al. 2020) except a maximum of 10 aliquots counts were used to account for higher sample densities resulting from using larger volume nets as described in Schultz et al. (2019).

Data Processing

Fish catches were summarized to station-level CPUV by dividing the total catch of each species summed across all tows conducted at a station by the total tow volume. The potential for depletion across tow replicates was assumed to be negligible given the pelagic and dynamic nature of the waters sampled. In addition to Delta Smelt, five other fish species were incorporated into either dependent or independent variables in this analysis (Figure 1-2). Although many other species may share the same pelagic habitat and food preferences as Delta Smelt during portions of their life histories, we selected the most encountered forage fish species from the fish survey to represent potential competitors of Delta Smelt (Figure 1-3). Northern anchovies were excluded from the competitors variable despite a high frequency of capture because of their preference for much higher salinities than Delta Smelt. The competitors variable (Competitors_All) therefore included Threadfin and American Shad, Mississippi Silverside and two other smelt species; Wakasagi and Longfin Smelt (*Spirinchus thaleichthys*). When modeling combined Delta Smelt and Wakasagi catch as the response variable, Wakasagi were removed from the independent competitor variable (Competitors_NoWakasagi).

Fish catch data were zero inflated with more zero-catch values than would be expected from common statistical distributions (e.g., log-normal, Poisson, negative binomial). This may reflect highly patchy distributions, or distinct processes governing presence/absence and abundance. In such cases a two-step approach may be appropriate, where presence/absence and abundance are modeled separately. We therefore considered each response variable first as a binary metric of presence and absence by assigning a value of one to all fish study tows with a non-zero catch of the relevant group. Subsequently, a censored dataset was created for each response grouping that retained only positive detections for the species/ group of interest. In total we considered six potential response variables: Delta Smelt alone, Delta Smelt and their most analogous surrogate, Wakasagi (an introduced smelt species from Japan that are of the same genus as Delta Smelt and appear to be increasing in abundance in the upperstream portions of Bay-Delta; Davis et al. 2022), and a broader group of small pelagic species (Delta Smelt combined with Competitors_All as described above) were treated as either a continuous (CPUV) or binary (presence/absence) variable.

Zooplankton were classified at various taxonomic levels, the broadest level comprising three groups, Mysidae, Amphipoda and mesozooplankton (zooplankton 0.2 to 20 millimeters (mm) total length (TL). This grouping was then narrowed down to only include taxa that were verified to be Delta Smelt prey items based on prior diet studies (Slater and Baxter 2014, Slater et al. 2019). Second, mesozooplankton was further separated into Calanoida, Cyclopoida, and Cladocera. These three groupings were again narrowed down to individual species/genus for known or hypothesized Delta Smelt preferred prey items. Finally, we further differentiated the selected calanoid and cyclopoid species by life stage (adult versus copepodite). In total, we evaluated five models for each response variable, with models 1-5 containing increasingly granular treatment of the invertebrate data (Table 1-1).

Since invertebrate and fish sampling were not always perfectly paired in time and space, many detections of Wakasagi and Delta Smelt occurred when the invertebrate study was not sampling. Invertebrate data were therefore averaged across time and space to increase the number of paired samples; each fish sample was paired with invertebrate samples collected up to one week prior and in the same subregion, which were summarized to taxon-specific mean CPUV values. Invertebrate sampling occurred in multiple habitats (shoal, channel surface and channel deep) at each site, so catch was average across all habitats sampled at a site. After processing and joining the datasets as described above, a total of 2,246 unique station/date combinations remained, and this joined dataset was used in all subsequent analyses. Training and testing datasets for each response variable were then created using the 'createDataPartition' function from the R package 'caret' which attempts to balance the classes within each set (Kuhn 2022). For presence/absence models all 2,246 instances were used while models using CPUV in the response were trained using censored data including only instances when the species or species group was detected (Delta Smelt: N = 104, DS/Wakasagi: N = 284; N = 1,517). In all cases 70% of the data was used for training with 30% reserved for testing and cross validation.

Model Fitting

We first developed a range of candidate models incorporating combinations of abiotic habitat features and food variables derived from the invertebrate sampling data and based on reported Delta Smelt food and habitat preferences from prior studies (Slater et al. 2019, Slater and Baxter 2014, Feyrer et al. 2011). Abiotic covariates were consistent across models and included water quality parameters collected during fish sampling (see above), a 14-day backward-looking moving average of Delta Outflow (California Data Exchange Center (CDEC)), week of year and region (Figure 1-1). Food variables differed across models and ranged from the very coarse aggregation with only three variables – all mysids, all amphipods, and all zooplankton – to the much more disaggregated grouping with individual Delta Smelt prey taxa included (All covariates and their sources are summarized in Table 1-2). The influence of these covariate sets on each response variable was modeled using the gradient boosting machine algorithm as implemented in the 'gbm' and 'dismo' R packages (Commonly referred to as Boosted Regression Tree or BRT models). Implementation of BRT models requires limited user inputs, but tuning of several hyperparameters can help to limit overfitting (Schratz et al. 2019). We therefore utilized a grid search approach to identify optimal values for learning rate – the weight given to each new tree in the BRT model – and tree complexity – the number of levels allowed in each tree. BRT models were fitted using the 'gbm' function with learning rate values between 0.1 and 0.0005 and tree complexity values of 2-5 (Greenwell et al. 2020). Combinations of hyperparameters were then compared based on root mean square error (RMSE) and the lowest error combination was used in subsequent model fitting. Each model was then refit using the 'gbm.step' function from the R package 'dismo' which automates an iterative approach to identify the optimal number of trees to include in the BRT model for a given complexity and learning rate (Hijmans et al. 2021).

Model Validation

After fitting, models were evaluated using a variety of cross-validation and diagnostic techniques. The 'gbm' function calculates and reports internal cross-validation metrics during fitting including the area under the receiver operating curve (AUC) for classification models and cross-validation correlation for regression models. AUC measures both sensitivity and specificity of a classification model and values greater than 0.8 generally indicate strong discrimination (Hosmer and Lemeshow 2000). We then generated predictions from each model using the reserved training data. For the presence/absence models, predicted classifications were generated using a range for threshold values between 0.3 and 0.7, and predicted classifications were then compared with observations using the 'confusionMatrix' function from the R package 'caret', which calculates a suite of predictive performance indices including accuracy, no-information rate, Cohen's kappa coefficient, sensitivity, and specificity. For CPUV models, relationships between observed and predicted values were compared visually, using scatterplots, and by calculating Pearson correlation coefficients.

Describing, comparing, and visualizing the influence of food and habitat on the presence/absence or CPUV of Delta Smelt and their potential surrogates was the primary objective of this analysis. The most influential covariates were identified by comparing variable importance; the contribution of response variables is calculated internally by the 'gbm' and 'gbm.step' functions by permuting the model repeatedly with a single covariate excluded and calculating the proportional change in residual error. The strength, direction, and form of the relationships between dependent and independent variables were evaluated by constructing partial dependence plots using the 'plot.gbm' function, which show the conditional effect of a single covariate when all others are held at their mean or reference values. The strength of interactions between covariates was evaluated using the 'gbm.interaction' function from the 'dismo' package. Finally, Shapley values were estimated for each prediction from the testing datasets using the 'fastshap' (Greenwell 2021). Shapley values indicate the contribution of each covariate to a given prediction and provide further insight into the importance and dependencies of the covariates (Winter 2002).

Results

In total, 8,875 tows from the fish study and 4,526 tows from the invertebrate study were included in the final, paired dataset, and after summarizing to the station level, resulted in 2,246 paired samples. Among these samples, there were 104 positive detections for Delta Smelt, 284 for Delta Smelt/Wakasagi and 1,517 for small pelagic fish. Prevalence was therefore 4.6% for Delta Smelt, 12.6% when combined with Wakasagi and 67.5% for small pelagic fish. After censoring non-detections, the average CPUV was 3.19×10^{-5}, 9.77×10^{-5} and 1.24×10^{-3}, for Delta Smelt, Delta Smelt/Wakasagi and small pelagic fish, respectively. Mean CPUV for the three primary invertebrate groupings, amphipods, mysids, and zooplankton, was 9.12×10^{-1}, 3.85×10^{0} and 5.23×10^{3}, respectively. In all cases, most of the catch was comprised of potential Delta Smelt food items (95%, 99%, and 79% considered potential food for amphipods, mysids, and zooplankton, respectively). Summary statistics by region for specific zooplankton, mysid and amphipod taxa are given in Table 1-2.

Delta Smelt Models

Some validation metrics indicated good fits for the models of Delta Smelt presence and absence. For example, regardless of at which level invertebrate data were aggregated, model accuracy was 0.96. However, cross-validation indicated that for each of the models in this set, the no-information p-value was substantially greater than 0.05, which meant that simply predicting absence in all cases would result in greater model accuracy (Table 1-3). Given this indication of poor predictive performance and the low prevalence of Delta Smelt, we elected not to model censored CPUV for this response variable and interpret the model results cautiously. Examination of variable importance scores and partial dependence plots nevertheless indicated an interesting and unexpected association with hypothesized competitors that warranted further evaluation. We hypothesized that Delta Smelt would try to avoid other small pelagic fishes to reduce competition for food resources, and that this relationship may be difficult to detect in our dataset (i.e., we expected a weak, negative relationship between Delta Smelt presence and competitor abundance). Contrary to these expectations, competitor CPUV was consistently the most important variable in this set of models (Figure 1-4), and the relationship with Delta Smelt presence was strong, positive, and essentially linear. This association was confirmed visually by comparing boxplots of hypothesized competitor species' CPUV binned by Delta Smelt presence and abundance (Figure 1-5). Other variables with high importance rankings were salinity and outflow; food variables were generally ranked with lower importance across the various levels of invertebrate aggregation. The relationship between Delta Smelt presence and salinity was nonlinear with low probability of presence predicted at the lowest values, a rapid increase between ~0.1 and 1ppt, and intermediate plateau at higher salinities (Figure 1-6A).

Delta Smelt and Wakasagi Presence/Absence

Given the positive and linear association between Delta Smelt detection and the abundance of their hypothesized competitors, we evaluated whether combining Delta Smelt detections with those of one or more proxy species could help to improve model performance while still providing results relevant to threatened species management. Because they are the closest relatives of Delta Smelt among the small pelagic fish group, Wakasagi were the most directly analogous proxy species (Davis et al. 2022). As with the Delta Smelt-only models, there was little evidence of any preference for one level of invertebrate aggregation over another (Table 1-3); prediction accuracy was similar across all models in this set, though very slightly higher with intermediate levels of aggregation in the food variables. In contrast with the Delta Smelt-only models, the inclusion of Wakasagi in the response resulted in model predictions that were both highly accurate, and outperformed a consistent prediction of absence (i.e., no-information rate p-value < 0.05). Despite this improvement in predictive performance, the models of combined Delta Smelt and Wakasagi detections nevertheless indicated the same dominant drivers of distribution, with competitors and salinity consistently having the highest variable importance rankings. Indeed, the inclusion of Wakasagi resulted in an even stronger influence of competitors and further reduced the relative importance of food variables (Figure 1-7B); this relationship was again positive and linear. Meanwhile, the influence of salinity differed somewhat from the Delta Smelt results with a stronger peak in predicted presence in the 0.1-1ppt range (Figure 1-6B). Outflow was ranked lower than in the Delta Smelt models, but nevertheless retained a positive and mostly linear relationship with the probability of presence.

Small Pelagic Fish Presence/Absence

The outsized importance of competitors in explaining the distribution of Delta Smelt, both alone and in combination with Wakasagi, was unexpected, but suggested that a broader suite of small pelagic fish species may reflect to some degree the habitat preferences of Delta Smelt. We therefore progressed further in our use of proxy species, next modeling the presence and absence of the full small pelagic fish community. No further competitor variable was available for this analysis, so the expectation was that these models would help to clarify the relative importance of abiotic conditions and food availability in determining fish distribution. For each of the small pelagic fish presence/absence models, regardless of the level of taxonomic aggregation in the food covariates, the four most important variables were always abiotic and included salinity, temperature, outflow and turbidity (Figure 1-4). The probability of presence was positively related to outflow and turbidity, negatively related to temperature, and again, related to salinity in a nonlinear fashion (Figure 1-6C-E.). In contrast with the Delta Smelt/Wakasagi models, the relationship had a broader peak and with the lowest probability of presence occurring at the highest salinities.

Abundance

Abundance of Delta Smelt/Wakasagi and small pelagic fish was modeled with datasets censored for presence. Cross-validation correlations and their significance varied little across levels of food aggregation for either for small pelagic fish ($R^2 = 0.47 – 0.49$; $p < 0.001$) or Delta Smelt/Wakasagi ($R^2 = 0.24 – 0.28$; $p < 0.001$). Results of the abundance models shared some similarities with the presence/absence models in terms of variable importance rankings and the general shapes of the relationships (e.g., for salinity and competitor density), but also had some interesting differences. Zooplankton, especially *Sinocalanus doerrii*, became one of, or the most, important covariates in the small pelagic CPUV models (Figure 1-8), with a positive and mostly linear effect of these food covariates (Figure 1-9B-C). A similar pattern was observed for the combined Delta Smelt and Wakasagi CPUV response variable, with *S. doerrii* density having the highest relative influence (Figure 1-8; Figure 1-9B-C).

Regional Dynamics

The choice to exclude finer regional classifications from the candidate covariates was intentional, with the reasoning that allowing the biotic and abiotic characteristics to capture regional differences would produce more generalizable and informative results. However, examination of model predictions across finer-scale regions can provide insight into whether the influence of a given variable is consistent throughout the Bay-Delta, or if there are instead localized dynamics. Calculation of Shapley values, which reflect the contribution of each covariate to each model prediction, allowed for comparison of model dynamics on a subregional basis, even though subregion was not included as a covariate. Of interest was how the model explained the generally higher frequency of detection and CPUV in the Sacramento River Deep Water Ship Channel (SRDWSC; Figure1), which was previously identified as a hotspot for Delta Smelt population remnants (Merz et al. 2011). Based on examination of regional patterns in Shapley values, it appears that competitor CPUV contributes consistently to model predictions across regions (Figure 1-10, Row 1), but that higher competitor catch densities are much more common in the SRDWSC. In contrast, the density of *S. doerrii* only contributed appreciably to model predictions for stations within the SRDWSC, and the covariate values had little overlap between regions (Figure 1-10, Row 2.); the catch density for *S. doerrii* at SRDWSC stations was consistently higher than in other areas. Lastly, despite substantial overlap in the ranges of salinity values between regions, the contribution of this covariate to model predictions appears larger within the SRDWSC region and interestingly, the majority of Delta Smelt or Wakasagi detections occurred within a very narrow salinity range (Figure 1-10, Row 3).

Discussion

Our study sought to use machine learning based modeling approaches to characterize the importance of abiotic and biotic habitat characteristics for a rare species, but initial results produced a model with poor predictive ability. In an effort to circuvent some of the issues associated with very sparse detection data, we therefore updated the analyses to include a surrogate species, Wakasagi, which did improve predictive power, and generated a better supported model. Contrary to our expectations, both models indicated that the abundance of the small pelagic fish community (which we interpreted as competitors for the same food resources) had the highest importance over abiotic and food variables, as well as a *positive*relationship with both target and surrogate presence. When we used the small pelagic fish community presence/absence as the response variable, abiotic variables had a higher influence over biotic variables. However, when we used the small pelagic fish community CPUV as the response variable, food abundance variables and salinity stood out as strong predictors. We interpret these results to suggest that abiotic variables limit habitat that fish species can occupy (and therefore presence/absence of many small pelagic fishes) but biotic variables (e.g., food) may play a more important role in determining their abundance, given that abiotic conditions are suitable. Additionally, our study results suggest that actions that benefit the broader small pelagic fish community may also benefit Delta Smelt.

The relative importance of the competitor variable stood out in both Delta Smelt and the combined Delta Smelt and Wakasagi models, far outweighing all other variables considered. These strong positive relationships are surprising, given previous research of Delta Smelt habitat use that evaluated the effect competitors; Hendrix et al. (2022) found a slight negative relationship between Threadfin Shad (a species we included in our competitor variable) and Delta Smelt occupancy. However, Hendrix et al. (2022) found a positive relationship between Delta Smelt occupancy and predation intensity, which was interpreted as some degree of overlap between Delta Smelt and predator habitat. In contrast, comparisons between Delta Smelt and Wakasagi have shown overlap in ecological niches, distribution, and environmental tolerances (Davis et al. 2022, Swanson et al. 2000), while analysis of the declines of other pelagic fish species in the Bay-Delta have indicated similar responses to changes in certain environmental variables (such as salinity and turbidity; Thomson et al. 2010, Feyrer et al. 2007 and changes to the prey community (Feyrer et al. 2003). Our results therefore suggest that Delta Smelt shares an ecological niche with other small pelagic fish species to some degree.

The strong positive associations between Delta Smelt and Wakasagi with the small pelagic fish competitor variable may reflect associations between the species at smaller spatial scales and could result from interspecific shoaling behavior. Originally, Delta Smelt were not believed to be a strongly shoaling species (Moyle 2002), but shoaling behavior has been observed in controlled experimental settings (Davis et al. 2019, Ferrari et al. 2014). Shoaling behavior has multiple benefits, ranging from lowering cortisol levels and metabolic rates to increased predator avoidance (Chrétien et al. 2021, Hasenbein et al. 2016, Nadler et al. 2016). Given the decline in the Delta Smelt population, individuals may associate with similarly sized and shaped fish that share an ecological niche since conspecifics are either not present or present at only a low density, which may explain the strong association with other small pelagic species. Other fish species have been shown to readily shoal with morphologically similar species regardless of if the other species were native or introduced (Camacho-Cervantes et al. 2014).

Larger groups of fish can also find patchy food resources more quickly, however this may lead to increased food competition in mixed species shoals, unless food preferences differ (as reviewed in Pavlov and Kasumyan 2000). There is evidence that some of the other small pelagic fish species (Threadfin Shad) prefer similar prey as Delta Smelt, mainly pelagic copepods (Feyrer et al. 2003). However, results from our study suggest the small pelagic fish community may have been consuming different species of pelagic copepods. In our study, *S. doerrii* had a high relative influence on the CPUV of small pelagic fish, whereas other studies have shown that Delta Smelt feed primarily on *Pseudodiaptomus forbesi* during the summer and autumn season. While Delta Smelt do consume *S. doerrii*, it is primarily during the adult life stage in the winter; a period not evaluated here (Slater et al. 2019). Given the general decline of zooplankton in the Delta, changes in community composition, and seasonal shifts in peak abundances of important prey species (Winder and Jassby 2011, Merz et al. 2016), each fish species could now be targeting different available prey resources. Feyrer et al. (2003) observed expanded niche breadth and lower prey overlap in the tidal marsh fish community when the dominant food resource (mysids) greatly declined in abundance, consistent with the niche overlap hypothesis (Pianka 1974). The apparent importance of *S. doerrii* may also be more of a modeling artifact than a reflection of actual ecological dynamics. Given that high densities of this species were found almost exclusively in the SRDWSC (Figure 1-10), it is possible that *S. doerrii* are being used by the models as a surrogate for some other, unobserved, characteristic of this known hotspot for both Delta Smelt and Wakasagi (Davis et al. 2022), or the correlations between invertebrate prey and fish abundance could reflect shared habitat requirements of predator and prey rather than tracking of preferred prey. Direct comparisons of diets for fish caught concurrently with Delta Smelt could help to clarify if Delta Smelt and small pelagic fishes are consuming different species of copepods in the same environment.

While considerable overlap exists between regions where both Delta Smelt CPUV and the combined competitor CPUV were high, certain regions appear to support the small pelagic fish community but not Delta Smelt; primarily portions of the lower Sacramento River and surprisingly, the Cache Slough region (Figure 1-3). Our results may reflect more recent changes in the Cache Slough region since historically Cache Slough has yielded catches of Delta Smelt across multiple life stages (Merz et al. 2011, Sommer et al. 2009). The invasion of *Egeria densa*, a submerged aquatic vegetation species, may have reduced habitat for Delta Smelt by contributing to reductions in turbidity throughout this region (Lacy et al. 2021, Hestir et al. 2016), and increasing predation pressure by providing habitat for largemouth bass (*Micropterus salmoides*), and other non-native predatory species (Conrad et al. 2016, Brown and Michniuk 2007). Increases in surface water temperatures have also reduced habitat for Delta Smelt particularly in the more upstream regions (Halverson et al. 2022), including Cache Slough and the SRDWSC. However, thermal stratification in deep channels may provide a temperature refuge for Delta Smelt, particularly in the Sacramento Deepwater Shiping Channel SRDWSC (Mahardja et al. 2022), which may explain why Delta Smelt CPUV was higher in the deeper waters of the SRDWSC than the shallower habitat of Cache Slough. Shad species have a higher thermal tolerance (critical thermal maxima of 35°C for juvenile American Shad [Bayse et al. 2020], 33.3°C for Threadfin Shad [Monirian et al. 2010]) indicating these species may be better able to survive higher temperature conditions in Cache Slough and therefore account for the relatively high values of the small pelagic CPUV in this region.

Across a wide range of aquatic ecosystems, salinity is an important abiotic determinant of fish assemblages and distribution (Thiel et al. 1995, Ostrand and Wilde 2001, Barletta et al. 2005, Shaha et al. 2022, D 'Amen et al. 2023), and in our study it was the abiotic variable with the highest relative influence across all models. While the Delta Smelt and Wakasagi model had a narrower, more distinct peak in the 0.1-1 ppt range, the small pelagic fish community exhibited a wider peak encompassing a wider salinity range, likely reflecting the diversity of salinities within which individual species are found. For example, Threadfin Shad and American Shad are found primarily in freshwater and exhibit a negative probability of occurrence with increasing salinity (Feyrer et al. 2007, O' Connor et al. 2012), but juvenile Longfin Smelt are found at higher salinities (~2 ppt, Kimmerer et al. 2009) and Mississippi Silversides' probability of occurrence increases with higher salinity up to 3 ppt (Mahardja et al. 2016).

Across all three presence/absence models and the small pelagic fish CPUV model, there was a positive relationship with outflow (a 14 day backward moving average of outflow). Freshwater outflow actions have been utilized as part of management actions to benefit Delta Smelt by improving abiotic habitat for Delta Smelt and providing subsidies of zooplankton prey from upstream (Lee et al. 2023, Hassrick et al. 2023, Frantzich et al. 2021). However, results have been mixed; flow actions did not always result in increased Delta Smelt abundances (FLOAT-MAST 2020). Our results support previous research and modeling efforts arguing that increased outflow can benefit Delta Smelt (Castillo 2019, Feyrer et al. 2011). While the focus has been on increasing and improving habitat for Delta Smelt, our results suggest that increased outflow – or perhaps more accurately, the broader set of Bay-Delta conditions associated with wetter years (Mahardja et al. 2022) – benefits other small pelagic fish species as well. Further research is needed to better explain and explore the mechanisms related to outflow and how this benefits the small pelagic fish community, since the effects of increased outflow and the overall better conditions during higher precipitation year may be difficult to disentangle. For example, wetter conditions and higher flows tend to be associated with cooler water temperatures from spring through fall which likely benefits pelagic fishes, independent of habitat and food considerations (Bashevkin and Mahardja 2022).

Collectively, our results demonstrate a strong pattern of association between the threatened Delta Smelt and a group of relatively abundant small pelagic fish species, suggesting the potential for use of surrogate species to improve the predictive power of ecological models and facilitate more reliable evaluation of management actions. However, use of surrogate species in endangered species conservation and management is not without challenges. Caro et al. (2005) examined the use of surrogate species using a theoretical framework, concluding that in an ideal scenario, a surrogate species and the target species of interest will have a similar disturbance threshold; that is, the population growth rate for both species will move from positive to negative with a similar magnitude of a given disturbance. The authors then describe the information to evaluate suitability of a surrogate, which include 1) the relationship between the disturbance, the demographic response and the disturbance threshold, 2) the key traits or set of traits that is linked to the disturbance threshold, 3) the relationship between the key trait of the target (i.e., Delta Smelt) and surrogate species (e.g., do they have the same or similar thermal tolerances).

Although the ideal scenario is unlikely to be achieved in all cases for Delta Smelt and its potential surrogates because of multiple disturbances and diversity within the small pelagic community, decades of published research (e.g., Mac Nally et al. 2010, Moyle et al. 2016) and long-term monitoring data (Bashevkin et al. 2022) on the Bay-Delta pelagic fish community can facilitate further investigation of surrogate suitability for evaluating specific disturbances. Caro et al. (2005) identify three specific scenarios where using a substitute species may fail: when the target species is under greater disturbance than the surrogate species, when no relationship exists between the surrogate species' disturbance threshold and the key trait being measured, and when the relationship between the disturbance threshold and key trait no longer holds in the surrogate species or is different compared to the target species. To help avoid these potential pitfalls, we recommend that prior to use of surrogate species in any formal management or decision-making process, analyses like those presented here be extended to include publicly available data from the multiple long-term pelagic fish monitoring programs conducted by the California Department of Fish and Wildlife and others. The durability of these relationships across a longer time period and the generalizability of the relationships across subregions within the Bay-Delta should be evaluated as a logical next step.

This call for further research should preclude targeted use of surrogate species to inform near-term Delta Smelt recovery needs. The impacts of many historic and ongoing management actions intended to recover Delta Smelt remain uncertain because of the infrequency with which they are now detected in surveys. Consideration of impacts on the broader small pelagic fish community in re-evaluation of past actions, and monitoring of ongoing actions should increase the ability to detect biologically meaningful impacts. An additional near-term application of Delta Smelt surrogate species could be to inform release locations for cultured Delta Smelt which are now being introduced into the Bay-Delta after captive breeding and rearing. Identification of optimal release locations is challenged by the dynamic nature of the Bay-Delta and the multiple criteria that influence growth and survival. Releasing cultured Delta Smelt in the vicinity of high densities of other small pelagic fish could be trialed in an adaptive management framework, and the performance of these releases compared with those located based on alternative criteria.

Although use of surrogate species introduces its own challenges and uncertainties, infrequent detection is a pervasive problem in the study and management of rare species which can obscure the effects of recovery actions and lead to paralysis in management systems. Decision-makers tasked with allocating large amounts of financial and other resources towards threatened and endangered species recovery may interpret a lack of sensitivity to detect the impacts of management actions as ineffectiveness of those actions, and result in reluctance to implement alternatives. An ability to demonstrate measurable change can therefore be critical to such recovery efforts, and surrogate species can thus represent potentially viable alternative to continued infrequent detections of target species and the resulting high levels of management uncertainty.

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Tables

Table 1-1. Overview of Models

Response Variable	Competitor Covariate	Abiotic Covariates	Model #	Food Covariates
Delta Smelt Binary or DS/Wakasagi Binary or Small Pelagic Binary or DS/Wakasagi CPUV or Small Pelagic CPUV	All Competitors (Threadfin Shad, American Shad, Longfin Smelt, Mississippi Silverside) or Competitors without Wakasagi or None	Delta Outflow (14-day moving average), Salinity, Turbidity, Temperature, Chlorophyl a, Week of year, Coarse Region	1	Total Zooplankton CPUV, Total Mysid CPUV, Total Amphipod CPUV,
			2	DS Food Zooplankton CPUV, DS Food Mysid CPUV, DS Food Amphipod CPUV,
			3	DS Food Calanoids CPUV, DS Food Cladocerans CPUV, DS Food Cyclopoids CPUV, DS Food Mysid CPUV, DS Food Amphipod CPUV,
			4	*Pseudodiaptomus forbesi* CPUV, *Eurytemora affinis* CPUV, *Sinocalanus doerrii* CPUV, *Acartiella sinensis* CPUV, *Tortanus spp.* CPUV, *Limnoithona tetraspina* CPUV, *Bosmina longirostris* CPUV, *Sididae spp.* CPUV, *Daphniidae_spp.* CPUV,
			5	*P. forbesi* adult CPUV, *P. forbesi* copepodite CPUV, *E. affinis* adult CPUV, *E. affinis* copepodite CPUV, *S. doerrii* adult CPUV, *S. doerrii* copepodite CPUV, *A. sinensis* adult CPUV, *A. sinensis* copepodite CPUV, *Tortanus spp.* adult CPUV, *Tortanus spp.* copepodite CPUV, *Limnoithona tetraspina* CPUV, *Bosmina longirostris* CPUV, *Sididae spp.* CPUV, *Daphniidae_spp.* CPUV,

Table 1-2. Model variables with summary statistics.

Variable Code	Unit and Source	Far West	West	North
Delta_Smelt	Catch/ 10,000 $m^{3,a}$	0 (0-0)	0 (0-0)	0 (0-0.61)
Combined_CPUV	Catch/ 10,000 $m^{3,a}$	0 (0-0)	0 (0-1.3)	0 (0-13)
Small_Pelagics	Catch/ 10,000 $m^{3,a}$	0 (0-4.38)	1.2 (0-83.15)	3.3 (0-880)
Chl_a	Chlorophyl a[a]	2.1 (0.62-4.3)	2.25 (0.84-5)	1.5 (0.6-3.3)
Salinity	Salinity (PPT)[a]	12 (5.375-16)	5.05 (0.08455-12)	0.065 (0.01-0.57)
TemperatureTop	°C[a]	19 (14-22)	20 (13-23)	21 (12-24)
Turbidity	NTU[a]	18 (5.9-42)	17 (5.1-46)	5.2 (1.3-27)
Outflow_14	Daily Mean Delta Outflow (CFS)[b]	14,000 (8,100-24,750)	14,000 (8,000-24,000)	13,000 (8,000-25,000)
LH_Week	Weeks since April 15	14.5 (3-23)	15 (3-24)	14 (3-23.85)
Competitors_NoWak	Catch/ 10,000 $m^{3,a}$	0 (0-3.85)	1.2 (0-83.15)	3.3 (0-868.50)
Competitors_All	Catch/ 10,000 $m^{3,a}$	0 (0-3.85)	1.2 (0-83.15)	3.3 (0-880)
Amphipods_Food	Catch/ $m^{3,c}$	0.0064 (0-0.07)	0.091 (0-3.48)	0.36 (0.0133-2)
Amphipods_Total	Catch/ $m^{3,c}$	0.075 (0.0067-0.2625)	0.18 (0.006355-3.73)	0.36 (0.0133-2)
Mysids_Food	Catch/ $m^{3,c}$	0.565 (0.04-6.2)	1.2 (0.014-29)	0.022 (0-14)
Mysids_Total	Catch/ $m^{3,c}$	0.78 (0.06925-6.6)	1.2 (0.017-29)	0.025 (0-14)
Zooplankton_Food	Catch/ $m^{3,c}$	620 (140-2,400)	1,300 (440-4,515)	3,300 (540-23,000)
Zooplankton_Total	Catch/ $m^{3,c}$	1,900 (492.5-7,700)	1,800 (677-6,415)	3,900 (777.5-29,000)
A_sinensis	Catch/ $m^{3,c}$	280 (25-1,400)	580 (130-2,200)	0.96 (0-350)
A_sinensis_adult	Catch/ $m^{3,c}$	60 (8.6-460)	320 (56-1,100)	0.67 (0-310)
A_sinensis_cope	Catch/ $m^{3,c}$	180 (18-927.5)	220 (1.9-1,100)	0 (0-29.85)
B_longirostris	Catch/ $m^{3,c}$	0 (0-0.27)	0 (0-16)	18 (2.8-3,024)
Calanoids_Food	Catch/ $m^{3,c}$	520 (140-1,600)	1,200 (408.5-3,700)	3,100 (490-10,000)
Cladocerans_Food	Catch/ $m^{3,c}$	0 (0-2.8)	0.48 (0-55.15)	150 (16-8,500)
Cyclopoids_Food	Catch/ $m^{3,c}$	7.65 (0.1725-740)	21.5 (0.25-1400)	0 (0-31)
Daphniidae_spp	Catch/ $m^{3,c}$	0 (0-0.17)	0.033 (0-8.95)	23 (1.6-3,100)
E_affinis	Catch/ $m^{3,c}$	0.535 (0-17.75)	2.2 (0-380)	6.2 (0-580)

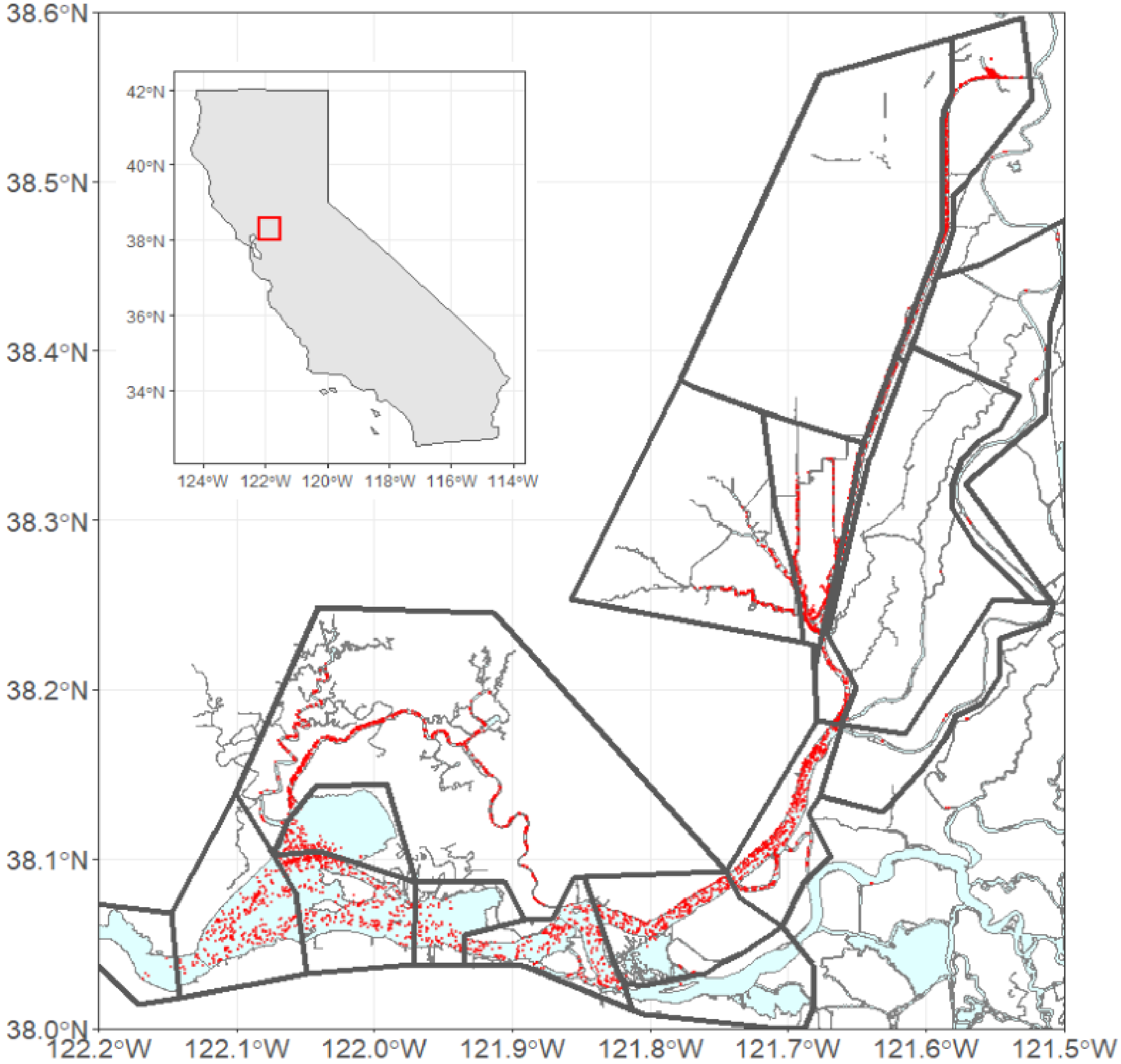
Variable Code	Unit and Source	Far West	West	North
E_affinis_adult	Catch/ m^{3,c}	0 (0-0.97)	0.29 (0-59)	0.9 (0-66)
E_affinis_cope	Catch/ m^{3,c}	0.485 (0-17)	1.8 (0-341.5)	5.15 (0-520)
L_tetraspina	Catch/ m^{3,c}	7.65 (0.1725-740)	21.5 (0.25-1,400)	0 (0-31)
P_forbesi	Catch/ m^{3,c}	9.4 (1.7-115)	96 (3.9-3,100)	2,600 (290-8,600)
P_forbesi_adult	Catch/ m^{3,c}	5.1 (1.6-79)	55 (2.485-1,330)	510 (38.3-1,884)
P_forbesi_cope	Catch/ m^{3,c}	2.3 (0-35)	40 (0.31-1,600)	1,800 (120-7,700)
S_doerrii	Catch/ m^{3,c}	0 (0-0.096)	0 (0-4.98)	5.5 (0.14-3,000)
S_doerrii_adult	Catch/ m^{3,c}	0 (0-0.072)	0 (0-3.7)	2 (0-1,300)
S_doerrii_cope	Catch/ m^{3,c}	0 (0-0.072)	0 (0-1.6)	2.3 (0-1,500)
Sididae_spp	Catch/ m^{3,c}	0 (0-1.4)	0.0545 (0-25.15)	61 (1.3-3,385)
Tortanus_adult	Catch/ m^{3,c}	32 (0.54-97.75)	6.3 (0-68)	0 (0-0.097)
Tortanus_cope	Catch/ m^{3,c}	155 (8.5-530)	16 (0-461.5)	0 (0-0.016)
Tortanus_spp	Catch/ m^{3,c}	190 (9.35-595)	24 (0-520)	0 (0-0.16)

Notes: [a] Collected by fish survey, averaged to station level, [b] Obtained from DAYFLOW report (https://data.cnra.ca.gov/dataset/dayflow), [c] Collected by invertebrate survey, averaged by subregion and period.

Table 1-3. Summary of Model Cross-Validation Statistics

Response	Model #	Accuracy	Kappa	No Information p-value
Delta Smelt	1	0.96	0.124	0.398
Delta Smelt	2	0.96	0.124	0.398
Delta Smelt	3	0.96	0.124	0.398
Delta Smelt	4	0.96	0.124	0.398
Delta Smelt	5	0.96	0.124	0.398
DS/Wakasagi	1	0.92	0.629	0.000
DS/Wakasagi	2	0.92	0.645	0.000
DS/Wakasagi	3	0.92	0.631	0.000
DS/Wakasagi	4	0.92	0.634	0.000
DS/Wakasagi	5	0.92	0.643	0.000
Small Pelagic Fish	1	0.78	0.478	0.000
Small Pelagic Fish	2	0.78	0.455	0.000
Small Pelagic Fish	3	0.78	0.484	0.000
Small Pelagic Fish	4	0.78	0.486	0.000
Small Pelagic Fish	5	0.78	0.476	0.000

Figures



Polygons show the sampling strata across which invertebrate data were averaged while points show the location of each fish sampling station.

Figure 1-1. Map of the Sacramento-San Joaquin Delta

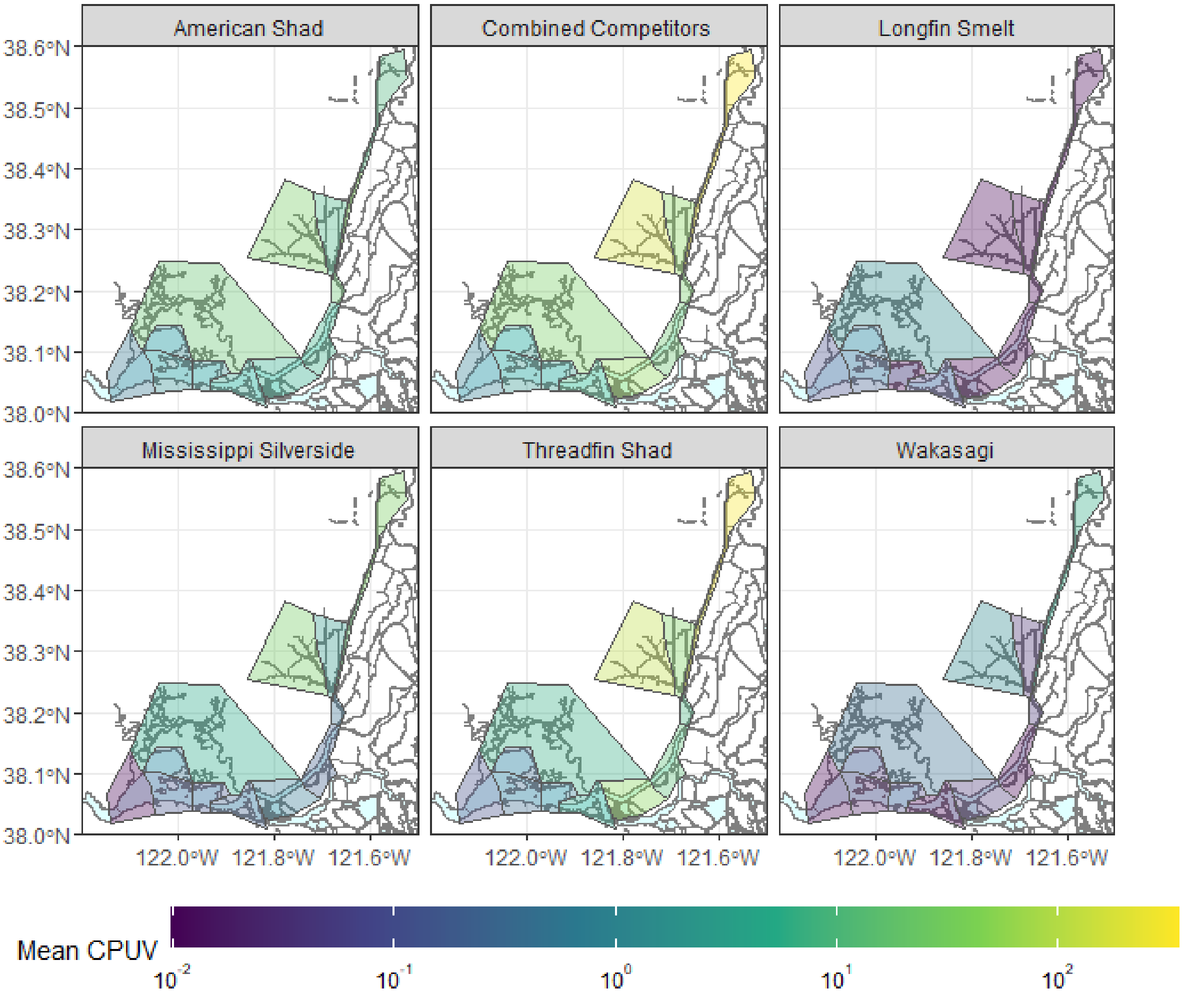


Figure 1-2. Comparison of Mean CPUV (catch/10,000 m^3) for Individual Competitor Species and All Small Pelagic Fish Combined

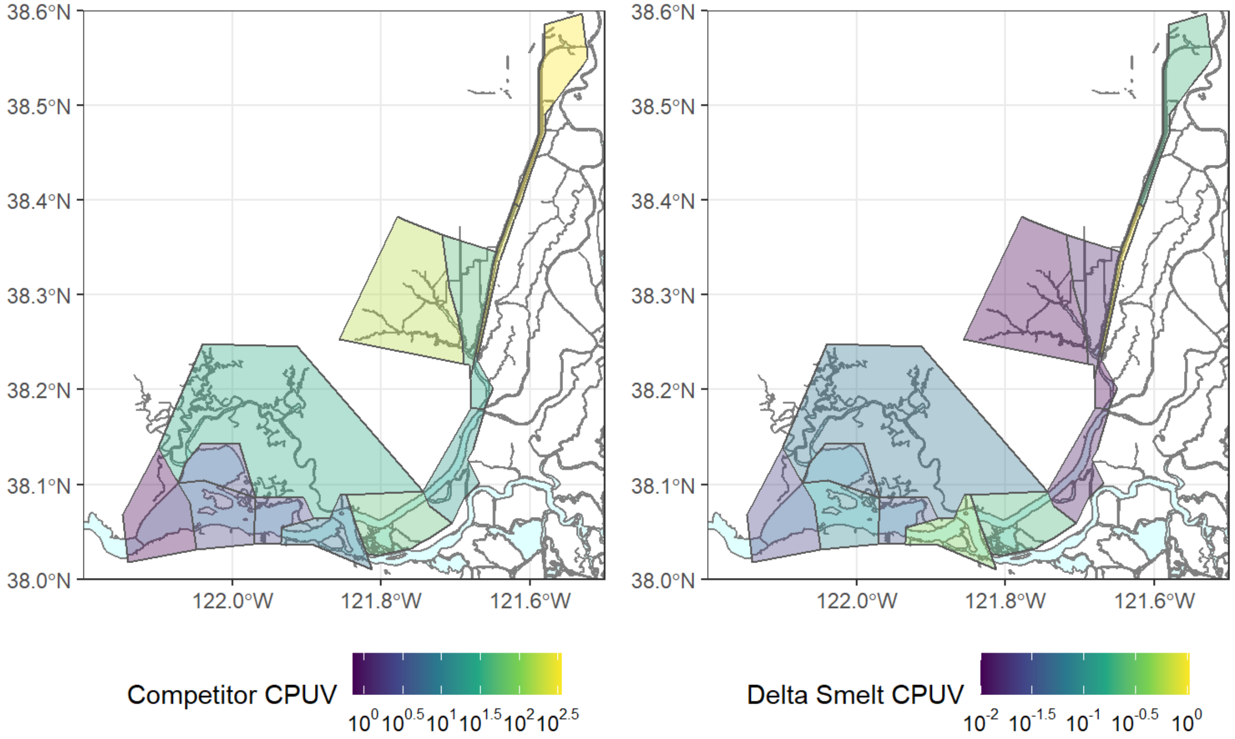
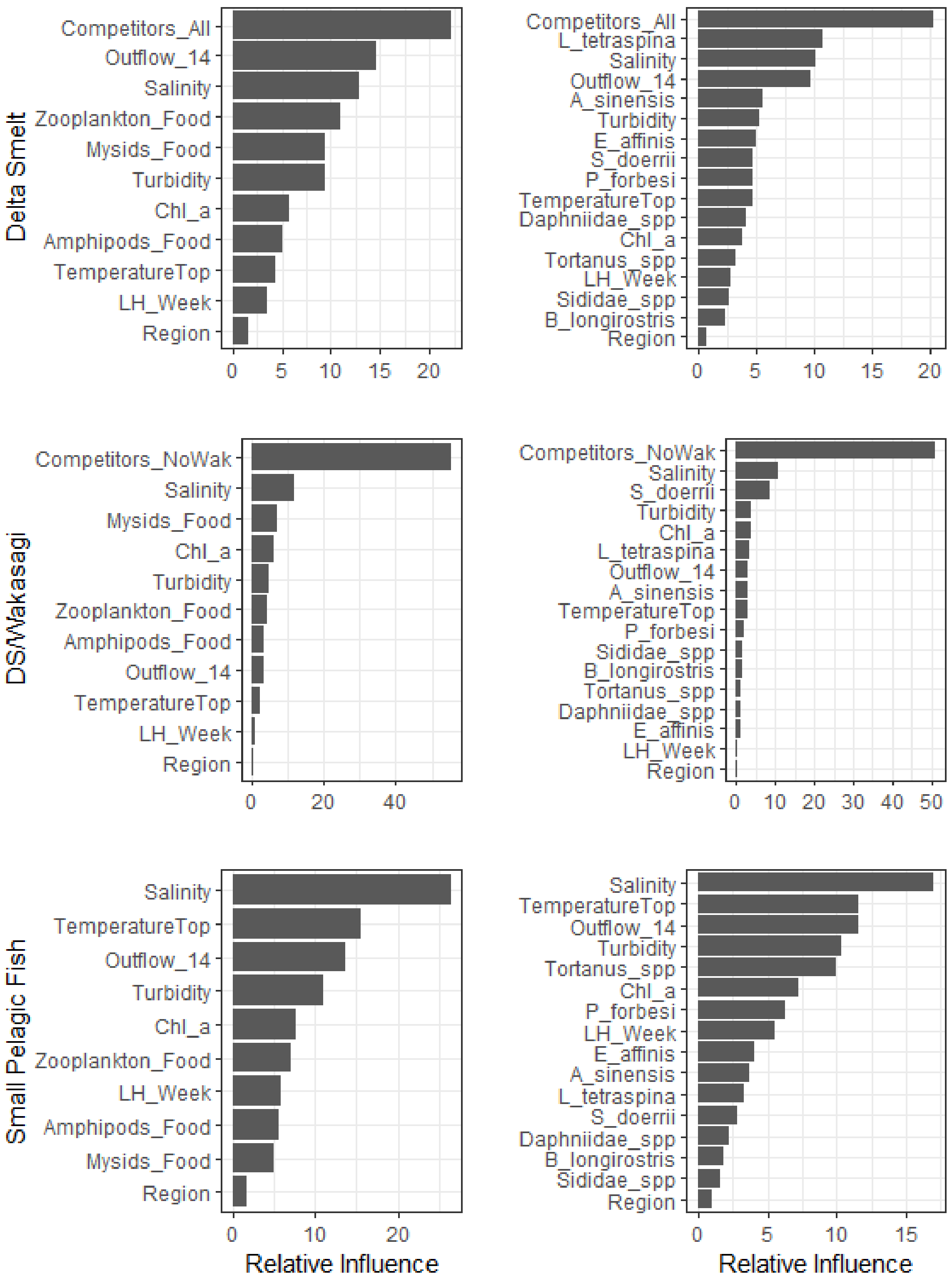
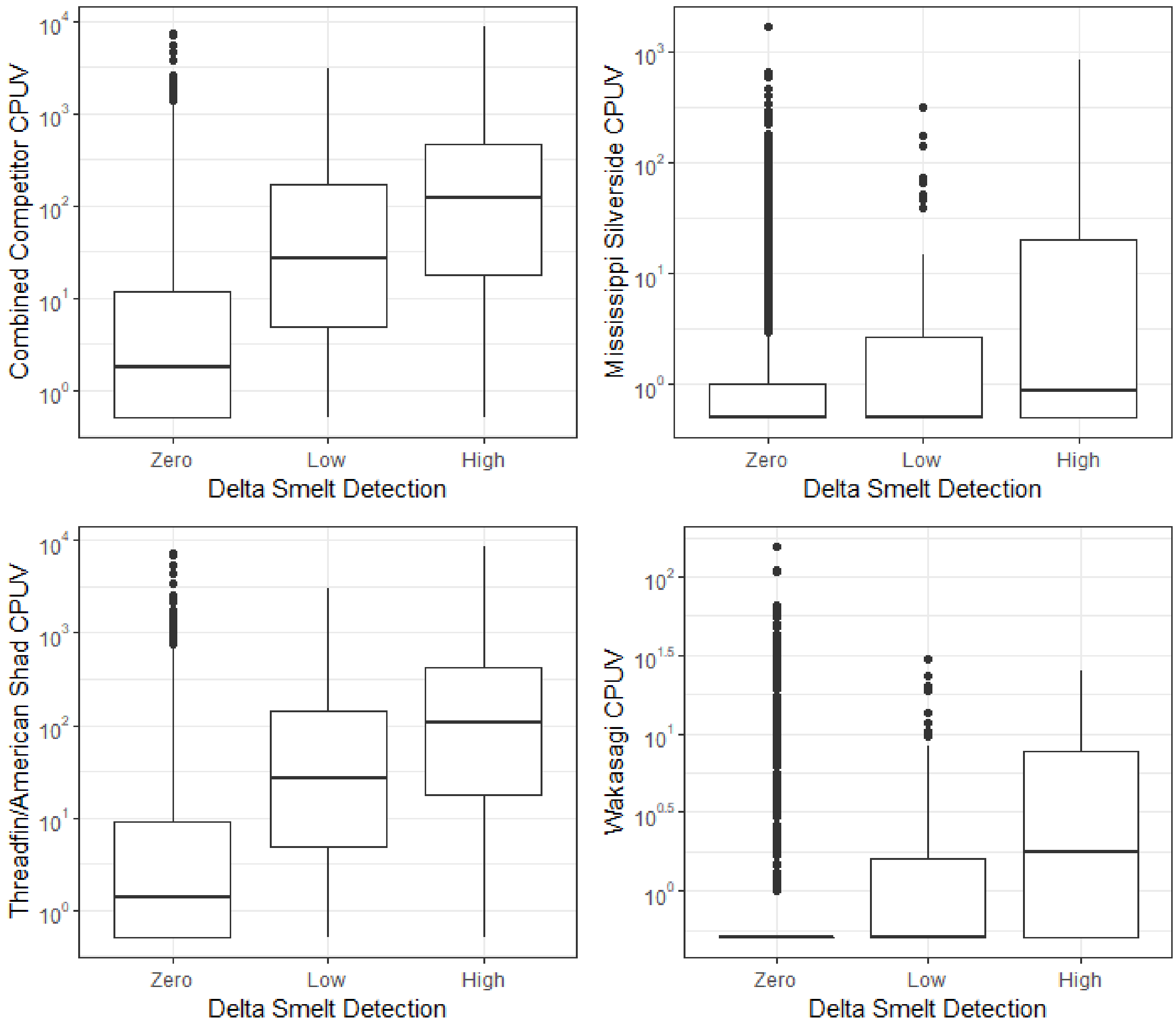


Figure 1-3. Comparison of Mean CPUV (catch/10,000 m^3) by Sampling Stratum for Delta Smelt and Their Small Pelagic Competitors



For each response variable, the first column shows rankings from models with relatively coarse aggregation of food items (Model 2) while the second column shows rankings from models with finer separation of food items (Model 4).

Figure 1-4. Variable Importance Rankings for Selected Presence/Absence Models



Low = Delta Smelt CPUV >0 and <2 fish/10,000 m^3, High = Delta Smelt CPUV ≥ 2 fish/10,000m^3.

Figure 1-5. Boxplots of Competitor Catch Density Relative to Binned Delta Smelt Catch

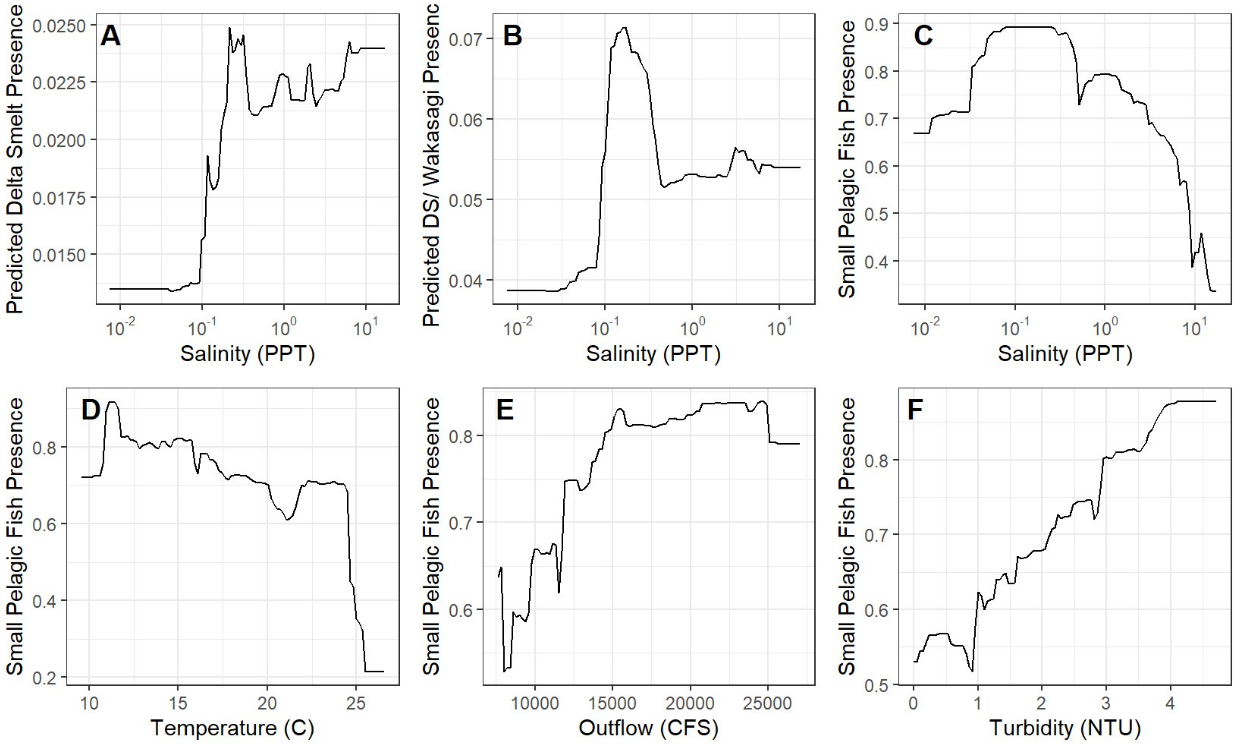


Figure 1-6. Conditional Effect of Important Covariates on A) Delta Smelt Presence, B) Combined Delta Smelt and Wakasagi Presence, and C-F) Small Pelagic Fish Presence

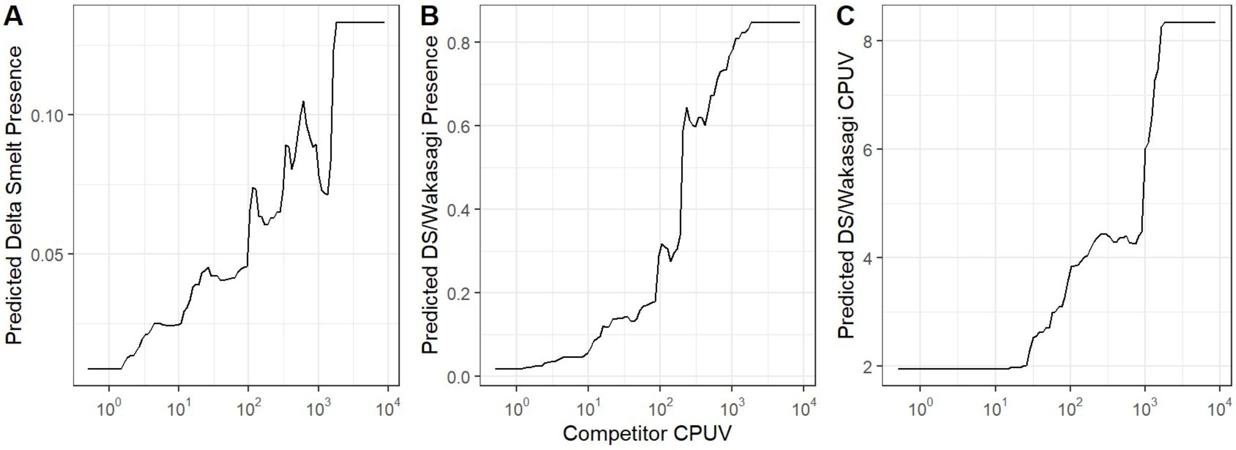
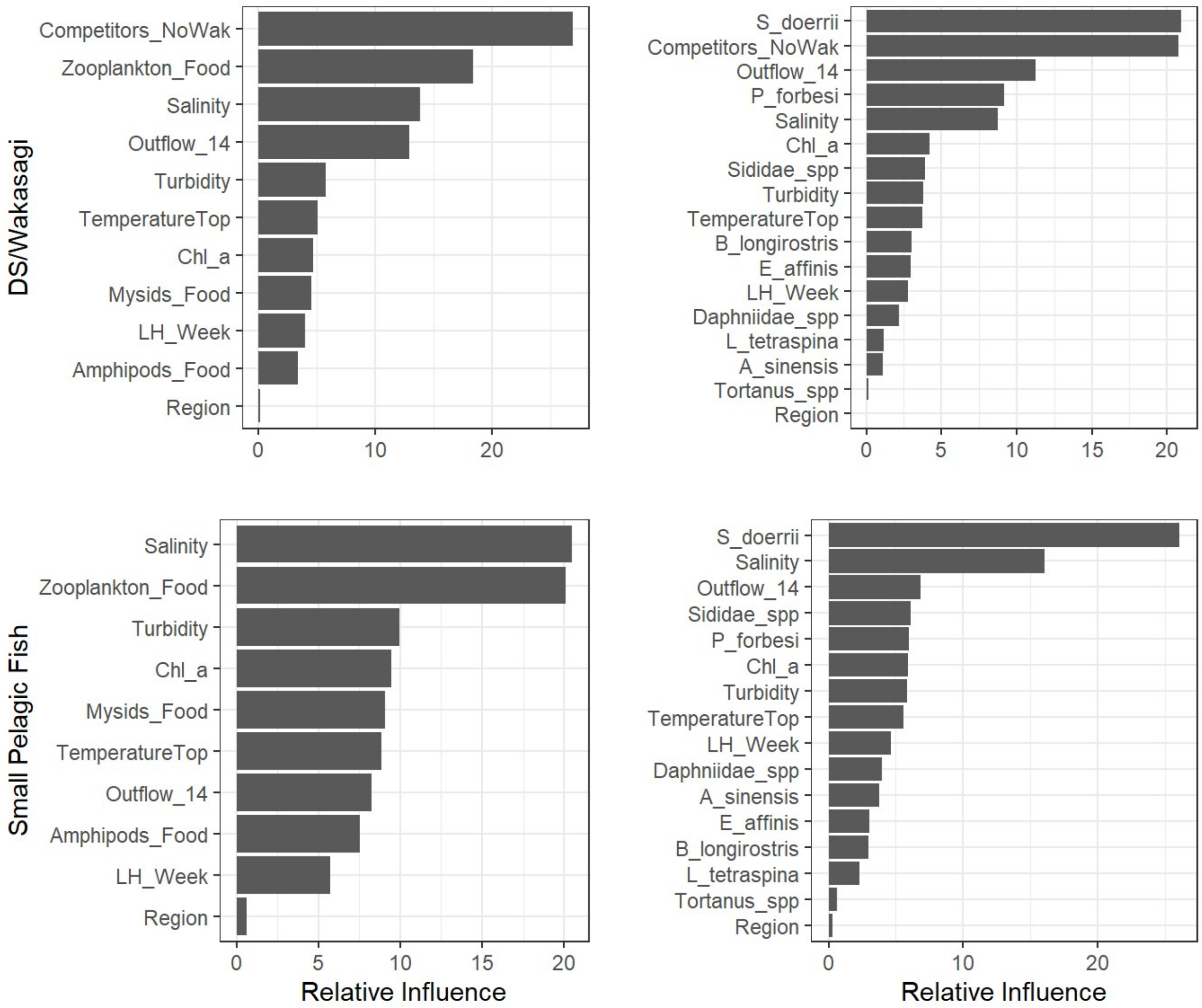


Figure 1-7. Conditional Effect of Competitor Density on A) Delta Smelt Detection probability, B) Delta Smelt or Wakasagi Detection Probability, and C) Delta Smelt/Wakasagi CPUV



For each response variable, the first column shows rankings from models with relatively coarse aggregation of food items (Model 2) while the second column shows rankings from models with finer separation of food items (Model 4).

Figure 1-8. Variable Importance Rankings for Selected CPUV Models

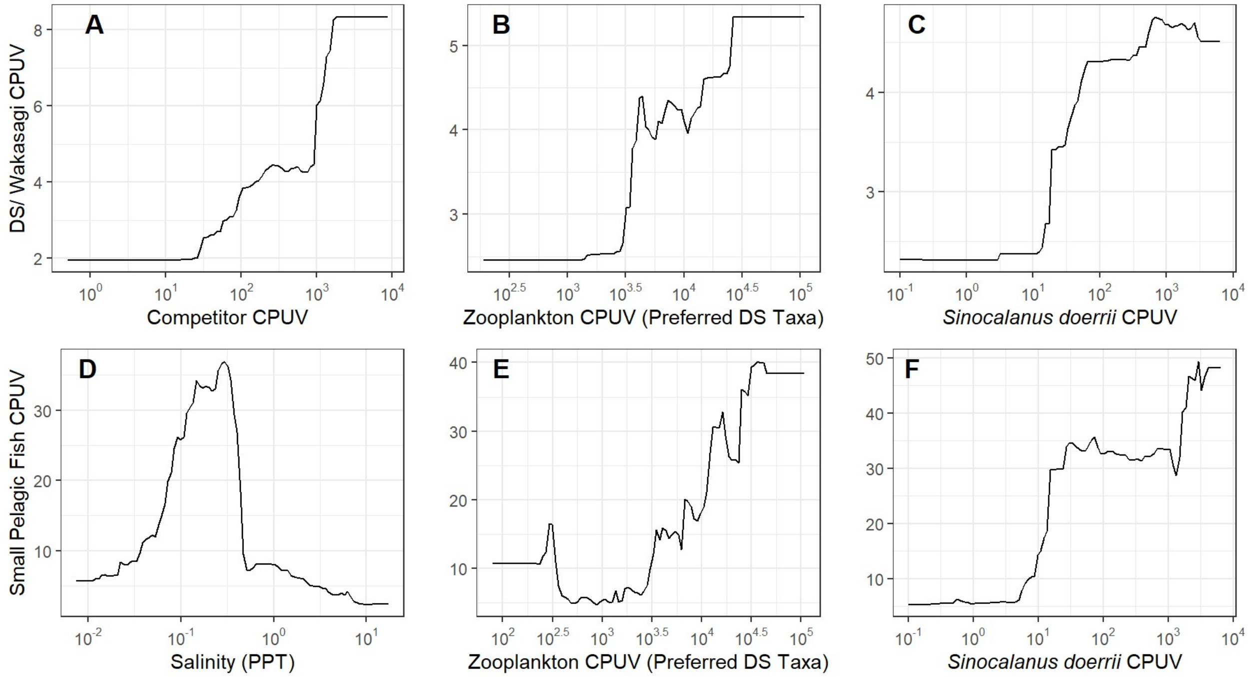
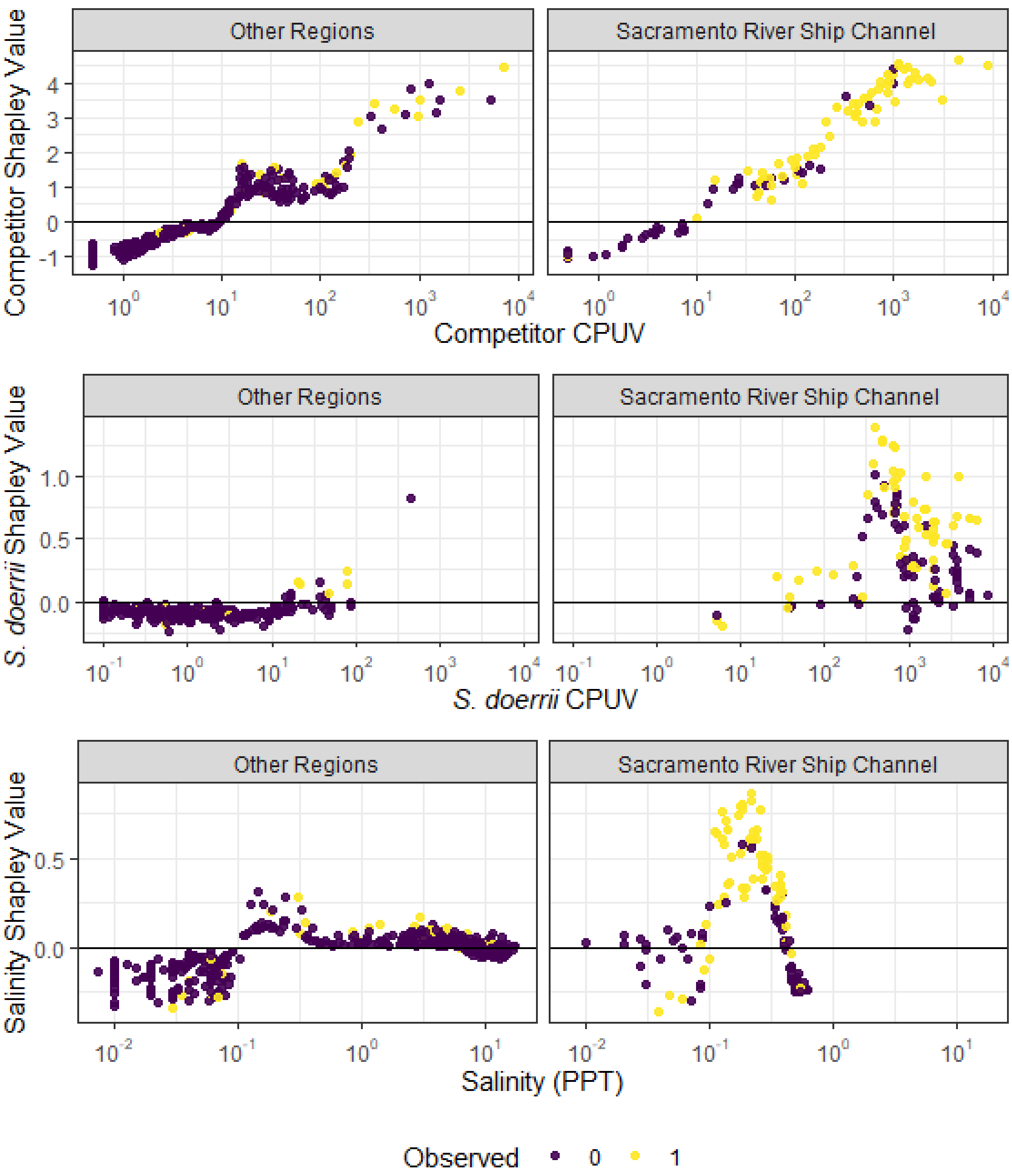


Figure 1-9. Conditional Effect of Important Covariates on A-C) Combined Delta Smelt and Wakasagi CPUV, and D-F) Small Pelagic Fish CPUV



Shapley values reflect the contribution of a covariate to the individual model predictions. Colors show observed presence and absence of Delta Smelt or Wakasagi.

Figure 1-10. Shapley Values Relative to Selected Covariate Values for Delta Smelt/Wakasagi Model 4 with Comparison of the Sacramento River Deep Water Ship Channel to other Sampled Regions

Chapter 2. Phytoplankton Community Structure and Nutrient Conditions in the Upper San Francisco Estuary During Managed Flow Action Periods

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Abstract

Phytoplankton are a primary component of the aquatic food web in the San Francisco Estuary (SFE) and the Sacramento-San Joaquin River Delta. Various changes to the ecosystem such as the introduction of invasive clams, nutrient enrichment, and changes in flow regimes have caused shifts in the phytoplankton communities, leading to harmful algal blooms, reduced primary productivity, and declines in fish populations. As part of efforts to improve habitat for the endangered Delta Smelt, managed flow actions are used to move the position of the low salinity zone seaward during high precipitation years in the fall, which may affect phytoplankton abundances and assemblages. We sampled nutrients, chlorophyll-*a*, phytoplankton, and surface colonies of *Microcystis* spp. in the upper SFE from 2017 through 2021 to assess changes and hypotheses related to phytoplankton biomass and assemblage, water quality, and presence of *Microcystis* spp. (a potentially toxic cyanobacteria taxa) during managed flow actions. Chlorophyll-*a*, a proxy for phytoplankton biomass, was lower during flow action years. However, the phytoplankton community was composed of a greater proportion of diatoms, whereas in no-action years (when the management action did not occur), the phytoplankton community was dominated by cyanobacteria and cryptophytes. Nutrient concentrations were not significantly different between action and non-action years. Our study also observed a higher presence of *Microcystis* spp. across all regions during no-action years. Increased flows, whether from increased precipitation or targeted flow actions, likely affected residence times in the SFE which changed the phytoplankton community and presence of *Microcystis* spp. A higher proportion of diatoms in the phytoplankton community likely benefits the zooplankton community however, the difficulty in distinguishing effects from higher precipitation and targeted flow actions warrants further study and validation of mechanisms underlying changes in the phytoplankton community.

Introduction

As the principal primary producers in many estuarine systems, phytoplankton concentration and community structure affect the entire aquatic food web (Capriulo et al. 2002, Froneman 2006, Danielsdottir et al. 2007, Hoffman et al. 2008, Allinger et al. 2013). Anthropogenic influences can alter environmental conditions such as salinity, light penetration, residence times, and nutrient concentrations, which are important factors in phytoplankton growth and community structure. The changing phytoplankton biomass and community composition can subsequently affect the quality or quantity of food for consumers (Danielsdottir et al. 2007, Vargas et al. 2006), and alter the growth of harmful algal blooms (Torre et al. 2019). It is imperative to monitor phytoplankton to fully understand the effects of directed changes to estuarine conditions intended to improve ecosystem health.

The San Francisco Estuary (SFE) is the largest estuary on the west coast and the center of California's major water hub, where the consequences of land use have dramatically altered the hydrology and primary productivity in the region. In the semi-arid, Mediterranean climate of California, most agricultural and municipal fresh water is sourced from the Sacramento-San Joaquin River Delta (herein referred to as the Delta, Luoma et al. 2015) which affects flow rates and water quality in the region (Beck et al. 2018). Agricultural runoff and wastewater treatment further impact nutrient dynamics of the system through discharge of effluent (Sobota et al. 2009). Furthermore, flow rates in the Delta are heavily managed for water resource needs, flood prevention, hydroelectric power generation, and protection of native fish species (Miller et al. 2012). These modifications can alter the distribution and function of phytoplankton communities (Cloern 2007, Jassby 2008).

Following the introduction of the invasive overbite clam *Potamocorbula amurensis* in the mid 1980's, there have been several shifts in phytoplankton community structure (Ball and Arthur 1979, Lehman 2022) which may be linked to a range of ecosystem perturbations. Potential contributors to the shifts in primary productivity include grazing from *P. amurensis* and zooplankton (Kimmerer and Thompson 2014), modified river flow, and nitrogen loading (Alpine and Cloern 1992, Glibert et al. 2014). Modified flows can alter water residence time, subsequently creating conditions for certain phytoplankton taxa to flourish (Lehman et al. 2022, Stumpner et al. 2020). Additionally, salinity and water temperature are key drivers in phytoplankton community structure across the brackish to freshwater gradient of the upper SFE (Cloern 2018, Lehman 2022). In recent decades, there has been a shift in phytoplankton community structure in the upper SFE, towards smaller benthic diatoms in the more brackish regions and small-celled cyanobacteria and flagellates in the freshwater regions (Lehman 2022). While nutrient concentrations are not considered limiting in the upper SFE, certain taxa may have competitive advantages with alterations to nitrogen supply; for example, ammonium is efficiently utilized and readily uptaken by the cyanobacteria *Microcystis* spp. (Lehman et al. 2021).

Since the mid-1980s, the SFE has been considered a High Nutrient Low Chlorophyll (HNLC) estuary (Cloern 2001) with limiting concentrations of light and nonlimiting concentrations of nitrogen and phosphorus (Wilkerson et al. 2006, Cloern et al. 2020). From 1975 to 2005, wastewater discharge was found to be the dominant source of ammonium loading into the upper SFE (Jassby 2008) leading to increases in the nitrogen to phosphorus ratio (Lehman 2022). The physiological responses of diatom growth to varying concentrations of nitrate and ammonium in the SFE remain a source of great debate (Cloern 2021, Glibert et al. 2022), as diatoms are largely considered high quality food for secondary consumers. Increased flow was hypothesized as one potential mechanism to alter concentrations of nutrients in the SFE, as outflow was expected to dilute concentrations of ammonium and increase concentrations of nitrate through flushes into the LSZ from nitrate rich regions such as Cache Slough and the San Joaquin River (Brown et al. 2014).

Shifts in phytoplankton community composition may affect nutritional quality and trophic transfer efficiency (Cloern and Dufford 2005). Diatoms are considered an important primary producer due to their large cell diameter, high carbon content (Chow-Fraser and Wong 1985; Lehman 2004), and essential fatty acid content (Lehman 2022). Cryptophytes are also generally rich in polyunsaturated fatty acids, which may improve survival and growth rates in zooplankton and fish (Brett and Müller-Navarra, 1997, Khanaychenko et al. 2018, Galloway et al. 2014, Brett et al. 2006). In contrast, many cyanobacteria are known to have detrimental effects on the wider food web due to toxicity, eutrophic oxygen depletion, and shading of competitor phytoplankton (Ibelings and Havens 2008).

A particularly alarming trend in recent years is the increase in magnitude and duration of summer blooms of *Microcystis aeruginosa* (Lehman et al. 2005; 2017; 2022), a colonial cyanobacteria that can produce toxins that potentially impact fish and zooplankton in the Delta (Ger et al. 2010, Lehman et al. 2010, Acuña et al. 2012,). In the Delta, *Microcystis* spp. blooms were first observed in 1999, although the genus was observed in phytoplankton assemblages as early as 1975 (Lehman et al. 2005). Summer blooms of *Microcystis* spp. are now commonplace in the Delta when high surface water temperatures reach over 25 degrees Celsius (°C) (Mioni et al. 2011). Harmful *Microcystis* spp. blooms can rapidly and efficiently uptake ammonium when environmental conditions are favorable, altering water quality in the region. *Microcystis* spp. can also form surface scums in regions with high water residence time, limiting light to other phytoplankton and resisting grazing by zooplankton (Ibelings et al. 1991, Ger et al. 2018). Higher *Microcystis* spp. cell density has been shown to be correlated with low stream flows in the SFE (Lehman et al. 2008), and flow actions are hypothesized to help with control of potential blooms (Brown et al. 2014).

Several ongoing or planned management actions involving flow in the Delta are intended to benefit native fish species (NMFS 2009, USFWS 2008, NMFS 2019, USFWS 2019, Frantzich et al. 2021) including the endangered Delta Smelt (*Hypomesus transpacificus*). Managed flow actions are designed to increase favorable Delta Smelt habitat by releasing reservoir water from upstream or restricting water exports to allow for better and wider overlap of important habitat characteristics such as low-salinity water, food resources, turbidity, and geomorphologic complexity (Baxter et al. 2015). For example, the flow-related Fall *X2* action is intended to improve Delta Smelt fall habitat by managing the position of *X2*, which is the distance of the 2 parts per thousand (ppt) bottom water isohaline (measured in kilometers) upstream of the Golden Gate Bridge (Jassby et al. 1995). In above-normal water years, the September and October *X2* position is maintained to no more than 81 kilometers (km), and in wet years the fall *X2* action is enacted to move the *X2* seaward to no more than 74 km (Figure 2-1). An *X2* position near 74 km results in increases of low-salinity habitat due to superimposition with the broad shoals of Suisun Bay (Brown et al. 2014; Kimmerer et al. 2013). Such conditions existed in the wet year of 2011, which coincided with greater catches of Delta Smelt in Suisun Bay during the Fall Midwater Trawl annual monitoring survey (Bever et al. 2016), however there was not a similar effect during the wet years of 2017 and 2019 (Schultz et al. 2019, FLOAT-MAST 2020).

Increased flow during a Fall *X2* action could impact nutrient dynamics and the biomass and composition of the phytoplankton assemblage through several mechanisms. The change in salinity may shift communities from marine to freshwater taxa (Muylaert et al. 2009), and reductions in temperature due to cool water releases from upstream reservoirs may promote an increase in diatom growth and overall phytoplankton biomass in the Delta (Lehman 2004). Residence times in the Delta vary with flow thereby altering the distribution of nutrients through variable rates of loss to uptake by primary producers and other microbial pathways (Dahm et al. 2016). Alterations of nitrogen sources through the Fall *X2* action may influence phytoplankton assemblages. The potential dilution of ammonium during flow actions on agricultural and wastewater treatment point sources (Novick et al. 2015, Brown et al. 2014) may differentially affect constituents of the microalgal assemblage (Glibert et al. 2011) and rates of primary production (Liu et al. 2018).

Increased flow is expected to increase nitrate concentrations because at higher outflows more nitrate would be flushed into the LSZ from nitrate rich regions such as Cache Slough and the San Joaquin River (Brown et al. 2014). Flow conditions may also impact formation of *Microcystis* spp. colonies (Lehman et al. 2008).

Our paper addresses how the Fall *X2* action and the hydrological conditions in wet water years (herein referred to as action years), affected the phytoplankton community and biomass in the upper SFE relative to conditions in drier, non-action years (herein referred to as non-action years). We examined the correlations between phytoplankton assemblages, water quality conditions and nutrient distributions during the fall season. During action years, when outflow is higher and the position of *X2* is more seaward, Brown et al. (2014) predicted that:

- Biomass, as measured by chlorophyll-*a*, will be higher, and there will be more diatoms, and fewer cyanobacteria.
- The concentration of nitrate will be higher, and concentration of ammonium will be lower.
- There will be fewer floating *Microcystis* spp. colonies in the upper SFE.

Methods

Study Area and General Sampling Design Location and Sampling Period

Our study area consisted of five regions within the upper SFE (Figure 2-1). We sampled from September to November in 2017 and 2018, and April to November in 2019 – 2022. All five regions were sampled within a one-week period, with sampling on alternating weeks in 2017 and weekly sampling in 2018 – 2022. We sampled two sites per week within each region in 2017 and three per week in each region from 2018 through 2022. Sites were selected via a generalized random tessellation stratified survey design (Starcevich et al. 2016; Stevens Jr and Olsen 2004). At each site, we collected phytoplankton, chlorophyll-*a*, nutrient samples, and water quality parameters. Only data from September to November was used for analysis.

Sampling Methods

We measured water quality parameters 1m below the water surface using a handheld multiparameter sonde, YSI EXO2 (Yellow Springs Inc, Yellow Springs, OH). At each site, we measured water temperature (°C), salinity (calculated from conductivity and temperature; ppt), turbidity (NTU), chlorophyll-*a* (microgram per liter (µg/L)), and dissolved oxygen (milligram per liter (mg/L)).

We collected whole water samples from one meter below the surface using nylon tubing into a 2-liter (L) vacuum flask. Prior to collection of the sample, the vessel was stopped, and we rinsed the flask three times. After rinsing, we collected one liter of sample water into the vacuum flask.

We filtered sample water for nutrient analysis via gentle vacuum pressure (<10 mm mercury (Hg)) through 0.2-micrometer (µm) pore size, 47-mm diameter polycarbonate filters into clean 120-ml High Density Polyethylene bottles. Nutrient samples were kept refrigerated until later analysis for nitrate (NO_3^-), ammonium (NH_4^+), phosphate (PO_4^{3-}) and dissolved organic carbon (DOC) within 2 weeks of collection.

We transferred 45 milliliters (mL) of whole water into a centrifuge tube for phytoplankton identification. We added 1 to 5-mL of Lugol's solution to stain and preserve the samples. Phytoplankton samples were stored in the dark prior to identification and enumeration.

Lab Analysis Methods

We analyzed phytoplankton samples using light microscopy following the methods by Lund et al. (1958). Subsamples were settled for 12 to 24-h using Utermoehl settling chambers. We identified phytoplankton to genera or to the lowest taxonomic unit possible. We analyzed the settled well slides over horizontal transects, identifying and counting each cell (or trichome with filamentous cyanobacteria), in each transect until a target of 400 total cells counted or 100 cells of a single genus was met. We counted the entire subsample if neither target was met. We did not count any cells smaller than 5 µm, as these cell sizes were not considered to be quality food for consumers when the study was designed. Final phytoplankton counts were recorded in units of cells per milliliter. We characterized harmful algal bloom (HAB) organisms using the IOC/UNESCO taxonomic reference list (Moestrup 2009), which only includes taxa associated with toxic blooms (Hallegraeff 2003).

Filtered sample water was analyzed by the UC Davis Ecosystem, Ecology, and Limnology Lab using colorimetric assays for ammonium (NH_4^+, micromole per liter (µmol/L)), nitrate (NO_3^-, µmol/L), and phosphorous (PO_4^{3-}, µmol/L) (Murphy and Riley 1962, Verdouw et al. 1978, Doane and Horwáth 2003).

To account for the often-poor representation of *Microcystis* spp. in whole water samples, due to their colonial morphology, we also incorporated visual observations of surface water. In 2017, we sampled water from the surface with a 5-gallon bucket, and noted the presence or absence of *Microcystis* spp. In 2018 – 2022, we collected *Microcystis* spp. by towing a 12.7-cm diameter plankton net with 50-µm mesh for two minutes at the surface. The collected sample was then assessed visually by ranking the density of surface colonies in a ~2.5 L bucket, with 1 as an absence of *Microcystis* spp. and 5 as a contiguous *Microcystis* spp. colony forming mats or scum.

Statistical Analysis

We investigated differences in nutrient concentrations between years and regions using Analysis of Variance (ANOVA) without interaction effects in R statistical computing environment (R version 4.3.1, R Core Team 2023), which we used in all analyses described below. We used Bonferroni corrected estimated marginal means (EMM, 'emmeans' package version 1.5.3, Lenth et al. 2020) to compare differences in nutrient concentrations among regions.

Using a generalized linear model (GLM) with an identity-linked Gamma distribution we evaluated chlorophyll-*a* as an estimate of phytoplankton biomass with predictor variables. The full model included the following predictor variables: region, habitat, year, *X2*, environmental parameters (temperature, salinity, turbidity, and dissolved oxygen) and nutrient concentrations (nitrate, ammonium, and phosphate). After full model selection with every possible combination of the predictor variables, we selected the most parsimonious model via the corrected Akaike Information Criteria (AIC).

Non-metric Multidimensional Scaling (NMDS) was used to examine phytoplankton community structure in the five regions of the upper SFE for both action and non-action years. We used the R package "Vegan" (Oksanen et al. 2022) for NMDS analyses of community structure. For each NMDS, we fit environmental variables into the ordination space using the "envfit" function. The analysis included continuous environmental variables and convex hulls relating factors including action year status (Action and No-Action) and region to the community structure.

The Redfield ratio of 16 moles of Nitrogen for every mole of Phosphorus was used as a screening criterion for possible nutrient limitation (Redfield 1934). Large deviations from the Redfield ratio are evidence that the depleted nutrient is being consumed and may limit phytoplankton productivity and biomass.

Results

Samples were collected from 407 sites, with 394 collected from the channel habitats and 11 collected from shoal habitats, where channel habitats were not available.

Water Flow and Abiotic Water Quality Variables

Water exports were reduced to implement the flow action in 2017 and 2019 when flows in the Sacramento and San Joaquin River were higher, resulting in a higher Net Delta Outflow Index (NDOI) and eastward X2 compared to the no-action years (Figure 2-2). The action years were characterized as wet years based on the Sacramento Valley Index water year type, and *X2* averaged 76-km from September through November. Outflow in action years was higher, with a mean NDOI of 10,642 cubic feet per second (cfs). In contrast, no-action years ranged from below normal to critical water year types and had an average *X2* of 86 km, and an average NDOI of 6,294 CFS from September through November.

Water temperature was not significantly different in all regions (Figure 2-4). There were significant differences in salinity between action and non-action years for all regions except for Sacramento Deep Water Ship Channel (SRDWSC) and the Cache Slough and Liberty Island (CSC) region. Differences in salinity between action and non-action years were especially pronounced in the Suisun Bay and Suisun Marsh regions. Turbidity was significantly higher in Suisun Marsh during action years.

Phytoplankton Biomass

Chlorophyll-*a* was significantly higher in no-action years than action years in the Lower Sacramento River, Suisun Bay, and Suisun Marsh regions (Figure 2-4). Chlorophyll-*a* was generally higher in no-action years, particularly in the terminal sloughs and channels of Suisun Marsh and the upper portions of the Sacramento River Deep Water Ship Channel (SRDWSC) (Figure 2-7).

The most parsimonious chlorophyll-*a* model included ammonium and phosphate concentration, water temperature, salinity, and *X2*, with interaction effects between year status (action or non-action) and region (Figure 2-6, Table 2-2). Region, ammonium, and phosphate were statistically significant ($p<0.05$) as individual terms and there was a significant interaction between year status and region. The differences between action and non-action years were the most pronounced in the downstream sites of Suisun Bay, Suisun Marsh, where the interaction was significant. Ammonium had a negative relationship chlorophyll-*a*, while phosphate had a positive relationship. Some of the other individual terms such as temperature, salinity and X2 appeared to correlate with chlorophyll a, but these relationships were not validated by the model.

Phytoplankton Diversity

Most phytoplankton were identified within 5 taxonomic groups – diatoms (*Bacillariophyceae*), cyanobacteria (*Cyanophyceae*), dinoflagellates (*Dinophyceae*), green algae (*Chlorophyceae* and *Charophyceae*), and cryptophytes (*Cryptophyceae*). Taxa that did not fit into these taxonomic groups (e.g., *Spirotrichea*, *Raphidophytes*, *Haptophytes*, *Ochrophytes*, and *Euglenozoa*) were classified as "Other."

The NMDS results indicated multiple environmental variables correlated with phytoplankton community structure with certain taxa exhibiting different presence between action and no-action years (Table 2-3, Figure 2-5). Ammonium, chlorophyll-*a*, dissolved oxygen, pH, salinity, temperature, turbidity and *X2* location, action year status, and region were all significant, however most r^2 values were relatively small except for *X2* and action year status ($r^2 = 0.153$ and 0.102 respectively, Table 2-3). We did not observe any trends of the six taxonomic groups associated with the environmental parameters. Phytoplankton community structure did not show a strong trend by region, however there were differences due to action year status in the NMDS (Figure 2-5 and Figure S5-2-1); *Planktothrix* and *Geitlerinema* were more commonly present during action years, while *Microcystis*, *Selenastraceae*, Cryptophyta, *Strombidium*, *Bacillaria*, *Diploneis*, *Stephanodiscaceae*, and *Fragilariopsis* were associated with no-action years. Multiple taxa were present in both action and no-action years.

There were also notable changes in overall abundance of taxonomic groups in both region and action year status (Figure 2-10). In no-action years, there was a higher abundance of cyanobacteria and cryptophyte species, particularly in the CSC region (Figure 2-9). Diatoms had the highest proportion of the community structure in Suisun Marsh across both action and no-action years. Dinoflagellates were the most common group in the Lower Sacramento River in action years and were present in all other regions during action years but were relatively rare in no-action years. Green algae were also more prevalent during no-action years in the Lower Sacramento River, Suisun Bay, and Suisun Marsh.

Nutrient and Water Quality Data

Nutrient concentrations were not statistically significant different between action and no-action years, but there were regional differences in nutrient concentrations (Figure 2-3). In the SRDWSC and CSC, nitrate concentrations were generally lower in comparison to downstream regions (Figure 2-3). During no-action years ammonium was significantly higher in Suisun Marsh than SRDWSC, CSC, and Suisun Bay. Phosphate concentrations were the least variable throughout all regions and were highest in the SRDWSC.

Nitrogen to phosphate ratios were closer to the 16:1 Redfield ratio in the downstream regions but were below the Redfield ratio in the CSC and SRDWSC across both action and no-action years (Figure 2-6). The ratio of nitrate to ammonium was similar between action and no-action years for most regions, except for the Lower Sacramento River which had higher nitrates relative to ammonium during no-action years.

Harmful Algal Blooms

Microcystis spp. were more common in no-action years than action years (Figure 9). Presence was least likely in the two freshwater regions - CSC and the SRDWSC. The probability of seeing low amounts of *Microcystis* spp. in these two regions increased during the no-action years. The probability of absence of *Microcystis* spp. was lower during no-action years in the Lower Sacramento River and the probability of low, medium, and high increased during no-action years. In Suisun Marsh, the probabilities remained unchanged across action and no-action years. The probability of absence of *Microcystis* spp. decreased in Suisun Bay during no-action years, while probability of medium, and high presence of *Microcystis* spp. slightly increased during no-action years.

Discussion

Phytoplankton biomass (indicated by concentrations of chlorophyll-*a*) in Suisun Bay and Suisun Marsh was lower during the action years, but diatoms composed a larger proportion of the phytoplankton community. During no-action years, lower flow rates may have allowed for a proliferation of cyanobacteria and cryptophytes over diatom growth. Our study did not observe any significant differences in nutrients between action and no-action years but did observe higher nitrate in downstream regions in both action and no-action years.

Phytoplankton Biomass

Phytoplankton biomass was lower in action years compared to no-action years throughout most of the study regions, except in the SRDWSC, contrary to our hypotheses. The lower phytoplankton biomass in Suisun Bay and Marsh during the action years suggests the primary producer stock was not improved by the action in these target regions. The higher chlorophyll-*a* concentrations in the downstream regions during no-action years may be due to the retention of phytoplankton with increased residence time resulting from lower flow rates (Cloern et al. 1985) and lower turbidity reducing shading effects on growth (Gameiro et al 2011). Chlorophyll-*a* concentrations have been shown to increase with longer residence time in other estuaries (Gameiro et al. 2004, Wan et al. 2013) and parts of the SFE (Stumpner et al. 2020, Smits et al. 2023). Nutrient concentrations were similar between action and no-action years in these regions so nutrient concentrations in these regions likely do not explain the differences between action and no-action years.

There was an inverse relationship with ammonium and chlorophyll biomass that was higher in no-action years (Figure 2-8), but the driver of the relationship between chlorophyll biomass and ammonium remains uncertain. Declines in biomass as ammonium concentrations increase may support the ammonium suppression hypothesis by Dugdale et al. (2007), however, it may also be due to the draw down of ammonium coincident with a phytoplankton bloom. Ammonium concentrations were highest in the Lower Sacramento River in action years and were likely influenced by SRWTP discharge into the Sacramento River at Freeport. With the tertiary treatment upgrade at the SRWTP in 2021, ammonium discharge from the largest point source in the northern Delta declined (Glibert et al. 2022, Randall 2023), with subsequent increases in nitrate concentration in the northern Delta. Nitrate was not selected in the most parsimonious model, so was not predicted to influence phytoplankton biomass.

Alternatively, phosphate had a positive relationship with chlorophyll biomass in our model and may have been driven by the low N:P ratio in the SRDWSC. The SRDWSC was a unique region with respect to biomass, with the highest chlorophyll-*a* concentrations in action years and almost unchanged concentrations between action and no-action years. This region also had the lowest Redfield (nitrogen: phosphorus) ratio during both action and no-action years – suggesting nitrogen limitation in the region (Turner et al. 2003, Ptacnik et al. 2010). Seasonal limitation of nitrogen in the SRDWSC appears to be strongest in the landward edges of the channel where water exchange is lowest and linked to stratification (Loken et al. 2022, Wilkerson et al. 2015). Productivity in the SRDWSC may be highest near the transition of a nutrient limited environment at the landward terminal end to a light-limited state on the seaward end of the channel (Loken et al. 2022). The high residence times in the region may allow for the phytoplankton biomass to deplete sources of nitrogen (Smits et al. 2023). Studying the phytoplankton dynamics in this region may help inform future restoration efforts, as this region is one of the few remaining areas in which Delta Smelt are still frequently caught and supports some of the highest zooplankton abundances in the upper SFE (Feyrer et al. 2017, Lee et al. 2023).

Phytoplankton Diversity and Abundance

There was a difference in phytoplankton assemblages between action and no-action years as each region's ordination encompassed a smaller proportion of taxa during the no-action year (Figure 2-5) which was most strongly driven by the *X2* variable. We believe that the *X2* variable is likely related to flow velocity rather than the position of *X2*; a more seaward *X2* is indicative of higher flow rates during the fall season (see figure 2) and vice versa. Additionally, the r^2 value of the *X2* variable ($r^2 = 0.153$) was the highest amongst all the other variables but can be considered low, suggesting that what is affecting the phytoplankton community is related to *X2* but not strongly explained by *X2*. We believe that flow is likely one of the main drivers of assemblage changes that we observed. Advection of phytoplankton during high flow conditions likely reduces diversity, as certain morphological traits (shape, buoyancy regulation and motility) may allow certain phytoplankton taxa to retain position in higher flow conditions as seen in other systems (Fraisse et al. 2013). Increased flow may also affect algal competition for light (Zhang et al. 2015).

We expected any differences in the assemblages would be due to the salinity in different regions. Although salinity did predict the phytoplankton assemblage the relationship was weak. Previous studies in the SFE reported successional patterns of phytoplankton based on a salinity gradient. However, these studies included either more marine sites (Cloern 2018, median salinity 24.3 ppt) or more freshwater sites (Lehman 2022), while the average salinity ranges in our study were relatively narrower in comparison (0.08 – 11.0 ppt). Several taxa including *Cocconeis* spp., Cryptophyta, and *Chaetoceros* spp. were common throughout the study region regardless of salinity and region.

Action years were characterized by a higher proportion of diatoms and dinoflagellates, while cyanobacteria and cryptophytes increased in abundance during no-action years. This assemblage shift correlates with shifts in increased chlorophyll-*a* concentrations, and decreased outflow. The higher flow rates in action years may have selected for larger celled phytoplankton such as diatoms and against smaller celled phytoplankton, however, our study did not evaluate the presence of picoplankton (cell sizes smaller than 5μm). In the action year of 2017, cyanobacteria had a correlation with increased outflow in studies that included interior portions of the Delta collected throughout the year (Perry et al. 2023, Lehman et al. 2022). This was due to the prevalence of picocyanobacteria (*Eucapsis* spp., *Syneccochus* spp., *Chrococcu*s spp.) which accounted for 80% of the cyanobacteria in the Perry et al. (2023) dataset in 2017.

One possible explanation for the higher proportion of diatoms in action years is that higher outflow traps diatom assemblages within Suisun Bay through estuarine circulation patterns in Suisun Bay and Marsh where less dense taxa are transported seaward but denser diatoms sink and are transported back upstream via the landward flowing bottom current (Cloern et al. 1983). Despite this potential effect of circulation on the assemblage structure, chlorophyll-*a* concentrations were lower in the target regions of Lower Sacramento River, Suisun Bay, and Suisun Marsh. While chlorophyll-*a* in the CSC was lower during action years, this may be an effect of higher precipitation and flow from further upstream floodplains and not directly because of the Fall *X2* action.

Higher proportion of diatoms observed in Suisun Bay and Marsh in action years may potentially be well-suited as prey items for herbivorous zooplankton, such as *Pseudodaiptomous forbesi*, the most abundant calanoid copepod in the upper SFE during the fall and the main prey item for planktivorious fish (Lee et al. 2023, Slater and Baxter 2014, Slater et al. 2019, Bryant and Arnold 2007). Diatoms are both rich in fatty acids and considered high quality food for secondary consumers. *P. forbesi* in the Columbia River showed a feeding preference for diatoms and ciliates (Bowen et al. 2015) and in the SFE, diatoms had the highest relative DNA read abundance in the guts of *P. forbesi* (Wong 2024).

However, not all diatoms may be beneficial for zooplankton. A single-taxa bloom of the chain forming diatom *Aulacoseira granulata* did not stimulate production of *P. forbesi* in the upper SFE (Jungbluth et al. 2020) and experimental studies in marine systems have demonstrated the potential deleterious effects of polyunsaturated aldehydes found in several species of *Thalassiosira* spp. and *Chaetoceros* spp. on copepod early development (see Table 2-1 in Ianora and Miralto 2010). Polyunsaturated aldehydes degrade the fatty acid content in the cell and have increased production under nutrient limited environments (Ribalet et al. 2007), and therefore may be less of a concern in a HNLC estuary. Additionally other studies in the upper SFE show *P. forbesi* do consume potentially toxic phytoplankton such as cyanobacteria (Holmes and Kimmerer 2022).

Dissolved Nutrients

Increased outflow during action years did not appear to significantly alter concentrations of nutrients in the target regions of Suisun Bay and Suisun Marsh. While our results show differences in phytoplankton communities between action and no-action years, we believe the community differences are likely a result of lower residence times during action years because nutrient concentrations did not significantly vary between the year types. Phosphate and nitrogen concentrations were generally moderate, and unlikely to limit phytoplankton growth or to drive eutrophication of the estuary. Due to rate of tidal mixing and the rarity of blooms in the upper SFE, the risk of eutrophication is low. We conclude that the action is unlikely to affect the (HNLC) status of the SFE. However, potentially toxic microcystins are more prevalent in high ammonium concentration conditions (Chen et al. 2019), so monitoring nutrients is crucial to helping understand the effects of phytoplankton on the ecological community.

While not statistically significant, there was a notable decrease in ammonium in the Lower Sacramento River region during no-action years that is likely due to the tertiary treatment upgrade of the Sacramento Regional Wastewater Treatment Plant (SRWTP) which reduced ammonium loading. In late spring of 2021 (a no-action year), the SRWTP upgrade went fully operational to significantly reduce ammonium loading from the effluent into the Delta. Since phase II of the upgrade, ammonium concentrations downstream of the treatment plant in Rio Visa have lowered post upgrade (Randall 2023). The effect of the treatment upgrades on available ammonium was strongest at monitoring station near the treatment facility in Freeport, suggesting that the SRWTP was the major point source of ammonium for the region around Cache Slough and that the concentrations were diluted with higher flow (Glibert et al. 2022). Other downstream regions such as Suisun Bay and Suisun Marsh may not have seen any benefits from the SRWTP upgrade due to dilution associated with distance from the SRWTP, and because excess ammonium is still being supplied to the region from the secondary treatment facility in Suisun Bay.

Harmful Algal Blooms

No harmful algal blooms were observed in our study regions; however, *Microcystis* spp. surface colonies were common in both action and no-action years in all regions. The *Microcystis* spp. colonies were broken up and occurred at relatively low abundance compared with observations from the San Joaquin River (Lehman et al. 2020). *Microcystis* spp. was much more prevalent during the no-action years, especially in the Lower Sacramento River. Since *Microcystis* spp. thrives in warm, low-velocity freshwater in the Delta (Mioni et al. 2011) the increased flow during action years selected against the conditions that would promote *Microcystis* spp. blooms. Most *Microcystis* spp. blooms in the Delta originate in the San Joaquin River (Lehman et al. 2017), so evaluating the growth and transport of those blooms in the Southern Delta under both high flow rates and low export rates will help to assess the impacts of flow actions on HAB events.

Although the *Microcystis* spp. observed in Suisun Bay and Marsh was not in bloom conditions, the colonies can continue to produce toxins up to a salinity of 4 ppt (Black et al. 2011). The toxic impact of the observed *Microcystis* spp. cannot be determined without measuring microcystins. The degraded cyanobacterial colonies may augment the phytoplankton biomass and provide a food source for zooplankton, some of which can consume decomposed *Microcystis* spp. (Hanazato and Yasuno 1987).

There are many other taxa in our study region that have the potential to produce harmful algal blooms, including other cyanobacteria (*Aphanizomenon* spp.) and dinoflagellates taxa (*Gonyaulux* spp.). These taxa had a relatively low abundance in our study and since we did not see them in bloom formation it is unlikely that these species produced large concentrations of toxic secondary metabolites. Similarly, the lack of bloom formation and the relatively high dissolved oxygen concentrations demonstrate a low risk of eutrophication from phytoplankton blooms. Toxic blooms do occur in the San Francisco Estuary, for example the *Heterosigma akashiwo* (raphidophyte) bloom in 2022 caused mass mortality in fish populations but was restricted to the more marine regions west of San Pablo Bay (OPC 2022), and outside of our study region and timeframe.

Conclusions and Management Implications

While not directly measured by our study, higher flows during action years likely decreased residence times, which has been shown to have a large effect on phytoplankton biomass in other estuaries (Wan et al. 2013, Gameiro et al. 2004) and parts of the SFE (Stumpner et al. 2020, Smits et al. 2023). If management goals are targeted to improve total phytoplankton biomass in the Suisun Bay and Suisun Marsh regions, fall flow actions are not likely to be beneficial. Alternatively, if goals are to improve food quality for zooplankton (e.g. diatoms), then the flow actions during action years likely selected for diatoms and against potentially toxic phytoplankton such as cyanobacteria. Cyanobacteria presence increased in no-action years, which are considered less nutritious food and produces toxic secondary metabolites. Considering some of these changes in community composition, flow augmentation may be considered a success. However, recent research into the diets of copepods such as *P. forbesi* demonstrated that these zooplankters do readily consume cyanobacteria (Holmes and Kimmerer 2022) and certain diatom blooms only provided a minor stimulus to the growth of *P. forbesi* (Jungbluth et al. 2020).

While important, nutrients likely did not have as large of an impact on phytoplankton biomass and community compared to increased flow and its relationship with residence time. Our study found that nutrient concentrations remained relatively similar across action and no-action years for the target regions of Suisun Bay and Marsh, yet chlorophyll-*a* and the phytoplankton community were different across the two different year types. In regions with higher residence times (e.g. SRDWSC), nutrient dynamics likely play a larger role in phytoplankton biomass and composition, as evidenced by the excess phosphate in the SRDWSC.

The flow action was associated with a decreased presence of *Microcystis* spp. in target regions of Suisun Bay and Suisun Marsh, but this association is likely due to an effect of water year type. Blooms of *Microcystis* spp. in the upper SFE are frequently initiated in the summer when residence time and water temperature thresholds are met (Lehman et al. 2017). These blooms are concentrated in the southern regions of the Delta and San Joaquin River (Lehman et al. 2022) and are subsequently transported seaward to other regions of the estuary through the confluence of the San Joaquin River and Sacramento River. While there were noticeable changes in presence of *Microcystis* spp. in the Lower Sacramento River region between action years and no-action years, this association likely due to the regions proximity to these cyanobacteria hotspots.

While our study has answered some questions about the Fall *X2* flow augmentation, it was difficult to distinguish between effects of flow augmentation and effects of climatic conditions. In many ways, flow augmentation and climatic conditions are closely tied together, as flow augmentation is not possible during drier water year types. There were statistically significant effects of the flow action in the target regions of Suisun Bay and Suisun Marsh where the same effect was not observed in the landward regions of SRDWSC and CSC.

Grazing patterns of secondary consumers such as *Potamocorbula amurensis*, are an additional confounding factor when investigating the effects of outflow on phytoplankton communities, which may be strongest in late summer and into fall in Suisun Bay (Kimmerer and Thompson 2014). Due to their seasonal development, abundance of *P. amurensis* and their subsequent grazing rates are lowest in the winter-spring period (Zierdt Smith et al. 2023, Crauder et al. 2016). Periods of high outflow in spring can cause stratification of the water column in Suisun Bay, and if the clam grazing rate is low relative to the rate of cell growth, there may be an opportunity for a spring bloom of diatoms, and subsequent important opportunity for secondary consumers of interest (Cloern 1982, 1984, 1991, Lucas et al. 1998). Outflow actions during spring and summer may have stronger effects than observed in the fall but need to be further studied.

Finally, we advocate for more work to be done looking at phytoplankton taxonomy. Some areas of interest may be looking at colony formation or morphological features since these features may affect how phytoplankton are retained in the water column or advected from the system during times with higher flow. Additionally, measuring biovolume may give a more accurate understanding of how species assemblages affect measurements of chlorophyll-*a* and variability in size.

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Tables

Table 2-1. Mean and Standard Deviation in Parentheses of Water Quality Parameters by Region from September Through November

Year	Strata	Chl-a µg/L	Salinity ppt	Temp. °C	Turbidity NTU	DO mg/L	NH4 µmol/L	NO3 µmol/L	PO4 µmol/L
Action	Cache Slough Complex	1.21 (1.08)	0.08 (0.03)	15.1 (2.02)	5.46 (5.80)	9.03 (0.57)	5.24 (4.71)	3.05 (1.97)	0.57 (0.21)
	Sacramento Deep Water Ship Channel	2.5 (1.19)	0.287 (0.17)	16.9 (2.3)	12.55 (13.64)	8.82 (0.54)	3.49 (4.2)	2.18 (1.85)	0.78 (0.43)
	Lower Sacramento River	0.86 (0.50)	0.11 (0.12)	14.8 (1.41)	4.35 (3.41)	9.09 (0.31)	8.37 (4.57)	5.35 (1.22)	0.45 (0.14)
	Suisun Bay	1.35 (0.67)	5.14 (3.63)	16.1 (1.8)	13.16 (6.38)	8.86 (0.35)	5.9 (5.49)	4.79 (0.82)	0.56 (0.09)
	Suisun Marsh	1.66 (1.14)	5.36 (2.1)	15.7 (1.76)	27.83 (16.88)	8.42 (0.69)	5.22 (3.25)	4.23 (0.87)	0.53 (0.19)
No-Action	Cache Slough Complex	1.7 (0.85)	0.13 (0.14)	16.3 (1.94)	4.53 (3.52)	8.07 (1.61)	3.57 (4.02)	3.51 (3.04)	0.92 (0.84)
	Sacramento Deep Water Ship Channel	2.59 (1.43)	0.30 (0.17)	16.8 (2.76)	14.09 (13.94)	8.79 (0.48)	4.29 (8.78)	2.91 (2.45)	0.95 (0.36)
	Lower Sacramento River	1.39 (0.50)	1.26 (1.55)	16.6 (2.51)	6.47 (5.65)	8.57 (0.54)	4.63 (6.04)	5.12 (2.11)	0.60 (0.21)
	Suisun Bay	2.67 (1.71)	11 (4.10)	16.4 (2.38)	6.15 (5.50)	8.14 (0.43)	4.97 (4.76)	4.88 (1.45)	0.71 (0.32)
	Suisun Marsh	3.63 (1.89)	9.26 (3.39)	16.3 (2.42)	14.51 (15.97)	7.81 (0.85)	5.62 (3.95)	4.92 (1.67)	0.73 (0.87)

Key:

°C = degrees Celsius; µg/L = micrograms per liter; µmol/L = micromoles per liter; Chl-a = Chlorophyll-a; DO = dissolved oxygen; mg/L = milligrams per liter; NH4 = ammonium ion; NO3 = nitrate ion; NTU = nephelometric turbidity unit; PO4 = phosphate; ppt – parts per thousand

Table 2-2. Generalized Linear Model Summary for Action Year Status in Individual Terms and Interaction Terms

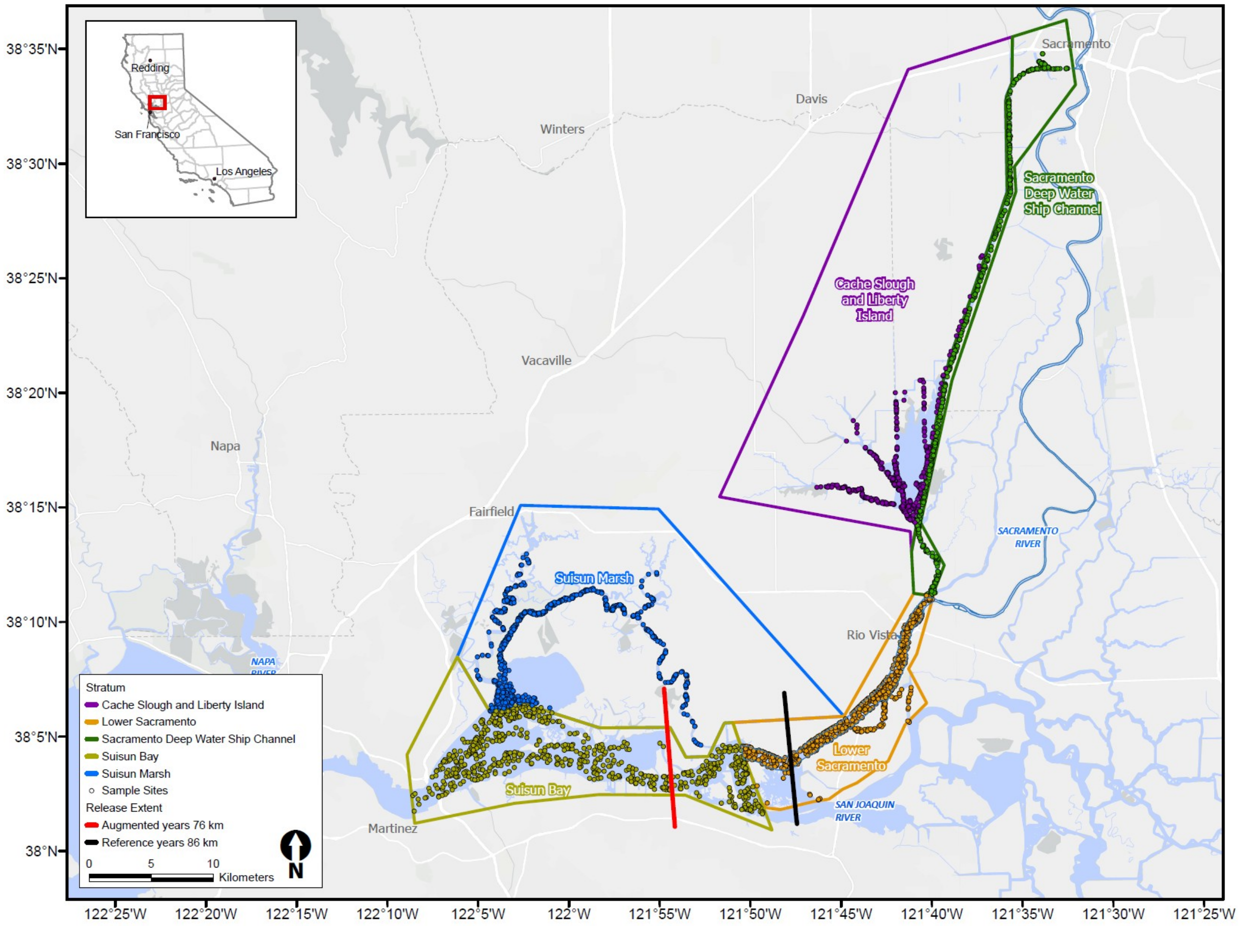
Covariate Type	Parameter	Estimate	Standard Error	t-value	p-value
Individual Terms	(Intercept)	6.16	1.822	3.381	0.001
	ActionYear, No-Action	0.046	0.396	0.117	0.907
	Cache Slough and Liberty Island	-1.364	0.308	-4.423	1.24E-05
	Lower Sacramento River	-1.577	0.334	-4.724	3.17E-06
	Suisun Bay	-1.276	0.302	-4.229	2.89E-05
	Suisun Marsh	-1.091	0.338	-3.229	0.001
	Temperature	-0.038	0.021	-1.749	0.081
	Salinity	0.042	0.021	1.955	0.051
	NH4	-0.05	0.021	-2.365	0.018
	PO4	0.826	0.123	6.686	7.37E-11
	X2	-0.042	0.024	-1.738	0.083
Interaction Terms	ActionYear: Cache Slough and Liberty Island	0.374	0.378	0.988	0.324
	ActionYear: Lower Sacramento River	0.683	0.389	1.755	0.08
	ActionYear: Suisun Bay	1.128	0.38	2.97	0.003
	ActionYear: Suisun Marsh	1.806	0.401	4.499	8.87E-06
	ActionYear: NH4	0.005	0.024	0.201	0.841

Key: NH4 = ammonium ion; NO3 = nitrate ion; NTU = nephelometric turbidity unit; PO4 = phosphate

Table 2-3. R^2 and p Values for Correlation Between NMDS Configuration of Phytoplankton Taxonomic Groups and Environmental Parameters

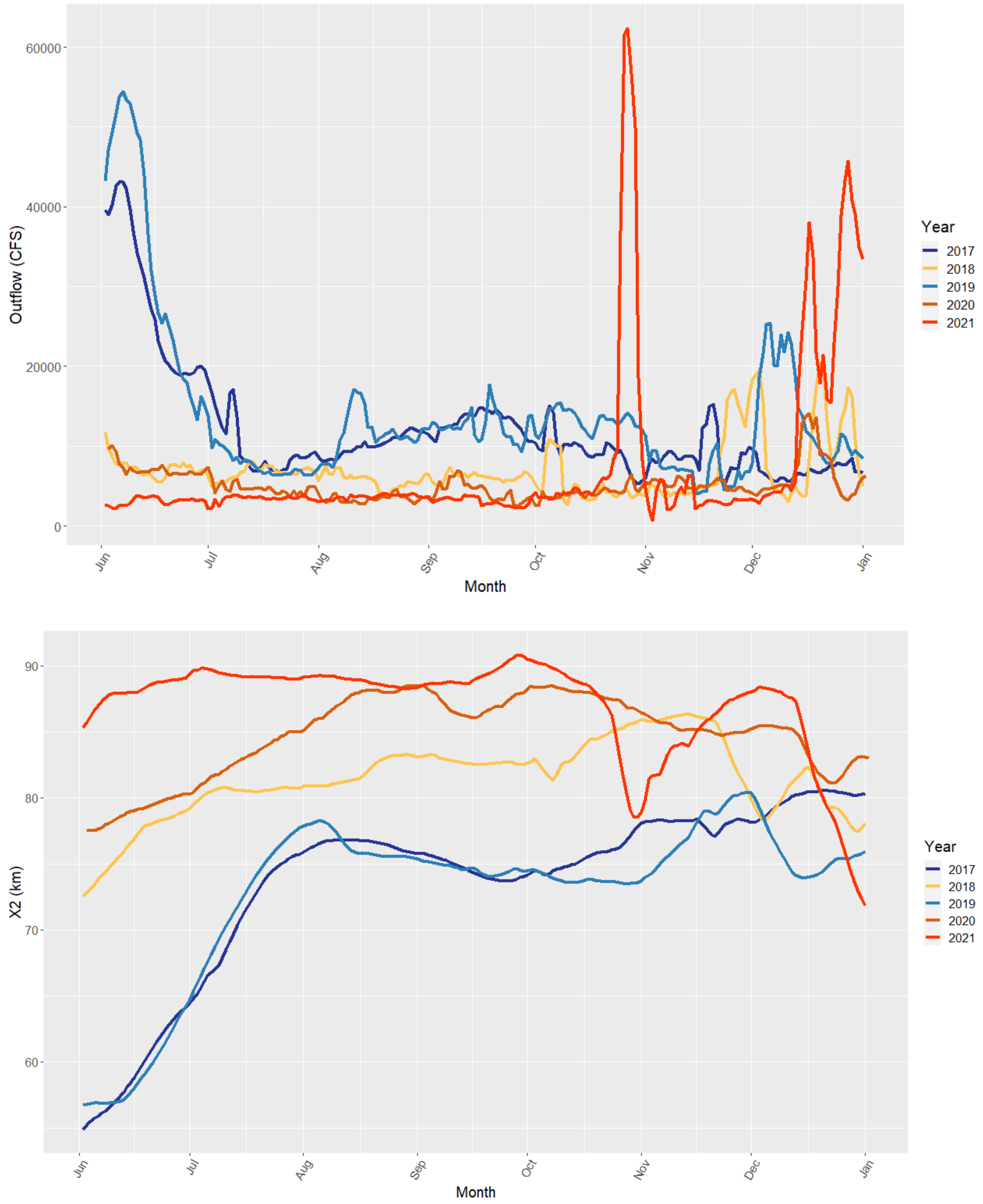
Parameter Type	Parameter	NMDS1	NMDS2	r^2	P-Value
Vector	Chlorophyll a	-0.999	-0.049	0.026	0.004
	DO	0.623	-0.783	0.054	0.0001
	DOC	0.433	-0.902	0.006	0.329
	NH4	0.631	-0.776	0.018	0.023
	NO3	-0.761	-0.649	0.005	0.389
	pH	0.793	-0.609	0.02	0.018
	PO4	-0.474	0.881	0.002	0.663
	Salinity	-0.529	0.849	0.029	0.003
	Temperature	-0.965	0.261	0.017	0.031
	Turbidity	-0.119	0.993	0.03	0.003
	X2	-0.98	0.2	0.153	0.0001
Factor	Action Year	-	-	0.102	0.0001
	Region	-	-	0.031	0.0011

Figures



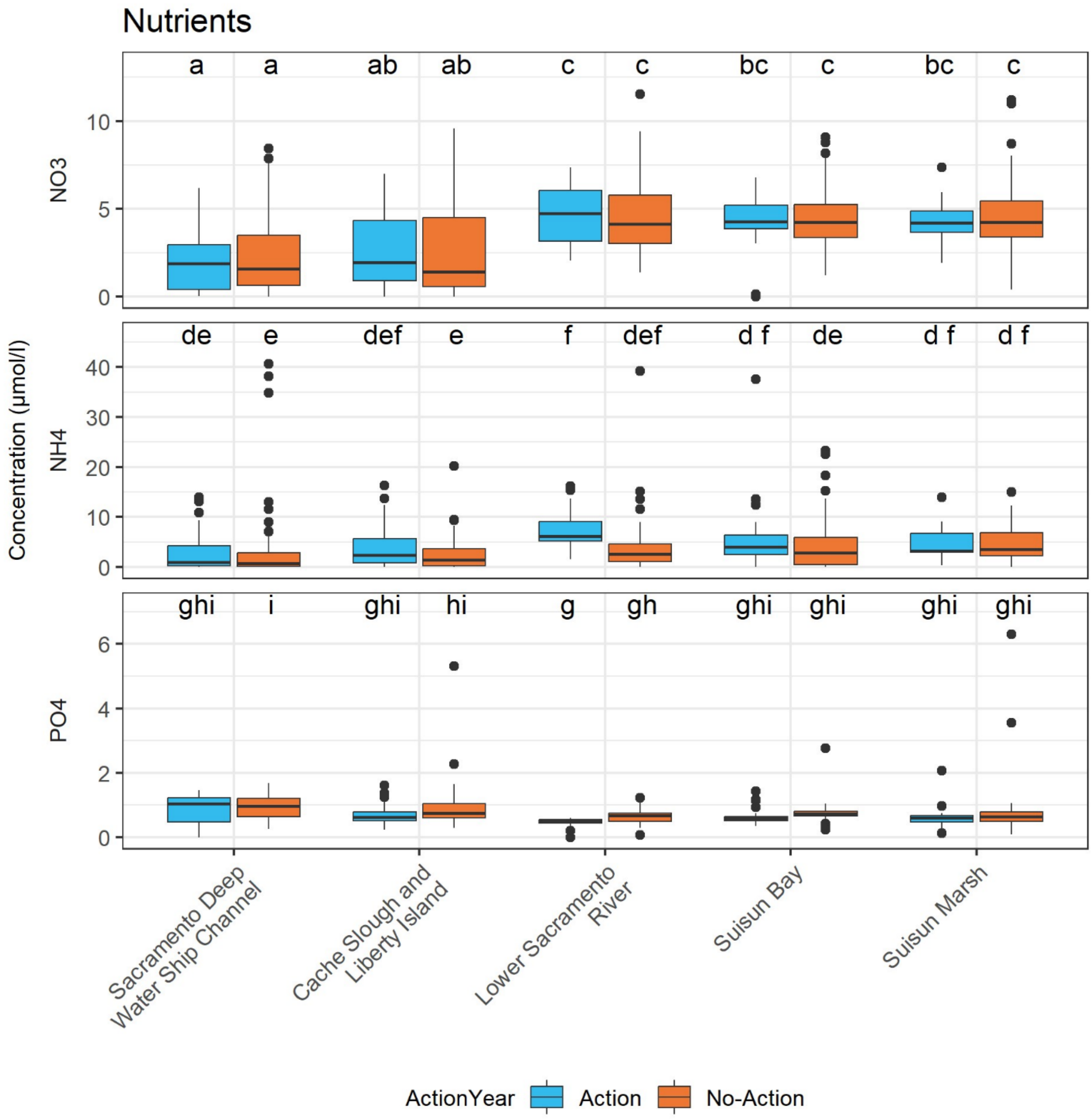
Red line indicates average X2 position in the fall during action years and the black line indicates average X2 position during no-action years.

Figure 2-1. Map of the Regions and Locations Sampled in the Upper San Francisco Estuary and Average Position of X2 in Action and No-Action Years



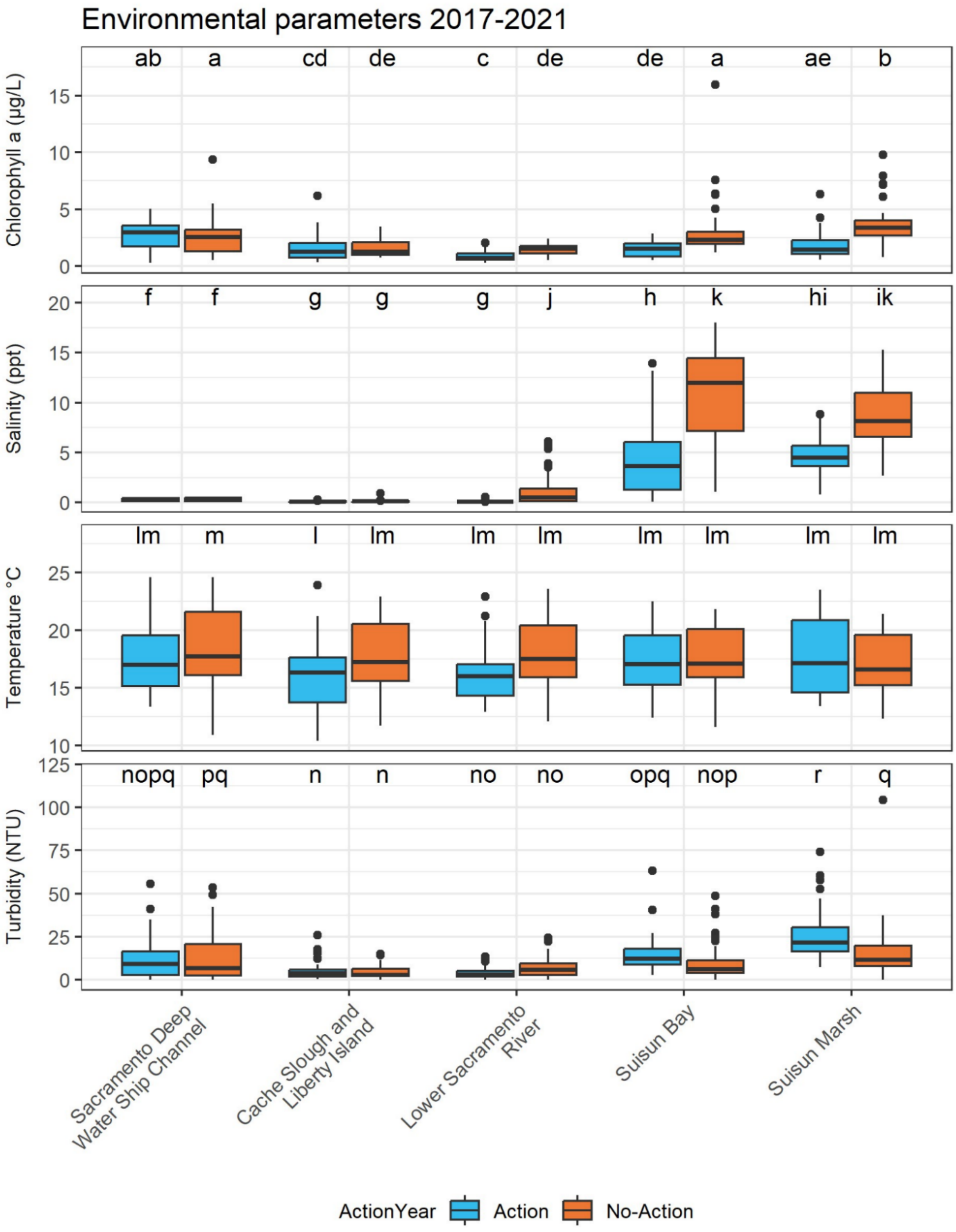
Measurements from DWR dayflow data. Cooler colors indicate action years, while warmer colors indicate no-action years.

Figure 2-2. Outflow and X2 from June Through January 2017 – 2021



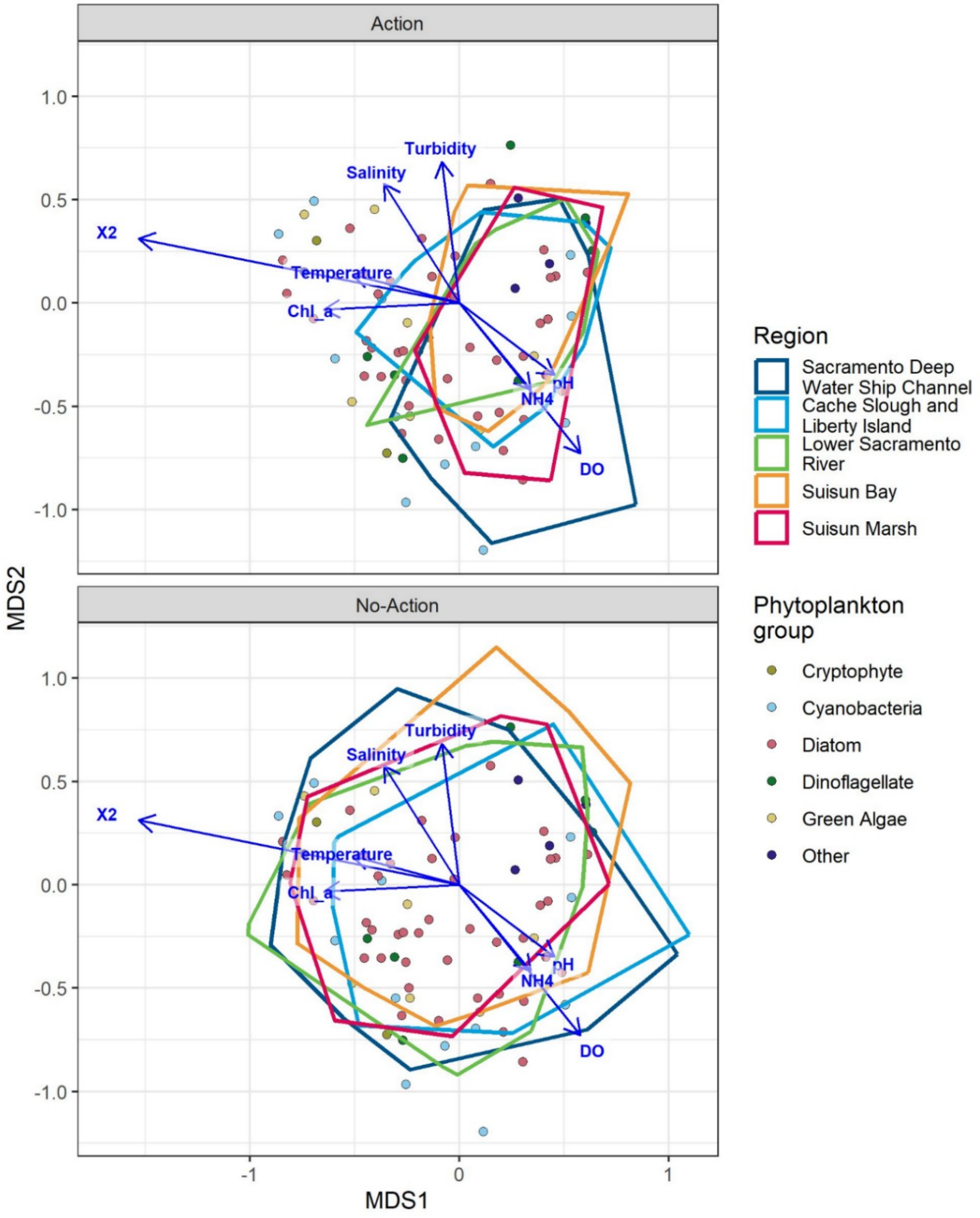
Shared letters denote no significant difference observed between regions and years.

Figure 2-3. Nutrient Concentrations Between Action (blue) and No-Action (orange) Years and Study Regions in the Upper SFE



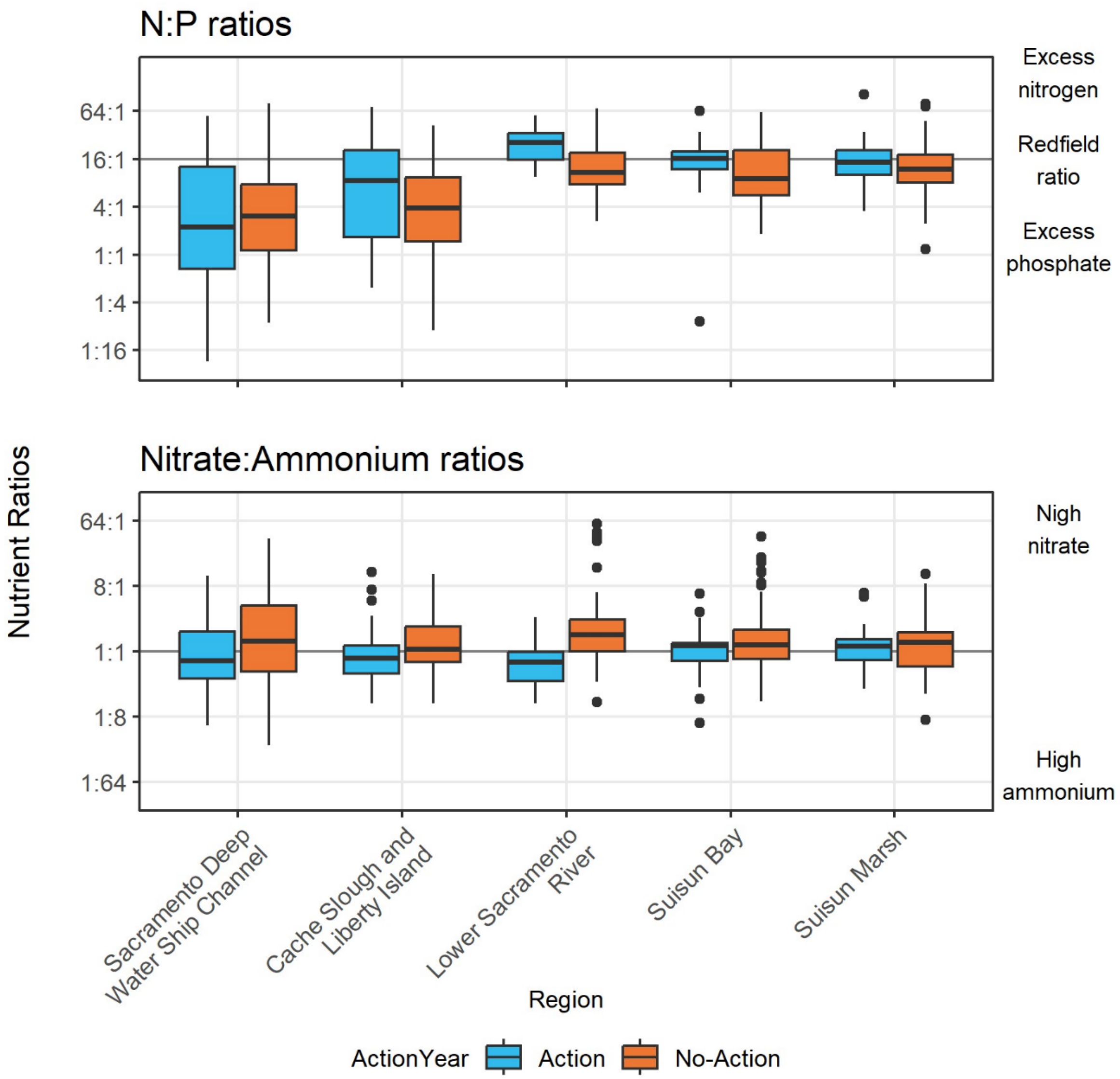
Shared letters denote no significant difference observed between regions and years. Outliers are omitted from this plot

Figure 2-4. Environmental Parameters Between Action (blue) and No-Action (orange) Years and Study Regions in the Upper SFE



The length and direction of the arrows indicate the strength and direction of the linear correlation of environmental variables with ordination scores. The polygonal hulls encompass all sites within each region.

Figure 2-5. Non-Metric Multidimensional Scaling (NMDS) Ordination of Phytoplankton Community Structure in the upper San Francisco Estuary for Action Year Status



The thin solid line represents the 16:1 Redfield ratio of 16 nitrogen : 1 phosphate.

Figure 2-6. Ratios of Dissolved Nutrients Between Action (blue) and No-Action (orange) Years and Regions of the Upper SFE, with Dissolved Inorganic Nitrogen to Phosphate (top) and Nitrate to Ammonium (bottom)

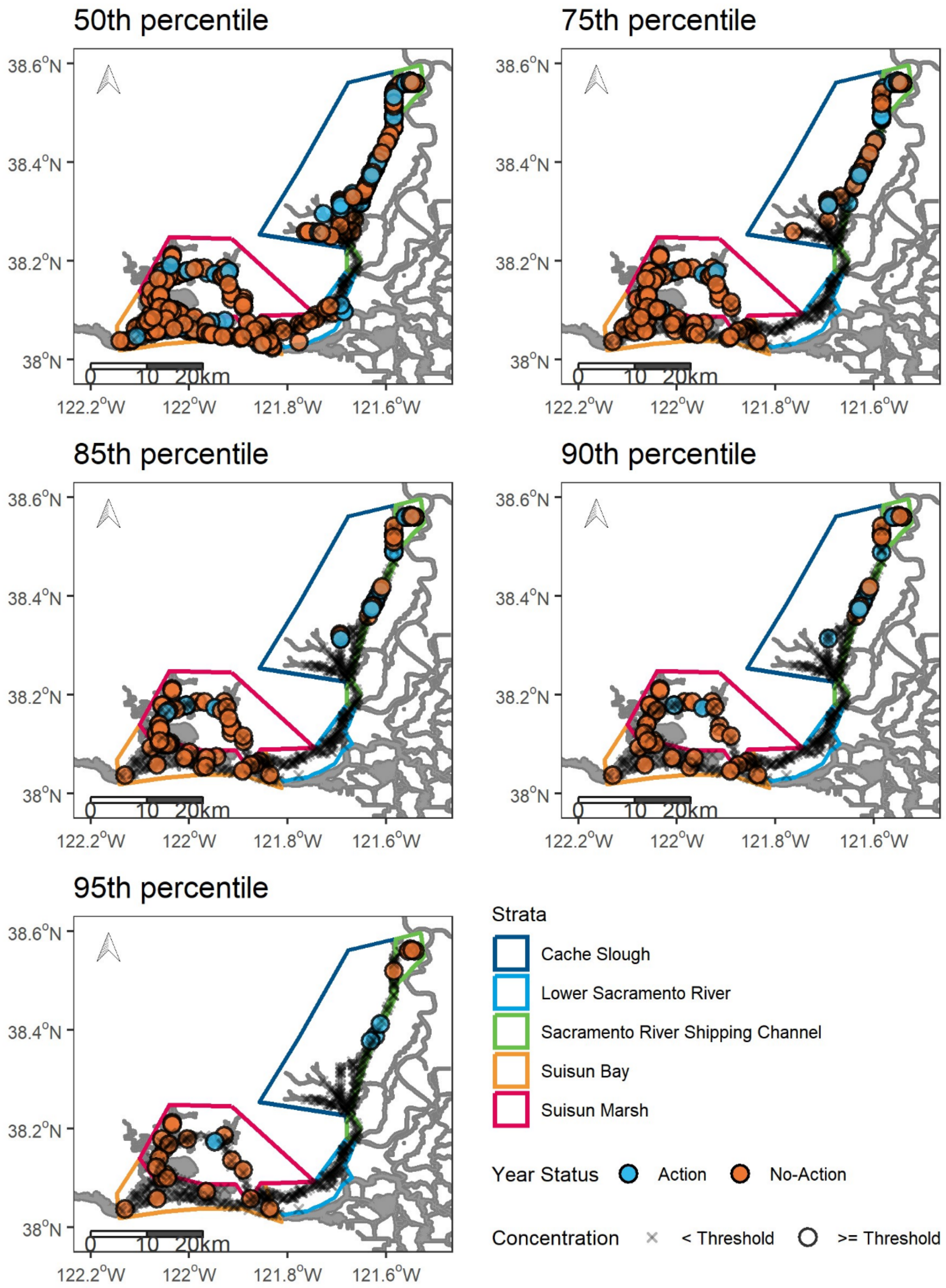
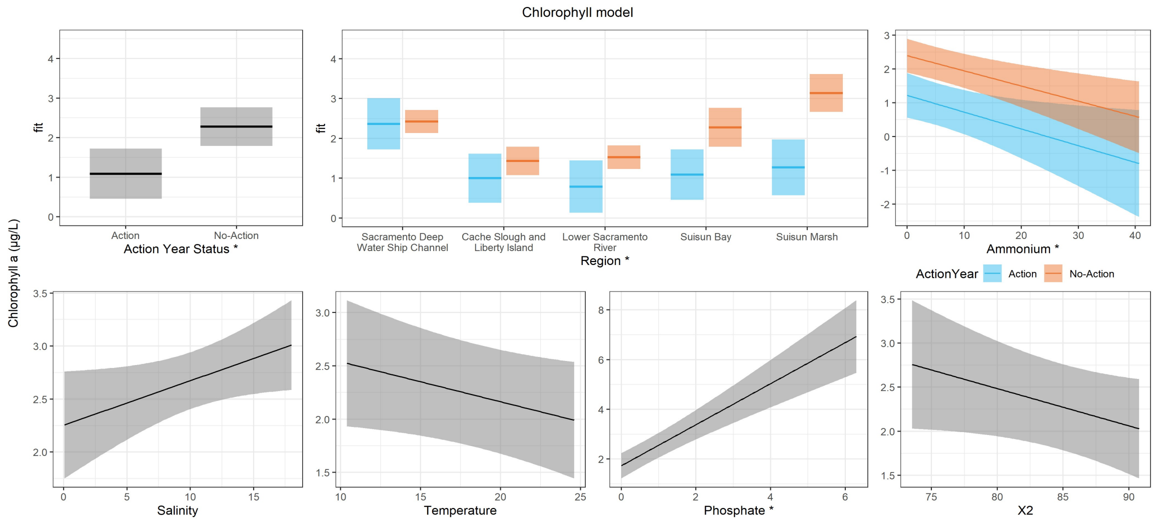
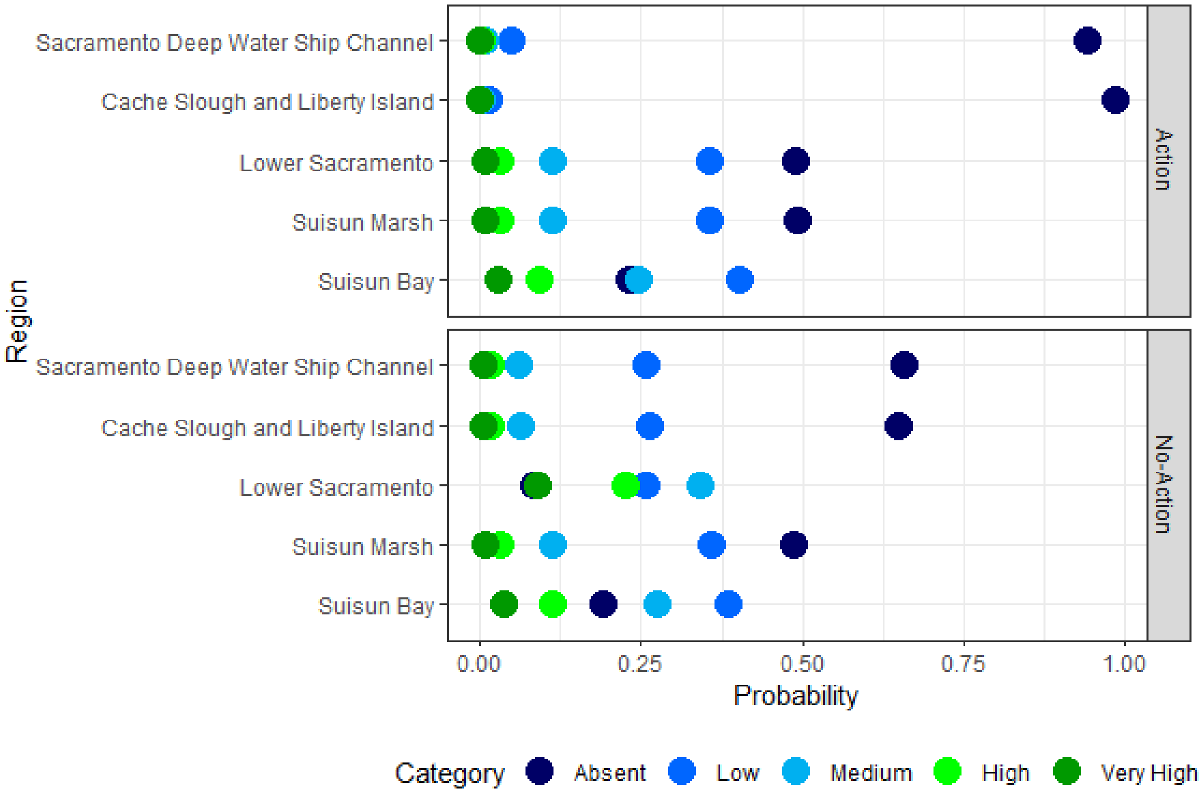


Figure 2-7. Chlorophyll Concentrations Between Action (action) and No-Action (no-action) Years in the Five Regions of the Upper SFE



* indicates statistically significant variables. Parameters with two colors indicate model selection of the interaction between the parameter and the action year status.

Figure 2-8. Chlorophyll-a Generalized Linear Model with Action Year Status, Region, Temperature, Salinity, Ammonium, Phosphate, and X2 with 95% Confidence Intervals



Regions with shared letters are not significantly distinct from each other.

Figure 2-9. Proportion of Sites with Colonial Microcystis spp. Observed in Each Region and Year

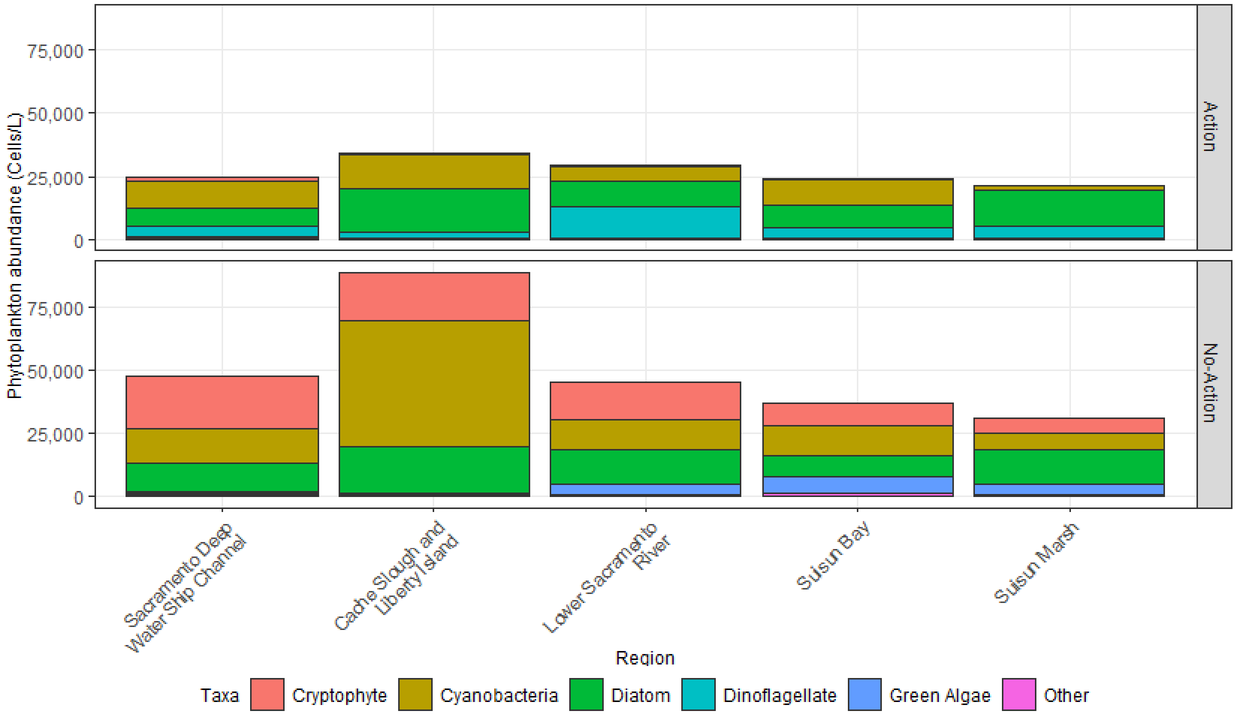


Figure 2-10. Mean Abundance of Each Phytoplankton Group by Year and Region as (A) Proportion of the Total Phytoplankton Abundance and (B) Scaled to Mean Chlorophyll-a to Highlight the Respective Contribution of Each Group to the Phytoplankton Biomass

Supplemental Information

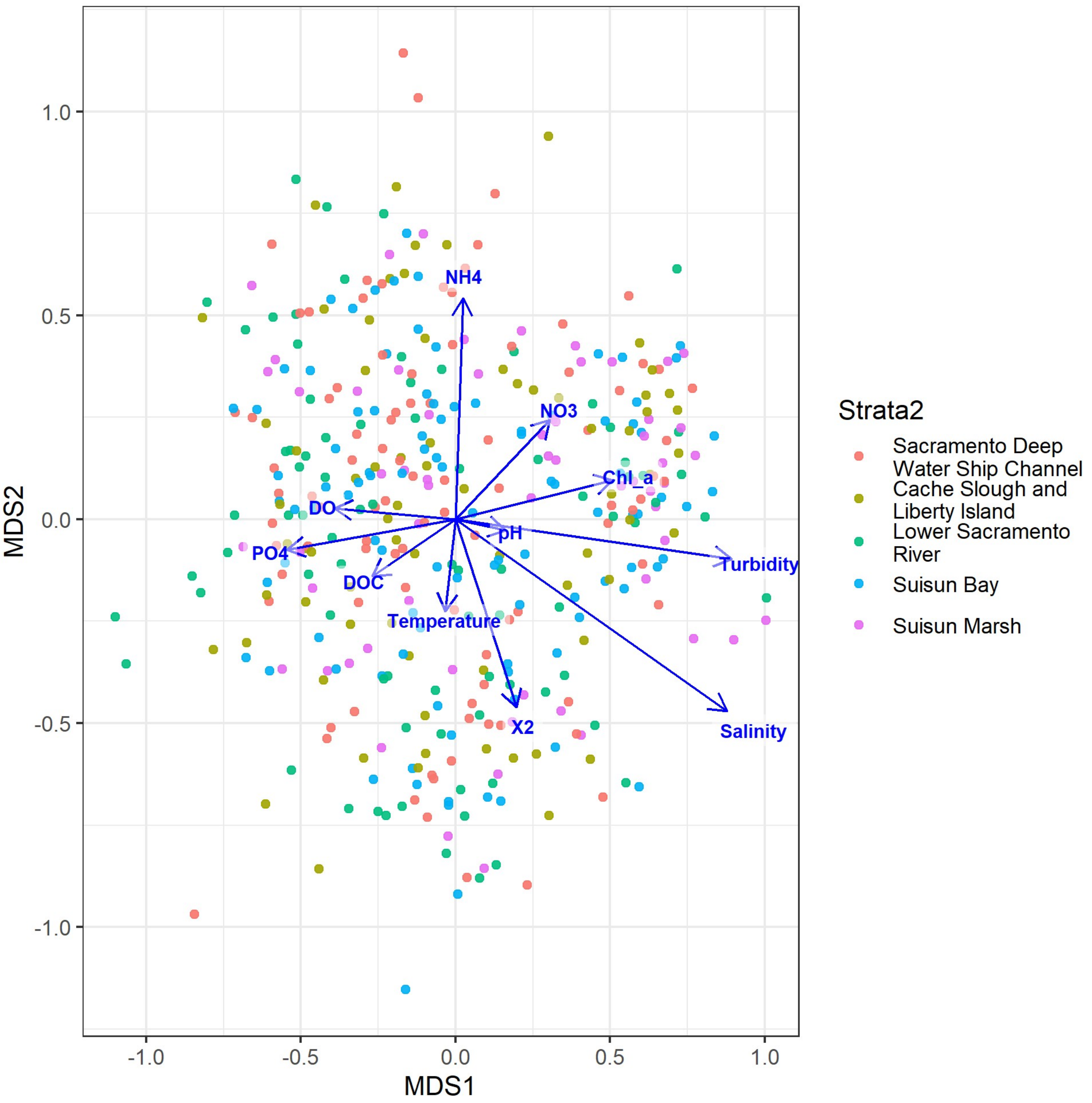


Figure S2-1. NMDS Ordination of Phytoplankton Community Structure and Environmental Parameters by Region

Chapter 3. Implementing Rapid Genetic Identification of Delta Smelt in a Non-Genetics Agency Laboratory to Support Species Monitoring

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Abstract

Genetic-based species identification is an essential tool for managers in the San Francisco Bay-Delta. Methods leveraging species-specific DNA patterns can distinguish between morphologically similar organisms like the endangered Delta Smelt (*Hypomesus transpacificus*) and the non-native Wakasagi (*Hypomesus nipponensis*). Although these methods provide unambiguous species identification, they are usually only performed in specialized genetics laboratories, resulting in a delay in obtaining timely data for management decision-making. Here we describe the collaborative implementation of a rapid genetic method, SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing), between UC Davis researchers and agency scientists at the US Fish and Wildlife Service (USFWS)-Lodi Fish and Wildlife Office (LFWO). Using pre-assembled, ready-to-use SHERLOCK assay kits, USFWS-LFWO personnel used non-invasive mucus swab sampling and performed SHERLOCK directly on swabbed material, without a DNA extraction step, to determine genetic-based species identification of individual smelt (*Hypomesus spp.*) specimens caught by the gear-based monitoring program using net trawling. These data were obtained by USFWS personnel in a non-genetics laboratory using a relatively low-cost, portable fluorescence reader device. The results were then confirmed by the specialized genetics collaborating laboratory (University of California, Davis). Because of the assay's ease of use, the lack of needing extensive and expensive infrastructure, and the small laboratory footprint of the assay reagents and equipment, we suggest that this example of SHERLOCK implementation for species identification at a government agency laboratory, with no previous experience with in-house genetic identification, is generalizable to other agencies and institutions. Implementing SHERLOCK at agency laboratories will ultimately improve turnaround time for producing data critical to management decisions and accelerate progress towards the long-term goal of making the technology accessible to all Bay-Delta scientists.

Introduction

Accurate and timely differentiation between listed species and closely related, morphologically similar non-listed species co-occurring in the same habitat is critical for effective management. In the San Francisco Bay-Delta estuary, three morphologically similar osmerid fish species are present: Delta Smelt (*Hypomesus transpacificus*) and Longfin Smelt (*Spirinchus thaleichthys*), both federally listed under the Endangered Species Act, and the non-native Wakasagi (*Hypomesus nipponensis*). Multiple government agency monitoring programs use gear-based methods to generate data on abundance and status and trends for these and other Bay-Delta species, providing critical information for adaptive management (Mahardja et al. 2021). The Enhanced Delta Smelt Monitoring Program (EDSM), operated by the US Fish and Wildlife Service, is currently the primary fish survey used to understand the abundance and distribution of the endangered Delta Smelt (United States Fish and Wildlife Service et al. 2023). Information provided from the EDSM survey can inform management on the efficacy of recovery actions for Delta Smelt, such as export reduction at large water pumping infrastructure to reduce entrainment (Smith et al. 2021) and the augmentation of Bay-Delta outflow in the dry period of California (Polansky et al. 2024).

However, most monitoring programs, including EDSM, rely primarily on morphology-based species identification. Morphology-based identification can be performed in the field or laboratory but requires personnel training and expertise that takes time to acquire. In addition, accurate identification can be challenging in some contexts, especially for early developmental stages like larvae or juveniles. Delta Smelt and Wakasagi are the most problematic Bay-Delta osmerids to differentiate (Stagg et al. 2023), reflecting their close taxonomic and phylogenetic relationship as congeners (Asadi Aghbolaghi et al. 2024). Morphological differentiation between the two species requires counting of chromatophores, which is challenging in the field, and made even more difficult when specimens found are dead and undergoing decomposition (Benjamin et al. 2018). Furthermore, hybridization can occur between the species, which has been observed with maternal Wakasagi and paternal Delta Smelt parentage (Fisch et al. 2014, Benjamin et al. 2018). Previous studies of hybridization have suggested that these events are relatively rare, but the frequency of hybridization might not be static, and could increase or decrease over time and with changing conditions (e.g., climate change). Changes in the ecosystem and management practice (*e.g.*, recent higher abundance of Wakasagi and releases of hatchery Delta Smelt for population supplementation [Davis et al. 2022, Baerwald et al. 2023b]) might also affect hybridization dynamics.

Genetic-based methods provide unambiguous species identification that can improve accuracy compared to morphological-based methods used in isolation. In some cases, such as when genetically distinct populations are visually indistinguishable from one another, it may even be a necessity (Dowling et al. 2015, Baerwald et al. 2023a). For Bay-Delta osmerids, species-specific single nucleotide polymorphisms (SNP) present on the maternally inherited mitochondrial genome have been leveraged for quantitative polymerase chain reaction (qPCR) (Baerwald et al. 2011). A panel of 24 nuclear SNPs has also been developed for species identification for Delta Smelt, Wakasagi, and Longfin Smelt, and for detecting hybrids (Benjamin et al. 2018). These methods are generally reliable and definitive, but there are some limitations in the context of management. One limitation is time. The multi-step process for each, beginning with delivery of samples to the laboratory and subsequent DNA extraction, can take at least several days, and more usually weeks or months with typical service laboratory workloads, delaying data delivery to agency scientists and managers. In addition, these methods require relatively extensive laboratory training to correctly extract the DNA, perform the genetic assay (usually in a specialized laboratory using expensive equipment), analyze results, and report the data. To date, the process has involved relatively minor but invasive sampling of Delta Smelt, Wakasagi, and Longfin Smelt (caudal fin clipping). A genetic-based species identification method that: 1) is rapid and easy to perform without requiring specialized personnel or expensive equipment; 2) is minimally invasive, non-destructive, and preserves specimens for other analyses; 3) does not require a separate time-consuming DNA extraction step; and 4) can deliver accurate species identification data very rapidly, would be an asset to managers and researchers.

We (Baerwald et al. 2020) have developed three separate species-specific genetic detection assays for Delta Smelt, Longfin Smelt, and Wakasagi using SHERLOCK (Specific High-sensitivity Enzymatic Reporter Unlocking), a CRISPR-based genetic method. SHERLOCK was initially developed as a diagnostic tool for human pathogen detection (Gootenberg et al. 2017), but its ability to detect species-specific genetic variants makes it ideal for identification of any organism, and even for intraspecific genetic identification (Baerwald et al. 2023a). These SHERLOCK assays are highly specific, sensitive, and can be configured in a portable field assay format, allowing fast data collection when there is a "fish in hand" (Baerwald et al. 2020). Sufficient DNA can be obtained non-destructively by collecting swabs of external mucus from individual fish, and these samples can be used directly as templates in the SHERLOCK reaction without a separate DNA extraction step. Here we describe the collaborative implementation of SHERLOCK between the Genomic Variation Laboratory at UC Davis (hereafter referred to as "the genetic laboratory") and agency scientists at USFWS-Lodi Fish and Wildlife Office that operates the EDSM survey (hereafter referred to as "the field office"). The goal of the project was to establish independent SHERLOCK genetic identification capability at the field office, via a pilot phase process where both groups performed SHERLOCK genetic identifications on new, previously not assayed *Hypomesus* mucus swab specimens obtained during gear-based monitoring. To achieve this overall goal, we set three objectives: testing SHERLOCK frozen reaction stability over time, validating SHERLOCK assays on existing mucus samples from frozen Delta Smelt and Wakasagi, and implementation of SHERLOCK in the field office to test unknown specimens on a rolling basis, as new *Hypomesus* spp. were caught by the monitoring program during the Winter 2023-24 field season.

Materials and Methods

Frozen SHERLOCK Reaction Stability Testing

To confirm stability of pre-frozen SHERLOCK assays over time, which would allow transfer and storage of multiple aliquots from the genetic lab to the field office that could be used over an entire field sampling season, performance of frozen reaction aliquots was assessed in a time course experiment. Sequences for the recombinase polymerase amplification(RPA) primers and CRISPR RNA (crRNA) for Wakasagi and Delta Smelt SHERLOCK assays are shown in Table 3-1 (all sequences are the same as in Baerwald et al. 2020 except for the Wakasagi assay forward RPA primer, which was redesigned for increased assay sensitivity). All reaction components (reaction setup similar to Baerwald et al. 2023a) except the DNA templates (or water for negative controls) for the Wakasagi assay were assembled as shown in Table 3-2. Lyophilized RPA reagent (TwistDX) was rehydrated with 60 microliters (µL) of this reaction master mix, and aliquots of 18 µL were dispensed into 0.2 milliliter (mL) polymerase chain reaction (PCR) 8-strip tubes, which were stored without any freeze/thaws in a -80 degrees Celsius (°C) freezer until testing was performed. For testing, tubes were thawed on ice and 2 µL of synthetic Wakasagi DNA (gBlock; approximately 5,500 and 550 copies per reaction for testing higher and lower concentrations) were added to each 18 µL aliquot; each concentration was tested in triplicate reaction replicates ("technical replicates").

For negative controls, 2 µL of nuclease-free water was tested in a single reaction replicate. Testing was performed over six months in one-month intervals by incubating the full reactions at 37°C for one hour on an Axxin T16-ISO fluorescence reader.

SHERLOCK Assay Validation on Mucus Samples from Frozen Specimens

A mucus swabbing protocol was developed for staff with no previous experience in genetic laboratory methods (Figure 3-1). A sterile Puritan rayon-tipped applicator was used to swab the lateral length of each fish five times. If fish were stored frozen, they were partially thawed on ice before performing swabbing. The tip of the swab was then submerged in 300 µL of 1X phosphate-buffered saline (PBS) and swirled for ten seconds to disperse DNA, and the tip was discarded. The PBS buffer solution was then used for SHERLOCK and stored in a -20°C freezer. In certain cases where more DNA is required, a fin clip is collected from the specimen. Caudal fin clips roughly 5mm in size were taken from frozen samples using a bleach-sterilized scalpel, preserved in 100% ethanol, and extracted with the Qiagen DNeasy Blood and Tissue kit following manufacturer's instructions, including overnight incubation with Proteinase K at 50°C. To test mucus or fin clip samples, SHERLOCK reaction setup was identical to above, except 2 µL of swabbed DNA in PBS (or tissue-extracted DNA, or synthetic control DNA) was added to each experimental well. Each sample was assayed with at least three technical replicates for each SHERLOCK assay. The addition of gBlock and UltraPure water was used as a positive and negative control in each experiment, respectively. The reaction was incubated at 37°C for one hour in the Axxin T16-ISO fluorescence reader (Figure 3-1). Alternatively, for running samples in a multiwell plate format, SHERLOCK can also be run on the BioTek Synergy H1 microplate reader. In this case, the reaction is prepared in the same way as it is for the Axxin reader, but with a smaller total reaction volume. Aliquots of 9µL SHERLOCK reaction master mix are dispensed into black opaque 384-well plates and 1 µL of swabbed DNA is added to each experimental well, with at least three technical replicates for each sample for each assay. The reaction is incubated at 37°C for one hour in the BioTek fluorescence reader. To provide additional confirmation of mitochondrial genetic species ID, we performed Sanger sequencing of the CytB gene region (same region used for SHERLOCK) on some samples. For swab samples, 200 µL of swab material in PBS was used for DNA purification using the Qiagen DNeasy Blood and Tissue Kit following the manufacturer's protocol. A species-specific portion of the mitochondrial Cytochrome B gene was amplified using PCR and sent for sequencing. PCR primers with the addition of M13 tails were used to amplify the Cytochrome B gene (Table 3-3). The samples were then amplified in a thermocycler for 37 cycles of, 95°C for 20 seconds, 54°C for 20 seconds, and one minute at 72°C. Successful amplification was confirmed with agarose gel electrophoresis and PCR products were sent to Quintara Biosciences for Sanger sequencing. The sequences were analyzed using the program Molecular Evolutionary Genetics Analysis (MEGA X; Kumar et al. 2018). Species ID was determined by using NCBI's Basic Local Alignment Search Tool (BLAST) to reference sequences of known species. To further confirm mitochondrial genetic identification, Taqman qPCR assays were performed on a subset of samples using Delta Smelt- and Wakasagi-specific primers and probes as described in Baerwald et al. (2011). Each sample was run in triplicate reactions, and negative and positive controls were included in each experiment (plate). Quantification cycle (Cq) values were determined using Bio-Rad CFX Maestro software. A DNA extract or swab sample was considered positive if it had an average Cq value <40 for all replicates.

SHERLOCK Training and Assay Implementation at the Field Office

To enable independent capability to perform genetic ID at the field office, reagents, supplies, and a fluorescence reader instrument (Axxin T-16) were supplied to the field office. A detailed protocol designed for the end-user at USFWS was also provided. For the Delta Smelt and Wakasagi SHERLOCK assays, aliquots of pre-frozen master mixes were prepared at the genetic lab, transferred to the field office on dry ice, and stored immediately in a -80°C freezer. Aliquots of positive control DNAs for each assay were also transferred and stored at -20°C. Kits containing all components needed for swabbing, in the field or in the lab, were pre-assembled and distributed to field crews. Putative Delta Smelt, identified based on morphology, were caught and swabbed by EDSM personnel. To perform genetic species identification at the field office, a streamlined rapid workflow was developed, consisting of 1) minimally invasive/non-destructive mucus swabbing and resuspension of collected material in pre-aliquoted tubes of 1X PBS; 2) addition of 2 μL resuspended mucus swab material into pre-aliquoted SHERLOCK reaction master mix in 8-strip tubes (thawed on ice); 3) initiation of SHERLOCK reaction by placing 8-strip tubes into fluorescence reader machine (Axxin T-16) and running program to measure signal over one hour incubation at 37°C; 4) data collection by manual inspection of fluorescence curves and noting software-generated calls for positive/negative reaction output (Figure 3-1).

Results

Stability of Pre-Frozen SHERLOCK Reaction Aliquots Over Time in -80°C Storage

To confirm that frozen SHERLOCK reactions could be transferred in batches large enough for multiple experiments in a field season, pre-assembled reaction aliquots (Wakasagi assay) containing all components except target DNA were prepared. SHERLOCK fluorescence endpoint values (normalized fluorescence value after 60 minutes incubation) remained similar to T=0 baseline throughout all time points, up to six months (T=6) after storage at -80°C (Figure 3-2).

Validation Testing of Mucus Swabs from Frozen *Hypomesus* Specimens

Mucus swabs from 19 frozen Delta Smelt and 40 frozen Wakasagi (Table 3-4 and Table 3-5) caught in previous seasons and stored at -20°C, were tested with both Delta Smelt and Wakasagi SHERLOCK assays (triplicate reactions for each) to validate performance on this sample type. For the Delta Smelt swabs, 19/19 (100%) amplified above background in the Delta Smelt assay (Figure 3-3A). However, 7/19 (37%) showed partial positive amplification in the Wakasagi assay (Figure 3-3B). Follow-up testing of these seven specimens with qPCR and Sanger DNA sequencing confirmed Delta Smelt maternal genetic ID (Table 3-5 and data not shown). Consistent amplification was observed for Wakasagi mucus swabs in the Wakasagi SHERLOCK assay and no amplification above background was detected in the Delta Smelt assay (Figure 3-3 C,D).

SHERLOCK Implementation in the Field Office: Winter 2023-2024 Genetic ID Results for *Hypomesus* Specimens Morphologically Identified as Delta Smelt

Mucus swabs from six untagged putative Delta Smelt caught in EDSM trawls during winter 2023-2024 were analyzed using SHERLOCK (Delta Smelt and Wakasagi assays) at the field office. For some of these specimens, multiple mucus swabs were taken and analyzed. Aliquots of the swabs were transferred to the genetic laboratory, and SHERLOCK was performed to confirm the results. For all six specimens, similar results were found between labs (Table 3-6). Five specimens were positively identified as Delta Smelt using SHERLOCK, confirming the initial visual ID. One fish (S-254) that was visually identified as Delta Smelt showed partial amplification (only some technical replicates) in the Delta Smelt SHERLOCK assay, and all replicates showed positive amplification in the Wakasagi SHERLOCK assay. The swabs were rerun on SHERLOCK multiple times, producing similar results (combined results shown in Table 3-6). Follow-up Sanger DNA sequencing was performed to confirm species ID. Specimens that were consistently identified as Delta Smelt by the field office and the genetic lab by SHERLOCK showed DNA sequence matches to Delta Smelt reference sequences (Table 3-7). In contrast, S-254 showed a 100% match to Wakasagi. A custom hybrid SNP panel assay (Benjamin et al. 2018) was performed as a follow-up on S-254, and the pattern of nuclear SNP genotypes observed was consistent with an F1 Wakasagi-Delta Smelt hybrid (data not shown).

Discussion

Genetic-based species identification methods are a tool for management agencies that can provide increased accuracy and confidence in assigning species IDs to specimens, especially when there is some ambiguity in morphology-based assignment. However, these genetic methods generally require that samples are sent to a specialized laboratory for processing, leading to a delay in obtaining data. Often the process also requires multiple subsequent steps (e.g., sample collection, DNA extraction, performing assays, analyzing data, and data transfer/reporting). When collecting data about the status, trends, abundance and distribution of listed species, acquiring the independent capability to rapidly obtain genetic ID results “in-house” (i.e., at the field monitoring office/station, even if a genetics lab is not co-located), would allow for rapid genetic species identification and increased confidence of key datasets for decision-making.

In this study we tested the implementation of SHERLOCK genetic identification to support the monitoring of the endangered Delta Smelt in the San Francisco Bay-Delta. The overarching goal was to increase the accuracy and efficiency of species identification for monitoring programs by leveraging a user-friendly and relatively low-cost genetic identification method, SHERLOCK. More accurate and timely information from monitoring should help management bodies/agencies make the proper decisions, for example supporting the water projects to better operate their pumping facilities to reduce the entrainment of the endangered Delta Smelt (Smith et al. 2021), as well as to evaluate the success of flow actions meant to increase their habitat (Sommer et al. 2020). Both Delta Smelt and Wakasagi exhibit a high degree of phenotypic plasticity, with intraspecific morphological differences even within California (Stagg et al. 2023). Over the past few years, hatchery-raised Delta Smelt have been released into the estuary to supplement the wild population (United States Fish and Wildlife Service 2020). Hatchery Delta Smelt display some key morphological differences to their wild counterparts (Jenkins et al. 2020), and it has been posited that the vast majority of Delta Smelt captured in the wild in recent years are of recent hatchery ancestry. Anecdotal evidence suggests that wild-born larval and early juvenile osmerids in recent years have become more difficult to identify down to species (USFWS staff, personal communications), highlighting the fairly urgent need to consider genetic methods for verifying species identification.

Our conclusions from this pilot implementation of SHERLOCK at USFWS-LFWO are that 1) SHERLOCK-based genetic ID can be successfully performed independently by a field office with minimal laboratory space; 2) SHERLOCK results from the field staff were in concordance with results ran in a specialized genetic laboratory; 3) the combined SHERLOCK results were validated by DNA sequencing; and 4) SHERLOCK in combination with another genetic assay (hybrid SNP panel) can help identify hybrid smelts. In our validation dataset, where we used frozen Delta Smelt and Wakasagi specimens caught in previous years, we found some evidence for partial cross-amplification of Delta Smelt swabs using the Wakasagi SHERLOCK assay. Since the pattern is unidirectional, accurate species identification is still possible, however future work should prioritize improving accuracy of the assay. Factors like cross-contamination of mucus swabs when multiple specimens are collected could also be another factor to consider (Tilley et al. 2024), as multiple species are often caught together during trawling and multiple specimens might be handled together during morphological identification. Field protocol adjustments might reduce potential cross contamination. In the current study, we utilized replicate sampling at two levels (multiple swabs per specimen and multiple SHERLOCK reactions per swab), in part to account for possible cross contamination. For all specimens tested with SHERLOCK, triplicate SHERLOCK reactions were performed. For a subset of specimens (including most of the 2023-2024 specimens tested by the field office), multiple swabs were collected from the same fish. Variability between replicates can be used to flag samples for additional follow-up testing (e.g., Sanger sequencing or hybrid SNP panel genotyping).

Our study provides a model for adoption of rapid, semi-portable genetic identification methods within institutions that do not have existing genetic laboratories on-site. These might include government agencies and also academic research groups not focusing on genetic work, non-governmental organizations (NGO), businesses (e.g., food industry), and others.

The SHERLOCK rapid approach has some limitations. The method cannot identify a completely unknown fish specimen, such as when a new invasive fish species is introduced into a system, unless there are assays designed for species known to be likely invaders. For these cases, traditional methods like DNA barcoding are more appropriate. The SHERLOCK approach is most suitable when there are a few, closely related species that need to be distinguished, and there is existing knowledge about genetic variants that can robustly and consistently be used. In the case of our study, the SHERLOCK assays for Delta Smelt and Wakasagi were designed based on existing knowledge of published and well-validated species-specific SNPs (Baerwald et al. 2011, 2020). For successful transfer of SHERLOCK to a non-genetics laboratory, several components are needed, including dedicated or temporary laboratory space (ideally where specimens are not usually handled, and/or that can be cleaned and decontaminated), a fluorescent reader machine like the Axxin T-16, and a -80°C freezer for pre-frozen master mix aliquots. In addition, development of a step-by-step protocol and training of personnel is needed, as well as technical support during pilot implementation, and a supply of reagents. For the latter, developing and validating lyophilized master mix aliquots that do not require freezer storage and have long shelf life could be implemented. Overall, utilizing rapid genetic identification at agency laboratories as demonstrated here will improve turnaround time for producing data critical to management decisions. The early adoption of SHERLOCK described here will hopefully accelerate progress towards making this technology accessible to all scientists, beyond specialized genetics laboratories.

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Tables

Table 3-1. SHERLOCK RPA Primers, crRNA, and gBlock Sequences

DSM RPA Forward primer	**TAATACGACTCACTATAG**GGAATGGCCAACCTTCGGAAA
DSM RPA Reverse primer	GARATATTRGAGGGTGCAGG
DSM crRNA	GGGGAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACACAACAGCGUCGUUGGUAAUUUUCAGGA
DSM gBlock	AAATCTAATACGACTCACTATAGGGAATTCAACTACAAGAACCCTAATGGCCAACCTTCGGAAAACCCATCCCCTCCTGAAAATTACCAACGACGCTCTTGTTGATCTGCCTGCACCCTCCAATATTTCTATCTGATGAAACTTTGGCT
WAG RPA Forward primer	**TAATACGACTCACTATAG**GGATTTACCTGCACCCTCCAATATTTC
WAG RPA Reverse primer	CAGTATAGTGCATAGCCAAAA
WAG crRNA	GGGGAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACCACACCCCAAGAAGGGAUCCAAAGUUUC
WAG gBlock	AATTCTAATACGACTCACTATAGGGCGGAAAACCCACCCCCTCCTAAAAATTACCAATGACGCCCTAGTTGATTTACCTGCACCCTCCAATATTTCAATCTGATGAAACTTTGGATCCCTTCTTGGGCTGTGTCTTATTATCCAAATCCTTACGGGCCTCTTTTTGGCTATGCACTATACTGCTGAGACTGCTACCGCTTTT

In the forward RPA primers, the T7 sequence is bolded. In the crRNA sequence, the protospacer sequence is underlined. DSM RPA primers, and DSM and WAG crRNAs, are identical to Baerwald et al. (2020). DSM, Delta Smelt. WAG, Wakasagi.

Table 3-2. SHERLOCK Assay Reaction Components, Per RPA Pellet

Reagent	Volume (µL)
UltraPure water	45.84
5X Buffer	21.71
RNase Inhibitor Murine	2.72
RNase H	2.17
T7 RNA polymerase	2.17
rNTPs	8.69
Species-specific primer mix	1.01
MgOAc	5.15
DTT	0.61
Species-specific crRNA	2.27
LwaCas13	0.4
PolyU Reporter	10.1

Table 3-3. PCR Primers Targeting Cytochrome B Gene for Sanger Sequencing

Primer name	Primers (M13 tails bolded)
L14724_DSM_M13F	CAGGAAACAGCTATGACGGACCAGTGACTTGAAAAACCACCGTTG
H15149_DSM_M13R	TGTAAAACGACGGCCAGTGCCCCCCAAAACGACATCTGTCCTCA

Table 3-4. Validation Wakasagi Swab Specimens

ID #	Survey	Collection date	Length, mm	SHERLOCK species ID
W-50	EDSM	8/27/2018	66	WAG
W-51	EDSM	8/27/2018	61	WAG
W-52	EDSM	9/27/2018	81	WAG
W-53	EDSM	10/9/2018	92	WAG
W-54	EDSM	10/10/2018	99	WAG
W-55	EDSM	11/23/2018	96	WAG
W-56	EDSM	11/23/2018	99	WAG
W-57	EDSM	12/17/2018	97	WAG
W-58	EDSM	7/1/2019	35	WAG
W-59	EDSM	7/9/2019	38	WAG
W-60	EDSM	7/22/2019	43	WAG
W-61	EDSM	7/30/2019	64	WAG
W-62	EDSM	8/6/2019	40	WAG
W-63	EDSM	10/16/2019	86	WAG
W-64	EDSM	11/6/2019	93	WAG
W-65	EDSM	11/21/2019	106	WAG
W-66	EDSM	12/10/2019	89	WAG
W-67	EDSM	2/13/2020	94	WAG
W-68	EDSM	5/20/2020	45	WAG
W-69	EDSM	7/8/2020	57	WAG
W-70	EDSM	7/30/2020	51	WAG
W-71	EDSM	8/13/2020	68	WAG
W-72	EDSM	10/22/2020	90	WAG

ID #	Survey	Collection date	Length, mm	SHERLOCK species ID
W-73	EDSM	11/25/2020	103	WAG
W-74	EDSM	12/14/2020	87	WAG
W-75	EDSM	7/13/2021	52	WAG
W-76	EDSM	7/13/2021	58	WAG
W-77	EDSM	7/13/2021	60	WAG
W-78	EDSM	7/22/2021	66	WAG
W-79	EDSM	9/28/2021	84	WAG
W-80	EDSM	10/5/2021	83	WAG
W-81	EDSM	10/15/2021	81	WAG
W-82	EDSM	10/21/2021	82	WAG
W-83	KDTR	2/15/2022	67	WAG
W-84	SEIN	3/29/2022	68	WAG
W-85	20mm	6/7/2022	48	WAG
W-86	EDSM	7/11/2022	40	WAG
W-87	EDSM	7/15/2022	60	WAG
W-88	EDSM	8/1/2022	74	WAG
W-89	EDSM	9/21/2022	70	WAG

Key: WAG = Wakasagi

Table 3-5. Validation Delta Smelt swab specimens

ID #	Survey	Collection date	SHERLOCK species ID	qPCR species ID	Sanger sequencing species ID
S17	EDSM	1/19/2023	DSM	DSM	nd
S402	FCCL	1/19/2023	DSM	DSM	DSM
S403	FCCL	2/2/2023	DSM	DSM	nd
S404	FCCL	2/2/2023	DSM	nd	nd
S181	SKT	2/9/2023	DSM/WAG	DSM	DSM
S182	SKT	2/9/2023	DSM/WAG	DSM	nd
S18	EDSM	2/9/2023	DSM	nd	nd
S19	EDSM	2/9/2023	DSM	nd	DSM
S209	EDSM	2/14/2023	DSM/WAG	DSM	nd
S210	EDSM	2/14/2023	DSM/WAG	DSM	DSM
S211	EDSM	2/14/2023	DSM	nd	nd
S212	EDSM	2/14/2023	DSM	nd	DSM
S213	EDSM	2/14/2023	DSM	nd	DSM
S214	EDSM	2/14/2023	DSM/WAG	DSM	DSM
S215	EDSM	2/14/2023	DSM/WAG	DSM	nd
S216	EDSM	2/14/2023	DSM/WAG	DSM	nd
S217	EDSM	2/14/2023	DSM	nd	DSM
S218	EDSM	2/14/2023	DSM	nd	nd
S219	EDSM	2/14/2023	DSM	nd	nd

Key: nd = no data; DSM = Delta Smelt; WAG = Wakasagi

Table 3-6. Summary of SHERLOCK and Sanger DNA Data for Six *Hypomesus* Specimens Collected by The field Office in Late 2023-Early 2024.

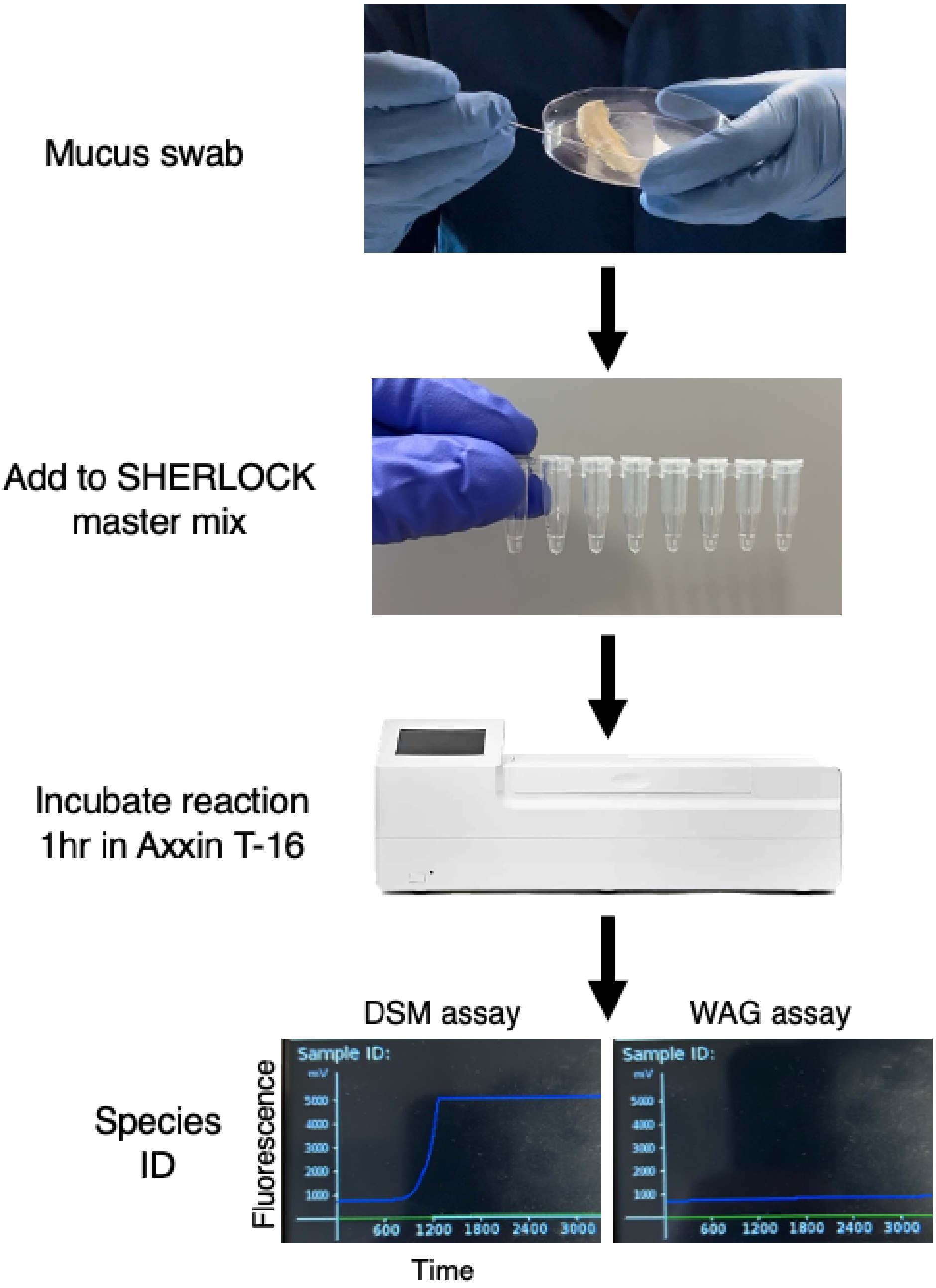
DSM ID #	Length (mm)	SHERLOCK DSM % Positive (USFWS)	SHERLOCK WAG % Positive (USFWS)	SHERLOCK DSM % Positive (GVL)	SHERLOCK WAG % Positive (WAG)	Sanger sequencing species ID	Sanger sequencing % match
S252	54	100%	0%	100%	0%	DSM	100%
S253	48	100%	22%	100%	0%	DSM	99.35%
S254	70	33%	100%	50%	100%	WAG	100%
S519	60	100%	0%	100%	0%	DSM	100%
S520	53	100%	0%	100%	17%	DSM	100%
S529	57	100%	0%	100%	0%	nd	N/A

% Positive is the percentage of all replicates tested (including all swabs and all SHERLOCK reaction replicates) that showed positive amplification above threshold in the assay indicated. Sequencing % match is percent identity to top BLASTn hit. nd: no data. N/A: not applicable. DSM, Delta Smelt. WAG, Wakasagi.

Table 3-7. DNA Sequences for Five Smelt Specimens Collected by USFWS-LFWO in Winter 2023-2024 Sampling Season

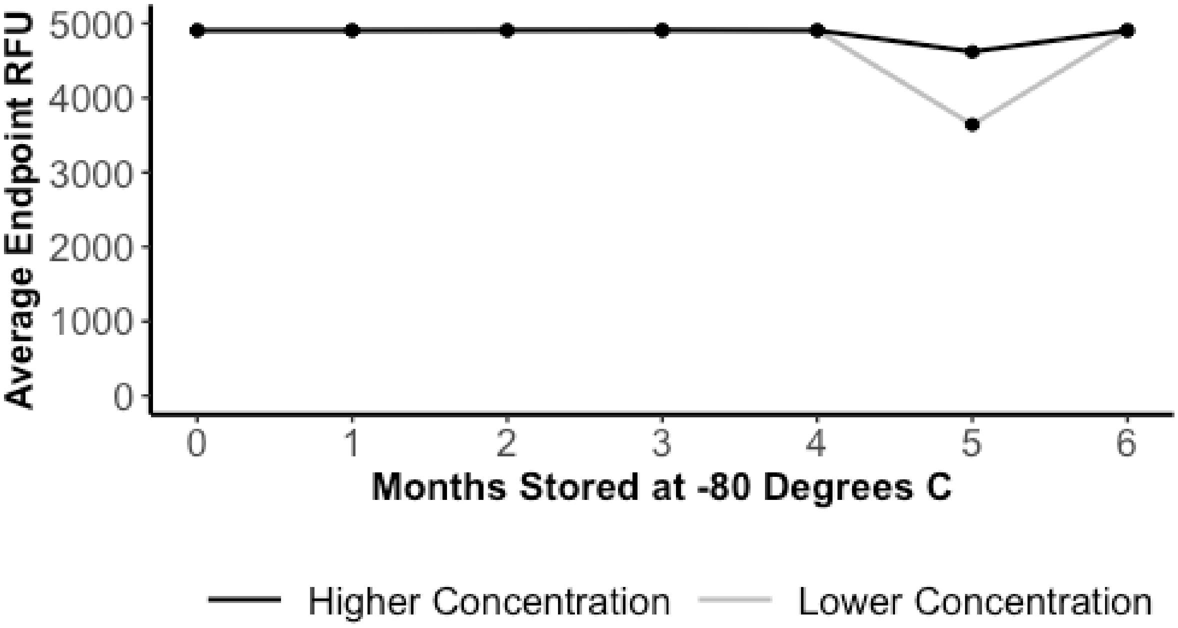
Specimen ID #	DNA sequence
S-252	TTAATTCAACTACAAGAACCCTAATGGCCAACCTTCGGAAAACCCATCCCCTCCTGAAAATTACCAACGACGCTCTTGTTGATCTGCCTGCACCCTCCAATATTTCTATCTGATGAAACTTTGGCTCCCTCCTTGGACTATGTCTTATTATTCAAATCCTCACAGGCCTATTCCTAGCCATGCACTACACTGCCGAGACTGCTACAGCATTTTCTTCTGTAGTACACTTATGCCGGGACGTTAATTACGGGTGACTAATCCGGAACATGCACGCTAACGGAGCATCTTTCTTCTTTATTTGTATTTATCTTCATATTGGCCGAGGTCTTTACTACGGCTCCTTCCTTTATAAGGAAACCTGAAACATCGGCGTAGTCCTTCTCCTTTTGGTTATAATGACTGCCTTTGTTGGCTATGTTCTTCCC
S-253	TTAATTCAACTACAAGAACCCTAATGGCCAACCTTCGGAAAACCCATCCCCTCCTGAAAATTACCAACGACGCTCTTGTTGATCTGCCTGCACCCTCCAATATTTCTATCTGATGAAACTTTGGCTCCCTCCTTGGACTATGTCTTATTATTCAGATCCTCACAGGCCTGTTCCTAGCCATGCACTACACTGCCGAGACTGCTACAGCATTTTCTTCTGTAGTACACTTATGCCGGGACGTTAATTACGGGTGACTAATCCGGAACATGCACGCTAACGGAGCATCTTTCTTCTTTATTTGTATTTATCTTCATATTGGCCGAGGCCTTTACTACGGCTCCTTCCTTTATAAGGAAACCTGAAACATCGGCGTAGTCCTTCTCCTTTTGGTTATAATGACTGCCTTTGTTGGCTATGTTCTTCCC
S-254	TCAATTCAACTACAAGAACCCTAATGGCCAACCTTCGGAAAACCCACCCCCTCCTAAAAATTACCAATGACGCCCTAGTTGATTTACCTGCACCCTCCAATATTTCAATCTGATGAAACTTTGGATCCCTTCTTGGGCTGTGTCTTATTATCCAAATCCTTACGGGCCTCTTTTTGGCTATGCACTATACTGCTGAGACTGCTACCGCTTTTTCCTCTGTTGTTCACCTCTGCCGAGACGTTAATTACGGCTGACTAATCCGTAACATGCACGCTAACGGAGCATCTTTCTTCTTTATTTGCATTTACCTTCATATTGGCCGAGGTCTTTATTACGGCTCATTCCTGTACAAGGAAACTTGAAACATCGGTGTGGTTCTACTACTTTTAGTCATAATGACCGCTTTTGTGGGCTATGTTCTTCCC
S-519	TTAATTCAACTACAAGAACCCTAATGGCCAACCTTCGGAAAACCCATCCCCTCCTGAAAATTACCAACGACGCTCTTGTTGATCTGCCTGCACCCTCCAATATTTCTATCTGATGAAACTTTGGCTCCCTCCTTGGACTATGTCTTATTATTCAAATCCTCACAGGCCTATTCCTAGCCATGCACTACACTGCCGAGACTGCTACAGCATTTTCTTCTGTAGTACACTTATGCCGGGACGTTAATTACGGGTGACTAATCCGGAACATGCACGCTAACGGAGCATCTTTCTTCTTTATTTGTATTTATCTTCATATTGGCCGAGGTCTTTACTACGGCTCCTTCCTTTATAAGGAAACCTGAAACATCGGCGTAGTCCTTCTCCTTTTGGTTATAATGACTGCCTTTGTTGGCTATGTTCTTCCC
S-520	TTAATTCAACTACAAGAACCCTAATGGCCAACCTTCGGAAAACCCATCCCCTCCTGAAAATTACCAACGACGCTCTTGTTGATCTGCCTGCACCCTCCAATATTTCTATCTGATGAAACTTTGGCTCCCTCCTTGGACTATGTCTTATTATTCAAATCCTCACAGGCCTATTCCTAGCCATGCACTACACTGCCGAGACTGCTACAGCATTTTCTTCTGTAGTACACTTATGCCGGGACGTTAATTACGGGTGACTAATCCGGAACATGCACGCTAACGGAGCATCTTTCTTCTTTATTTGTATTTATCTTCATATTGGCCGAGGTCTTTACTACGGCTCCTTCCTTTATAAGGAAACCTGAAACATCGGCGTAGTCCTTCTCCTTTTGGTTATAATGACTGCCTTTGTTGGCTATGTTCTTCCC

Figures



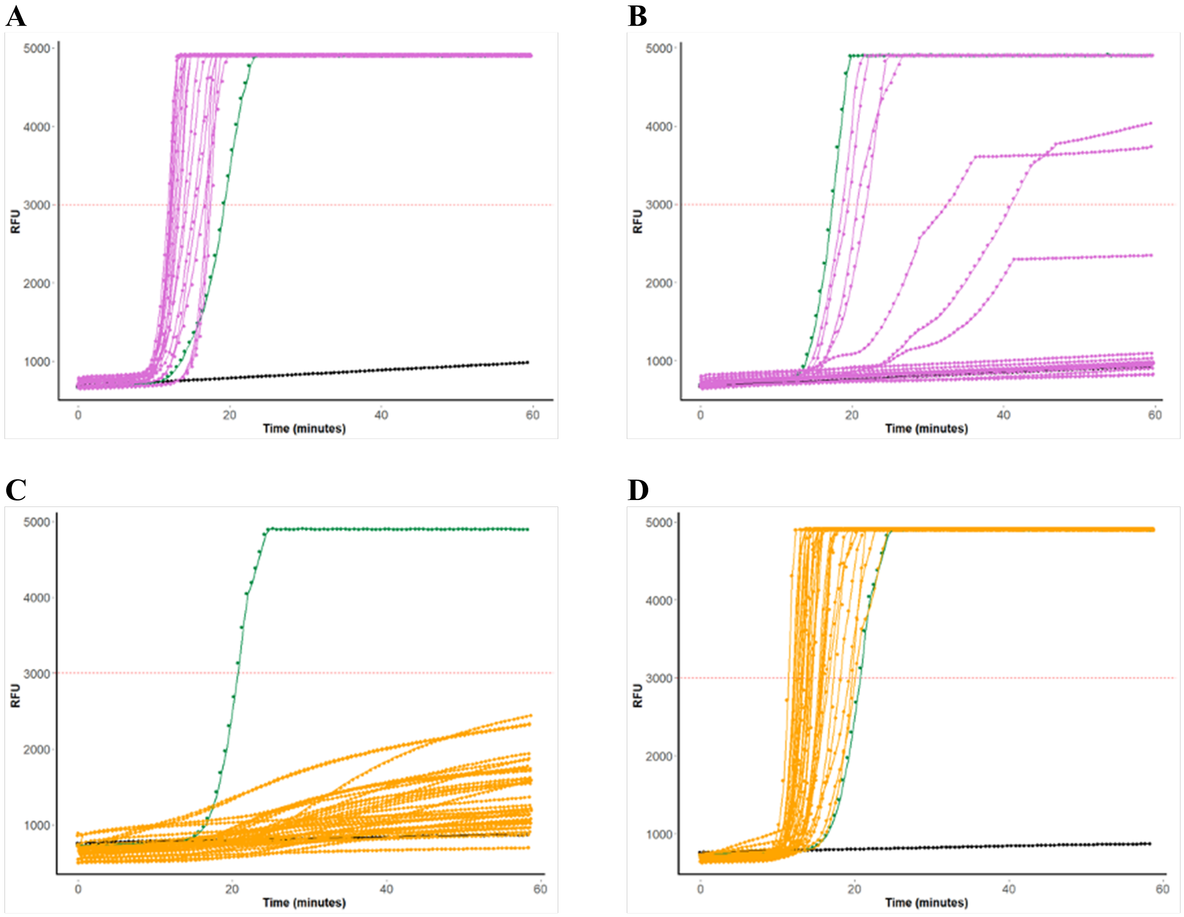
A custom workflow was designed for performing SHERLOCK genetic identification using mucus swabs without DNA extraction. First, minimally invasive swabbing is performed on specimens and transferred to PBS liquid buffer. Second, a small aliquot of the mucus liquid buffer is pipetted into pre-made, ready to use SHERLOCK reaction aliquots. Third, up to 16 reactions (including samples run in triplicate and positive and negative controls) are run for one hour on a portable fluorescence reader. Finally, data are visualized for both Delta Smelt (DSM) and Wakasagi (WAG) assays to determine maternal mitochondrial genetic ID for each specimen.

Figure 3-1. Workflow for Rapid and Minimally Invasive Species Identification Using SHERLOCK



Average endpoint relative fluorescence units (RFUs) for positive control DNAs, assayed every month with pre-frozen SHERLOCK Wakasagi assay aliquots, are plotted over a six-month period. Two positive control (gBlock) WAG DNA concentrations were tested: ~5,500 DNA copies ("Higher Concentration" – black line) and 550 DNA copies per reaction ("Lower Concentration" – gray line). Average RFU values for triplicate reactions are plotted.

Figure 3-2. Stability of Pre-Frozen SHERLOCK Reaction Mixes Over Time at -80℃



DSM and WAG SHERLOCK assays were tested for specificity using 19 swabbed Delta Smelt samples and 40 swabbed Wakasagi samples. **A,B** Amplification curves for 19 Delta Smelt swabs (purple), no template negative control (black), and positive control gBlock (green) in the DSM (**A**) and WAG (**B**) SHERLOCK assays. **C,D** Amplification curves for 40 Wakasagi swabs (orange) in the DSM (**C**) and WAG (**D**) SHERLOCK assays.

All curves represent averages of all replicate reactions for each sample or positive/negative control.

Figure 3-3. SHERLOCK Validation Testing of Wakasagi and Delta Smelt Frozen Specimens

Chapter 4. Otolith-Based Comparisons of Wild Versus Hatchery-Origin Delta Smelt

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Abstract

The rapid decline of California's endangered, endemic Delta Smelt (*Hypomesus transpacificus)* has called for immediate and effective conservation actions. Since 2021, up to 92,000 cultured Delta Smelt have been released annually into their natural habitats within the Sacramento-San Joaquin River Delta, with ecological surveys capturing both hatchery-produced ("cultured") and wild-spawned ("wild") fish throughout each year. Cultured fish have constituted the bulk of the catch in recent years, highlighting their critical role in conserving the species. Key questions remain, however, regarding biological differences between cultured and wild fish, their relative health, and how managers can identify cultured and wild individuals collected in the field. We applied otolith-based tools to contrast the health and environmental histories of wild and cultured Delta Smelt collected in situ from 2019-2023. Specifically, we quantified differences in otolith development, otolith-somatic proportionality, ontogenetic patterns in growth, and otolith geochemistry. We then developed and tested mixture discriminant analysis models for discriminating between cultured and wild Delta Smelt based on their geochemical profiles.

Cultured and wild fish exhibited similar otolith-somatic proportionality, whereas cultured fish exhibited higher frequencies of abnormal (vateritic) otolith development. Growth varied ontogenetically in both groups; however, growth rates were highest in wild fish during the early life history (e.g., from 10-50 days-post-hatch, days post hatch (dph), but higher in cultured fish during later life stages (e.g., > 100 dph). Geochemical profiles of $^{87}Sr/^{86}Sr$ were distinct among cultured and wild fish, resulting in > 95% classification success to each group. Together, these results highlight the utility of otolith-based approaches for identifying wild and cultured Delta Smelt, and for quantifying differences in their health and environmental history. Such data are key to understanding population dynamics and for informing the management and conservation of imperiled species.

Introduction

Delta Smelt Status & Supplementation

Fisheries management and conservation efforts often rely upon the ability to discriminate between wild-spawned ("wild") versus hatchery-produced ("cultured") individuals (Nielsen et al. 1997, Naish et al. 2007, Willmes et al. 2018a, 2021). This distinction is essential for evaluating the effectiveness of population augmentation programs designed to support commercial and recreational fisheries, such as Fall-run Chinook Salmon (*Oncorhynchus tshawytscha*). By understanding the origin of individual fish, managers can more effectively measure the impact of these programs on fish stocks and adjust strategies accordingly. Similarly, the ability to discriminate between wild and cultured fish is critical to supplementation programs designed to rebuild fish populations that are at imminent risk of extinction, such as the endangered Delta Smelt (*Hypomesus transpacificus*) (Lessard et al. 2018). Accurate discrimination between wild and cultured fish supports targeted supplementation efforts, allowing conservation programs to track survival rates, movement patterns, habitat use, and overall health of supplemented individuals. Such insights enable adaptive management practices that can improve the fitness of cultured fish and enhance the resilience of wild populations over time.

The Delta Smelt is a small, migratory estuarine fish that is endemic to the Sacramento-San Joaquin River Delta (Delta) of the San Francisco Estuary, California, United States (Moyle et al. 1992). It was once one of the most abundant pelagic fishes throughout the upper San Francisco Estuary, likely serving as an important forage fish in pelagic estuarine food webs (Moyle et al. 2016). Over the last several decades, however, its population has plunged to record-low abundance levels, leaving it critically endangered (NatureServe 2012) and highly vulnerable to extinction (Moyle et al. 2016, 2018, Hobbs et al. 2017). These fish are now rarely observed by numerous long-term monitoring programs (Tempel et al. 2021), with annual abundance indices commonly reported as 'zero' (Polansky et al. 2019), although occasional observations confirm that they are not yet extinct.

In response to this decline, several key management and conservation initiatives have been suggested and implemented by federal and state resource agencies, including wetland restoration (Hammock et al. 2019), improved management of freshwater outflows (Sommer et al. 2020), and hatchery-based conservation and supplementation programs (Lindberg et al. 2013, USFWS 2019). Beginning in 2021, approximately 45,000 to 95,000 adult cultured Delta Smelt have been experimentally released annually into their natural habitats within the Sacramento-San Joaquin River Delta, with all cultured fish being marked to facilitate the identification of hatchery-origin versus wild-spawned individuals (USFWS 2020). Both cultured and wild-spawned individuals have subsequently been captured, indicating some degree of success in forestalling extinction; however, many questions remain regarding the relative fitness of cultured Delta Smelt, and how to efficiently mark and distinguish large numbers of cultured individuals from wild fish as hatchery production and supplementation are expanded.

Otoliths in Fish and Fisheries

Otoliths ("ear stones") are calcium carbonate ($CaCO_3$) structures in fish that grow continuously throughout an individual's lifetime (Campana and Neilson 1985). These structures are critical to a fish's ability to sense orientation, acceleration, and sound; and they are valuable to fisheries scientists for inferring lifelong growth and movements of migratory species (Campana 1999, Thorrold & Campana 2001). For example, small repeating continuous and discontinuous zones (i.e., rings) often provide daily resolution of ages in young fish, whereas seasonal opaque and translucent banding patterns (.i.e., annuli) often provide annual aging resolution; both of which can be used to reconstruct the age and life-long growth history of individual fish (Pannella 1971). Furthermore, analyzing the chemical composition of otoliths across these banding patterns allows for the reconstruction of past environmental and migratory histories (Reis-Santos et al. 2008, Hobbs et al. 2019).

Otoliths as Natural Tags

Otoliths can also be used to differentiate between wild and cultured individuals based on variation in their shapes, accretion patterns, and chemistry (Gibson-Reinemer et al. 2009, Koeberle et al. 2020, Yoo et al. 2020, Willmes et al. 2021). In contrast to commonly used physical tagging methods such as adipose fin clipping and subcutaneous tag insertion, which are laborious and invasive, otoliths can provide a 'natural tag' to discriminate between wild and cultured fish, thus eliminating the time, costs, and increased mortality associated with individually marking tens of thousands of cultured fish that are used for population supplementation (Willmes et al. 2018a). Hence, the examination of Delta Smelt otoliths using increment and geochemical analyses could provide valuable tools for discriminating between wild and cultured fish collected in the field, and for quantifying differences in their growth and migratory life histories (Hobbs et al. 2019, Lewis et al. 2021).

Otolith Crystalline Structure

The crystalline structure of otoliths can also serve as an indicator of fish health. Sagittal otoliths of teleost fishes are composed primarily of the aragonite polymorph of $CaCO_3$. Several studies, however, have reported a significant increase in the vaterite polymorph of $CaCO_3$ in otoliths of cultured fish compared to wild-spawned counterparts (Reimer et al. 2016, Lewis et al. 2022). This increased frequency and prevalence of vaterite in cultured fish is manifested by the irregular shape, texture, and increased transparency of vateritic otoliths due to its effects on accretion and the transmission of light. The causes of these developmental abnormalities in cultured fish remain uncertain, but may be attributed to high stocking densities, temperature variation, diet composition, and growth—factors that often differ between hatchery and natural environments (Sweeting et al. 2004, Reimer et al. 2017). Such developmental abnormalities can negatively affect the functioning of a fish's inner ear, thus impacting its ability to hear, balance, and respond to its environment, and decreasing its fitness (Reimer et al. 2016, Austad et al. 2021). Furthermore, for fisheries scientists, these crystalline anomalies can alter otolith-based reconstructions of a fish's age, growth, and migratory life history based on increment and geochemical analyses (Lewis et al. 2022). In other applications, however, the unique appearance and chemical signatures of vateritic otoliths can also be used to help discriminate between wild and hatchery-origin fish (Melancon et al. 2005, Budnik et al. 2020).

Otolith-Somatic (O-S) Proportionality

Otolith size and shape can also vary ontogenetically and among populations. For example, different fish populations and stocks of a fish species may exhibit distinct otolith shapes, which can be useful for identifying an individual's origins (Begg & Brown 2000, Mérigot et al. 2007, Radhakrishnan et al. 2012) and distinguishing between wild and cultured individuals (Koeberle et al. 2020). Furthermore, constant otolith-to-somatic (o-s) proportionality across ontogenetic stages is a key assumption for back-calculation of prior growth rates from otolith increments using models such as the biological intercept model (BIM) (Campana 1990, Hobbs et al. 2007). However, individuals within a species that experience distinct environmental and feeding conditions could exhibit variable o-s relationships (Stormer & Juanes 2016). Thus, comparisons of o-s proportionality between cultured and wild populations can be valuable for developing tools to further facilitate group identification and for evaluating comparability in reconstructed growth estimates from otolith increment analysis.

Approach & objective

Here, we quantified differences in the otolith size, structure, accretion rate, and chemistry between cultured and wild Delta Smelt that were collected from the upper San Francisco Estuary, California, USA by state and federal agencies. Otolith increment profiles were quantified to examine differences in stage-specific growth rates, and crystalline structure was quantified to assess differences in vestibular development. Variation in otolith-somatic proportionality was assessed to quantify differences in otolith shape and assess comparability of otolith-based growth estimates. Last, strontium isotope (^{87}Sr/^{86}Sr) profiles were quantified to contrast differences in geochemical profiles that could be used to identify cultured and wild fish and to reconstruct movements of cultured fish post-release into the wild. In aggregate, we present new data on the relative health and fitness of cultured versus wild Delta Smelt and provide new tools that could be used to help identify the origins of wild-caught specimens. Such results are valuable for informing culture and supplementation programs and maximizing their effectiveness in supporting fisheries and conservation.

Methods

Fish Collection and Archival

Wild and cultured Delta Smelt were collected from the upper San Francisco Estuary and archived by a variety of collaborating federal and state agencies including the US Fish and Wildlife Service (USFWS) Enhanced Delta Smelt Monitoring (EDSM) and Chipp's Island Survey (CHIP) programs, the California Department of Fish and Wildlife (CDFW) Spring Kodiak Trawl (SKT) survey, the US Bureau of Reclamation's (Reclamation) Central Valley Project (CVP) salvage facility, and the California Department of Water Resources (DWR) State Water Project (SWP) salvage facility (Table S1). EDSM and SKT surveys both use a Kodiak Trawl net, CHIPP uses a midwater trawl net, and the CVP and SWP use screens to salvage fish that have become entrained in their pumping facilities. Fish were identified to the species level by the respective program, stored in liquid nitrogen, and transported to UC Davis for dissection and archival of multiple tissues, including otoliths.

All hatchery-origin fish that were released into the environment were produced at the UC Davis Fish Conservation and Culture Laboratory (FCCL) and marked in one of two ways (100% proportional tagging), either by removal ("clipping") of the adipose fin and/or subcutaneous injection of colored visual identification elastomer (VIE) tags. Thus, fish lacking an adipose fin ("ad-clipped") and/or containing a VIE tag were identified as "cultured." In contrast, fish with both an intact adipose fin and lacking a VIE tag were identified as "wild." A total of 279 Delta Smelt collected from 2019-2023 were available for the study, including 163 wild and 116 cultured fish. Most (151) wild Delta Smelt were collected in 2019-2021, prior to supplementation; whereas all cultured fish were collected in 2022-2023, after supplementation had commenced (Table 4-1). Sample sizes differed among analyses given the availability and quality of otoliths and associated metadata for each fish (Table S2).

Otolith Crystalline Structure

The crystalline structure of each Delta Smelt otolith was visually assessed following Lewis et al. (2021) to quantify variation between cultured and wild fish in healthy otolith development. Whole, intact sagittal otoliths from 162 wild and 116 cultured individuals were extracted, digitally imaged, and visually scored from I to IV, with I containing ≤ 1% vaterite, II containing 1% to ≤ 15%

vaterite, III containing 15% to ≤ 30% vaterite, and IV containing > 30% vaterite (Lewis et al. 2022). Each fish was assigned a vaterite score based on the highest category of the left or right otolith (maximum vaterite content). Logistic regression was used to quantify the probability of a fish containing relatively "high" (V_H, category III-IV) versus low (V_L, I-II) vaterite content as the interactive effects of fish origin (cultured, wild) and fork length (FL) (Equation 1).

Equation 1: $\mathrm{glm}(V_H(1,0) \sim \mathrm{Origin}*\mathrm{FL}, \mathrm{family} = \mathrm{binomial}(\mathrm{link}= \text{"logit"}), \mathrm{data})$

Otolith-Somatic Proportionality

Otolith size and fish size were measured and contrasted to verify consistency and comparability of otolith-based reconstructions of early growth histories for wild and cultured Delta Smelt. A total of 15 cultured and 148 wild individuals were selected to examine variation in otolith-somatic (o-s) proportionality. Sample sizes reflected specimens within the size range at which otolith increment analysis has been previously validated (< 60 millimeters (mm), Xieu et al. 2021) and for which intact otoliths and metadata were available (Table S2). Only 15 cultured fish met these criteria due to their typical release at much later life stages. Nevertheless, this comparison allowed us to assess whether o-s proportionality appeared similar between each group, specifically during the early life history (e.g., < 250 days-post-hatch, dph) where increment comparisons are most valuable.

Otolith size was quantified as the maximum dorsal-ventral diameter (d-v, in µm) using digital image analysis in the ImageJ software package (Abramoff et al. 2006, Xieu et al. 2021). Otolith-somatic proportionality was examined by modeling otolith size as a function of the interactive effects of fish size and origin (heterogeneous slopes), while assuming a fixed intercept of 8.6 micrometers (µm) and 5.8 mm, corresponding with the mean otolith size and fish length at hatch, respectively. The fixed intercept was used to constrain the slopes to more biologically meaningful and comparable values given the limited size ranges of individuals in the model. Prior to analysis, adjusted otolith size (O_a) and fork length (L_a) were calculated by subtracting the mean values at hatch, with O_a modeled as the interactive effect of L_a and fish origin, while assuming a fixed y-intercept of 0 (Equation 2). The difference in slopes between cultured and wild fish was examined using the *emtrends* function in the *emmeans* package in R (Lenth 2024).

Equation 2: $\mathrm{lm}(O_a \sim L_a{:}\mathrm{Origin} - 1, \mathrm{data})$

Otolith Age and Growth

Otoliths from 112 cultured and 162 wild Delta Smelt were polished, imaged, and daily increments quantified following Xieu et al. (2021). Sample sizes reflected fish for which high-quality images of otolith thin sections (revealing the daily increments from the core to edge) were available (Table S2). Daily increment profiles were used to estimate the age (number of increments) and relative daily growth histories (increment widths) of each fish. During older life stages, daily increments become unreliable due to compaction during annulus formation (Xieu et al. 2021); thus, daily age and growth profiles were reconstructed up to the first 250 dph. A generalized additive model (GAM, k =8) was used to model otolith growth rates (G_o) as the interactive smooth effects of ontogeny (daily age) and origin using the "mgcv" package in R (Pedersen et al. 2019) (Equation 3). The factor smooth (fs)

GAM was preferred because it allowed us to model different complex growth patterns (smooths) for each group while assuming a shared smoothing parameter between groups (Pedersen et al. 2019).

Equation 3: gam(G_o ~ s(age*origin, bs = "fs"), data)

Otolith Geochemistry

Otolith $^{87}Sr/^{86}Sr$ core-to-edge geochemical profiles were collected for 113 cultured and 160 wild Delta Smelt. Sample sizes reflected fish for which suitable, intact otolith thin sections were available for mounting on petrographic slides for geochemical analysis (Table S2). Strontium isotope ratios ($^{87}Sr/^{86}Sr$) were quantified continuously from core to edge of each otolith using Laser Ablation Multi-Collector Inductively Coupled Plasma Mass Spectrometry (LA-MC-ICPMS) at the UC Davis Interdisciplinary Center for Plasma Mass Spectrometry. Laser and ICPMS settings followed those previously used to examine migratory patterns in Delta Smelt (Hobbs et al. 2019), including a square spot size 40 µm (length and width), scan speed of 5 µm/s, and frequency of 10 Hz. ICPMS data were reduced using the IsoFishR package in R (Willmes et al. 2018b).

Geochemical data were modeled with mixture discriminant analysis (MDA) in the "mda" package in R (Hastie & Tibshirani 2023). MDA is a discriminant analysis function that can be used to distinguish between groups that exhibit multiple discontinuous multivariate clusters, such as wild Delta Smelt that exhibit both migrant and resident phenotypes (Hobbs et al. 2019) that are geochemically distinct from each other and possibly from cultured individuals. The MDA model predicted the origin (cultured or wild) of each fish based on the full profile of each of its $^{87}Sr/^{86}Sr$ values (Sr_n) (Equation 4). Two separate MDAs were run. The first was based on the first 100 µm (n = 100) of each $^{87}Sr/^{86}Sr$ profile (MDA_{100}), focusing on the early life history and including all individuals with chemistry data. The second was based on the first 400 µm (n = 400) of each $^{87}Sr/^{86}Sr$ profile (MDA_{400}), which omitted 16 smaller-sized fish but included more complex migratory dispersal patterns (Table S2). The performance of each model was evaluated using 10-fold stratified cross validation with the numbers and proportions of correct and incorrect classifications reported in a confusion matrix.

Equation 4: mda(Origin ~ $Sr_1 + Sr_2 + \ldots Sr_n$, data)

Results

Otolith Crystalline Structure

Overall, vaterite content of Delta Smelt otoliths ranged from 0% to nearly 100%, with the majority (81.7%) of the fish examined in this study exhibiting less than 1% vaterite (Category I) in both otoliths (Table 4-2, Figure 4-1). However, 18.3% of Delta Smelt exhibited notable quantities of vaterite in at least one otolith, including 7.2%, 3.6%, and 7.6% of fish assigned to Vaterite Categories II, III, and IV, respectively (Table 4-2, Figure 4-1). Vaterite content was asymmetrical among otoliths, with approximately 6% of fish exhibiting vaterite in both otoliths.

Vaterite prevalence differed significantly between cultured and wild fish ($\chi^2_3 = 55.7$, $p < 0.01$), with 3.7% of wild Delta Smelt exhibiting detectable vaterite, whereas 38.8% of cultured Delta Smelt exhibited at least one vateritic otolith (Table 4-2, Figure 4-1). Similarly, only 1.9% of wild fish exhibited relatively high quantities of vaterite (Categories III-IV), whereas 24.1% of cultured individuals contained at least one highly vateritic otolith (Figure 4-1). The probability of a fish exhibiting relative high proportions of vaterite (Categories III-IV) varied significantly as a function of origin and the interaction between origin and fish length (Table 4-3); however, no overall effect of length was observed nor was there a significant interaction between origin and length. Thus, overall, being of hatchery-origin appeared to be the best predictor of a fish exhibiting abnormal otolith development.

Otolith-Somatic Proportionality

Otolith size varied significantly and linearly in relation to fish size, with no measurable difference in slopes between cultured (slope = 25.97 ± 0.52) and wild (slope = 26.47 ± 0.20) individuals (Table 4-4, Figure 4-2). Overall, fish size explained 70% of the variance in otolith size, with results generally matching those observed by (Xieu et al. 2021). For individuals larger than 60 mm, some evidence of non-linearity was observed; however, due to annulus formation, this age group lies at sizes beyond that for which increment analysis is used to estimate growth rates in Delta Smelt (Xieu et al. 2021). Thus, within the relevant size range, o-s proportionality appeared similar between cultured and wild Delta Smelt, confirming the comparability of otolith-based growth estimates between the two groups.

Age and Growth

Otolith-based age estimates indicated that the mean age of wild Delta Smelt in our collection was 125 dph, substantially younger than the age at which cultured fish were released into the environment (approximately 270-320 dph). Total ages of cultured fish could not be estimated from otoliths due to ring compaction during annulus formation. Nevertheless, otolith-increment analysis allowed for the comparison of relative daily growth rates between cultured and wild Delta Smelt during the early life history, here defined as up to 250 dph or approximately 55 mm FL (Xieu et al. 2021).

In general, Delta Smelt exhibit a pronounced ontogenetic trend in growth rate, characterized by a peak in growth at around 50 days, followed by a subsequent decline in growth as fish continue to mature (Xieu et al. 2021). Although wild and cultured Delta Smelt in this study exhibited a similar general growth pattern, key differences in otolith growth profiles were observed between the two groups (Table 4-5, Figure 4-3). For example, growth was more variable in wild fish, with mean increment widths ranging from 2-7 μm/d versus 2-4 μm/d in cultured fish. A generalized additive model indicated that the additive and interactive effects of age and origin explained 78.1% of the variation in growth rate when including a significant age-origin interaction term (Table 4-5). This age-by-origin interaction was due to an inversion of the relative growth rates between wild and cultured fish as fish matured beyond approximately 100 dph. For example, mean growth rates during the early life history were up to 75% greater in wild versus cultured Delta Smelt, whereas growth rates were approximately 50% greater in cultured versus wild individuals during later life stages (Figure 4-3).

Otolith Geochemistry

Cultured and wild Delta Smelt exhibited distinct otolith geochemical profiles (Figure 4-4a). Cultured fish exhibited relatively stable $^{87}Sr/^{86}Sr$ values of approximately 0.7069 to 0.7072 throughout their lives, reflecting the stable environment provided by the culture facility. In contrast, most wild Delta Smelt exhibited lower $^{87}Sr/^{86}Sr$ values of approximately 0.7062 to 0.7063 in the early life history, reflecting the lower Sacramento River region of their Delta habitat (Hobbs et al. 2019). Younger wild fish remained in freshwater throughout most of their lives, while a fraction of older individuals exhibited dispersal to low-salinity brackish habitats, with $^{87}Sr/^{86}Sr$ values increasing ontogenetically from approximately 0.7063 to values ranging from 0.7080 to 0.7085 (approximately 1-2 parts per thousand (ppt) salinity) (Figure 4-4a).

These differences in otolith geochemical profiles facilitated the accurate classification of Delta Smelt as being of wild or hatchery origins (Table 4-6, Figure 4-4b,c). For example, MDA analysis and 10-fold cross-validation using the first 100 μm of each $^{87}Sr/^{86}Sr$ profile (Figure 4-4b), focusing only on the early life history, resulted in 96.7% classification success, with only 3/113 cultured fish misclassified as wild, and 6/160 wild fish misclassified as cultured. The same analysis applied to the first 400 μm of each $^{87}Sr/^{86}Sr$ profile (Figure 4-4c), including more complex dispersal patterns during older life stages (precluding some smaller individuals), resulted in 95.3% classification success, with 0/113 cultured fish misclassified as wild, and 12/144 wild fish misclassified as cultured.

Discussion

Summary of main findings

The supplementation of wild fish populations with hatchery-produced individuals has long been a key tool in fisheries management and conservation. Here, we contrasted several otolith-based metrics between wild and cultured Delta Smelt, providing valuable insights regarding the health and success of cultured individuals released into the wild. Specifically, cultured fish exhibited similar o-s proportionality, a higher frequency and intensity of otolith abnormalities, slower larval growth rates, and enhanced juvenile growth rates. Additionally, otolith geochemistry was highly effective for identifying fish collected from the field as hatchery- or wild-origin, achieving greater than 95% classification success. These otolith-based approaches offer a powerful toolkit for contrasting differences in development, growth, and environmental histories, and for identifying cultured Delta Smelt collected by field surveys. Such data are needed to support key decisions regarding management of the wild Delta Smelt population and operation of the culture facility to support population supplementation. More broadly, our results demonstrate how otolith-based tools can inform managers regarding the status of wild fish populations, the benefits of supplementation, and the relative fitness of cultured and wild members of managed fish populations.

Otolith Crystalline Structure

Vaterite formation in otoliths, while rare in wild fish, occurs at significantly higher rates in hatchery-reared populations, potentially impairing key survival functions such as balance, hearing, and predator avoidance (Oxman et al. 2007, Reimer et al. 2016, Vignon & Aymes 2020). We found that vaterite deposition in otoliths of cultured Delta Smelt was significantly higher than in the wild population. For example, otoliths of only 3.7% of wild Delta Smelt contained some vaterite and just 1.9% of wild fish exhibited otoliths with relatively high amounts of vaterite. In contrast, 38.8% and 24.1% of cultured fish exhibited observable and high levels of vaterite, respectively. Vaterite prevalence in cultured Delta Smelt was similar to that observed in studies of other cultured fish populations (Reimer et al. 2016, 2017, Austad et al. 2021, Lewis et al. 2022). Together, these results indicate that hatchery conditions may induce vaterite formation in cultured fish.

Hatchery conditions, including high stocking density and *ad libitum* feeding, are proposed mechanisms for increased vaterite formation in cultured fish populations (Reimer et al. 2017), although the exact mechanisms remain inconclusive (Chalan et al. 2022). Delta Smelt are fed *ad libitum* to maximize growth rates and are reared at relatively high densities (Lindberg et al. 2013), thus our observed elevated growth rates in hatchery (versus wild) fish during later stages of development (e.g., > 100 dph), when hatchery-based growth and stocking conditions possibly contribute to vaterite formation, may further support these hatchery conditions as mechanisms for vaterite formation. Future studies should investigate whether variation in stocking and feeding regimes in the hatchery might mitigate vaterite formation in cultured Delta Smelt. Furthermore, assessing how vaterite in Delta Smelt otoliths impacts swimming performance, predator avoidance, and survival is warranted.

The low incidence of vaterite in wild Delta Smelt could be due to several possible mechanisms. While it is possible that vaterite formation is expressed less in wild Delta Smelt relative to cultured Delta Smelt due to reasons stated above, it is also possible that wild Delta Smelt express vaterite at similar rates as cultured individuals, but that wild individuals with vateritic otoliths are more quickly selected out of the wild population (e.g., by predation or poor foraging success). To assess the causes and consequences of vaterite formation, future studies need to quantify relative rates of vaterite expression between cultured and wild Delta Smelt, and to experimentally test the effects of vaterite on fitness and survival, both in the laboratory and the wild.

Otolith-Somatic Proportionality

The quantification and comparison of o-s size relationships is key to the accurate application and interpretation of otolith-based growth reconstructions in fishes (Campana 1990). This is because the use of otolith increment widths is dependent upon a common and stable relationship between otolith accretion rates and somatic growth rates. In Delta Smelt, we found that otolith-somatic proportionality was ontogenetically stable and similar between cultured and wild Delta Smelt. Thus hatchery conditions, including water temperature, salinity, and food ration, do not appear to affect the relative rates of somatic growth and otolith accretion in Delta Smelt. Given that hatchery effects have been observed in other species (Mugiya and Tanaka 1992, Stormer & Juanes 2016), this validation step is key to the application of otoliths in contrasting cultured and wild populations.

The relatively small sample size of cultured fish within the appropriate size class (< 60 mm) for otolith growth reconstructions likely reduced our power to detect a significant difference between the two groups. Optimally, more cultured fish within the appropriate size range would be preferable, but were not available given the supplementation strategy. However, the application of the fixed intercept (based on Xieu et al, 2021) allowed us to constrain the slopes such that they were biologically meaningful, and this improved our ability to provide a reliable comparison of slopes between the two groups of Delta Smelt. Given this design, the o-s proportionality relationship for cultured fish in this study was nearly identical to that of the wild fish, indicating that increment analysis is likely to provide comparable estimates of relative growth rates between the two groups.

Otolith Age & Growth

Cultured and wild Delta Smelt exhibited distinct ontogenetic patterns in growth, with cultured fish growing more slowly during the early life history, but faster during later life stages, relative to wild individuals. This interactive effect of age and origin on the growth of wild and hatchery Delta Smelt could be attributed to several plausible mechanisms. During early life stages, wild Delta Smelt may benefit from favorable conditions that facilitate enhanced foraging and growth relative to fish reared in a hatchery setting. For example, water temperatures in their natural habitats are cool and favorable during the winter-spring and likely contain a higher diversity of available smaller-sized natural prey (i.e., microplankton) of larval Delta Smelt (Nobriga 2002) than tanks within the culture facility, which receive commercially available rotifers and *Artemia sp.* nauplii. Other hatchery-specific conditions could also reduce the growth of cultured fish including stocking density, salinity, water quality, turbidity, and handling effects. Alternatively, selective pressures in the wild (e.g., intra- and interspecific competition for food, and size-selective predation) are often strongest during the early life history and likely favor wild larvae that exhibit the fastest growth rates (Houde 2008), whereas such selection pressures are likely reduced under culture conditions.

As Delta Smelt mature in the wild, they encounter increasingly harsh environmental conditions in the late summer and early fall, including increasing water temperatures, reduced water quality, and reduced prey availability. For example, water temperatures for subadult Delta Smelt regularly reach unsuitable levels (e.g., 23 degrees Celsius (°C) to 25°C) (Nobriga et al. 2008, Bashevkin et al. 2022, Mahardja et al. 2022) which have been shown to lead to metabolic suppression, reduced body condition, and depressed foraging and growth, both in the laboratory (Komoroske et al. 2014, 2015) and in the wild (Hammock et al. 2015, 2017, Lewis et al. 2021). Similarly, the Delta ecosystem has transitioned into a food limited system, particularly for pelagic organisms such as Delta Smelt during late-summer and early fall, resulting in a demographic bottleneck for Delta Smelt (Sommer et al. 2007, Hammock et al. 2015, 2017, 2022). Thus, during later life stages, the stable, cool, and food-rich conditions provided in the culture facility may result in enhanced growth of cultured Delta Smelt relative to wild individuals that are exposed to environmental stress during inhospitable summer-fall conditions.

Overall, these results indicate that culture conditions appear less favorable during early (larval to post-larval) life stages and more favorable during later life stages (juvenile to sub-adult). Thus, the culture facility appears to provide juvenile and sub-adult Delta Smelt a refuge from stressful *in situ* conditions in the late-summer and fall which might otherwise lead to high mortality and extinction. Furthermore, these results suggest that culture conditions for larval Delta Smelt, the stages at which growth and mortality have the largest impact on population dynamics and hatchery production, could be improved to further enhance growth and production of Delta Smelt at the conservation hatchery.

Otolith Chemistry Classification & Clustering

Geochemical profiles allowed us to classify Delta Smelt as wild or cultured with greater than 95% accuracy. Thus, otolith geochemistry can serve as an effective tool for informing population assessments and the effectiveness of supplementation. Although 100% of cultured fish are physically tagged (e.g., adipose clipping and VIE tagging), some amount of tag loss or incomplete fin clipping can result in misidentification of fish origins based on visual tagging methods. Otolith geochemistry offers a reliable secondary method for verifying the origin of fish captured in monitoring surveys. Furthermore, as hatchery production increases beyond the culture facility's ability to achieve 100% physical tagging, the application of otolith geochemistry for reconstructing the origins of field-caught fish is likely to become increasingly valuable.

Studies of other fish populations have also demonstrated relatively high (e.g., 80-100%) classification success using geochemical approaches (Barnett-Johnson et al. 2008, Zitek et al. 2010, Marklevitz et al. 2011); however, geochemical approaches must first be validated to ensure proper interpretation (Wolff et al. 2013). The application of geochemical tools to physically tagged (known-cultured) and untagged (known-wild) Delta Smelt collected from the field provided a unique opportunity to develop and validate MDA models using otolith geochemistry as a tool for identifying the origins of Delta Smelt. MDA analysis based on the first 100 μm or 400 μm of each geochemical profile achieved similar classification success (97% and 95%, respectively), with a slight improvement using the shorter profile segment. This suggests that the complex migratory patterns observed in the geochemical profiles of older migratory individuals (Hobbs et al. 2019) could potentially complicate classifications, but any such effect appeared fairly negligible, with both approaches achieving high classification success.

Conclusions

The validation (Hobbs et al. 2007, Lewis et al. 2021) and application (Hobbs et al. 2019, Lewis et al. 2021, 2022) of otolith-based tools have greatly advanced our understanding of the biology, life-history, and environmental needs of Delta Smelt. Given that population supplementation with cultured fish is a key conservation strategy that is likely to expand into the future, the calibration and application of an otolith-based toolkit to evaluate and inform culture practices, release methods, and the status of the wild population have become paramount. Here, we show that abnormal vateritic otolith development is elevated in cultured individuals, that ontogenetic patterns in growth are distinct between wild and cultured individuals, and that geochemical environmental reconstructions using otolith ^{87}Sr/^{86}Sr analysis can be used to accurately discriminate between wild and cultured individuals collected from the field. In aggregate, these otolith-based approaches allow us to reconstruct multiple valuable demographic metrics for wild and cultured Delta Smelt, including their health, origins, and movements; all of which will remain key to informing culture practices, supplementation strategies, and assessing the status of the remaining wild Delta Smelt population.

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Tables

Table 4-1. Total Number of Archived Delta Smelt Available for the Study by Year and Known Origin, Assuming Complete (100%) Fractional Tagging (fin clip and/or elastomer) of Cultured Individuals That Were Released Into the Wild

Year	Wild	Cultured	Total
2019	126	0	126
2020	24	0	24
2021	1	0	1
2022	9	75	84
2023	3	41	44
Total	**163**	**116**	**279**

Only a single wild-origin fish was collected in 2021, prior to supplementation.

Table 4-2. Total Number of Delta Smelt Assigned to Each Vaterite Category by Year and Origin

Year	Hatchery				Wild				Total
	I	II	III	IV	I	II	III	IV	
2019	-	-	-	-	121	2	1	1	125
2020	-	-	-	-	22	1	0	1	24
2021	-	-	-	-	1	0	0	0	1
2022	45	15	5	10	9	0	0	0	84
2023	26	2	4	9	3	0	0	0	44
Total	**71**	**17**	**9**	**19**	**156**	**3**	**1**	**2**	**278**

No cultured fish were collected in years prior to hatchery releases (2019-2021, dashes).

Table 4-3. Results of a Logistic Regression Model Examining the Probability of a Delta Smelt Exhibiting Relatively High Vaterite (category II-IV) as the Interactive Effects of Size (fork length) and Origin

Parameter	Estimate	SE	Z	P	Model P
Intercept	-0.34	1.7	-0.2	0.84	**<0.001**
Origin:Wild	-8.43	3.66	-2.31	**0.02**	
Fork length	-0.01	0.02	-0.47	0.64	
Origin:Wild*Fork Length	0.11	0.06	1.68	0.09	

Significant (> 0.05) P-values are in bold.

Table 4-4. Results of a Linear Model Examining Otolith-Somatic Proportionality of Hatchery and Wild Delta Smelt

Paremeter	Slope	SE	df	t	Model P	R^2
$\mathrm{FL}_{\mathrm{cultured}}$	25.97	0.52	14	50.16	**<0.001**	0.70
$\mathrm{FL}_{\mathrm{wild}}$	26.47	0.20	147	134.86	**<0.001**	
Difference	**-0.49**	**0.55**	**161**	**-0.89**	**0.374**	-

Parameter estimates (slopes) for each group and their difference are shown. Model outputs include standard error (SE), t-value (t), p-value (p), model coefficient of determination (R^2). Significant (> 0.05) P-values are in bold.

Table 4-5. Results of the Generalized Additive Model (GAM) Examining Otolith Growth as the Interactive Effects of Fish Age and Origin

Parameters and Statistics					R^2	P
Parametric	intercept	se	t	p	78.1	**<0.001**
	3.50	0.62	5.68	**<0.001**		
Smooth s(age, origin)	edf	rdf	f	p		
	14.93	15	11320	**<0.001**		

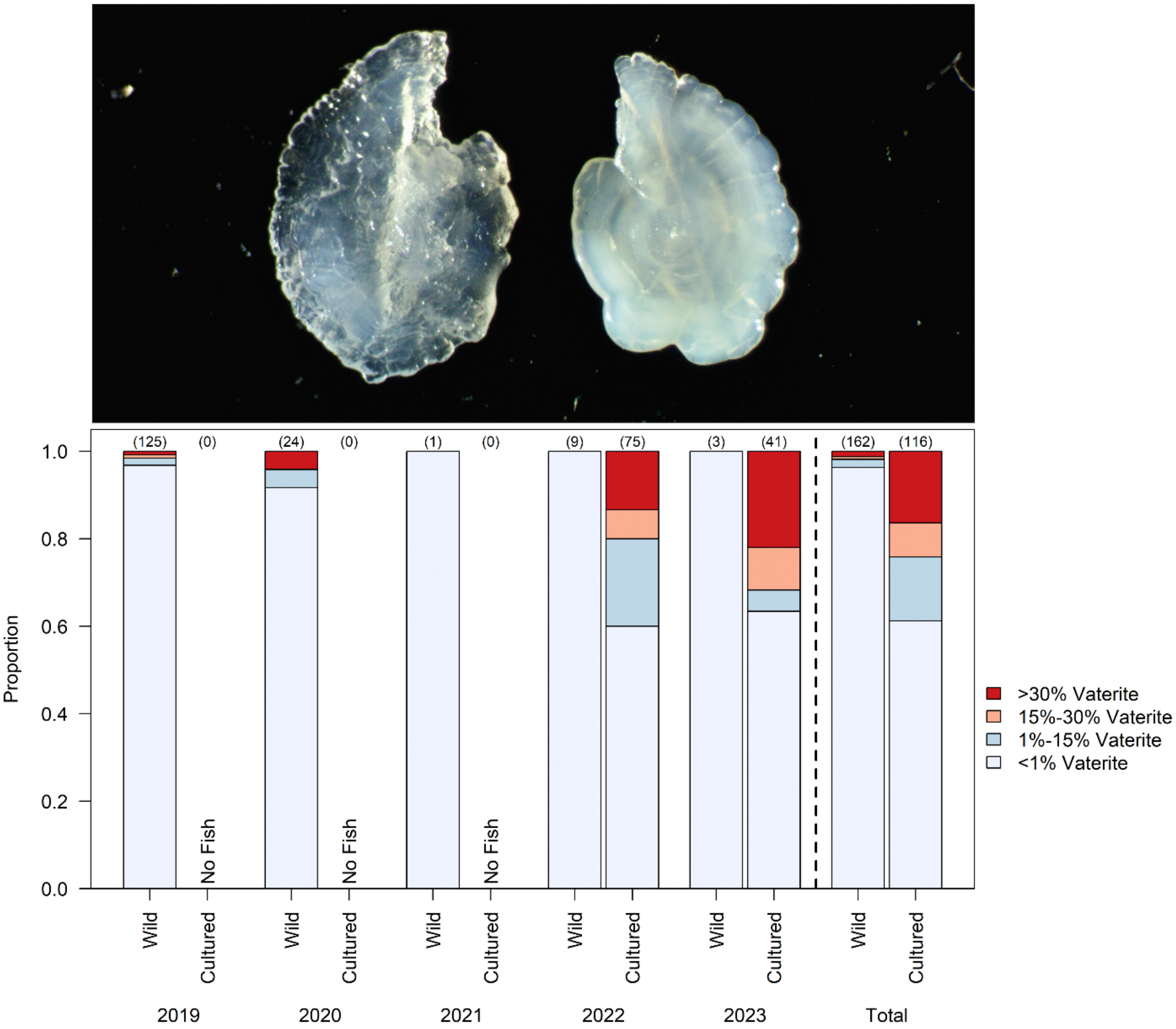
The intercept and its associated standard error (SE), t-value (t), p-value (p) are provided. The estimated degrees of freedom (edf), residual degrees of freedom (rdf), f-value (f), and p-value (p) are also shown for the smooth function s(age, origin), along with the coefficient of determination (R^2) and p-value (P) for the full model. Significant (> 0.05) p-values are in bold.

Table 4-6. Confusion Matrix for MDA Models Predicting Delta Smelt Origins Based on the First 100 µm or 400 µm of Each Fish's Otolith $^{87}\mathrm{Sr}/^{86}\mathrm{Sr}$ Profile

Origin		Predicted			
		100-µm		400-µm	
		Cultured	Wild	Cultured	Wild
Actual	Cultured	110	3	113	0
	Wild	6	154	12	132
Accuracy:		**96.7% (1.01)**		**95.3% (1.15)**	

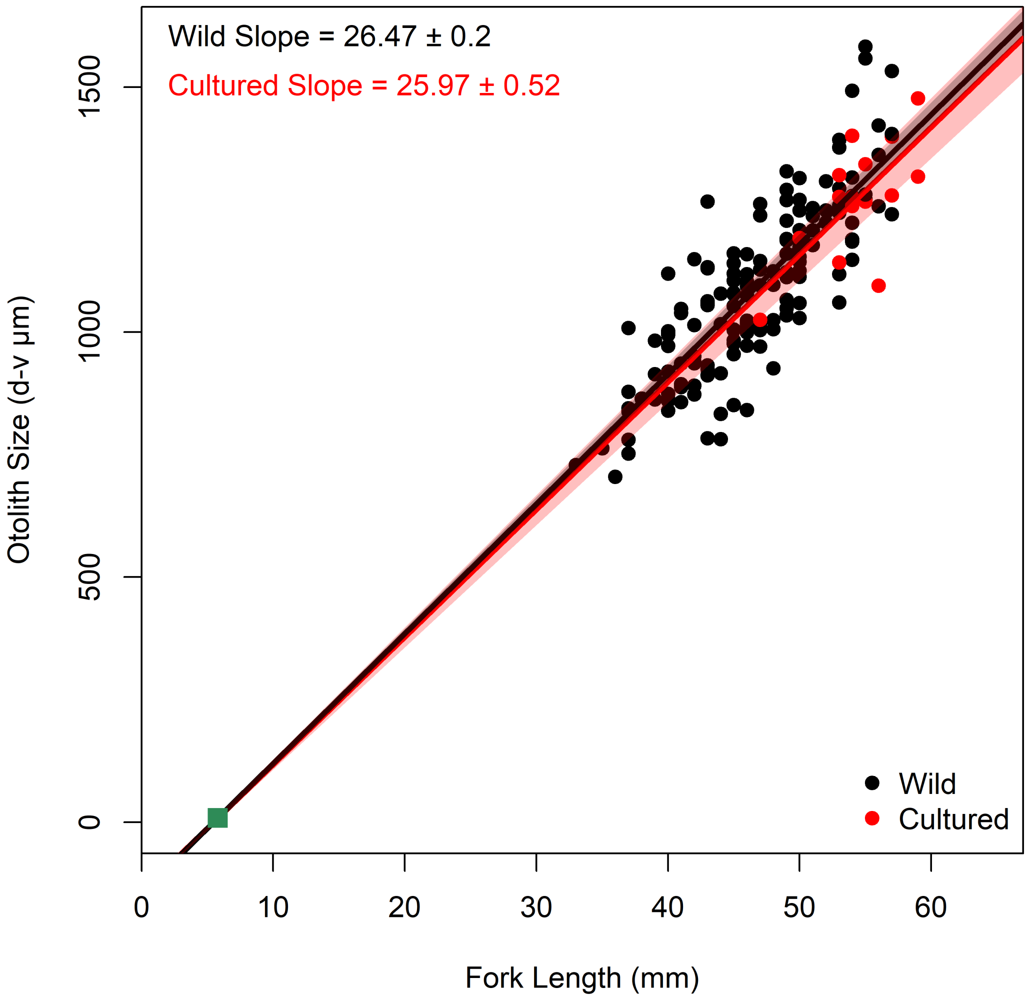
Values in cells reflect the total numbers of fish that were correctly (blue) and incorrectly (orange) assigned to each origin based on stratified 10-fold cross-validation of each model. Overall mean (se) accuracy (% classification success) is also provided for each model. Sample sizes differ between analyses due to the exclusion of smaller fish from the 400-µm model.

Figures



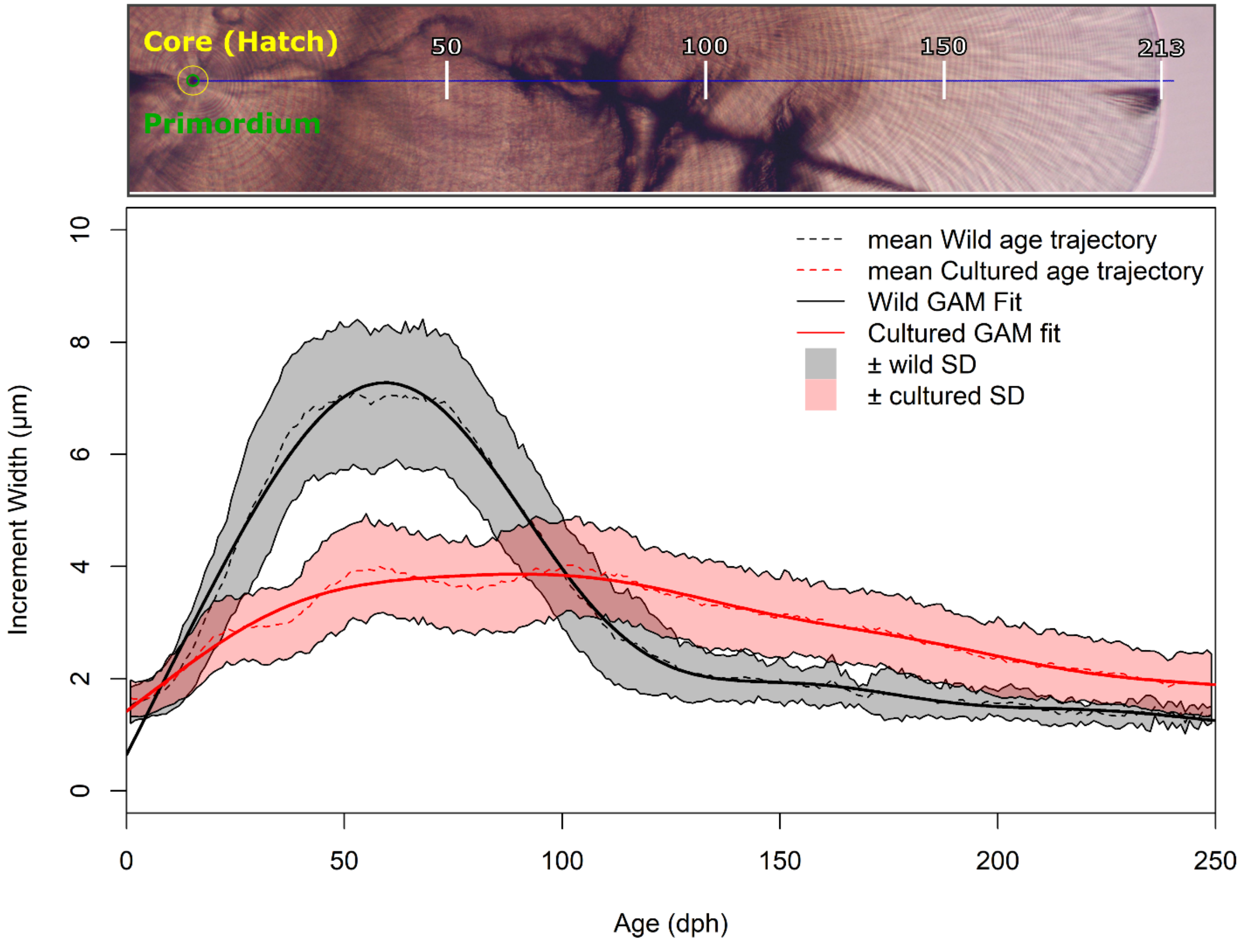
A pair of asymmetrical Delta Smelt otoliths (top) exhibiting 100% vaterite (abnormal, left) and 100% aragonite (0% vaterite, normal, right). (bottom) The proportion of fish assigned to each vaterite category by origin and year (sample sizes above each bar). Hatchery releases commenced in Fall 2021 with the first cultured Delta Smelt captured in the wild in January 2022.

Figure 4-1. Crystalline Structure of Cultured and Wild Delta Smelt Collected in Field Surveys



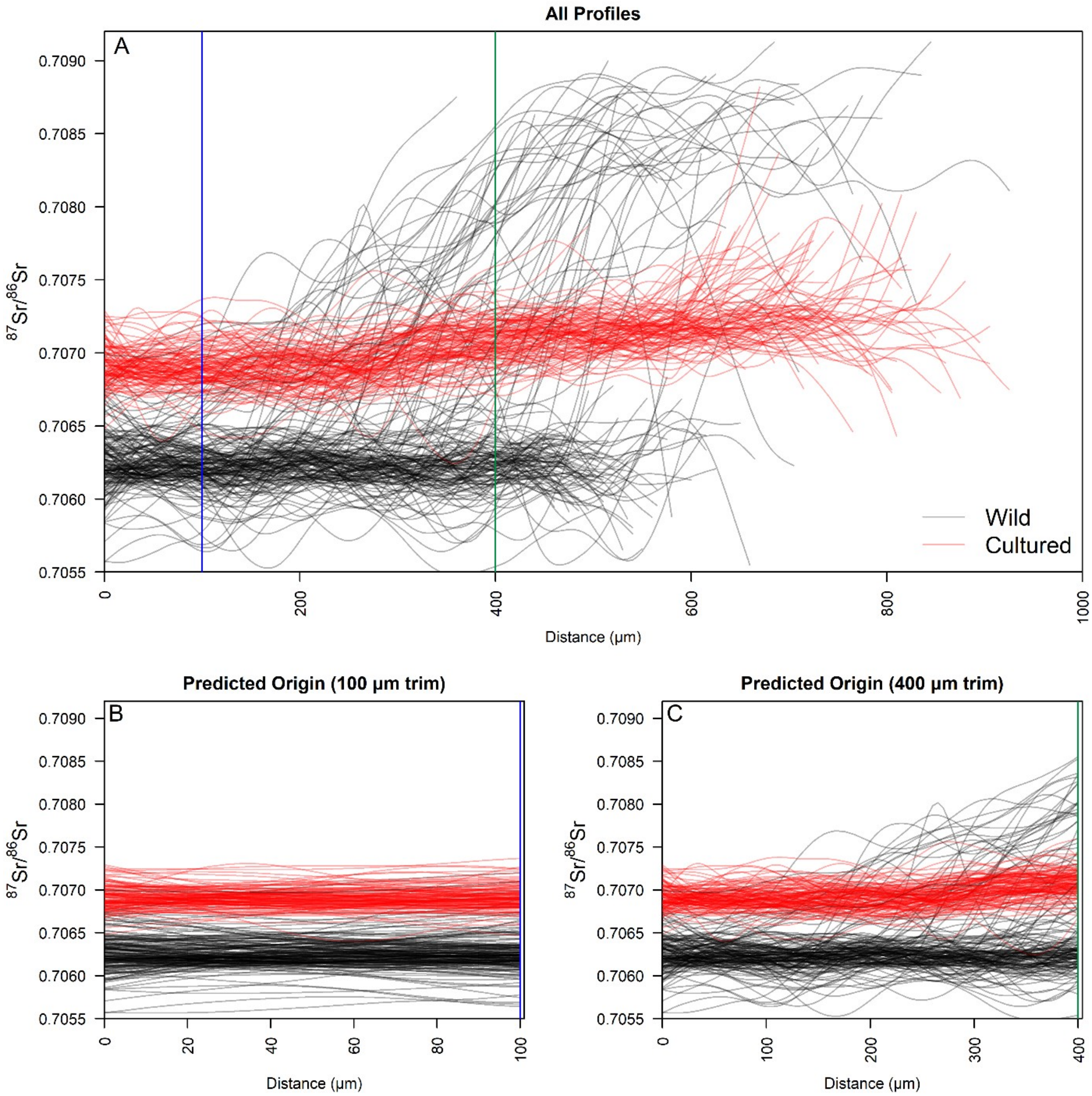
Best-fit linear regressions (solid lines) and 95% confidence intervals (shading) are shown (Table 4). Slopes ± s.e. are provided assuming a mean Delta Smelt length and otolith size at hatch of 5.8 mm and 8.5 µm, respectively (Xieu et al. 2021) (green rectangle). Only fish within the size ranges suitable for daily increment analysis (e.g., < 60 mm) were used to contrast o-s proportionality during the early life history (Xieu et al. 2021).

Figure 4-2. Otolith-Somatic Proportionality for Wild (black) and Cultured (red) Delta Smelt



Mean daily increment widths (dashed line) and standard deviations (shading) are provided, along with smoothed predictions from the generalized additive model (solid lines). Widths of increments beyond 250 dph are less reliable and not included in the analysis (Xieu et al. 2021).

Figure 4-3. Otolith Increment Profiles of Wild and Cultured Delta Smelt



(A) Full ^{87}Sr/^{86}Sr profiles of all individuals of known cultured (red) and wild (black) origins. Vertical dashed lines indicate the trim values for the 100-µm MDA (MDA_{100}) and 400-µm MDA (MDA_{400}) analyses used to classify individuals into each group. Trimmed profiles used in (B) MDA_{100} and (C) MDA_{400} analyses, with predicted group assignments as in (A). Ten-fold cross validation indicated 95% and 96% classification success for each model, respectively (Table 6).

Figure 4-4. Core-to-Edge ^{87}Sr/^{86}Sr Profiles of Wild and Cultured Delta Smelt

Supplementary Information

Table S4-1. Delta Smelt Included in the Study by Year and Survey

Year	EDSM	SKT	CHIP	CVP	SWP	Total
2019	119	7	0	0	0	126
2020	22	2	0	0	0	24
2021	1	0	0	0	0	1
2022	63	18	2	1	0	84
2023	25	4	3	8	4	44
Total	**230**	**31**	**5**	**9**	**4**	**279**

Fish were provided by collections conducted by the U.S. Fish and Wildlife Service Enhanced Delta Smelt Monitoring (EDSM) program, the California Department of Fish and Wildlife Spring Kodiak Trawl (SKT) survey, the U.S. Bureau of Reclamation's Central Valley Project (CVP) pumping facility, the California Department of Water Resources State Water Project (SWP) pumping facility, and the UC Davis Fish Conservation and Culture Laboratory (FCCL).

Table S4-2. Sample Sizes of Cultured and Wild Delta Smelt Included in Each Analysis

Analysis	Included			Excluded	Notes
	Cultured	Wild	Total		
total available	116	163	279	0	All available specimens with corresponding field and laboratory data (Table S1)
vaterite	116	162	278	1	1 fish with both otoliths badly broken (2019_EDSM_0432)
o-s proportionality	15	148	163	116	115 fish ≥ 60 mm excluded (only fish < 60 mm FL included) 1 fish with 2 broken otoliths (2019_EDSM_0432)
growth	112	162	274	5	3 fish with vateritic otoliths (2022_EDSM_0102, 2022_CHIP_0004, 2022_EDSM_0005) 2 fish with both otoliths lost/damaged (2019_EDSM_0432, 2023_EDSM_0211)
chemistry	113	160	273	6	3 fish with both otoliths lost or damaged (2019_EDSM_0456, 2023_EDSM_0211, 2019_EDSM_0432) 2 fish with vateritic otoliths (2022_EDSM_0102, 2022_EDSM_0005) 1 fish missing chemistry run file (2019_EDSM_0476)
100-µm MDA	113	160	273	6	All fish with chemistry data included
400-µm MDA	113	144	257	22	16 fish with max_dist < 400um excluded

Chapter 5. Sub-lethal Responses of Delta Smelt to Contaminants Under Different Flow Conditions

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Abstract

The Delta Smelt is a largely zooplanktivorous, endangered fish endemic to the San Francisco Estuary (Estuary). High flows increase the availability of fresh and brackish water habitat for Delta Smelt, but also may mobilize contaminants, potentially increasing toxicological stress. Here, we examine the association between contaminants and Delta Smelt health across contrasting water year types and flow-related management actions. Our study spanned the fall season of three years: one dry year (2018) bracketed by two wet years (2017 and 2019), and coincided with several management actions meant to benefit Delta Smelt. We collected field water from six sites in the estuary that encompass the fresh and low salinity habitat of Delta Smelt and analyzed the water for contaminant concentrations. After a 96-hour exposure to the field water, we assessed cultured Delta Smelt survival and the histopathological condition of the gill and liver. Insecticides, particularly fipronil metabolites, were the most prevalent contaminants detected in 2017 and 2018, and a variety of contaminants associated with the rice harvest were detected in 2019. No acute toxicity was observed during any exposure, but we observed negative effects in the livers of Delta Smelt exposed to agricultural water from the Toe Drain and Cache Slough during a 2019 pulse flow action, which coincided with elevated detections and concentrations of organic pesticides. Other noteworthy sub-lethal effects, likely occurring in response to contaminant mixtures, included severe gill lesions in Delta Smelt exposed to Decker Island water in 2019. In the drier year of 2018, lesions were generally mild or absent. Thus, the tradeoffs between increased habitat availability and contaminant loading may provide one explanation for why Delta Smelt abundance does not consistently respond positively to outflow.

Keywords

Delta Smelt, Toxicology, Contaminants, Histopathology, Delta, SFE, Analytical chemistry

Introduction

The San Francisco Estuary and adjacent Sacramento-San Joaquin Delta (hereafter the estuary) together comprise the largest estuary on the west coast of the United States. The estuary is a highly altered system, rife with anthropogenic changes resulting from the economic development of California. It receives flow from the Sacramento and San Joaquin rivers, provides irrigation and drinking water to millions of Californians, and serves as a migratory pathway for various anadromous and semi-anadromous fish species.

The Delta Smelt (*Hypomesus transpacificus*) is a small, mainly zooplanktivorous, and largely annual fish that is endemic to the estuary. Its distribution varies according to life stage, with the dominant phenotype rearing in the Low Salinity Zone (LSZ; 0.5-6 practical salinity unit (PSU)) and later migrating upstream to freshwater areas in the Delta to spawn (MAST 2015; Moyle et al. 2018; Hobbs et al. 2019). The species is listed as threatened by the U.S. Fish and Wildlife Service (USFWS) and endangered by the California Department of Fish and Wildlife (CDFW) due to its sustained low abundance for the past several decades (MAST 2015; Jin et al. 2018). For example, from 2017-2022, zero Delta Smelt were detected by the CDFW's Fall Midwater Trawl, a survey which previously collected thousands of Delta Smelt annually (CDFW 2023).

Because they reside in the fresh and low salinity areas of the estuary during summer and fall, the location and extent of Delta Smelt habitat is largely determined by the amount of freshwater flow through the system (Delta Science Program 2020). Thus, increases in freshwater flow may support recovery of the species. Fall outflow is of particular interest because Delta Smelt appears to go through a demographic and condition bottleneck as its available low salinity habitat shrinks with low flows at the end of the summer dry season (Moyle et al. 1992; Feyrer et al. 2011, Hammock et al. 2021). A number of water management strategies in the estuary are aimed at the recovery of the species. Specifically, Fall X2 enhances the habitat available for Delta Smelt. The position of "X2" refers to the distance (kilometer) from the Golden Gate Bridge to the salinity isohaline of two PSU. Historically, X2 has been a zone of high productivity and turbidity and typically represents the center of Delta Smelt distribution (Jassby et al. 1995; Kimmerer et al. 2013; Delta Science Program 2022). Fall X2 actions require the U.S. Bureau of Reclamation (Reclamation) and the CDWR to manage exports and reservoir releases (either actively or passively; USFWS 2008) so that Delta outflow maintains specific monthly average locations of X2 in the fall during 'wet' or 'above normal' water years when there is sufficient water available. The goal of Fall X2 is to push the LSZ westward to increase the overlap with the turbid, cooler environment of Suisun Bay and Suisun Marsh, thereby enhancing availability of high-quality habitat and prey for Delta Smelt (MAST 2015; CNRA 2017).

Food availability, which is intrinsically tied to habitat distribution, is an important factor related to the status of Delta Smelt. Accordingly, specific management actions, such as the North Delta Food Subsidy (NDSF; Sommer et al. 2020), aim to increase resource availability for Delta Smelt. The North Delta and the Yolo Bypass (a managed floodplain) in particular, maintain high levels of phytoplankton and are considered areas of high productivity (Mahardja et al. 2019). However, during the summer and fall, low flows keep this highly productive water within the Yolo Bypass region, and water conveyance can result in reverse flows in the area (Frantzich et al. 2021). In 2011 and 2012, fall phytoplankton blooms were observed downstream of the North Delta for the first time in over 20 years after larger than normal fall agricultural flow pulses (CDWR 2019). The NDFS aims to mirror the 2011-2012 pulse flow by rerouting freshwater from agricultural return water or from the Sacramento River through the Yolo Bypass, thereby moving nutrients and phytoplankton downstream of the Yolo Bypass (e.g., lower Cache Slough and lower Sacramento River). The goal of the NDFS is to reduce negative net flows in the Cache Slough Complex and consequently improve food web productivity and prey availability for Delta Smelt downstream towards the central Delta and in the more food-limited areas of the upper estuary (Frantzich et al. 2021).

Although freshwater flows may benefit Delta Smelt, in the estuary they also contain varying concentrations and types of contaminants from industrial, urban, and agricultural sources (Thompson et al. 2007; Smalling et al. 2013; Orlando et al. 2014; Jabusch et al 2018; De Parsia et al 2018; 2019), resulting in the inclusion of the estuary on the Clean Water Act Section 303(d) and the 305(d) List of Impaired Water Bodies (SWRCB 2018). For instance, effluent from municipal wastewater treatment plants (e.g., Sacramento, Stockton, and Vacaville) and untreated stormwater runoff discharges into the Delta. While some urban effluents (e.g., wastewater) are typically discharged continuously throughout the year, other sources are more seasonal. For example, irrigated agricultural land is linked to seasonal inputs of contaminants, as tail-water is discharged into the estuary during timed pulse flows in the dry summer months, while urban stormwater runoff discharges contaminants during wet winter months. The positive association between high outflow and contaminants (Chen et al. 2019; Commelin et al. 2022; Schoenenberger et al. 2022) has important implications for water quality and biota in the estuary.

While the increase of freshwater outflow has long been known to expand physical habitat availability for Delta Smelt (Jassby et al. 1995; Feyrer et al. 2007; Feyrer et al. 2011; Bever et al. 2016), a concurrent increase in contaminants may also negatively impact the species. For example, Teh et al. (2020) described substantial improvement in liver condition of wild-caught Delta Smelt as drought severity increased, suggesting that water quality improved during the drought, improving liver health of surviving fish. Tradeoffs between increased habitat availability and contaminant loading may partially explain why Delta Smelt abundance does not necessarily respond positively with outflow, if exposure to contaminants negatively affects Delta Smelt health and condition (Stevens and Miller 1983, Dege and Brown 2003, Miller et al. 2012, FLOAT-MAST 2020). Thus, understanding the cumulative effects of outflow and contaminants on Delta Smelt viability is integral for developing optimal management practices aimed at recovering Delta Smelt populations.

To examine the influence of regional variation in water quality and potential increases in contaminants associated with increased flow, we evaluated the survival, health, and condition of cultured Delta Smelt exposed to water collected from six locations in the estuary. Our study occurred during the fall season of two wet years (2017 and 2019) and one dry year (2018) and coincided with Fall X2 and NDFS actions. Chemical analyses were conducted to determine the presence and concentrations of contaminants in the collected water and to examine whether contaminants were associated with detrimental effects on Delta Smelt. Our assessment of Delta Smelt included survival and histopathology of the liver and gills, a method which can detect a multitude of sub-lethal stressors (Hadi and Alwan 2012; Devi and Mishra 2013; Cao et al. 2018). Our (*a priori*) hypotheses were that 1) Delta Smelt would exhibit site-specific gill and liver damage associated with contaminant exposure; 2) lesions would be more prevalent and/or severe during the wetter years of 2017 and 2019 compared to the drier year of 2018; and 3) lesions and contaminants would be more prevalent and/or severe during flow actions.

Materials and Methods

Sampling Design and Water Collections

Water collections took place every two weeks from October to December in 2017 and from September to November in 2018 and 2019. Six fixed sampling sites were selected to span freshwater and low salinity habitat of Delta Smelt (Merz et al 2011; Hammock et al. 2015). Selected sites were the Toe Drain, Cache Slough [Cache], Sacramento River at Isleton [Isleton], Sacramento River at Decker Island [Decker Island], Montezuma Slough [Montezuma], and Grizzly Bay (Figure 5-1, Table 5-1). In 2017, not all sites were collected for all toxicity exposures; Toe Drain, Isleton, and Decker Island were not included in Exposure 1, and Grizzly Bay was not included in Exposure 2. Water was collected from all site locations for subsequent toxicity exposures in 2018 and 2019.

Up to 80 liters (L) of ambient water from each site were collected with a bilge pump as sub-surface grabs and stored in four 20 L plastic cubitainers (I-CHEM, Fisher Scientific) for use in Delta Smelt toxicity exposure tests. Additional sub-samples were collected in 1 L glass amber bottles (I-CHEM, Fisher Scientific) and 1 L plastic bottles (I-CHEM, Fisher Scientific) for chemical analyses and water quality measurements, respectively. All samples were kept in cold (0-6 degrees Celsius (°C)), dark conditions until use.

Water Years and Outflow Actions

The 2017 water year was classified as ’wet’ by CDWR, which triggered an X2 action that coincided with toxicity exposures 1-4. Average Delta outflow during this period ranged from a high of 292 cubic meters per second (m^3/sec) for waters collected for Exposure 1 and subsequently decreased to a low of 164 m^3/sec for waters collected for Exposure 5 (Table 5-1; Figure 5-2; https://cdec.water.ca.gov). The 2018 water year was classified as ‘below normal’ and as such, no X2 action was implemented. Delta outflow ranged from 117 to 292 m^3/sec during these exposures (Figure 5-2). The NDFS took place in early fall of 2018, where rice drainage agricultural tail-water from Colusa Basin Drain was rerouted through the Yolo Bypass between August 28 and September 26, resulting in peak mean flows in the Toe Drain at 17 m^3/sec (Twardochleb et al. 2021). This NDFS action coincided with Delta Smelt Exposure 1 (Table 5-1; Figure 5-2). The 2019 water year was classified as ‘wet,’ triggering an X2 action that coincided with all Delta Smelt exposures. Delta outflow ranged from 204 to 412 m^3/sec during these exposures (Table 5-1; Figure 5-2), and in early fall, rice field drainage agricultural return water was redirected during the NDFS between August 26 and September 21 (Twardochleb et al. 2021). This NDFS action increased peak mean flows through the Toe Drain to 23 m^3/sec and coincided with Delta Smelt Exposures 1 and 2 (Figure 5-2).

Chemical Analyses

Chemical analyses varied across years. In 2017 and 2019, organic compound analyses included Gas Chromatography coupled with Quadrupole Time-Of-Flight Mass Spectrometry (GC-QTOF-MS; Agilent model 7200) and Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS; Agilent model 6530) for targeted pesticide analyses (e.g., for synthetic pyrethroid insecticides). Upon receipt, 1 L water samples were passed through solid phase extraction cartridges (Oasis HLB) and sequentially eluted with solvents to produce two extracts, one for Liquid

Chromatography (LC) and one for Gas Chromatography (GC) analysis following methods outlined in Moschet et al. (2017). Depending on the year, extracts were analyzed on up to four instrument platforms: LC with positive and negative electrospray ionization modes (ESI+ and ESI-) and GC with both electron ionization (EI) and negative chemical ionization (NCI). In 2018 we focused only on GC-NCI-targeted analyses, following the aforementioned methods.

Delta Smelt Toxicity Testing

Sub-adult Delta Smelt were used in each of the toxicity exposures and were obtained from the UC Davis Fish Conservation and Culture Laboratory (FCCL; Byron, CA). FCCL cultures a population of Delta Smelt that is annually outbred with fish collected from the wild, serves as a refuge population, and has recently been used to supplement the wild population (Fisch et al. 2012; Lindberg et al. 2013; Hung et al. 2022). Cultured Delta Smelt are maintained in pre-conditioned surface water from the California Aqueduct that is treated for solids removal and disinfected with ultraviolet, but almost certainly contains dissolved contaminants. One day before the start of the exposure tests, Delta Smelt were placed into replicate buckets filled with temperature-controlled FCCL culture water for a 24-hr acclimation period. At the toxicity exposure initiation, water in the buckets was replaced with collected ambient field water, replenished culture water (i.e., control), or high salinity control water. The freshwater control (FCCL culture water) was included as a comparison for the freshwater sites (Toe Drain, Cache, Isleton, Decker Island), while the "high salinity" control (HSC) was included as a comparison for the brackish water sites (Montezuma, Grizzly Bay), thereby accounting for possible salinity stress or benefits to Delta Smelt (See Table S5-1, Supplemental, for site conductivities). HSC water consisted of FCCL culture water amended with Instant Ocean® (Spectrum Brands) to match the salinity of Grizzly Bay (the site with the highest salinity) which ranged from 2.8-10.8 PSU. Given that the Delta Smelt in this study were likely exposed to contaminants (i.e., from the California Aqueduct) prior to their use in the experiments, as well as during the experiments in the control water, we conducted chemical analyses on the control water in 2018 and 2019, although analyses were not conducted in 2017 (Table S5-2, Supplemental).

All toxicity exposure tests had durations of 96 hours and were conducted indoors at FCCL using a static water system. Experimental replicates consisted of 20-L black plastic buckets with lids (Encore Plastics). Lids were loosely placed on the replicate buckets to minimize light while allowing room for constant aeration (Delta Smelt are sensitive to light; Lindberg et al. 2013). Tests in 2017 and 2019 included four replicate buckets containing 8 L of water and five fish each, for a total of 20 fish per treatment, 160 fish per exposure (20 fish for each of eight treatments [six sites and two controls]), and 800 fish per year (five exposures of 160 fish each). Due to limited Delta Smelt availability for toxicity testing in 2018, experimental replicates were reduced from four to three, with five fish per replicate for a total of 15 fish per treatment, 120 fish per exposure, and 600 fish total. Test replicates were kept in a temperature-controlled water bath maintained at 16°C using a chiller and pump system to circulate the water, and test temperatures deviated from 16°C by no more than +/- 2°C during acclimation and toxicity exposures. Other water quality parameters (e.g., pH, DO, ammonia-nitrogen) were maintained within optimal physiological ranges. Mortalities were removed and recorded daily by FCCL staff. At the end of each 96-hour exposure period, surviving fish were euthanized with an overdose of buffered tricaine methane sulfonate and fixed in 10% buffered formalin for histopathology and other sub-lethal analyses.

Histopathology

Following the exposure tests, histopathology was performed on the gills and livers of Delta Smelt. Histological assessment can be used as a biomarker of environmental contamination (Au 2004). Generally, liver and gill histopathological changes are sensitive and responsive but not specific to pollutant exposure. The occurrence of similar lesion types under a wide range of stressors and contaminants (Mallatt 1985) and chemical interactions (e.g., synergism/antagonism) precludes our ability to directly study the cause-effect relationship between specific pollutants and lesions (Au 2004). Because the liver is the primary location for metabolic and detoxification processes and the gills are one of the first exposure routes for waterborne contaminants, these are the primary tissues used for the assessment of morphological alterations. We anticipated that gills would respond more quickly to external stressors than the liver (Teh et al. 2020), making gill histology of particular interest because the toxicity exposures were brief.

The left gill arches and the whole liver were excised and placed in 10% neutral buffered formalin and processed according to Teh at al. (2016). Briefly, tissues were embedded in paraffin, sectioned to 3-µm thickness and stained with hematoxylin and eosin. For liver and gill tissues, lesions were scored qualitatively from 0-3, where 0 = lesion not present, 1 = mild, 2 = moderate, and 3 = severe (multiple lesion types were scored per tissue; see descriptions in Teh et al. 2020). To provide an aggregate metric of the liver and gill condition, a histopathological index was calculated by summing the scores for each lesion type for each organ for an individual fish. These aggregate lesion scores from individual fish were then used in our statistical analyses. Thus, for each treatment, the average gill or liver lesion score represents the average degree of damage to each organ, with higher scores indicating more damage (Hammock et al. 2015, Teh et al. 2020).

Lesion scores of liver and gill tissues were statistically compared within individual project years with a Kruskal-Wallis Rank Sum test, with Site and Toxicity Exposure as predictors, followed by a Steel-Dwass multiple comparisons test if the Kruskal-Wallis test was significant. In each project year, a subset of fish was used for histological analyses (n = 368 fish in 2017, n = 236 fish in 2018, and n = 160 fish in 2019). To test for differences among years, liver and gill data were analyzed with a Kruskal-Wallis Rank Sum test with Year as the predictor, followed by a Dunn's multiple comparisons test if the Kruskal-Wallis test was significant.

Results

Analytical Chemistry

Of the 90 water samples collected over the three-year study, we identified 16 different compounds among 270 detections. The greatest number of detections occurred in 2017 (166), followed by 2019 (74), with 2018 having the fewest detections (30; Figure 5-3). However, we note that we analyzed for fewer chemical classes in 2018 than in 2017 and 2019. Thus, the lower number of chemical detections observed during 2018 is presumably a direct result of that change. Because of the difference in the analyses conducted, we cannot make annual comparisons across all three years, but we can compare 2017 and 2019. Overall, analyte concentrations were higher in 2019 compared to 2017, but the specific pesticides that were detected varied across years (Figure 5-3).

In 2017, the majority of detected compounds were insecticides (81%). Fipronil and its metabolites made up 66% of all detections during this year (Table S5-3, Supplemental). Fipronil and its degradates fipronil-desulfinyl, fipronil-sulfide, and fipronil-sulfone were detected consistently at concentrations ranging from 0.05-0.29 nanograms per liter (ng/L). The herbicide hexazinone and fungicide azoxystrobin were the second-most frequently detected compounds. Concentrations of azoxystrobin ranged from 4.67-23.67 ng L^{-1}, with the highest concentration detected at Isleton during Exposure 4 (Figure 5-3A). In 2017 fungicides and herbicides respectively comprised 11% and 12% of all detections. The insecticide methoxyfenozide was detected at least twice at each site throughout this study period, with concentrations ranging from 3.74-12.61 ng/L. Chlorpyrifos was detected in water samples collected from Isleton in Exposure 3 and Toe Drain, Cache, and Decker Island in Exposure 4, with concentrations ranging from 0.11 to 0.23 ng/L.

In 2018, we focused only on GC-NCI-targeted analytes; thus, we observed a low number of organic contaminants, with only fipronil and its degradates detected consistently across exposures (Figure 5-3B). Fipronil-desulfinyl was detected in 93% of samples with concentrations ranging from 0.1 to 0.5 ng/L. Fipronil and fipronil-sulfone were detected in the Toe Drain during Exposures 2 and 3 at 6.5 ng/L and 1.7 ng/L, respectively (Table S4, Supplemental).

In 2019, we saw a difference in the composition of analytes detected and contaminant concentrations were generally higher when compared to previous project years. For example, fipronil metabolites were only detected four times: during Exposure 1, fipronil-desulfinyl concentrations were 0.20, 0.35 and 0.29 ng/L at Cache, Montezuma, and Grizzly Bay, respectively, and fipronil-sulfone concentration was 0.23 ng/L at Montezuma. The fungicide azoxystrobin and the insecticide DEET were particularly prevalent in the 2019 water samples (Figure 5-3C). Azoxystrobin had a 100% detection frequency, with the highest concentration of 1,601 ng/L in the Toe Drain during Exposure 1 (Table S5-5, Supplemental). DEET (N,N-diethyl-*m*-toluamide) was detected at all sites in almost all exposures in 2019, with a maximum concentration of 33 ng/L in Exposure 3 at Cache. Exposures 1 and 2 had the highest concentrations and greatest variety of contaminants, including DEET, chlorantraniliprole, azoxystrobin, methoxyfenozide, metolachlor, and thiobencarb (Figure 5-3C). The presence of certain contaminants (e.g., chlorantraniliprole, azoxystrobin, metolachlor) was particularly notable in water from the Toe Drain and Cache in Exposures 1 and 2, which also coincided with the NDFS (Figure 5-4). Methoxyfenozide was also detected in Decker Island during Exposure 2.

Toxicity Testing and Histopathology

No acute toxicity was observed in Delta Smelt exposed to water from any of the six sampling locations across the three-year study period. Fish survival rates were high, exhibiting at least 96% survival across the three years (Figure S5-1A, Supplemental). As detected by histopathology, fish condition was variable, especially in the gills. When combining observations across exposure tests and sites, gill lesion scores differed significantly across years (Kruskal-Wallis χ^2: 72.23, df: 2, $P<0.0001$; Figure 5-5, Figure S5-1B, Supplemental), with higher scores in 2017 compared to 2018 ($P<0.0001$) and 2019 ($P=0.0208$). Liver lesion scores also differed across years (Kruskal-Wallis χ^2: 213.9, df: 2, $P<0.0001$; Figure 5-5, Figure S5-1C, Supplemental), with higher lesion scores in 2017 compared to both 2018 ($P<0.0001$) and 2019 ($P<0.0001$). For 2017, the presence of mild gill and liver lesions extended to the controls (Figure 5-5A, D). For 2017, we cannot explain why Delta Smelt in the HSC exhibited elevated gill lesion scores while those in the freshwater Control did not. It was apparently unrelated to salinity stress because the difference in salinity between control and HSC was similar in 2017 and 2019 and we did not see elevated gill lesion scores.

Gill lesion scores significantly differed in 2017 across sites (Kruskal-Wallis, χ^2: 32.28, df: 7, $P<0.0001$). Elevated gill lesions were observed in fish exposed to all ambient sites when compared to the freshwater Control, including fish in the HSC (Figure 5-5A). Chloride cell hyperplasia and mucous cell hyperplasia were the most frequently observed type of gill lesions, especially during Exposures 1 and 3 (Figure 5-6A). In Exposures 1 and 3 lesions were also observed in HSC fish, indicating the possibility that there was a contaminant present in the ambient water used for culturing the Delta Smelt (i.e., and used for the Controls); however, we did not measure analytical chemistry on the control water in 2017, thus we cannot confirm this. Chloride cell hyperplasia was observed in 88% of fish exposed to water from Cache, 21% of fish exposed to the Toe Drain, and in 63% of fish exposed to Montezuma. Mucous cell hyperplasia was observed in 26% of fish exposed to Cache, 38% of fish exposed to Toe Drain, and 20% of fish exposed to Montezuma. In particular, fish exposed to Cache water (Figure S5-2, Supplemental) tended to have high lesion scores, including half of the replicate fish with severe mucous cell hyperplasia in Exposure 3 (Figure S5-3B supplemental) as well as one fish with severe gill aneurysm or telangiectasia in Exposure 2 (Figure S5-3C, Supplemental).

Liver lesion scores were elevated in fish exposed to all sites and controls, but did not vary across sites in 2017 (Kruskal-Wallis, χ^2: 8.69, df: 7, P=0.2755). Lipidosis was observed in most Delta Smelt livers, including some control fish, suggesting that cultured fish had liver lesions prior to their use in the experiments in 2017. In Exposure 1, moderate and severe lipidosis was prevalent in fish exposed to water collected from Grizzly Bay (75%), Montezuma (75%), and Cache (38%), although this severity of lipidosis also occurred with high frequency in fish from the HSC (50%). Severe lipidosis was more prevalent in Delta Smelt exposed to water collected from Montezuma (50%) and Decker Island (63%) in Exposure 5, including one fish with severe lipidosis and moderate sinusoidal congestion.

Overall, gill lesions were mild or absent in 2018, with no differences across sites (Kruskal-Wallis, χ^2: 4.56, df: 7, P=0.7138; Figure 5-5B, Figure 5-6B). However, moderate chloride cell hyperplasia was intermittently observed in the gills of fish exposed to field water. In the liver, lesions were generally absent and did not differ across sites (Kruskal-Wallis, χ^2: 4.65, df: 7, P=0.7032; Figure 5-5E, Figure 5-6E).

In 2019, gill lesion scores differed across sites (Kruskal-Wallis, χ^2: 26.70, df: 7, P=0.0004). Delta Smelt exposed to Decker Island had higher gill lesion scores than any other site or control (Kruskal-Wallis χ^2: 26.7, df: 7, P=0.0004; Figure 5-5C). In fish exposed to Decker Island water, lesions such as mucous cell hyperplasia and epithelial cell hyperplasia were prevalent (Figure S5-4, Supplemental) and most notable in Exposures 1, 2, 3, and 5 (Figure 5-6C). Mucous cell hyperplasia and epithelial cell hyperplasia were evident in several fish exposed to Isleton and Grizzly Bay during Exposure 2.

Liver lesion scores also differed across sites in 2019 (Kruskal-Wallis χ^2: 18.65, df: 7, P=0.0093). Observed lesions were generally mild, or in the case of both controls, absent. Delta Smelt exposed to water collected from Cache exhibited higher lesion scores than the freshwater control (P=0.0430; Figure 5-5F), but were not statistically different from the other sites. The highest liver lesion scores in fish exposed to Cache water were observed during Exposures 1 and 2, coinciding with the NDFS (Figure 5-6F). Moderate to severe lipidosis was observed in 25% of fish exposed to the Toe Drain, Cache, and Decker Island in Exposure 1.

Discussion

This study evaluated the toxicity of estuary waters to cultured sub-adult Delta Smelt under different fall flow conditions. Generally, detected organic contaminants were always present, but type and concentration varied across our six study sites and were dynamic in each site within and across years, suggesting contaminant exposure and therefore hazard risk to freshwater biota varied through space and time. We observed low concentrations of organic compounds similar to those previously reported in the estuary during this season (Orlando et al. 2013; DeParsia 2018; DeParsia 2019; Orlando et al. 2020). Our study did not capture any storm events, which would likely be associated with increased pesticide concentrations. Nonetheless, we observed gill and liver damage in Delta Smelt following 96-hr exposure periods, indicating the potential for mixtures of low levels of contaminants to have sub-lethal effects on our study organism under a short-term exposure.

The chemicals detected in our study have been associated with negative health effects in fish at sub-lethal concentrations. For example, exposure to fipronil caused DNA damage in *Rhamdia quelen* (Ghisi et al. 2011) and oxidative damage in *Prochilodus lineatus* (Deiu et al. 2021). Chlorpyrifos has been noted to reduce hepatic glycogen in *Oreochromis niloticus* (Majumder and Kaviraj 2019) as well as gill damage in *Mugil cephalus* (Marigoudar et al. 2018). Azoxystrobin has caused oxidative stress and genotoxicity in *Austaloheros facetus* (Crupkin et al. 2021) and endocrine disruption in *Danio rerio* (Jiang et al. 2018). In our study, detected concentrations of these organic compounds were lower than those in the literature, however we still observed sub-lethal effects. Although the direct effects of contaminant exposure are difficult to measure in the environment, synergistic and/or additive interactions can cause sub-lethal toxic effects to tissues and affect physiological processes, emphasizing the need to identify the scope of mixture effects of these stressors on native fish species (Brooks et al. 2011; Fong et al. 2016).

Hypothesis 1: Site-Specific Responses to Contaminants

We observed numerous instances of site-specific detections of contaminants, some of which coincided with increased gill or liver lesions, partially supporting our first hypothesis. For example, elevated liver lesion scores in fish exposed to water from Toe Drain and Cache in early 2019 (i.e., Exposures 1 and 2) likely correspond to the mixture of insecticides, fungicides, and herbicides that were detected at higher concentrations than at other sites (e.g., azoxystrobin, methoxyfenozide, and thiobencarb). Despite concentrations of certain organic compounds being relatively high at Toe Drain and Cache compared to our other sites, these concentrations are nonetheless below benchmark concern levels (e.g., USEPA Aquatic Benchmark levels for freshwater vertebrates; USEPA 2022). Thus, our results suggest the benchmark concern levels may be too conservative, given the mixture of contaminants detected during our study. Based on our data, it appears that sub-lethal effects on wild Delta Smelt populations in the estuary vary by region and contaminant and we suspect that mixture effects were a likely contributor to the toxicity observed in our study.

While lesions appeared to be associated with contaminant prevalence in some cases, chemical detections and concentrations did not always correspond to elevated lesion scores. For instance, fish exposed to Decker Island water in 2019 consistently exhibited severe gill lesions, but we did not detect any individual contaminant at a concentration likely to cause such detrimental effects. Additionally, other contaminants not measured by our study may have contributed to the observed toxicity (Fong et al. 2016). However, gill lesions are not necessarily caused by any individual contaminant; rather the types and severity of gill lesions are often determined by exposure to low and moderate contaminant concentrations (Polesksic and Mitrovoic-Tutundzic 1994). For example, lesions such as epithelial hyperplasia with lamellar fusion and telangiectasia are typically attributed to a wide range of contaminants, including organophosphates, carbamates and herbicides, and mild to moderate mucus secretion is typically a protective response to contaminant exposure (Au 2004; Matey et al. 2011). Such gill lesions were frequently observed in Delta Smelt exposed to Decker

Island water in 2019. Therefore, Delta Smelt may have been adversely affected by the interaction of a diverse suite of contaminants present at these sites, rather than the presence of any single chemical.

The prevalence of elevated gill lesions and liver lipidosis in sites from the freshwater region of the estuary (e.g., Toe Drain, Cache, Decker Island) may have implications for dispersal and spawning of Delta Smelt in the wild. Specifically, Decker Island is located downstream of the confluence of Cache Slough Complex and Sacramento watershed and is the main corridor for dispersal for Delta Smelt to and from the North Delta, while Cache is critical spawning habitat for Delta Smelt (Bennett 2005, Sommer et al. 2011, Kurobe et al. 2022). The elevated lesion scores observed in Delta Smelt exposed to Cache, Toe Drain, and Decker Island water in 2019, suggest that these areas may be of elevated hazard risk for Delta Smelt.

Hypothesis 2: Water-Year Effects

The years 2017 and 2019 were considerably wetter than 2018; thus, we expected lesions to be more prevalent in these years than in 2018. This hypothesis was supported, as lesions were either mild or absent in fish in 2018. In contrast, during the two wet years we observed moderate to severe lipidosis and moderate sinusoidal congestion in the liver, as well as chloride cell hyperplasia and mucous cell hyperplasia in the gills. Consequently, we suggest that contaminant mobilization during wet years (e.g., Orlando et al. 2020) may offset habitat benefits, providing one explanation for why Delta Smelt abundance does not consistently increase with freshwater flow despite improved access to higher quality habitat during wetter periods (e.g., Stevens and Miller 1983, Dege and Brown 2003, Miller et al. 2012, FLOAT-MAST 2020; Mahardja et al. 2021).

Fipronil metabolite concentrations and frequency of detection did not follow our expectation of higher contaminant concentrations and detections during wetter years. Fipronil and its metabolites were detected in greater than 66% of samples collected in 2017 and over 90% of samples in 2018, but this detection frequency decreased to 6% in 2019. We believe this was due to the changing use of fipronil during our study rather than differences in hydrodynamics. Fipronil isn't registered for agricultural use in California (Sadaria et al. 2017), thus the probable source of fipronil and metabolite detections in 2017 and 2018 was from topical flea products entering municipal wastewater through home and commercial pet grooming (Sadaria et al. 2017, Sutton et al. 2019). As we didn't capture any storm events, this contaminant is largely entering the estuary from wastewater effluent (as opposed to stormwater runoff), thus its presence is unlikely to be related to water-year type. Instead, the decline in detections of fipronil in 2019 is more likely due to the label changes that were enacted by USEPA and CDPR, which considerably restricted fipronil applications after October 2018 (Messenger-Sikes and Windbiel-Rojas, 2018).

Given that we did not measure the full suite of analytes in 2018 that we did in 2017 and 2019, we cannot make comparisons of contaminant presence and concentration between wet and dry years; however, we did observe trends between the two wet years. Although fipronil and metabolite detection frequency varied greatly between 2017 and 2019, concentrations were similar in both years, consistently being detected below ng/L. Other compounds, such as chlorantraniliprole, methoxyfenozide, azoxystrobin, and hexazinone, were detected in similar concentrations and detection frequencies in both wet years. Chlorantraniliprole and methoxyfenozide were the fourth- and fifth-most applied insecticides in California by acreage for both 2017 and 2019, and azoxystrobin was the second-most applied fungicide in California by acreage during these years (CDPR 2017, 2019). Similarities between chemical classes, concentrations, and detection frequencies may be due to the antecedent conditions at these sites. Wetter conditions provide greater access to water for agriculture, leading to increased crop production and therefore, increased pesticide use and the potential for non-target exposure.

Hypothesis 3: Managed Flows and Contaminant Exposure

Our study overlapped with Fall X2 in 2017 and 2019, and the NDFS in 2018 and 2019. While these actions were intended to benefit Delta Smelt (CNRA 2016) by increasing freshwater flows, habitat access, and food availability in the downstream reaches of the estuary, we were interested in whether they could have unintended detrimental effects on the species through increased exposure to contaminants. During the NDFS action in 2019, we observed elevated contaminant concentrations and sub-lethal effects at sites in the North Delta (i.e., Toe Drain, Cache) compared to the downstream sites. Specifically, during the NDFS (Exposures 1 and 2), we observed a higher prevalence and severity of lipidosis in the livers of Delta Smelt exposed to the Toe Drain water and moderate to severe lipidosis in fish exposed to Cache water (e.g., Figure 5-6C, E). Additionally, we detected the highest number of chemicals at some of the highest concentrations in Exposure 1 (e.g., Figure 5-3C, Figure 5-4, Table S5-3, Supplemental). The waters collected from Toe Drain and Cache contained several contaminants that are associated with rice crops, such as azoxystrobin, methoxyfenozide, and thiobencarb (Orlando et al. 2020). Contaminant detections and liver lesion scores noticeably dropped between Exposures 2 and 3, coinciding with the end of the 2019 NDFS (e.g., Figure 5-4). Because the elevated liver lesion severity in Exposures 1 and 2 was largely restricted to fish exposed to water from upstream sites, the cause is unlikely to be an estuary-wide or water-year effect. Rather, these sub-lethal effects were likely caused by localized effects of the NDFS. Together, these results suggest that a pulse of contaminated source water may have impaired water quality in the North Delta (i.e., Toe Drain and Cache) during the NDFS, but these effects were transient and localized (i.e., they did not impact downstream sites or Exposures 3-5).

Although NDFS actions in 2018 and 2019 both used agricultural tail-water from rice field drainages, we did not detect negative effects on fish from the 2018 NDFS, which coincided with Exposure 1. Gill and liver lesion scores did not vary from Exposures 1 to 2 in the North Delta sites, nor did organic compound detections or concentrations. Moreover, observed gill and liver lesions were mild and, in some cases, absent. This annual difference is likely related to the amount of active agriculture taking place during the year. Water use and availability can vary dramatically between wet and dry water years, resulting in changes in acreage of fallow fields and of crops planted. For instance, between 2018 and 2019, pesticide-treated acreage in California increased 3.3% from 105 to 109 million acres (CDPR 2019), which may partially account for the differences we observed between the two years where the NDFS actions occurred. The increase in agriculture and concomitant increase in pesticide use in 2019 may have resulted in increased exposure of Delta Smelt to these contaminants. Thus, the water source used for the NDFS (i.e., agriculture return water vs. Sacramento River water) can have a significant impact on Delta Smelt health and condition. With respect to the NDFS action, our hypothesis that managed flow pulses would increase contaminants and lesions was partially supported, as NDFS actions were associated with increased sub-lethal effects in 2019 but not in 2018.

Determining whether the Fall X2 actions had adverse effects on Delta Smelt during our study is difficult because Fall X2 occurred in 2017 and 2019, but not 2018, such that this management action is conflated with water year type. A recent synthesis of Delta Smelt-related studies determined that high outflow by itself may not be sufficient in providing favorable habitat conditions, as other abiotic factors, such as temperature, can eclipse the habitat benefits provided by Fall X2 and a favorable LSZ location (FLOAT-MAST 2020). We note that the fall study periods all coincided with periods of base flow (i.e., low flow in general). A more thorough assessment of our hypothesis related to Fall X2 would need to include periods that do not conflate a Fall X2 action with a wet water year. However, this may not be possible, as Fall X2, a mandated action, does not occur during dry water years (USFWS 2008). A second possibility would be to examine the toxicity of water sources used for outflow augmentation, (e.g., runoff versus reservoir releases).

Conclusion

Our results demonstrate that low levels of contaminants are ubiquitous in estuary waters in the fall and even short-term exposure to these contaminants can elicit adverse health effects on Delta Smelt. Although our study included a limited number of years for comparison, our results suggest that habitat benefits attributed to wet years may be at least partially offset by elevated sub-lethal toxicity due to exposure to increased contaminants. Water year appears to have an effect on Delta Smelt toxicity, as Delta Smelt in the dry year of 2018 exhibited the fewest number lesions, which were generally mild or absent, and where the NDFS appeared to have no influence on Delta Smelt health and condition. In comparison, we observed negative effects in the livers of Delta Smelt exposed to the Toe Drain and Cache and elevated organic detections coinciding with the 2019 NDFS pulse flow, likely due to a combination of the antecedent agricultural conditions and pulse flow water source. Our results suggest contaminant loading is an important consideration in planning flow management actions, especially those actions using agricultural return water. Negative effects of contaminant exposure from freshwater flows outweighing the benefits provided by an increase in habitat availability, or other benefits of flow, suggest that improved contaminant management should be a consideration to reduce the costs of these actions. The inclusion of contaminant effects to Delta Smelt (and other imperiled species) can greatly benefit the conceptual models used in Agency decisions and aid in developing optimal management practices aimed at recovering Delta Smelt populations.

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Tables

Table 5-1. Summary of Sampling Events and Exposure Test Initiation Dates Across the Study

Year	Ave Delta Outflow (m^3/sec)	Flow Action	Collection Dates	Test Date	Test Exposure
2017	292.3	Fall X2	Oct. 11, 12	Oct. 13	1
2017	254.9	Fall X2	Oct. 24, 25	Oct. 27	2
2017	255.8	Fall X2	Nov. 8	Nov. 10	3
2017	201.6	Fall X2	Nov. 22	Nov. 24	4
2017	163.8	--	Dec. 5, 6	Dec. 8	5
2018	167.0	NDFS	Sept. 19, 20	Sept. 21	1
2018	292.3	--	Oct. 3, 4	Oct. 5	2
2018	117.4	--	Oct. 17, 18	Oct 19	3
2018	124.0	--	Oct. 31, Nov. 1	Nov. 2	4
2018	139.9	--	Nov. 14, 15	Nov. 16	5
2019	376.2	NDFS, Fall X2	Sept. 10, 11	Sept. 13	1
2019	290.1	NDFS, Fall X2	Sept. 24, 25	Sept. 27	2
2019	412.0	Fall X2	Oct. 10	Oct. 11	3
2019	372.5	Fall X2	Oct. 22, 24	Oct. 25	4
2019	203.7	Fall X2	Nov. 5, 6	Nov. 8	5

Figures

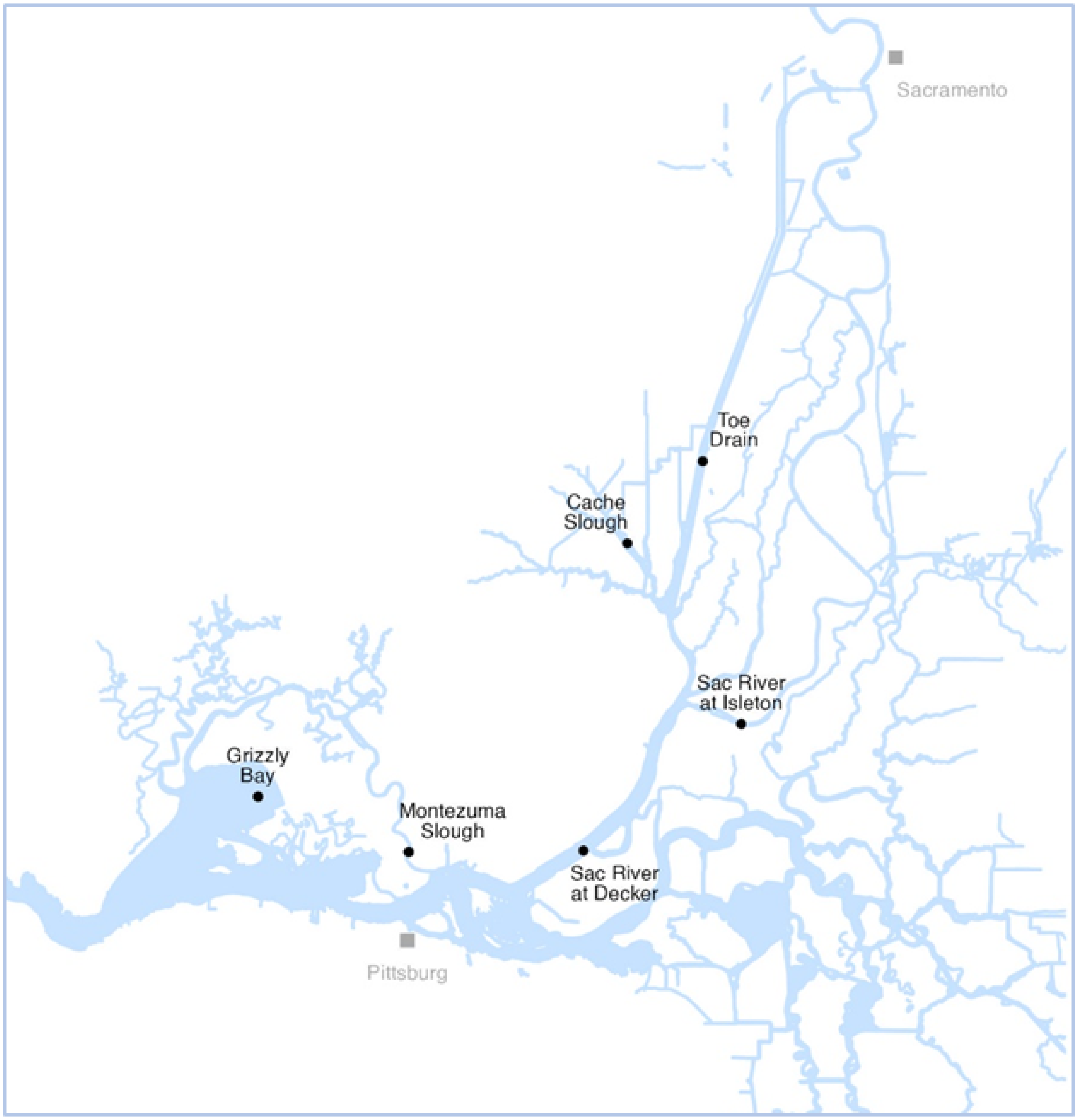
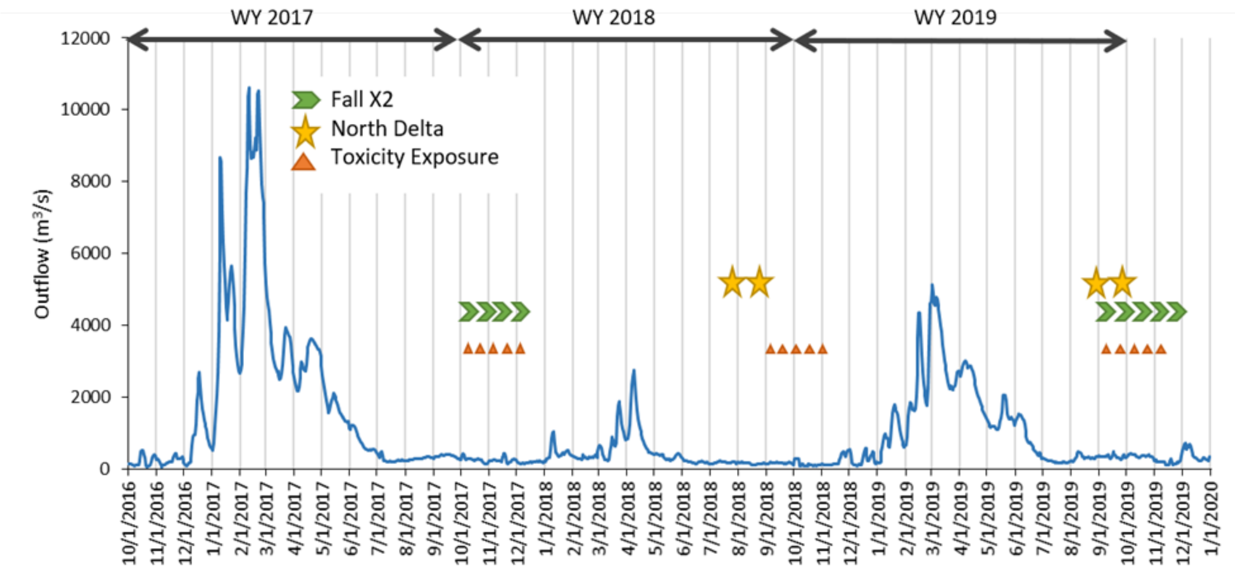
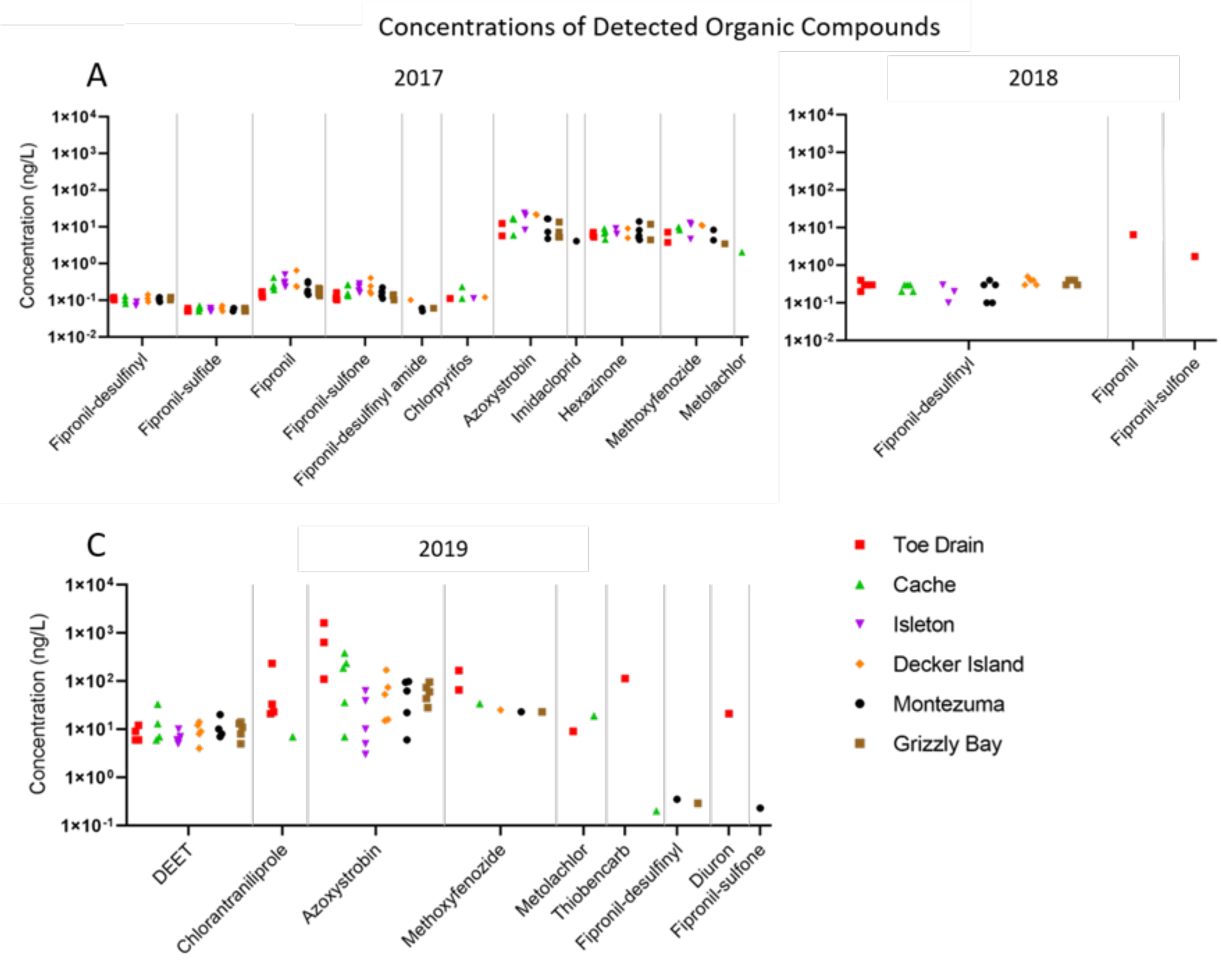


Figure 5-1. Map of Study Area and Sampling Sites in the Sacramento-San Joaquin River Delta and San Francisco Estuary, Located in California, USA



Outflow data obtained from Dayflow California Natural Resources Agency Open Data.

Figure 5-2. Net Delta Outflow (m^3/sec) Encompassing the Three-Year Project Period, Flow Actions, and Exposures



Note the different y-axis in Panel C.

Figure 5-3. Concentrations of Organic Compound Detections Shown for Each Exposure Test from the Six Sites for A) 2017, B) 2018, and C) 2019

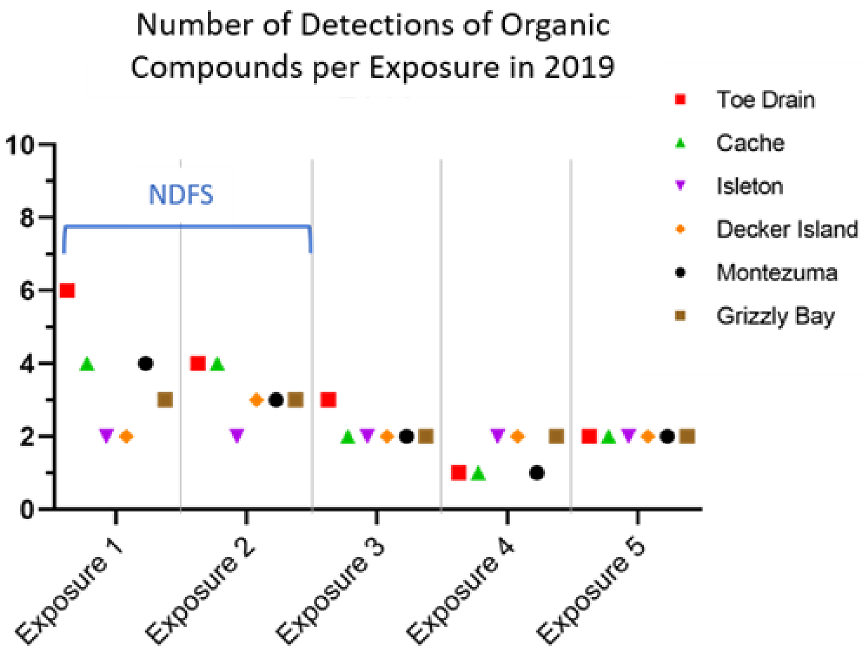
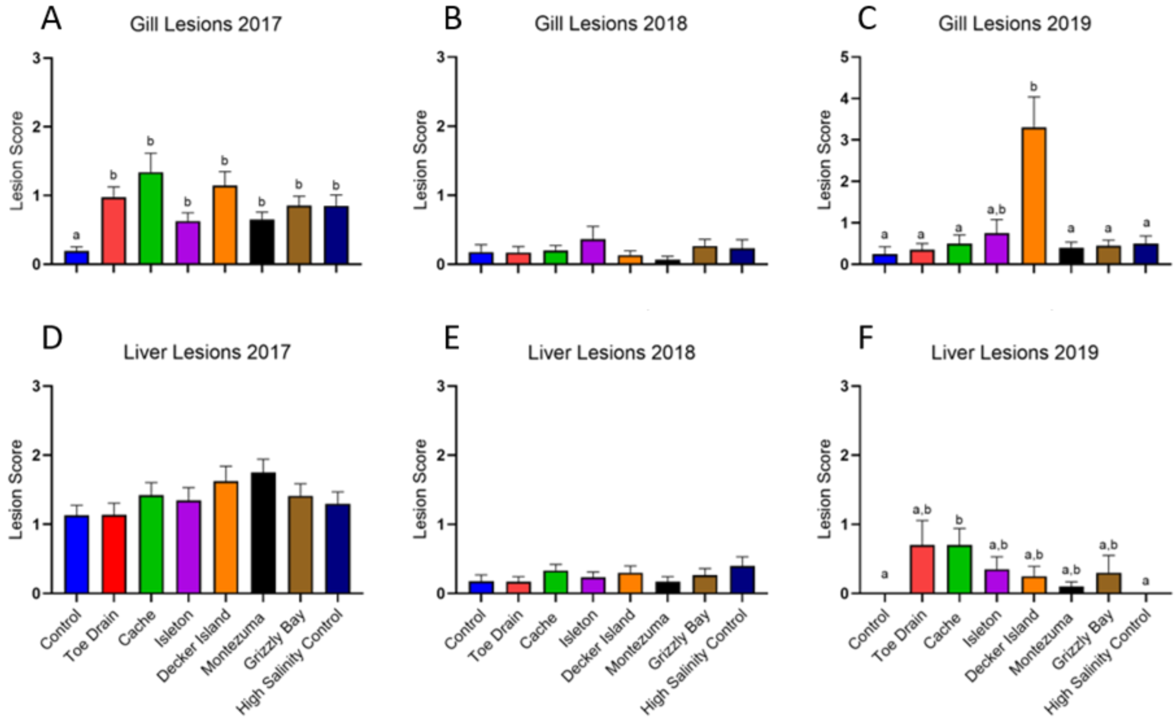
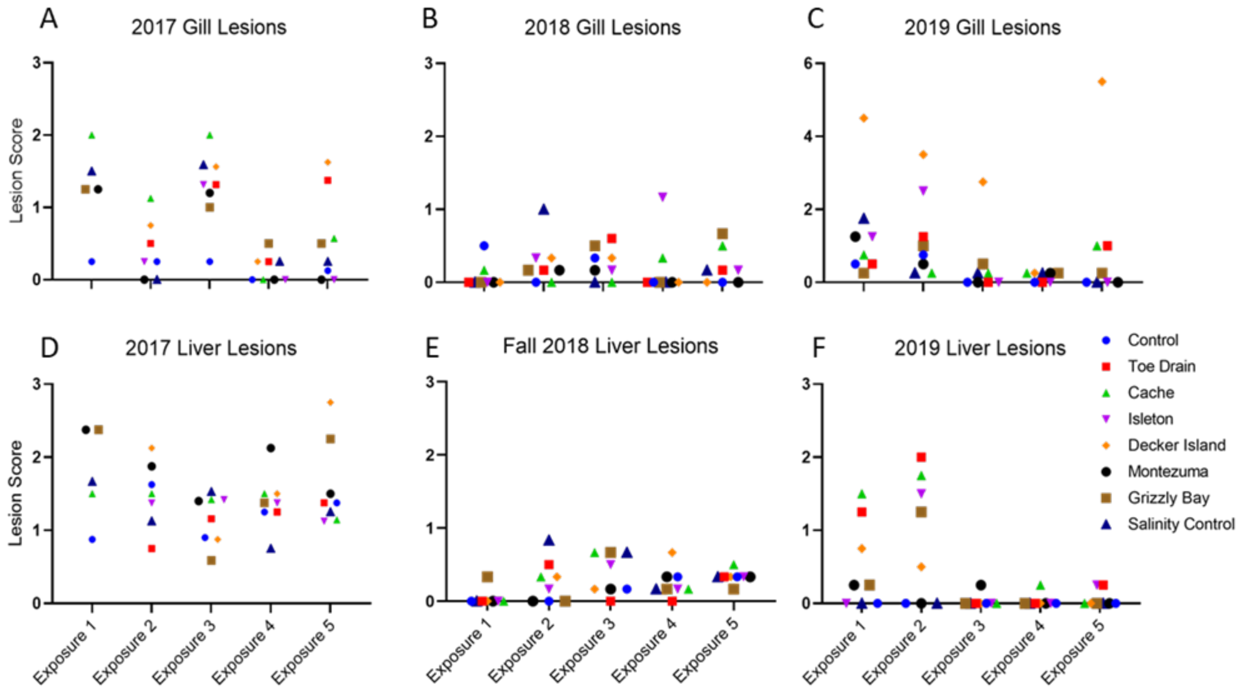


Figure 5-4. Number of Detections of Organic Compounds Per Exposure in 2019



Error bars denote standard error. Different letters indicate significant differences in lesion score across sites. Note the y-axis in Panel C differs from other panels.

Figure 5-5. Summary of Gill Lesion Scores for A) 2017, B) 2018, C) 2019, and Liver Lesion Scores for D) 2017, E) 2018, and F) 2019



Note the y-axis in Panel C differs from other panels.

Figure 5-6. Summary of Gill Lesion Scores for A) 2017, B) 2018, C) 2019, and Liver Lesion Scores for D) 2017, E) 2018, and F) 2019, Across Toxicity Exposures

Supplemental Information

Supplemental Tables

Table S5-1. Summary of Conductivities (µS/cm) of Individual Site Locations.

Exposure	Toe Drain	Cache Slough	Sac River Isleton	Sac River Decker Is.	Montezuma Slough	Grizzly Bay
2017						
1	NS	116	NS	NS	2,785	7,443
2	304	167	132	162	2,765	NS
3	284	178	142	138	3,312	7,544
4	380	163	125	147	3,542	7,344
5	194	203	162	268	3,590	11,113
2018						
1	525	202	125	317	2,577	11,457
2	502	218	126	1,737	8,720	14,663
3	212	169	171	2,651	8,924	14,550
4	207	176	153	2,752	6,977	18,310
5	198	182	155	1,809	4,794	16,554
2019						
1	518	196	159	154	184	4,005
2	567	219	135	146	1,476	7,947
3	248	192	100	134	830	7,387
4	210	190	108	128	214	5,167
5	161	172	103	274	3,079	9,702

NS: Not Sampled during this exposure

Table S5-2. Summary of Contaminants Detected in the Control in 2018 and 2019

Site	Exposure	Analyte	Result (ng/L)
Control 2018	3	Fipronil-desulfinyl	0.2
Control 2018	4	Fipronil-desulfinyl	0.1
Control 2019	1	Fipronil-desulfinyl	0.24
Control 2019	1	Cypermethrin	18.76
Control 2019	1	DEET	6
Control 2019	1	Azoxystrobin	29
Control 2019	2	DEET	10
Control 2019	2	Azoxystrobin	36
Control 2019	2	Manganese	5,000
Control 2019	3	DEET	6
Control 2019	3	Azoxystrobin	23
Control 2019	4	DEET	10
Control 2019	4	Azoxystrobin	18
Control 2019	5	DEET	9
Control 2019	5	Azoxystrobin	8

Table S5-3. Summary of Contaminants Detected Across the 2017 Study Period

Site	Exposure	Analyte	Result (ng/L)
Toe Drain	1	*Site not sampled during this exposure*	
Toe Drain	2	Fipronil-desulfinyl	0.1
Toe Drain	2	Fipronil-sulfide	0.05
Toe Drain	2	Fipronil	0.14
Toe Drain	2	Fipronil-sulfone	0.1
Toe Drain	2	Chlorpyrifos	0.11
Toe Drain	3	Fipronil-desulfinyl	0.11
Toe Drain	3	Fipronil-sulfide	0.05
Toe Drain	3	Fipronil	0.12
Toe Drain	3	Fipronil-sulfone	0.11
Toe Drain	3	Hexazinone	7.06
Toe Drain	4	Fipronil-desulfinyl	0.12
Toe Drain	4	Fipronil-sulfide	0.06
Toe Drain	4	Fipronil	0.17
Toe Drain	4	Fipronil-sulfone	0.12
Toe Drain	4	Chlorpyrifos	0.11
Toe Drain	4	Azoxystrobin	5.67
Toe Drain	4	Hexazinone	5.18
Toe Drain	4	Methoxyfenozide	3.74
Toe Drain	5	Fipronil-desulfinyl	0.11
Toe Drain	5	Fipronil-sulfide	0.06
Toe Drain	5	Fipronil	0.16
Toe Drain	5	Fipronil-sulfone	0.16
Toe Drain	5	Azoxystrobin	12.27
Toe Drain	5	Hexazinone	5.92
Toe Drain	5	Methoxyfenozide	7.15
Cache Slough	1	Fipronil-desulfinyl	0.08
Cache Slough	1	Fipronil-sulfide	0.05
Cache Slough	1	Fipronil	0.19
Cache Slough	1	Fipronil-sulfone	0.15
Cache Slough	1	Azoxystrobin	5.88
Cache Slough	1	Metolachlor	2.02
Cache Slough	2	Fipronil-desulfinyl	0.1
Cache Slough	2	Fipronil-sulfide	0.06
Cache Slough	2	Fipronil	0.23
Cache Slough	2	Fipronil-sulfone	0.13
Cache Slough	2	Chlorpyrifos	0.23
Cache Slough	2	Hexazinone	6.59
Cache Slough	3	Fipronil-desulfinyl	0.1
Cache Slough	3	Fipronil-sulfide	0.06
Cache Slough	3	Fipronil	0.23
Cache Slough	3	Fipronil-sulfone	0.14
Cache Slough	3	Hexazinone	8.95
Cache Slough	4	Fipronil-desulfinyl	0.13

Site	Exposure	Analyte	Result (ng/L)
Cache Slough	4	Fipronil-sulfide	0.06
Cache Slough	4	Fipronil	0.41
Cache Slough	4	Fipronil-sulfone	0.26
Cache Slough	4	Chlorpyrifos	0.11
Cache Slough	4	Azoxystrobin	15.91
Cache Slough	4	Hexazinone	7.48
Cache Slough	4	Methoxyfenozide	8.18
Cache Slough	5	Fipronil-desulfinyl	0.1
Cache Slough	5	Fipronil-sulfide	0.07
Cache Slough	5	Fipronil	0.25
Cache Slough	5	Fipronil-sulfone	0.26
Cache Slough	5	Azoxystrobin	17.15
Cache Slough	5	Hexazinone	4.48
Cache Slough	5	Methoxyfenozide	9.80
Sac. River at Isleton	1	*Site not sampled during this exposure*	
Sac. River at Isleton	2	Fipronil-desulfinyl	0.07
Sac. River at Isleton	2	Fipronil-sulfide	0.05
Sac. River at Isleton	2	Fipronil	0.23
Sac. River at Isleton	2	Fipronil-sulfone	0.18
Sac. River at Isleton	2	Hexazinone	6.37
Sac. River at Isleton	3	Fipronil-desulfinyl	0.07
Sac. River at Isleton	3	Fipronil-sulfide	0.05
Sac. River at Isleton	3	Fipronil	0.31
Sac. River at Isleton	3	Fipronil-sulfone	0.16
Sac. River at Isleton	3	Chlorpyrifos	0.11
Sac. River at Isleton	3	Hexazinone	8.95
Sac. River at Isleton	3	Methoxyfenozide	4.61
Sac. River at Isleton	4	Fipronil-desulfinyl	0.09
Sac. River at Isleton	4	Fipronil-sulfide	0.06
Sac. River at Isleton	4	Fipronil	0.49
Sac. River at Isleton	4	Fipronil-sulfone	0.28
Sac. River at Isleton	4	Azoxystrobin	23.67
Sac. River at Isleton	4	Methoxyfenozide	12.61
Sac. River at Isleton	5	Fipronil-desulfinyl	0.07
Sac. River at Isleton	5	Fipronil-sulfide	0.06
Sac. River at Isleton	5	Fipronil	0.28
Sac. River at Isleton	5	Fipronil-sulfone	0.23
Sac. River at Isleton	5	Azoxystrobin	20.30
Sac. River at Isleton	5	Methoxyfenozide	11.45
Sac. River at Decker Island	1	*Site not sampled during this exposure*	
Sac. River at Decker Island	2	Fipronil-desulfinyl	0.11
Sac. River at Decker Island	2	Fipronil-sulfide	0.06
Sac. River at Decker Island	2	Fipronil	0.24
Sac. River at Decker Island	2	Fipronil-sulfone	0.17
Sac. River at Decker Island	2	Hexazinone	4.92

Site	Exposure	Analyte	Result (ng/L)
Sac. River at Decker Island	3	Fipronil-desulfinyl	0.09
Sac. River at Decker Island	3	Fipronil-sulfide	0.05
Sac. River at Decker Island	3	Fipronil	0.23
Sac. River at Decker Island	3	Fipronil-sulfone	0.15
Sac. River at Decker Island	3	Hexazinone	8.94
Sac. River at Decker Island	4	Fipronil-desulfinyl	0.14
Sac. River at Decker Island	4	Fipronil-sulfide	0.07
Sac. River at Decker Island	4	Fipronil	0.64
Sac. River at Decker Island	4	Fipronil-sulfone	0.4
Sac. River at Decker Island	4	Fipronil-desulfinyl amide	0.1
Sac. River at Decker Island	4	Chlorpyrifos	0.12
Sac. River at Decker Island	4	Azoxystrobin	20.92
Sac. River at Decker Island	4	Methoxyfenozide	10.66
Sac. River at Decker Island	5	Fipronil-desulfinyl	0.09
Sac. River at Decker Island	5	Fipronil-sulfide	0.06
Sac. River at Decker Island	5	Fipronil	0.23
Sac. River at Decker Island	5	Fipronil-sulfone	0.24
Sac. River at Decker Island	5	Azoxystrobin	21.75
Sac. River at Decker Island	5	Methoxyfenozide	11.11
Montezuma Slough	1	Fipronil-desulfinyl	0.09
Montezuma Slough	1	Fipronil-sulfide	0.05
Montezuma Slough	1	Fipronil	0.14
Montezuma Slough	1	Fipronil-sulfone	0.11
Montezuma Slough	1	Fipronil-desulfinyl amide	0.06
Montezuma Slough	1	Azoxystrobin	16.34
Montezuma Slough	1	Hexazinone	13.82
Montezuma Slough	1	Methoxyfenozide	4.30
Montezuma Slough	2	Fipronil-desulfinyl	0.09
Montezuma Slough	2	Fipronil-sulfide	0.06
Montezuma Slough	2	Fipronil	0.18
Montezuma Slough	2	Fipronil-sulfone	0.13
Montezuma Slough	2	Fipronil-desulfinyl amide	0.05
Montezuma Slough	2	Imidacloprid	4.06
Montezuma Slough	2	Hexazinone	5.67
Montezuma Slough	3	Fipronil-desulfinyl	0.1
Montezuma Slough	3	Fipronil-sulfide	0.06
Montezuma Slough	3	Fipronil	0.16
Montezuma Slough	3	Fipronil-sulfone	0.17
Montezuma Slough	3	Azoxystrobin	4.68
Montezuma Slough	3	Hexazinone	8.07
Montezuma Slough	4	Fipronil-desulfinyl	0.12
Montezuma Slough	4	Fipronil-sulfide	0.06
Montezuma Slough	4	Fipronil	0.29
Montezuma Slough	4	Fipronil-sulfone	0.16
Montezuma Slough	4	Azoxystrobin	7.17

Site	Exposure	Analyte	Result (ng/L)
Montezuma Slough	4	Hexazinone	5.12
Montezuma Slough	5	Fipronil-desulfinyl	0.11
Montezuma Slough	5	Fipronil-sulfide	0.06
Montezuma Slough	5	Fipronil	0.32
Montezuma Slough	5	Fipronil-sulfone	0.22
Montezuma Slough	5	Fipronil-desulfinyl amide	0.06
Montezuma Slough	5	Azoxystrobin	16.44
Montezuma Slough	5	Hexazinone	4.39
Montezuma Slough	5	Methoxyfenozide	8.15
Grizzly Bay	1	Fipronil-desulfinyl	0.1
Grizzly Bay	1	Fipronil-sulfide	0.05
Grizzly Bay	1	Fipronil	0.13
Grizzly Bay	1	Azoxystrobin	13.45
Grizzly Bay	1	Hexazinone	11.74
Grizzly Bay	2	*Site not sampled during this exposure*	
Grizzly Bay	3	Fipronil-desulfinyl	0.1
Grizzly Bay	3	Fipronil-sulfide	0.05
Grizzly Bay	3	Azoxystrobin	5.16
Grizzly Bay	3	Hexazinone	4.37
Grizzly Bay	3	Fipronil	0.15
Grizzly Bay	3	Fipronil-sulfone	0.1
Grizzly Bay	4	Fipronil-desulfinyl	0.12
Grizzly Bay	4	Fipronil-sulfide	0.06
Grizzly Bay	4	Fipronil	0.2
Grizzly Bay	4	Fipronil-sulfone	0.13
Grizzly Bay	4	Azoxystrobin	5.37
Grizzly Bay	5	Fipronil-desulfinyl	0.12
Grizzly Bay	5	Fipronil-sulfide	0.06
Grizzly Bay	5	Fipronil	0.21
Grizzly Bay	5	Fipronil-sulfone	0.14
Grizzly Bay	5	Fipronil-desulfinyl amide	0.06
Grizzly Bay	5	Azoxystrobin	7.19
Grizzly Bay	5	Methoxyfenozide	3.39

Table S5-4. Summary of Contaminants Detected Across the 2018 Study Period

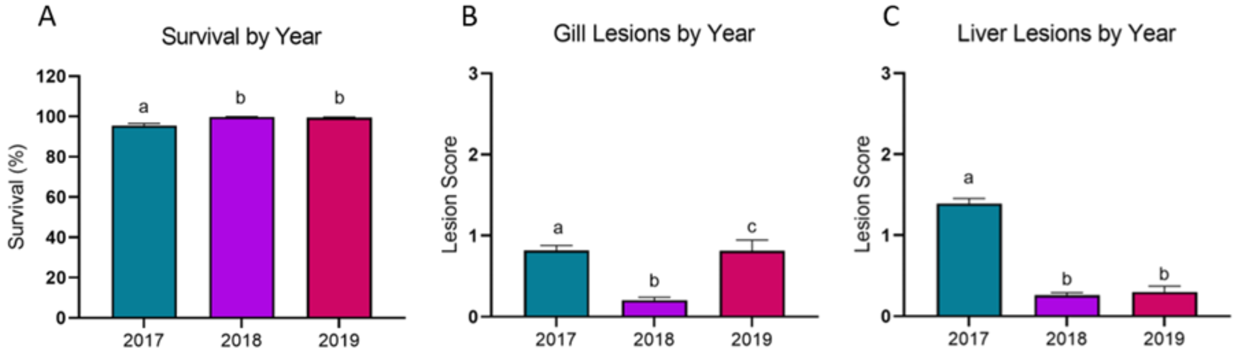
Site	Exposure	Analyte	Result (ng/L)
Toe Drain	1	Fipronil-desulfinyl	0.2
Toe Drain	2	Fipronil-desulfinyl	0.4
Toe Drain	2	Fipronil-sulfone	1.7
Toe Drain	2	Fipronil	6.5
Toe Drain	3	Fipronil-desulfinyl	0.3
Toe Drain	4	Fipronil-desulfinyl	0.3
Toe Drain	5	Fipronil-desulfinyl	0.3
Cache Slough	1	Fipronil-desulfinyl	0.2
Cache Slough	2	Fipronil-desulfinyl	0.2
Cache Slough	3	Fipronil-desulfinyl	0.3
Cache Slough	4	Fipronil-desulfinyl	0.3
Cache Slough	5	Fipronil-desulfinyl	0.3
Sac. River at Isleton	3	Fipronil-desulfinyl	0.1
Sac. River at Isleton	4	Fipronil-desulfinyl	0.3
Sac. River at Isleton	5	Fipronil-desulfinyl	0.2
Sac. River at Decker Island	1	Fipronil-desulfinyl	0.1
Sac. River at Decker Island	2	Fipronil-desulfinyl	0.1
Sac. River at Decker Island	3	Fipronil-desulfinyl	0.3
Sac. River at Decker Island	4	Fipronil-desulfinyl	0.3
Sac. River at Decker Island	5	Fipronil-desulfinyl	0.4
Montezuma Slough	1	Fipronil-desulfinyl	0.3
Montezuma Slough	2	Fipronil-desulfinyl	0.3
Montezuma Slough	3	Fipronil-desulfinyl	0.4
Montezuma Slough	4	Fipronil-desulfinyl	0.4
Montezuma Slough	5	Fipronil-desulfinyl	0.5
Grizzly Bay	1	Fipronil-desulfinyl	0.3
Grizzly Bay	2	Fipronil-desulfinyl	0.4
Grizzly Bay	3	Fipronil-desulfinyl	0.3
Grizzly Bay	4	Fipronil-desulfinyl	0.4
Grizzly Bay	5	Fipronil-desulfinyl	0.4

Table S5-5. Summary of Contaminants Detected Across the 2019 Study Period

Site	Exposure	Analyte	Result (ng/L)
Toe Drain	1	DEET	6
Toe Drain	1	Chlorantraniliprole	33
Toe Drain	1	Azoxystrobin	1601
Toe Drain	1	Methoxyfenozide	166
Toe Drain	1	Metolachlor	9
Toe Drain	1	Thiobencarb	113
Toe Drain	2	DEET	9
Toe Drain	2	Chlorantraniliprole	23
Toe Drain	2	Azoxystrobin	635
Toe Drain	2	Methoxyfenozide	65
Toe Drain	3	DEET	12
Toe Drain	3	Azoxystrobin	231
Toe Drain	3	Diuron	21
Toe Drain	4	Azoxystrobin	110
Toe Drain	5	DEET	6
Toe Drain	5	Azoxystrobin	21
Cache Slough	1	DEET	13
Cache Slough	1	Azoxystrobin	381
Cache Slough	1	Metolachlor	19
Cache Slough	1	Fipronil-desulfinyl	0.20
Cache Slough	2	DEET	7
Cache Slough	2	Chlorantraniliprole	7
Cache Slough	2	Azoxystrobin	186
Cache Slough	2	Methoxyfenozide	34
Cache Slough	3	DEET	33
Cache Slough	3	Azoxystrobin	234
Cache Slough	4	Azoxystrobin	36
Cache Slough	5	DEET	6
Cache Slough	5	Azoxystrobin	7
Sac. River at Isleton	1	DEET	7
Sac. River at Isleton	1	Azoxystrobin	63
Sac. River at Isleton	2	DEET	6
Sac. River at Isleton	2	Azoxystrobin	39
Sac. River at Isleton	3	DEET	10
Sac. River at Isleton	3	Azoxystrobin	10
Sac. River at Isleton	4	DEET	6
Sac. River at Isleton	4	Azoxystrobin	3
Sac. River at Isleton	5	DEET	5
Sac. River at Isleton	5	Azoxystrobin	5
Sac. River at Decker Island	1	DEET	8
Sac. River at Decker Island	1	Azoxystrobin	168
Sac. River at Decker Island	2	DEET	4
Sac. River at Decker Island	2	Azoxystrobin	74
Sac. River at Decker Island	2	Methoxyfenozide	25

Site	Exposure	Analyte	Result (ng/L)
Sac. River at Decker Island	3	DEET	14
Sac. River at Decker Island	3	Azoxystrobin	53
Sac. River at Decker Island	4	DEET	9
Sac. River at Decker Island	4	Azoxystrobin	16
Sac. River at Decker Island	5	DEET	12
Sac. River at Decker Island	5	Azoxystrobin	15
Montezuma Slough	1	DEET	20
Montezuma Slough	1	Azoxystrobin	94
Montezuma Slough	1	Fipronil-desulfinyl	0.35
Montezuma Slough	2	DEET	7
Montezuma Slough	2	Azoxystrobin	98
Montezuma Slough	2	Methoxyfenozide	23
Montezuma Slough	3	DEET	10
Montezuma Slough	3	Azoxystrobin	62
Montezuma Slough	4	Azoxystrobin	6
Montezuma Slough	5	DEET	8
Montezuma Slough	5	Azoxystrobin	22
Grizzly Bay	1	DEET	14
Grizzly Bay	1	Azoxystrobin	73
Grizzly Bay	1	Fipronil-desulfinyl	0.29
Grizzly Bay	2	DEET	11
Grizzly Bay	2	Azoxystrobin	95
Grizzly Bay	2	Methoxyfenozide	23
Grizzly Bay	3	DEET	8
Grizzly Bay	3	Azoxystrobin	43
Grizzly Bay	4	DEET	13
Grizzly Bay	4	Azoxystrobin	60
Grizzly Bay	5	DEET	5
Grizzly Bay	5	Azoxystrobin	28

Supplemental Figures



Error bars represent standard error. Bars with different letters indicate significant differences Survival was analyzed with a Welch's ANOVA followed by a Dunnett's T3 multiple comparison test. Gill and liver lesion scores were analyzed with a Kruskal-Wallis Rank Sums test followed by a Steel-Dwass multiple comparisons test.

Figure S5-1. Summary of Delta Smelt Endpoints Compared Among Years: A) Survival, B) Gill Lesions, and C) Liver Lesions

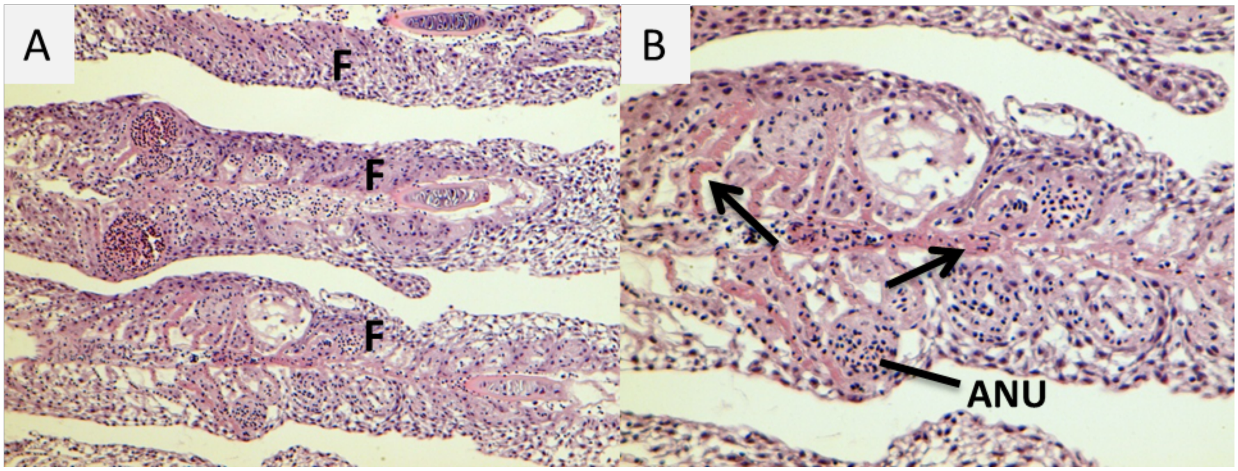
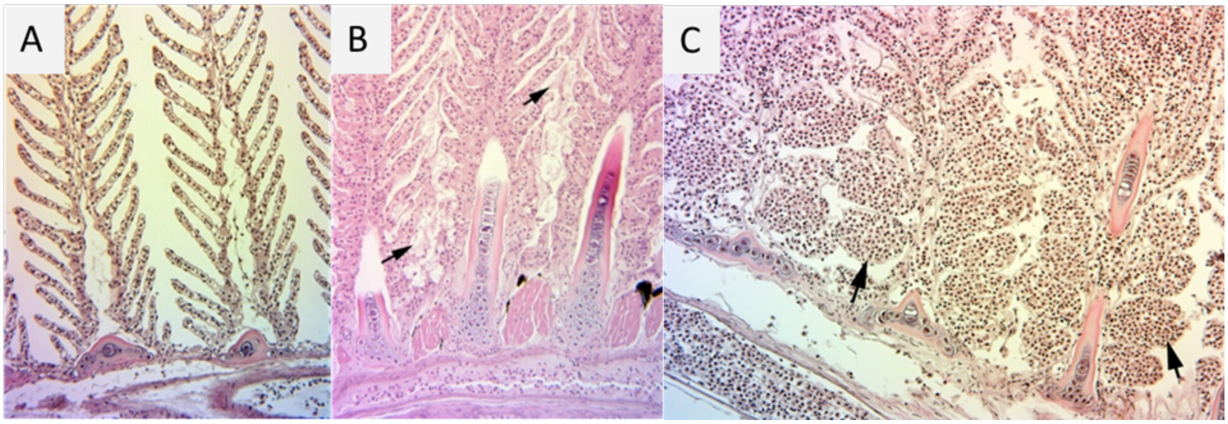


Figure S5-2. A) Severe Secondary Lamella Fusion (F) in 3 Gill Filaments at Low Magnification; B) Higher Magnification Showing Moderate Epithelial Cell Necrosis (arrows) and Aneurysm (ANU) in Delta Smelt Exposed to Water Collected from Cache Slough in a Toxicity Exposure Initiated on October 27, 2017



A) Typical regular thin gill lamellae structure of Delta Smelt exposed to FCCL water (Control). B) Severe mucous cell hyperplasia in gill exposed to Cache Slough water. Arrows point to mucus discharges filling the space of primary and secondary lamellae. C) Severe gill aneurysm or telangiectasia (arrows).

Figure S5-3. Delta Smelt Exposed to Water from Cache Slough in a Toxicity Exposure Initiated on November 10, 2017

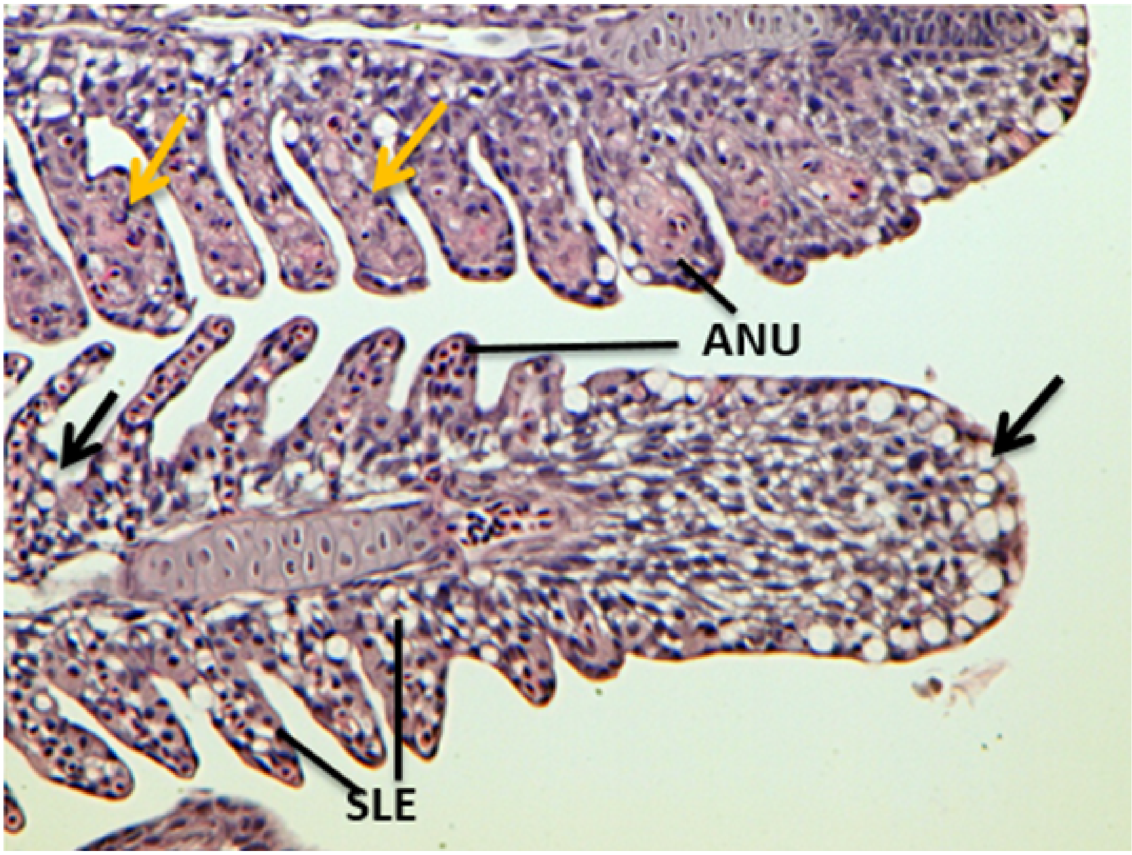


Figure S5-4. Mucous Cell Hyperplasia (black arrows), Epithelial Cell Necrosis (yellow arrows), Small Aneurysm (ANU), and Edema (SLE) in Secondary Lamella of Gill in Delta Smelt Exposed to Water Collected from the Sacramento River at Decker Island (200× magnification) in a Toxicity Exposure Initiated November 8, 2019

Chapter 6. Novel Field Enclosures Inform the Conservation of a Critically Endangered Fish

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Lay Summary: Nine deployments of caged, hatchery-reared Delta Smelt demonstrated that health and survival in the San Francisco Estuary are generally high but are also affected by season and region. Summer deployments are particularly challenging for the species. We also uncover variation in laboratory compared to field measures of thermal tolerance, with important management implications.

Abstract

Delta Smelt, an endemic, euryhaline osmerid of the San Francisco Bay Estuary (Estuary), California, USA, is an endangered fish. In an effort to establish methodology to understand how future management actions and population supplementation may benefit the species, hatchery-reared Delta Smelt were deployed in experimental cages throughout the Estuary in the winter, summer, and fall of 2019 to first determine how hatchery fish fare in a more natural environment. Except during winter, the caged fish were compared to reference fish at the hatchery of the same age. Effects of season and location of cage deployments on fish health (condition factor and histological condition of liver and gill), growth, thermal tolerance, and survival were evaluated. The results indicate both seasonal and location differences, with high survival in the winter (100%) and fall (88-92%), and in one case deployment of fish in the winter had improved condition compared to reference fish at the hatchery. Deployments were less successful in summer (67% survival), with one of the study sites having no surviving fish following high temperature exposure. Surviving Delta Smelt in summer and fall showed clear signs of nutritional stress by 29 days that may be related to biofouling of the cages limiting passive food inputs, restriction of natural foraging behavior by containment in the cages, and/or chronically low pelagic productivity in the Estuary overall. Field measurements of upper thermal tolerance (CTmax) following caging exposures suggest that laboratory measures of upper temperature tolerance may overestimate the realized tolerance in a more stochastic field environment. This study demonstrates the feasibility and utility of using cages as an experimental tool to better understand Delta Smelt responses to environmental changes in a more natural-field setting, and that hatchery Delta Smelt can survive throughout the year when caged in the wild at water temperatures below ca. 25°C.

Key Words: Delta Smelt, physiology, zooplankton, thermal tolerance, temperature, habitat, season, cage, wild, survival

Introduction

Species are increasingly at risk of extinction due to a suite of changing environmental conditions. Global extinction rates of eukaryotes average ~100 extinctions per million species years, roughly 1,000 times the pre-human species extinction rate (Pimm et al. 2014). The loss of biodiversity is more rapid in freshwater than in terrestrial ecosystems (Leidy and Moyle 2021). Fish in rivers and lakes in North America, for example, are becoming extinct at three times the global rate, or 305 extinctions per million species years (Pimm et al. 2014). Fish species in California are particularly imperiled; 5% of its native inland fishes are extinct and 78% are declining (Moyle et al. 2011). These declines are thought to be related to naturally narrow or fragmented ranges of many species in combination with profound anthropogenic influence (Moyle 2002). Such anthropogenic influences include habitat degradation and loss through channelization, damming, and water extraction, increased water temperature due to climate change, invasive species, reduced food availability and degraded water quality from nutrient and contaminant inputs (Moyle 2002; Mac Nally et al. 2010, Moyle et al. 2011).

Conservation actions designed to support imperiled fishes include direct restoration of habitat and/or indirect enhancements of key habitat attributes (e.g., access to historical habitat, food, improved water quality and passage) (Sommer et al. 2020b; Serra-Llobet et al. 2022). For example, a levee was moved away from the Consumnes River, California, reuniting the river with a portion of its historical floodplain, thereby increasing riparian vegetation and habitat for floodplain dependent species (Serra-Llobet et al. 2022). In a second example, a channelized reach of the Merced River that was dredged for gold was restored with a mixture of gravels and cobbles, resulting in increased Chinook Salmon (*Oncorhynchus tshawytscha*) spawning and use during both droughts and floods (Brown et al. 2022). However, similar efforts may be insufficient to support population resiliency for some species, making conservation measures such as reintroduction, translocation, and population supplementation necessary. In cases where a species is already rare or endangered, hatcheries may be developed to maintain a refugial population and those captive bred fish may be used to supplement the wild population (Johnson and Jensen 1991). However, questions quickly arise about how hatchery-reared fish will transition to stochastic environmental conditions in the wild, including whether the released fish will feed, grow, survive, and reproduce.

It can be challenging to evaluate the effectiveness of habitat restoration (Diefenderfer et al. 2021) and supplementation actions meant to benefit a rare species because field surveys, the commonly applied approach, may have only a handful of observations. However, observing hatchery populations in controlled but natural settings using enclosures, can provide a first step towards reintroduction or supplementation as well as being important tools for studying environmental preferences and life-history, contaminant exposure, and to inform adaptive management of species. For example, enclosures were used to identify limiting factors of endangered Razorback Suckers (*Xyrauchen texanus*) and Bonytail (*Gila elegans*) on the Green River, Utah (Christopherson et al. 2004). Similarly, cages have been used to test morphological variation in hatchery-reared, endangered June Suckers (*Chasmistes liorus*) in a more natural environment in Provo Bay of Utah Lake (Belk et al. 2008). Aha et al. (2021) used cages to compare habitats for rearing juvenile Chinook Salmon in Suisun Marsh, California, while Lusardi et al. (2020) examined relationships among growth, water temperature, and prey availability of Coho Salmon (*Oncorhynchus kisutch*) caged along a spring-fed river in the Shasta River basin. Enclosures can even be used as an alternative to hatcheries for breeding endangered fishes (Billman and Belk 2009), and may be a useful tool for 'soft-release' of supplementation populations into the environment to increase post-release survival (Tetzlaff et al. 2019; Baerwald et al. 2023). Thus, cages offer a compromise between the controlled conditions of laboratory experiments and the more realistic but uncontrolled conditions of natural experiments (*sensu* Diamond, 1983).

The Delta Smelt (*Hypomesus transpacificus*), is a small, pelagic, euryhaline osmerid that is endemic to the Sacramento-San Joaquin Delta and San Francisco Bay Estuary, California (referred to as 'the Estuary' hereafter). Delta Smelt were once one of the most abundant pelagic fishes in the Estuary but have declined steeply since the 1970's and are now exceedingly rare in the wild (Bennett 2005; Hung et al. 2022) due to a combination of anthropogenic stressors (e.g., habitat loss, water exports, food limitation, invasive species, reviewed in Yanagitsuru et al. 2022). Delta Smelt were listed under the federal and state Endangered Species Act (ESA) as threatened in 1993 (USFWS 1993) and uplisted to endangered on the California ESA in 2009 (CDFW 2023). Following the ESA-listing, a captive breeding program for the species began, with a founding population of wild-caught Delta Smelt in 2008 (Lindberg et al. 2013) resulting in nearly 15 years of a genetically managed refuge population. In addition to hatchery conservation efforts, resource managers have also adaptively managed a variety of habitat actions (Sommer et al. 2020a) to improve suitable habitat conditions and resiliency for Delta Smelt (Jassby et al. 1995; Nobriga et al. 2008; Hobbs et al. 2019; Yanagitsuru et al. 2022). These habitat actions include: tidal operation of a salinity control gate to freshen Suisun Marsh (Sommer et al. 2020b), increased freshwater flows to shift the salinity field seaward (Kimmerer et al. 2013; Feyrer et al. 2011; Hassrick et al. 2023), and redirecting Sacramento River water or agriculture drainage into the Yolo Bypass to export and/or increase plankton in the lower Sacramento River (Frantzich et al. 2021).

Given the scarcity of Delta Smelt in the wild, it is nearly impossible to evaluate the efficacy of these management actions using specimens collected during fish monitoring and survey efforts. Therefore in 2019, in situ enclosures for hatchery Delta Smelt were designed (Gille et al. in press) and deployed to ask whether hatchery Delta Smelt could transition to and survive in the wild and further, how habitat actions may benefit Delta Smelt (Baerwald et al. 2023). Enclosures were carefully tested and constructed around the unique physiological and behavioral requirements of Delta Smelt (Gille et al. in press), and the first deployment study (Baerwald et al. 2023) occurred at the same time as supplementation of the wild Delta Smelt population with hatchery-raised Delta Smelt was being considered. Questions regarding how to best release fish into the wild quickly emerged, including when, where and how fish should be introduced and acclimated in cages before release (soft vs. hard release).

The high survival of hatchery-reared Delta Smelt during the initial Baerwald et al. (2023) enclosure study paved the way for the use of enclosures as a tool to evaluate management actions but also provides an opportunity to field-acclimatize smelt in enclosures to assess key fitness-related traits such as growth, reproduction, survival, and tolerance (Boisclair and Sirois 1993; Fangue and Bennett 2003; Johnsson et al. 2000). Previous studies of thermal physiology of marine fishes suggest a difference between laboratory acclimation and field acclimatization, but effects may differ depending on the variability of the habitat or specific species (Fangue and Bennett 2003; Fangue et al. 2011). While environmental variables such as salinity and turbidity influence Delta Smelt distributions in the Estuary (Sommer and Mejia 2013), water temperature, as with other fishes (Fry 1947), is likely the most important factor impacting Delta Smelt physiology, behavior, and habitat use. Field catch and laboratory acclimation studies indicate Delta Smelt are particularly sensitive to temperature, with sublethal stress demonstrated above 20°C, food consumption limitations above 22°C, and limited survival above 25°C (Sommer and Mejia 2013; Komoroske et al. 2014; Jeffries et al. 2016; Davis et al. 2019a, 2019b; Smith and Nobriga 2023); however, no field-acclimatization studies have occurred since the initial Baerwald et al. (2023) study leaving gaps in our understanding. Continued increases in water temperature in the Estuary due to climate change or other anthropogenic factors will challenge Delta Smelt persistence, regardless of supplementation efforts, by compression of suitable thermal habitat (particularly in summer and fall) (Brown et al. 2016; Bashevkin et al. 2022; Mahardja et al. 2022). The ability to collect field-acclimatized information for Delta Smelt which may differ from laboratory-gained data in important ways may influence current assessments of where, for example, thermally suitable habitat for field deployed Delta Smelt might be.

The overall goal of this study was to evaluate the feasibility and utility of enclosure deployments in the Estuary including assessing the temporal and spatial effects on caged Delta Smelt physiology. We also compared thermal tolerance of field-acclimatized Delta Smelt to historical laboratory data of Delta Smelt to inform application of thermal physiology to management of the species. We investigated the following questions:

1. Does the season or location of cage deployments influence Delta Smelt health including gill and liver condition, growth, and survival?
2. Does the upper temperature tolerance of caged Delta Smelt vary across caging locations?
3. Are field measures of temperature tolerance congruent with previous laboratory studies?

These study questions were evaluated using cage deployments in winter, summer, and fall of 2019 at four sites throughout the range of Delta Smelt. We predicted that Delta Smelt would show signs of compromised health in summer and lower survival than during other seasons, and that condition would vary across locations in a single season. We also predicted differences in field- and laboratory-derived thermal tolerance measures, which are important to understand in order to inform resource managers that are often using thresholds from laboratory studies alone.

Methods

Field Sites and Deployments

Four study sites were selected in the Estuary: Rio Vista, Sacramento River Deepwater Ship Channel, Yolo Bypass, and Suisun Marsh. These sites represent different habitat types, and routine monitoring surveys have captured Delta Smelt at all sites in the last ten years (2013-2023) (Bashevkin et al. 2022;

USFWS 2023). The Rio Vista (RV) site is on the lower main stem of the Sacramento River, adjacent to the Rio Vista Army Base (Figure 6-1). The location is used by migratory Delta Smelt (the dominant phenotype) to reach freshwater spawning habitat in the winter/spring or brackish water rearing habitat in the summer (Moyle et al. 2016, Hobbs et al. 2019). The Sacramento River Deepwater Ship Channel (SRDWSC) is a freshwater, constructed channel that provides ships access to the Port of West Sacramento. It acts as a dead-end slough and is the primary location where Delta Smelt were found in the wild across multiple seasons in recent years (Merz et al. 2011; Mahardja et al. 2022; Davis et al. 2022). The 61-kilometer-long, 24,000 ha engineered Yolo Bypass (YB) is a major floodplain of the Sacramento River basin where Delta Smelt were historically detected year-round in its perennial eastern canal (Sommer et al. 2001, Moyle et al. 2016, Mahardja et al. 2019, Stompe et al. 2020), however, the last detection of Delta Smelt was in 2017 (Pien and Kwan 2022). Suisun Marsh (SM) comprises approximately 85,000 acres of tidal marsh, managed wetlands, and waterways in southern Solano County. It is the largest remaining wetland near San Francisco Bay and includes more than ten percent of Calfornia's total remaining wetland area. Suisun Marsh is a wildlife habitat of nationwide importance (Suisun Marsh Preservation Act of 1977), and is considered relatively high-quality habitat for Delta Smelt when salinity is appropriate (Hammock et al. 2015; Moyle et al. 2016). The University of California Davis Fish Conservation and Culture Laboratory (FCCL) in Byron, CA was also used as the hatchery reference site, where a captive refuge population of Delta Smelt has been maintained since 2008 (Lindberg et al. 2013).

There were three deployment periods in 2019, winter/spring (January to March, hereafter winter), summer (July to August), and fall (October to November). The availability of the enclosures increased during the study as more cages were constructed, allowing for more deployments. For the winter deployments, enclosures were deployed at RV (n = 3 cages) and at SRDWSC (n = 3) as in Baerwald et al. (2023), for summer deployments they were deployed at RV (n = 3) and YB (n = 1), and fall at RV (n = 3), YB (n = 3), and SM (n = 3; Figure 6-1, Figure 6-2b, Table 6-1). The winter deployment period was included to provide reliably cool water temperatures (range of 8-12°C, Table 6-2; Figure 6-3) and to correlate with the timing of future experimental releases for supplementation of Delta Smelt to the wild. Enclosure deployments were used in the summer and fall to support 1) the future evaluation of habitat and food management actions (e.g., overlap of suitable key physical attributes for Delta Smelt; salinity <6 practical salinity units (PSU), turbidity >12 Formazin Nephelometric Unit (FNU), and water temperature <22-25°C [Sommer and Mejia 2013; Smith and Nobriga 2023]), 2) potential efficacy of summer-fall supplementation releases, and 3) test enclosure effects and temperature tolerance of Delta Smelt following field acclimation (range of 15-26°C, Table 6-2; Figure 6-3).

Enclosure Design

Each enclosure was built as described in Baerwald et al. (2023) and allowed access to pelagic prey and exposure to ambient environmental conditions while preventing escape of Delta Smelt. Three replicate enclosures were deployed at each site (Figure 6-1) except for the summer at Yolo Bypass that only had one enclosure due to limitations in the enclosures available at the time. The enclosures were 1.22 meters (m) in height and 0.95 m in diameter (Figure 6-2). The winter and summer deployments used enclosures made of steel mesh painted with black marine-grade paint (Rust-oleum Topside). The fall deployments used enclosures made of aluminum mesh to make them lighter and were powder coated black to improve the surface coat quality. The steel or aluminum mesh consisted of 3.18-millimeter (mm) holes on 4.76 mm centers, providing 41% openness. For all deployments except for Yolo Bypass, three enclosures were attached to each other, in a line parallel to shore, using

3 m of 6.35 mm stainless steel cable (encased in PCV pipe for rigidity) between enclosures and anchoring system (described in Baerwald et al. 2023). The resulting system was able to move vertically with the tides while maintaining the same relative distance between enclosures (Figure 6-2). The single Yolo Bypass enclosure was tethered between the pontoons of the Yolo Bypass Fish Monitoring Program's rotary screw trap (Figure 6-1), which was not fishing during the period of deployment.

Species and pre-deployment preparation

Delta Smelt were obtained from the FCCL (i.e., Delta Smelt hatchery) and acclimated to seasonal water quality conditions and live prey (see below) prior to being transferred to the field in winter, summer, and fall. All procedures and experiments abided by animal welfare considerations (IACUC# 21610) and permit regulations (USFWS TE-027742, CDFW CESA MOU# 2081a-2018-0007-R3).

As described in Baerwald et al. (2023), the Delta Smelt for both winter deployments (Rio Vista and SRDWSC) were switched from temperature-controlled water (16°C) to unfiltered ambient water at 206 days post hatch (dph), from dry pelletized food to live *Artemia* at 207-210 dph and acclimated to higher flows (one day at 15 centimeters per second (cm/s), and the following days at 31 cm/s) at 223-224 dph. All fish also received Visible Implant Alphanumeric (VIA) tags for individual identification at 207 dph, were weighed, and fork length was measured (mean ± std; fork length 5.9 ± 0.5 centimeter(cm), weight 1.4 ± 0.4 g). 384 Delta Smelt were driven to Rio Vista on January 23, 2019 (243 dph) and 360 fish were driven to SRDWSC on February 27, 2019 (278 dph). See Table 6-1 for deployment details. All fish were transported in insulated, black 19L buckets with screw-top lids at a density of 34 (RV; 1.8 fish/L) and 30 (SRDWSC; 1.6 fish/L) fish per bucket (Baerwald et al. 2023). The buckets were filled with ambient water at the FCCL (i.e., from the CA aqueduct), which was raised to 5 PSU salinity using Instant Ocean and saturated with oxygen to decrease transport stress. Upon arrival at each site, buckets were loaded onto a boat and driven to the enclosures. Two buckets of fish were emptied into each enclosure (68 and 60 fish/enclosure, RV and SRDWSC, respectively) using a water-to-water transfer after providing one minute of water exchange between each bucket and the ambient water within each enclosure. These densities were selected to balance food availability with sufficient density to allow for normal shoaling behavior (Baerwald et al. 2023).

For the summer and fall deployments, Delta Smelt from the FCCL underwent a controlled temperature increase from 16°C to 18.5°C one week prior to deployments to acclimate them to the warmer temperatures at the field sites. These fish were unable to be VIA tagged due to their smaller size in summer (mean ± std; fork length 4.9 ± 0.4 cm, weight 0.82 ± 0.24 g). Instead, all fish were adipose fin clipped to allow identification of hatchery-origin fish in case of escape. A subsample of 60 fish that were not going into the field were weighed and measured one day prior to field deployment to provide average pre-deployment measurements. On July 30, 2019, 240 Delta Smelt were transported to RV (3 enclosures) and YB (1 enclosure; 194 dph). On October 9, 2019, 540 fish were transported to RV, YB, and SM (3 enclosures each site; 264 dph). Fish were transported and loaded into enclosures using the same methods as for the winter deployments with 30 fish per transport bucket and 60 fish/enclosure replicate. See Table 6-1 for deployment details.

Field Monitoring

Each enclosure was checked periodically throughout the deployment. During winter the enclosures were checked each weekday. Due to transport and temperature mortality concerns, for the summer and fall deployments, enclosures were checked daily during the first week and then weekly thereafter. During each check of the enclosures, the water surface was examined for mortalities (for survival analysis). Mortalities were measured for length and weight (when possible) and stored on ice prior to transferring to a -20ºC freezer. Mortalities with degraded bodies were not measured. Water quality and within cage water velocity, as well as zooplankton were collected adjacent to the enclosures.

Water quality

During each enclosure check, a YSI Pro DSS was used to collect discrete measurements of water temperature (°C), dissolved oxygen (DO, milligrams per liter (mg/L)), specific conductivity (µSiemens/cm), pH, and turbidity (FNU). Secchi depth (m) was also measured. Velocity spot samples (m/s) were collected inside cages using a Hach FH950 portable handheld velocity meter and averaged over three 10 s readings. In addition to discrete measures, continuous water quality data were collected from multiparameter YSI EXO2 sondes that were nearest to the respective enclosure sites. Sondes recorded water quality data every 15 minutes for the entire year at Beldon's Landings (BDL) in Suisun Marsh, Sacramento River at Rio Vista Bridge (RVB), Lisbon Weir (LIS) in the Yolo Bypass, and SRDWSC. Information about continuous sensors from the Department of Water Resources and US Geologic Survey are provided in Supplementary Table 6-1.

Zooplankton

Zooplankton samples were collected twice a week using a SEA-GEAR conical 0.5 m × 2 m plankton net with 53 micrometers (µm) mesh with a General Oceanics flowmeter suspended in the mouth (CDFW SCP S-182970002-20219-001). The net was towed below the surface of the water for two minutes, within 15 m of the enclosures. Occasionally, due to rough water conditions during the Winter deployment at Rio Vista, a smaller (0.3 m × 1 m, 53 µm mesh) plankton net was used instead and hand-towed 5-6.5 m along a dock 100 m upstream of the enclosures, however, because these methods both use the same mesh and include volume estimates, the data are comparable. Samples were stored in 1 L wide-mouth Nalgene bottles and preserved with 5% formalin and dyed with Rose Bengal. Samples were sent to BSA Environmental Services, Inc. (Beachwood, OH USA) for enumeration and identification of mesozooplankton and microzooplankton as described in Frantzich et al. (2021). Samples were identified to genus for cladocerans, order for harpacticoids, and species and lifestage for calanoid and cyclopoid copepods. Microzooplankton were also enumerated, including rotifers, barnacles, copepod nauplii, cladocera nauplii, unidentified nauplii, and ostracods.

Post-deployment Fish Sampling and Health Metrics

Following each deployment period, all surviving fish were collected from each enclosure and transported in 19 L insulated black buckets to shore (RV, SM, YB) or a laboratory (SRDWSC) where they were euthanized for growth measurements, dissections and/or tissue preservation. Each group of fish was euthanized with an overdose of buffered Tricaine Methanesulfonate (MS-222) after which they were weighed (grams (g)) and measured (mm fork length) for growth calculations (pre- and post-deployments). The carcass was preserved in 10% neutral buffered formalin. Formalin preserved bodies of up to 10 fish per enclosure were subsequently dissected in the laboratory, removing the stomach, liver, and left gill arches. Livers were weighed with an analytical balance and liver and gills were fixed in buffered formalin for histopathology. Stomachs were sent to the Wetland Ecosystem Team laboratory (Seattle, Washington USA) for gut content identification and later diet analysis. Following the summer and fall deployments but before euthanasia, a subsample of 6-9 fish per enclosure (summer) and 15 fish per enclosure (fall) were transported to shore for measures of critical thermal maximum described in the 'Temperature Tolerance' section below.

Diets

Diets of Delta Smelt collected from enclosures were assessed across sites and seasons. The total contents of each stomach were weighed (to 0.0001 g), identified to the lowest taxonomic resolution possible given the extent of digestion, and sorted into life stages when the diagnostic characteristics were identifiable.

Histopathology

Histopathology was performed on gill and liver tissues of Delta Smelt according to Teh at al. (1997). Briefly, tissues were dehydrated in an ethanol series and embedded in paraffin, sectioned to 3-µm thickness and stained with hematoxylin and eosin. Livers were screened for glycogen depletion, fatty vacuolar degeneration, single cell necrosis, inflamation, macrophage aggregate, cytoplasmic inclusion bodies, and sinusoid congestion as in Teh et al. (2020). Gills were screened for gill lamellar aneurysm, ionocyte hyperplasia, mucous cell hyperplasia, epithelial cell hyperplasia/hypertrophy, secondary lamela edema, gill epithelial cell necrosis, fusion, and inflamation (Teh et al. 2020). Lesions were scored on a scale of 0-3, where 0 = not present, 1 = mild, 2 = moderate, and 3 = severe. The liver and gill alterations and scoring criteria are described in detail in Teh et al. (2020).

To provide an overall metric of liver condition and a second overall metric for gill condition, liver lesions were summed to produce a liver lesion score and gill lesions were summed to produce a gill lesion score for each fish (Hammock et al. 2015, Teh et al. 2020). Liver lesion score for an individual was the summation of the liver alterations listed above except glycogen depletion, which was analyzed seperately because it is not a lesion. Gill lesion score was calculated as the summation of the gill alterations listed above (Teh et al. 2020).

Temperature Tolerance

Upper temperature tolerance was determined for cultured Delta Smelt after 4 weeks of field acclimatization in the enclosures using critical thermal methodology (CTmax; Beitinger et al. 2000), with modifications for field application. CTmaxima were conducted on a subset of caged Delta Smelt from Rio Vista (n=9 fish) during summer and Rio Vista (n=10 fish) and Suisun Marsh (n=10 fish) during fall. Each fish was removed from the field enclosure and placed into a 1.25 L chamber, contained in a water bath, at the water temperature of each location (summer RV = 21.0°C, fall RV = 14.2°C, fall SM = 13.5°C) for a 30-min acclimation period. After 30 min, the water bath was heated by two 800 W titanium heat bars, at a rate of 0.3°C per minute, following recommendations by Becker and Genoway (1979). After a loss of equilibrium, fish were quickly removed from their chambers and placed in recovery containers at site temperature; the temperature at which the loss of equilibrium was observed was recorded as the CTmax. In previous thermal tolerance studies of Delta Smelt, fish were given a 24-hour period to recover (Komoroske et al. 2014; Davis et al. 2019a). However, our study was done outside of a laboratory, and therefore, due to in-field constraints the recovery period was reduced to 4 hours. A handling control (n=5 fish) was conducted during the fall experiments at Rio Vista and Suisun Marsh to ensure that post-CTmax mortalities were due to high water temperature and not to handling. During the summer Rio Vista experiment, one fish was injured prior to acclimation and was not included in the final data set (n=8 fish).

To gain a better understanding of the thermal tolerance of Delta Smelt and to compare our work to previous CTmax laboratory studies, a comparative analysis of the upper thermal tolerance and acclimation potential (i.e., the rate at which fish can acquire thermal tolerance), of laboratory and field studies was completed. All known CTmax data for juvenile Delta Smelt was compiled into a single dataset and CTmax methodology was similar in all the studies. This data set included data from Swanson et al. (2000) on wild-caught Delta Smelt, data from hatchery reared smelt collected in the laboratory (Komoroske et al. (2014) and Davis et al. (2019a)) and the present study using field acclimatized fish exposed to natural variation in temperature and other factors such as turbidity and salinity.

Statistical Analysis

Survival is reported as the percentage of live fish collected at the end of each deployment out of the number introduced to each enclosure. We did not test effects of winter deployments (RV and DWSC) on growth metrics because these were assessed in Baerwald et al. (2023), but descriptive statistics (means, std, etc.) and figures are provided in the Supplementary Materials (Table S6-2 and Figure S6-2). Similarly to Baerwald et al. (2023) we used t-tests to assess whether weight, fork length, and condition factor (K) varied between pre-deployment subset Delta Smelt at FCCL and post-deployment fish at summer and fall sites.

To evaluate seasonal effects on caged fish, we analyzed proportional change in condition factor and change in weight at Rio Vista for each season, whereas to determine the effect of enclosure location we compared proportional change in condition and weight in the fall season between sites (i.e. RV, SM, YB, and FCCL). Because pre-deployment fish in the summer and fall were not the same fish used in the field enclosures, we could not calculate individual fish growth metrics. Therefore, we calculated pre-deployment averages for weight and K, then subtracted the averages from each individual's post-deployment weight and K. We accounted for differences between the starting size of fish across deployments by calcullating porportional changes in Weight and K, which we called deltaWeight and deltaK. Winter data were acquired from Baerwald et al. (2023), which had six enclosures in their design to test three cage protoypes (two replicates of small, large, and wrapped mesh sizing); however, the small and large results were similar, with the small mesh used in all later enclosure deployments in summer and fall. We filtered winter data to three enclosures (two small + one large) to match the number of enclosures in summer and fall and calculated deltaWeight and deltaK. We analyzed the effect of season and site with a random effect of enclosure on each individual's deltaWeight and deltaK using a mixed effects model (lmerTest package; Kuznetsova et al. 2017). We compared pairwise differences between seasons and sites using the emmeans package and adjusted the alpha value using the Bonferroni correction (Lenth et al. 2022).

Zooplankton and diet data were simplified to major taxonomic groups (amphipods, calanoid copepods, cyclopoid copepods, harpacticoid copepods, cladocera, Diptera, and 'other'). For each sample, abundance of each taxonomic group was divided by total catch in the sample to calculate relative abundance. To assess the difference in zooplankton communities available for fish consumption across time and space, we used a permutational multivariate analysis of variance (PERMANOVA) on the relative abundance of each major group of zooplankton in zooplankton tows across sites during the fall and across seasons at Rio Vista. To assess whether fish were consuming zooplankton in similar proportions to their environment, we performed two additional PERMANOVAs: one modeling community composition versus data type (diet versus zooplankton tow), season, and the interaction of datatype and season at RV; the final PERMANOVA modeled community composition versus data type, location and the interaction of data type and location during the fall. Empty stomachs were removed from analysis to meet the requirements of the test. Analyses were performed using R version 4.2.2 with the 'adonis' package from the 'vegan' package (Oksanen et al. 2020).

Liver lesion score, glycogen depletion, and gill lesion score for summer and fall fish were each analyzed with three Wilcoxon Tests, one for each endpoint. The winter season was not included because it lacked a FCCL control. A significant Wilcoxon Test in fall was followed by a Steel's Test (non-parametric equivalent of a Dunnett's Test) to compare each location to FCCL. The Steel Test was unnecesary for summer because there were only two locations (FCCL and Rio Vista). Two additional Wilcoxon tests were used to compare liver lesion score and glycogen depletion at Rio Vista fish across seasons. Significant tests were followed with Steel-Dwass tests to compare each of the seasons to one-another (Steel-Dwass is a nonparametric, Tukey HSD equivalent). Analyses were peformed in JMP Pro version 16.0.

Thermal tolerance (i.e. CTmax) of fall fish at RV and SM were analyzed with a t-test. RV during summer was not included in statistical analysis due to limited fish survival following the recovery period. We used linear regression to test the effect of acclimation temperature and study location (i.e., compare the field versus lab) on CTmax and acclimation rate. We estimated the acclimation rate of average CTmax data for Delta Smelt with CTmax as a function of acclimation temperature. We acknowledge the limitations of using mean CTmax values, however, individual data were not available for the previous studies.

Results

Field Monitoring

A summary of minimum, mean, and maximum water quality measures during field deployments are summarized in Table 6-2 (discrete) and visualized in Figure 6-3 (continuous) across the year. Water temperature was consistent with California's climate: cooler temperatures in winter and fall and the warmest temperatures in the summer. While Rio Vista, SRDWSC and Suisun Marsh were generally similar in water temperature regardless of season, the Yolo Bypass in summer was warmer, with an average temperature of 24.1°C, or 1.5°C higher than Rio Vista, and a maximum temperature over 3°C higher than Rio Vista. Yolo Bypass also experienced periods of lower dissolved oxygen compared to other sites. Salinity on average was higher in Suisun Marsh in fall compared to other sites (roughly 4 PSU compared to 0 PSU). Turbidity at Rio Vista was higher in the winter (average 47 FNU) compared to summer and fall deployment periods (average 5 FNU); however, during fall season turbidity was relatively higher at Suisun Marsh (22 FNU), then Yolo Bypass (17 FNU), and Rio Vista (12 FNU).

Survival and Growth

Percent survival across season and site are summarized in Table 6-1. Generally, survival varied with season, but was similar across sites within a season, with the exception of summer. At Rio Vista, the highest survival was observed in the winter season (100%) as compared to fall (89%) and summer (67%). Survival of fish at Yolo Bypass was higher in the fall (92%) compared to 0% survival in summer, when water temperature peaked at 26.2°C. During winter, survival at Rio Vista and SRDWSC differed by only ~2%, while in fall survival at Rio Vista, Suisun Marsh and Yolo Bypass differed by 3-4% (Table 6-1).

Comparative growth metrics before and after deployments in summer and fall at each site are summarized in the Supplementary Materials (Supp. Table S6-2 and Figure S6-2). Delta Smelt in summer had a significant decrease in condition factor after the deployments at both Rio Vista and FCCL (reference) compared to before (t-tests; $P<0.05$), with no change in weight of fish at Rio Vista or FCCL; however, fork length increased in FCCL fish. In fall, Delta Smelt condition factor, weight and fork length decreased after field deployments at all sites, with the exception of fork length of fish at Rio Vista, which showed no change in length post deployment (Supp. Table S6-2).

Mixed model results of the effects of season (at Rio Vista) and enclosure location (in the fall) on the change in weight and condition factor (i.e., deltaWeight and deltaK) of Delta Smelt are summarized in Table 6-3 and Figure 6-4. Season and site significantly affected the change in growth of Delta Smelt. At Rio Vista the most positive change in weight and condition occurred in the winter season, followed by summer, then fall (Figure 6-4A,B). In contrast, within the fall season the deltaK of fish differed by enclosure location (Figure 6-4D), but deltaWeight was similar at all fall sites including Rio Vista, Suisun Marsh, Yolo Bypass, and FCCL (Figure 6-4C). Post-hoc comparisons (detailed in the Supplementary Material) detected differences between reference fish at FCCL to Rio Vista and Suisun Marsh. The highest deltaK was at FCCL, followed by Yolo Bypass, whereas the highest deltaWeight was at FCCL followed by Rio Vista.

Zooplankton and Diets

There were major shifts in zooplankton community composition between seasons, with ‘season’ accounting for over 90% of the variance in the PERMANOVA model of zooplankton relative abundance by season at Rio Vista (Table 6-4, Figure 6-5). This was driven by higher relative abundance of cladocera in the winter and higher relative abundance of calanoid copepods in the summer (Figure 6-5). There were also major differences in zooplankton community composition between site, with ‘site’ accounting for over 90% of the variance in the PERMANOVA model of zooplankton relative abundance by site in the fall (Table 6-4, Figure 6-5). This was largely driven by more cyclopoid copepods in Suisun Marsh and more ostracods and cladocera in the Yolo Bypass (Figure 6-5).

There was a relatively small difference in Delta Smelt diets when compared with zooplankton community composition. The PERMANOVA model comparing Delta Smelt diets to zooplankton at Rio Vista demonstrated roughly 6% of the difference in relative abundance was due to it being a diet versus zooplankton sample, with 40% of the variation due to season (Table 6-4). For the model of smelt diet versus zooplankton in the fall, over 50% of the difference in relative abundance was due to location and only 6% due to it being a diet versus a zooplankton sample (Table 6-4). The largest difference between zooplankton and diet samples was the inclusion of Diptera and amphipods in the diet samples, which were absent in zooplankton samples.

Histopathology

The FCCL fish that were sampled immediately before and after the summer and fall deployments had livers and gills in good condition overall. Out of the 40 ‘before deployment’ fish collected prior to the summer and fall deployments, one fish had a liver with mild glycogen depletion, and one fish had a liver with mild liver inflammation. The livers of the other 38 fish had no lesions (Table 6-5). The gills of the ‘before deployment’ fish were also in good condition, with a mean gill lesion score of 0.48, or about one mild gill lesion for every other fish. These lesions were mainly mild inflammation or mild epithelial cell hyperplasia/hypertrophy. The ‘after deployment’ FCCL fish—

the reference fish in our study—also had livers and gills in good condition (Figure 6-6; Table 6-5). The biggest exception was mild to moderate glycogen depletion during fall (Figure 6B; Table 6-5).

Of the caged fish, by far the healthiest livers came from fish deployed to Rio Vista in winter (Figure 6-6, C; Table 6-5). During summer, livers collected from the Rio Vista fish were in considerably worse condition than FCCL ($P = 0.0018$; Figure 6-6, Table 6-5). In fall, Rio Vista, Yolo Bypass, and Suisun Marsh had significantly higher liver lesion scores than control, especially Suisun Marsh (Figure 6-6, Table 6-5, $P < 0.0001$, $P = 0.0001$, and $P < 0.0001$, respectively). Mean glycogen depletion was moderate to severe for all the field deployed populations besides Rio Vista in winter, which had essentially no glycogen depletion (Figure 6-7). Glycogen depletion was significantly higher for all summer and fall deployments compared to FCCL fish ($P < 0.0001$ for each comparison to the FCCL reference). The most common liver lesion overall was cytoplasmic inclusion bodies (Table 6-5). Suisun Marsh fish (from the fall deployment) also exhibited substantial single cell necrosis in the liver (Table 6-5).

Rio Vista varied significantly across seasons in terms of liver lesion score (Wilcoxon, Chi Square DF = 2, $P < 0.0001$; Figure 6-6). Based on a Steel-Dwass test, winter fish had a lower liver lesion score than fall ($P < 0.0001$), winter was lower than summer ($P < 0.0001$), but summer was not different from fall ($P = 0.7335$). Rio Vista also varied significantly across seasons in terms of liver glycogen depletion (Wilcoxon, Chi Square DF = 2, $P < 0.0001$; Figure 6-6). Based on a Steel-Dwass test, winter had more glycogen in the livers than fall and summer ($P < 0.0001$), and summer had slightly more glycogen than fall ($P = 0.03$).

In comparison to the livers, the gills were in relatively good condition (Figure 6-8). The only caged fish with gills in worse condition than the FCCL fish were those caged in Rio Vista and Yolo Bypass during fall ($P = 0.0312$ and 0.0461 for Rio Vista and Yolo Bypass, respectively). The most prevalent lesion at Rio Vista during fall was ionocyte hyperplasia, followed by mucous cell hyperplasia. At Yolo Bypass, the most prevalent gill lesion was epithilial necrosis.

Temperature Tolerance

Delta Smelt from Rio Vista enclosures during the summer had a mean (± SD) CTmax of 27.6 ± 3.0°C (Supplementary Figure S6-5); however, no fish survived to the 4-h recovery endpoint. All fish survived the 4-h recovery period in the fall experiments at Rio Vista and Suisun Marsh. The mean CTmax of fish in the fall were 25.6 ± 1.3°C and 26.5 ± 0.9°C, at Rio Vista and Suisun Marsh, respectively, and were marginally similar with a ~1°C difference ($t=-1.914$, $P=0.074$) (Figure 6-9).

Comparative laboratory and field thermal tolerance data (i.e., CTmax) are shown in Figure 6-9. A linear model found that Delta Smelt CTmax was affected by acclimation-temperature (Adj-$R^2=0.94$, $P<0.001$, Table 6-6). The acclimation rate (slope) was 0.26°C per 1°C ($t=7.14$, $P<0.001$), with CTmax conducted in a laboratory setting 2.22°C higher than CTmax of fish exposed to field conditions ($t=9.29$, $P<0.001$). No CTmax measures were taken at Yolo Bypass during the fall deployments and no deployments occurred during summer for Suisun Marsh; however, using the field-acclimation rate provided by the regression model (CTmax = 21.74 + 0.26 (acclimation temperature) and the mean field temperatures of 16.5°C (Yolo Bypass) and 23.4°C (Suisun Marsh) we can estimate the CTmax of 26.0°C for fish deployed at Yolo Bypass during fall and 27.8°C if fish were deployed in Suisun Marsh during the summer.

Discussion

Field deployments of hatchery Delta Smelt in custom enclosures were successful in evaluating seasonal conditions for the endangered Delta Smelt and meeting the study goal of evaluating the feasibility and utility of enclosure deployments. Consistent with our predictions, metrics of caged fish varied across deployment periods and sites, indicating both spatial and temporal effects on diet, growth, and health, and suggest differences in habitat quality for Delta Smelt in the wild. Inconsistent with our predictions, temperature tolerance was similar across sites, however, our findings showed field-acclimatized thermal tolerance differed from lab-acquired thermal tolerance. Together, the results suggest that regional and seasonal differences can be monitored using caged Delta Smelt, and using cages could help evaluate the effects of managed actions such as habitat restoration or augmented flows, or for field contaminant exposures. However, we also found signs of nutritional stress in the caged fish, perhaps because the cages prevented natural foraging behavior, and additionally encountered difficulty with biofouling of cages, particularly in summer and fall. Thus, as with any field experiment, results must be interpreted cautiously.

Survival and health

By caging Delta Smelt across three seasons and throughout the recently observed range of wild Delta Smelt, we showed that habitat conditions influenced survival and health. For example, caged fish at Rio Vista on the Sacramento River demonstrated a strong seasonal trend with 100% survival in the winter-spring with survival decreasing in the summer and fall by 33% and 11%, respectively (Table 6-1; Supplementary Figure 1). Enclosure location also presented trends in survival but was relatively dependent on the season, such that in cooler months survival was similar at both the terminal Sacramento River Deepwater Ship Channel and Rio Vista, but in warmer months, survival differed by as much as 100% or 4% (all three locations), in summer and fall, respectively. Low survival in the summer is consistent with studies regarding temperature and food limitation (Davis et al. 2022, Smith and Nobriga 2023), with one study showing similar mass-mortality of hatchery-reared Delta Smelt in early summer when exposed to semi-natural field temperatures of 27°C (Hung et al. 2019). Studies of Delta Smelt have indicated that juvenile and subadult Delta Smelt are sensitive to chronic food limitation during the summer and fall that make this life stage a ‘pinch point’ for the species (Smith and Nobriga 2023; Moyle et al. 2016; Bennett 2005; Maunder and Deriso 2011, Hammock et al. 2021).

After the four-week deployments in the wild, changes in growth of Delta Smelt were consistent with the survival results. Only fish in the winter at Rio Vista experienced positive growth and increased condition factor, suggesting conditions in the field during the winter were better for Delta Smelt than even the culture facility or later that winter at the SRDWSC. Whereas fish in the fall experienced the greatest decrease in weight and condition factor suggesting that conditions in the fall may be more limiting than in the winter to the growth and survival for wild Delta Smelt. The survival and growth correlation in the summer and fall, however, was somewhat influenced by location. Fish at Rio Vista had better survival in the fall compared to summer, likely influenced by higher summer temperatures, but fish in the fall also had more negative changes in weight and condition compared to summer. This finding may be a result of several factors such as age of fish and/or habitat quality. For example, summer fish were somewhat younger (~70 days) than the fall fish, which may have led to differences in direction of energy, with younger fish having greater growth rates than older fish that may start to become sexually mature (LaCava et al. 2023). Summer and fall fish were also tagged differently (adipose fin clipped vs. VIA tag) which may have led to differential trauma. However, significant impacts to growth or survival are not expected from tagging methods (Sandford et al. 2020). In addition to age, differences in water temperature between seasons likely influenced growth. For example, adult Delta Smelt would have lower growth rates than juveniles but the higher temperatures in the summer may have mitigated that ontogenetic effect. Mean summer temperatures were 22.7°C compared to a temperature in the fall of 15.6°C at Rio Vista, and warmer temperatures (without extreme temperature stress) can increase growth given sufficient prey as demonstrated in enclosure studies of juvenile Coho Salmon (Lusardi et al. 2020) and Chinook Salmon across different riverine and floodplain conditions (Jeffres et al. 2008). Within the fall season, all sites lost weight except for the Yolo Bypass, which was the only site that maintained similar weight to the FCCL control (Supplementary material Table 2). This result was surprising given previous findings of juvenile Chinook Salmon (Jeffres et al. 2008; Aha et al. 2021) and Longjaw Mudsucker (*Gillichthys mirabilis*; Forrester et al. 2003) that showed growth was affected by deployment location.

The histopathology results for the Delta Smelt caged during summer and fall also appear consistent with some combination of food limitation and high temperature stress. The timing, type, and severity of liver lesions observed in the summer and fall deployments are generally comparable to Delta Smelt held at 16°C without food for two weeks (Hammock et al. 2020), with caged fish generally exhibiting autophagosomes (Dhayalan et al. 2023) and moderate glycogen depletion in their livers. Signs of food limitation were markedly more severe for the fish caged at Suisun Marsh during fall compared to fish at FCCL. These fish exhibited prevalent single cell necrosis and severe glycogen depletion, in addition to autophagosomes. Single cell necrosis in liver is characteristic of Delta Smelt fasted for three weeks, and coincides with an increase in mortality due to food limitation (Hammock et al. 2020). In contrast, the gills of the deployed fish exhibited low prevalence and severity of lesions and were generally considered in good condition. The poor liver condition was probably not caused by deployment in the enclosures themselves, since one of the deployments (Rio Vista in winter) had fish in excellent condition. Thus, the histology results suggest that Delta Smelt deployed in enclosures during summer and fall received inadequate nutritional input, whereas Delta Smelt deployed to Rio Vista during winter received adequate nutritional input.

Of all the deployments, the livers of fish from Suisun Marsh were in the worst condition. Mean liver lesion score and single cell necrosis were the highest observed, and every fish examined from Suisun Marsh had severe glycogen depletion. However, fish caged in Suisun Marsh also had gills in excellent condition, leaving nutritional stress as the most likely cause for the poor condition of the livers. This was surprising, given that wild Delta Smelt collected from Suisun Marsh have historically exhibited relatively good condition (Hammock et al. 2015, 2021), and because Suisun Marsh is considered good habitat for native fishes as it contains a considerable area of relatively intact tidal wetands (Meng et al. 1994, Moyle et al. 2016; Baumsteiger et al. 2017). We suggest two explanations for this discrepancy. First, water temperature during fall was slightly higher in Suisun Marsh than the other locations, which would have increased metabolic demand and exacerbated any food limitation. Second, Suisun Marsh has low pelagic zooplankton biomass compared to the other regions in our study, especially considering the relatively good condition of resident Delta Smelt (Hammock et al. 2021). This suggests that Delta Smelt may be especially dependent on foraging behavior in the region, possibly eating prey that are not sampled by routine zooplankton monitoring. For example, Delta Smelt collected in proximity to tidal wetlands—nursery habitat for many fishes—are six times more likely to have larval fish in their guts than fish collected far from tidal wetlands (Hammock et al. 2019). Caging fish in Suisun Marsh for more than a few days may therefore be more problematic than in other regions, since pelagic prey are relatively scarce in the region and the enclosures prevent fish from compensating by foraging outside the enclosures. Thus, our results do not necessarily indicate that Suisun Marsh is poor habitat for Delta Smelt compared to other deployment locations, despite relatively low pelagic prey availability (Hammock et al. 2021).

Although the gills of fish deployed in enclosures were in good condition overall, there were signs of possible contaminant exposure at Yolo Bypass in the fall, which was the only deployment with elevated gill necrosis. While fish deployed in Rio Vista and Yolo Bypass during fall showed elevated ionocyte hyperplasia, that lesion varies strongly with salinity and may therefore be a response to changing water quality from the hatchery (FCCL) to the field (Teh et al. 2020). Although not analyzed in our study, chemical contaminants including heavy metals, current-use pesticides from agricultural applications, and legacy organochlorine insecticides (DDT and its metabolites) have been routinely detected in Yolo Bypass (Smalling et al. 2007; Jabusch et al. 2018; De Parsia et al. 2019; Orlando et al. 2020), including detections in juvenile Chinook Salmon tissue and their prey (Anzalone et al. 2022), and Delta Smelt (Stillway et al. 2024). We note that the lack of control samples from before and after the winter deployment makes the winter SRDWSC results hard to interpret, although they are consistent with the signs of nutritional and contamiant stress detected in wild Delta Smelt collected from the SRDWSC (Teh et al. 2020). However, even without the controls, the winter Rio Vista deployment demonstrates that Delta Smelt can be held in enclosures in the Delta at least one month without ill-effects, if pelagic conditions are suitable (i.e., sufficient prey for a given temperature).

In summary, negative field effects appeared to reduce the health and liver condition of caged Delta Smelt for all but the Rio Vista winter deployment. For all other deployments, the caged fish exhibited an array of indicators of starvation in Delta Smelt compared to the FCCL controls, including decreased weight, liver glycogen depletion, autophagosomes, and single cell necrosis in the liver. Each deployment occurred in areas with chronically low pelagic productivity, and the enclosures prevented a normally vagile fish from foraging more naturally. Moreover, the gills of the caged fish were in good condition, suggesting that the livers were probably less impacted by contaminants in the water, but by food limitation and/or contaminant exposure from the diet. Yolo Bypass in fall was the one exception, where fish had elevated levels of gill lesions, potentially due to contaminant exposure (Orlando et al. 2020; Stillway et al. 2024). Results of elevated liver lesions and depleted glycogen of caged Delta Smelt across habitats are consistent with food limitation studies of Delta Smelt and recaptured hatchery released Delta Smelt findings (Dhayalan et al. 2023). In the future, enclosures could be used to assess water quality and pelagic prey availability if supplemental food is provided to a subset of enclosures and biofouling is prevented.

Food availability and consumption

The zooplankton and diet analyses showed clear changes across region and season. Winter deployments were characterized primarily by cyclopoid copepods and cladocera in both diet and zooplankton samples, whereas calanoid copepods gained prominence in summer and fall in Rio Vista (Figure 6-5). This aligns with the population dynamics of the dominant calanoid copepod in the freshwater reaches of the Delta, *Pseudodiaptomus forbesi* (Kimmerer et al. 2017). Regionally, Suisun Marsh zooplankton was dominated by cyclopoid copepods in the fall, while Rio Vista and the Yolo Bypass had more calanoid copepods (Figure 6-5). This is likely due to dominance of the cyclopoid copepod *Limnoithona tetraspina*, which is one of the most abundant zooplankton in the low salinity zone in the fall (York et al. 2013) and shown in Supplementary Figure S6-3B. *L. tetraspina* is considered a less valuable prey item than calanoid copepods due to its small size (Slater and Baxter, 2014; Bouley and Kimmerer 2006). While cyclopoids were the most abundant prey item in the diets of caged smelt in Suisun Marsh (Figure 6-5), most of that was the genus *Acanthocyclops*, which was rare in the zooplankton community (Supplementary Figure S6-3 and Figure S6-4). Given that Suisun Marsh had the most severe signs of starvation of any deployment, the results may support the notion that *L. tetraspina* is not a valuable prey item.

Analyses comparing diet to zooplankton abundance found only a small amount of difference between diet and zooplankton samples across seasons and regions (Table 6-4). This was somewhat surprising because diet samples frequently included amphipods and diptera that were completely absent from the zooplankton samples. During the Yolo Bypass deployment, in particular, most of the diet contents were amphipods, whereas zooplankton samples were dominated by ostracods, copepods, and cladocera (Figure 6-5). The enclosures provided a substrate for biofouling and colonization by epibenthic and epiphytic organisms, including amphipods, which may explain the difference between the zooplankton tows and diet samples. These epibenthic and epiphytic organisms may be more abundant in the enclosures in warmer seasons (summer, fall) when biofouling was worse. Contrastingly, similar diet and community zooplankton compositions were observed in the cooler winter season. The low R^2 from the PERMANOVA may be due to the high variation in diet contents, and the low replication in zooplankton samples. Additional zooplankton samples and samples from the biofouling communities in future enclosure deployments may be needed to better characterize differences between diet and zooplankton communities.

Thermal Tolerance

Based on regression analyses, field-acclimatized hatchery Delta Smelt were roughly 2.2°C less tolerant than laboratory fish (Figure 6-9). Compared to static, controlled and homogenous laboratory conditions, the observed field conditions were more thermally variable (Figure 6-3) due to daily changes in air temperature and tides. Delta Smelt may have accrued greater energetic costs (i.e., stress) dealing with fluctuating daily temperatures. Thermal tolerance of Delta Smelt exposed to fluctuating thermal regimes in a laboratory has not been investigated; however, energetic costs of Atlantic Salmon (*Salmo salar*) under fluctuating thermal regimes were greater than exposures to the same mean temperature in a static regime (Beauregard et al. 2013). other water quality parameters (e.g., turbidity, conductivity, and dissolved oxygen) and biotic factors such as food limitation in the field may have also influenced thermal tolerance (Ern et al. 2016; Lee et al. 2016; Firth et al. 2021) as demonstrated by moderate to severe glycogen depleted livers (Figure 6-6B) and decreased weight (Table 6-3, Figure 6-4). Interactions between food limitation, glycogen, and tolerance have been shown to vary by species. For example, CTmax of Green Sturgeon (*Acipenser medirostris*) decreased with food-restriction whereas White Sturgeon (*Acipenser transmontanus*) CTmax was unchanged regardless of reduced growth (Lee et al. 2016; Rodgers et al. 2019), and CTmax of juvenile Coho Salmon increased in response to fluctuating warm temperature regimes independent of glycogen depletion (Corey et al. 2017). The daily minimum (i.e. nighttime) temperatures in the fluctuating field-acclimatization (i.e. caged Delta Smelt) may also have contributed to decreased thermal tolerance compared to laboratory-acclimated fish. For example, Delta Smelt acclimated to laboratory conditions of 16 or 17°C were held stable with a constant minimum value, compared to fish in the field enclosures that while acclimatized to a mean of 16 to 17°C (at Rio Vista and Suisun Marsh), actually had daily exposures to much cooler temperatures which may have influenced a reduced upper thermal tolerance. Increasing minimum temperatures (with the same maximum temperatures) in a fluctuating regime has shown to increase thermal tolerance in the western mosquito fish, *Gambusia affinis* (Otto 1974), though not for juvenile Coho Salmon (Corey et al. 2017). Lastly, it is important to note that the present study findings support the initial methodology decision to acclimate the caged fish to 18°C for a week at FCCL prior to field deployments. Prior to acclimation to 18°C, all Delta Smelt were kept at 12°C following aquaculture protocols required for tagging or adipose fin clipping. If fish remained at 12°C and were not warm acclimated to 18°C prior to deployments, significant mortality would likely have occurred with the summer temperatures exceeding the estimated tolerance limits of 12°C-acclimated fish at 24.9°C (calculated using the conservative field-acclimation rate equation).

Seasonal acclimatization, a common physiological mechanism, was also observed in Delta Smelt. Comparative results at Rio Vista showed that thermal tolerance of caged Delta Smelt in summer temperatures was greater than in the fall. During hot summer months, Delta Smelt likely re-direct energy to increasing heat shock response mechanisms (Iwama et al. 1999), whereas during fall, temperature regulation mechanisms may be reduced as the energetic cost of living may be reduced (Clarke 1993). However, it is important to remember that while thermal energetic costs may be reduced in cooler temperatures, Delta Smelt may still have elevated physiological costs depending on conductivity, turbidity, and food availability. No enclosures were deployed at Belden's Landing during the summer; however, the water temperature profile showed a mean of 23.4°C. Using the calculated acclimation rate for field Delta Smelt, we can estimate the upper thermal tolerance of fish caged at Belden's Landing during the summer to be 27.8°C. During the fall deployments, caged Delta Smelt experienced a mean temperature of 16.5°C. This drastic decrease in water temperature from summer to fall also likely led to a decrease in the thermal tolerance of the caged fish held at Belden's Landing. Rio Vista and Belden's Landing had similar fall water temperatures with a mean temperature of 15.6°C at Rio Vista and 16.5°C at Belden's Landing. Not surprisingly, the caged fish have similar thermal tolerances, with CTmax measures of 25.6°C (Rio Vista) and 26.5°C (Belden's Landing). Based on these CTmax trials, the mean experienced temperature seems to be a decent predictor for the thermal tolerance of Delta Smelt, but other water quality conditions must not be ignored. Belden's Landing is located on the Montezuma Slough, a tidal estuary, and has much higher salinities (measured as conductivity) than Rio Vista (Figure 6-3). Given wild Delta Smelt preference for low salinity habitats demonstrated by long-term field surveys (<2-6 PSU, Bennett 2005) we might hypothesize that the higher salinities at Belden's Landing to potentially cause them to re-direct energy from thermoregulation to osmoregulation, decreasing their CTmax values; however, CTmax remained similar or slighted elevated compared to Rio Vista. These results are consistent with previous laboratory studies demonstrating Delta Smelt's physiological tolerance and/or acclimation capacity to higher salinities > 10 to 34 PSU (Komoroske et al. 2014; Kammerer et al. 2016; Hammock et al. 2017; Hung et al. 2022), though inconsistent with historical field detections. An alternative hypothesis is that because smelt are hyperosmotic to the environment, a small increase in salinity (from 0 to 4 PSU) may provide energy-benefits reducing osmoregulatory demands. This energy budget hypothesis would need to be tested in a controlled laboratory setting to make any definite conclusions. Lastly, while we suspect the laboratory CTmax are 2.2°C higher than the field CTmax due to acclimation to controlled versus dynamic and stochastic environmental conditions that influences differential physiological and behavior performance, we also acknowledge that the reduced thermal tolerance in the field may be attributed to transport or food limitation stress. The peak stress levels of Delta Smelt measured by cortisol can occur around 30 minutes after handling stress (Pasparaski et al. 2022), suggesting the reduced field CTmax may have also been influenced by handling and transfer stress from the field to the immediate CTmax experiments that occurred about 1 hour following removal. More research may be warranted to understand the influence of transport or food limitation stress on upper temperature tolerance.

Conclusions and Implications

We conclude that hatchery Delta Smelt can successfully survive across most seasons and regions in the Estuary for extended periods of time within enclosures, though temperatures of 22°C are limiting. Physiological condition and survival depended on season and location, which should be considered in future decision making for deployments used to evaluate management actions and supplementation efforts. Additional locations should be considered in future deployments across seasons including historical tidal wetland habitat in Cache Slough Complex in the winter-spring, given the species might be migrating to upstream freshwater habitat, or testing locations with aspects of temperature refuge during warmer summer-fall months since temperature appears to drive the negative effects on survival and condition. We also found that although laboratory acclimated Delta Smelt have an overall greater thermal capacity, both laboratory and field-acclimatized fish have similar acclimation rates (0.26 °C per 1°C). This estimated thermal acclimation rate for juvenile Delta Smelt can be a tool used to predict the thermal tolerance and survival in specific habitats based on real-time water temperature measures. Thus, to maximize survival of hatchery propagated Delta Smelt in field enclosures (and supplementation strategies) that occur during warmer seasons, fish should be warm-acclimated in the laboratory for greater acquisition of thermal tolerance. Our study underscores the effectiveness of adopting an ecological physiological approach in understanding species responses. Through utilizing key ecological physiological metrics alongside cage deployments, we highlight the significance of these methods as important tools in the conservation efforts for Delta Smelt.

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Author Contributions

All authors contributed to this study as a whole and/or individual deployments, contributed to the writing and review of this manuscript, and have approved this final version. BED led the synthesis study and drafted the initial manuscript outline and general background sections. Specific methods and results sections were written by BGH, BED, NK, CP, HB, RH, and SA. BGH, SA, and ST conducted the histopathology and analysis. NK and RH conducted the zooplankton and diet analyses, and CP conducted the growth analysis. BED, HB, DC and NAF designed and conducted the thermal tolerance measures and analysis. MB, DG, DC, and NAF contributed to initial enclosure development study design, and implementation of deployments. TCH and LE were instrumental in fish rearing and pre-deployment acclimation and treatment of fish for the study and contributed to all FCCL control measures.

Conflict of Interests

There are no conflict of interests.

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Data Availability

The data generated for this research article vary in availability. While most data were original data generated in this research some data were from a third-part or open-data platform included in the synthesis. Continuous water quality is available on the California Data Exchange Center, survival and growth for winter enclosure deployments were reproduced from Baerwald et al. (2023) and available online, all other data is available upon request.

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Tables

Table 6-1. Deployment Dates, Age (days-post-hatch, DPH) of Fish when Put in Enclosures and Sampled, Number of Fish Deployed (n) and Estimated Percentage Survival of Delta Smelt for the Study

Location	Metric	Winter	Summer	Fall
Rio Vista (RV)	Dates	1/23-2/25/2019	7/30-8/28/2019	10/9-11/6/2019
	DPH	243-276	194-223	264-292
	n	136	180	180
	% Survival	100	67	89
Sacramento River Deepwater Ship Channel (SRDWSC)	Dates	2/27-3/27/2019	No enclosures	No enclosures
	DPH	278-306		
	n	120		
	% Survival	98		
Suisun Marsh (SM)	Dates	No enclosures	No enclosures	10/9-11/7/2019
	DPH			264-293
	n			180
	% Survival			88
Yolo Bypass (YB)	Dates	No enclosures	7/30-8/19/2019	10/9-11/7/2019
	DPH		194	264-293
	n		60	180
	% Survival		0	92
Fish Culture and Conservation Lab (FCCL)	Dates	1/23-2/25/2019 (1), 2/27-3/27/2019 (2)	7/29-8/29/2019	10/8-11/5/2019
	DPH	243-276 (1), 278-306 (2)	194-225	263-291
	n	60 (1), 60 (2)	90	90
	% Survival	83 (1), 85 (2)	98	99

Table 6-2. Discrete Water Quality Measures Taken at Enclosure Checks During the Deployments

Site and Season		Secchi (m)	Water Temperature (°C)	Dissolved oxygen (mg/L)	Specific conductivity (µS/cm)	pH	Turbidity (FNU)
Rio Vista Winter	Min	0.13	8.4	8.45	127	7.37	13.9
	Mean	0.31	10.13	9.54	213.95	7.73	46.59
	Max	0.68	11.8	10.9	283	8.21	99.63
SRDWSC Winter	Min	0.20	9.7	9.61	366	7.18	17.6
	Mean	0.32	11.8	10.26	528	8.08	27.1
	Max	0.47	14.1	10.59	695	8.76	38.2
RV Summer	Min	NA	21.99	7.52	125	7.6	2.57
	Mean	NA	22.77	7.89	131.12	7.78	4.93
	Max	NA	23.4	8.23	137.2	7.99	7.93

Site and Season		Secchi (m)	Water Temperature (°C)	Dissolved oxygen (mg/L)	Specific conductivity (µS/cm)	pH	Turbidity (FNU)
YB Summer	Min	0.25	22.6	7.09	184	8.17	14.3
	Mean	0.45	24.12	7.52	191.78	8.49	18.95
	Max	0.61	26.2	8.05	201	8.66	26.6
RV Fall	Min	NA	13.54	8.78	120.1	7.99	2.53
	Mean	NA	15.64	8.99	130.37	8.23	4.78
	Max	NA	16.2	9.19	134.8	8.48	12.27
YB Fall	Min	0.34	12.9	7.72	190	7.84	12.97
	Mean	0.43	15.7	8.81	226.89	8.12	17.29
	Max	0.55	16.9	9.95	272	8.45	22.03
SM Fall	Min	NA	14.4	7.56	7683	7.55	12.33
	Mean	NA	16.88	7.84	7955.76	7.68	21.62
	Max	NA	17.98	8.44	8802.1	7.81	33

The minimum, mean, and maximum values are summarized for each deployment location and season. RV is Rio Vista in the Sacramento River, SRDWSC is Sacramento River Deepwater Ship Channel, SM is Suisun Marsh, and YB is the Yolo Bypass.

Table 6-3. Mixed Effect Model Results for the Effect of Season (at Rio Vista) and Site (within fall) on the Change in Condition (Delta K) and Change in Weight (Delta Weight)

Model	Predictor	Estimate	Std. Error	df	t value	p-value
Seasons at Rio Vista						
Delta K	Winter (int)	0.060	0.014	5.656	4.353	0.005
	Summer	-0.124	0.020	6.843	-6.056	<0.001
	Fall	-0.268	0.020	5.897	-13.594	<0.001
Delta Weight	Winter (int)	0.167	0.027	5.472	6.254	0.001
	Summer	-0.244	0.040	6.578	-6.17	<0.001
	Fall	-0.396	0.038	5.696	-10.419	<0.001
Enclosure sites within fall season						
Delta K	FCCL (int)	-0.064	0.016	9.323	-4.102	0.002
	RV	-0.086	0.021	7.642	-4.051	0.004
	SM	-0.105	0.021	7.583	-4.974	0.001
	Yolo	-0.065	0.021	7.643	-3.075	0.016
Delta Weight	FCCL (int)	-0.351	0.081	8.721	-4.342	0.002
	RV	-0.044	0.109	7.371	-0.404	0.698
	SM	-0.196	0.109	7.323	-1.786	0.115
	Yolo	-0.120	0.109	7.371	-1.094	0.308

RV is Rio Vista in the Sacramento River, SM is Suisun Marsh, and YB is the Yolo Bypass.

Table 6-4. Permutational MANOVA Model Results Comparing Zooplankton Communities Across Seasons at Rio Vista, Across Site During the Fall (i.e. Rio Vista, Suisun Marsh, Yolo Bypass), and Comparing Zooplankton and Diet Composition Across Seasons

Model	Predictor	df	Sum of Squares	R2	Statistic	p-value
Rio Vista Zooplankton	Season	2	4.044	0.950	192.3403	0.001
	Residual	20	0.210	0.049		
	Total	22	4.255	1		
Fall Zooplankton	Site	2	0.598	0.916	76.05657	0.001
	Residual	14	0.055	0.084		
	Total	16	0.653	1		
Rio Vista diet vs. zooplankton	Diet/Zoops	1	2.118	0.0635	15.79482	0.001
	Season	2	13.644	0.409	50.86436	0.001
	Diet/Zoops*Season	2	1.687	0.051	6.849	0.001
	Residual	129	15.884	0.4765		
	Total	134	33.333	1		
Fall diet vs. zooplankton	Site	2	14.795	0.576	98.286	0.001
	Diet/Zoops	1	1.760	0.069	23.388	0.001
	Diet/Zoops*Site	2	2.282	0.089	15.160	0.001
	Residual	91	6.849	0.267		
	Total	96	25.686	1.000		

Table 6-5. Gill and Liver Condition Across Sites and Season

Lesion	FCCL			Rio Vista (n=59)			Yolo Bypass			Suisun Marsh			SRDWSC (n=60)		
	Mild	Mod	Sev	Mild	Mod	Sev	Mild	Mod	Sev	Mild	Mod	Sev	Mild	Mod	Sev
Winter															
Liver glycogen depletion	No data			9	15	2	No data			No data			2	27	24
Liver hydropic vacuolar degeneration				2	--	--							2	2	--
Liver single cell necrosis				2	--	--							8	--	--
Liver cytoplasmic inclusion bodies				1	--	--							19	14	7
Liver sinusoid congestion				--	--	--							1	--	--
Liver inflammation/macrophage aggregate				--	--	--							2	--	--
Lesion	**FCCL (n=18)**			**Rio Vista (n=21)**			**Yolo Bypass**			**Suisun Marsh**			**SRDWSC**		
Summer															
Gill cell necrosis	--	--	--	--	--		No data			No data			No data		
Gill ionocyte hyperplasia	--	--	--	--	--										
Gill mucous cell hyperplasia	--	--	--	--	--										
Gill epithelial cell hyperplasia/hypertrophy	--	--	--	--	--										
Gill inflammation	--	4	--	--	--										
Liver glycogen depletion	2	2	2	--	8	13									
Liver hydropic vacuolar degeneration	--	--	--	1	--										
Liver single cell necrosis	2	--	--	2	--										
Liver cytoplasmic inclusion bodies	1	1	--	6	4	4									
Liver inflammation/macrophage aggregate	--	--	--	1	--	--									

Lesion	FCCL			Rio Vista (n=59)			Yolo Bypass			Suisun Marsh			SRDWSC (n=60)		
	Mild	Mod	Sev	Mild	Mod	Sev	Mild	Mod	Sev	Mild	Mod	Sev	Mild	Mod	Sev
Fall															
Gill cell necrosis	--	--	--	1	--	--	6	4	--	--	--	--	No data		
Gill ionocyte hyperplasia	--	--	--	--	10	--	--	2	--	--	--	--			
Gill mucous cell hyperplasia	--	--	--	4	--	--	7	--	--	--	--	--			
Gill epithelial cell hyperplasia/hypertrophy	--	--	--	--	--	--	2	--	--	1	--	--			
Gill inflammation	--	--	--	3	--	--	--	--	--	1	--	--			
Liver glycogen depletion	1	--	--	--	5	40	1	--	44	--	--	44			
Liver hydropic vacuolar degeneration	--	--	--	10	--	--	3	--	--	--	--	7			
Liver single cell necrosis	--	--	--	1	--	1	--	--	--	1	1	7			
Liver cytoplasmic inclusion bodies	--	--	--	29	11	1	27	6	--	30	5	3			
Liver inflammation/macrophage aggregate	--	--	--	4	--	--	4	--	--	2	1	10			

Note that blanks in fields indicate zero (not observed).

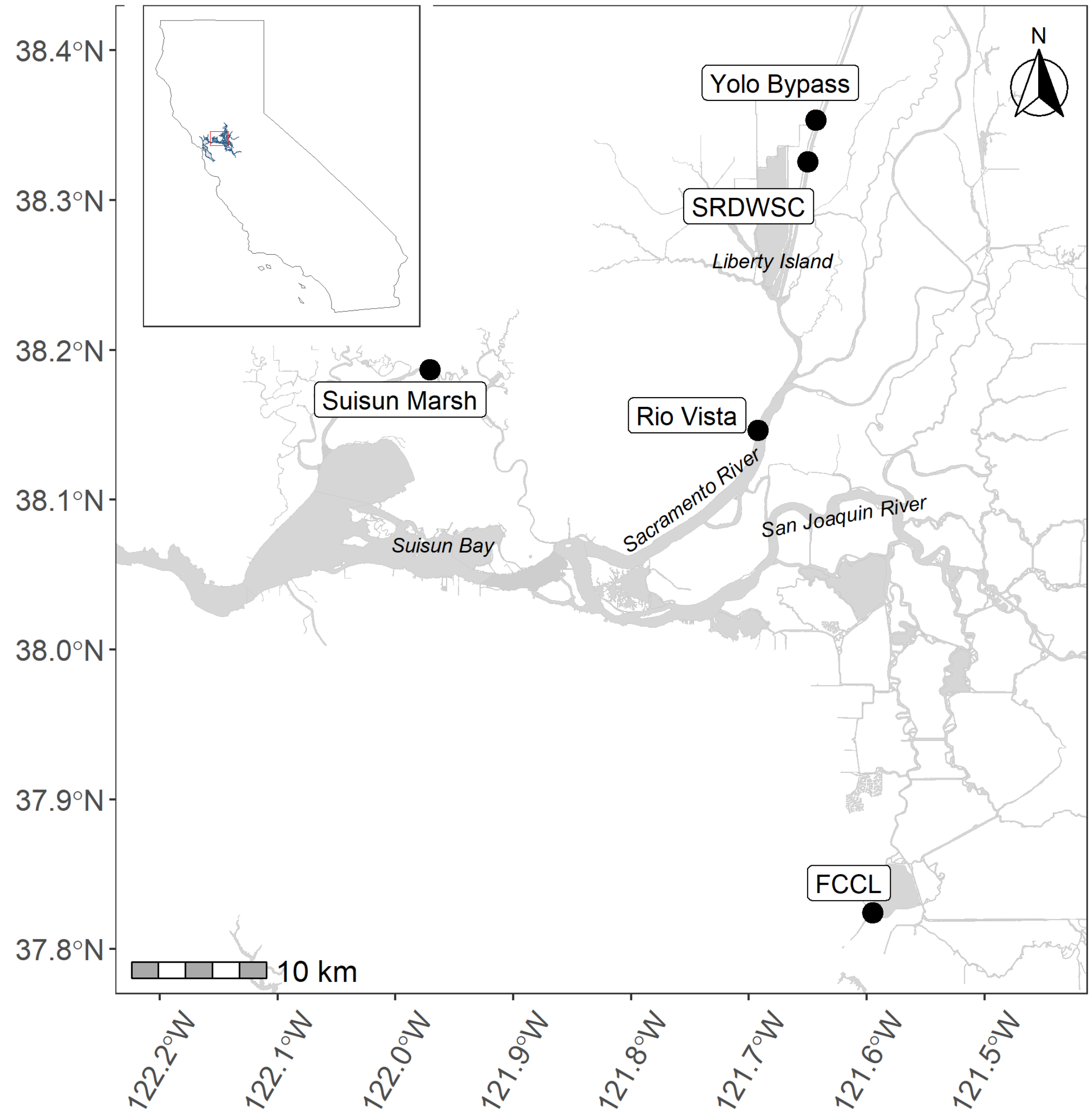
The count of fish observed with Mild, Moderate (Mod), and Severe (Sev) is summarized. Gills were not collected in Winter. Rio Vista gills were n=11 in summer (10 were autolyzed).

Table 6-6. Results of Linear Model of Delta Smelt Critical Thermal Maximum (CTmax) Versus Aclimation/Acclimatization Temperature and Location (field versus lab)

Factor	Estimate	Standard Error	T-value	p-value
Intercept - Field	21.738	0.702	30.988	<0.0001
Acclimation Temp	0.265	0.037	7.135	0.001
Location (Lab)	2.222	0.239	9.292	<0.0001

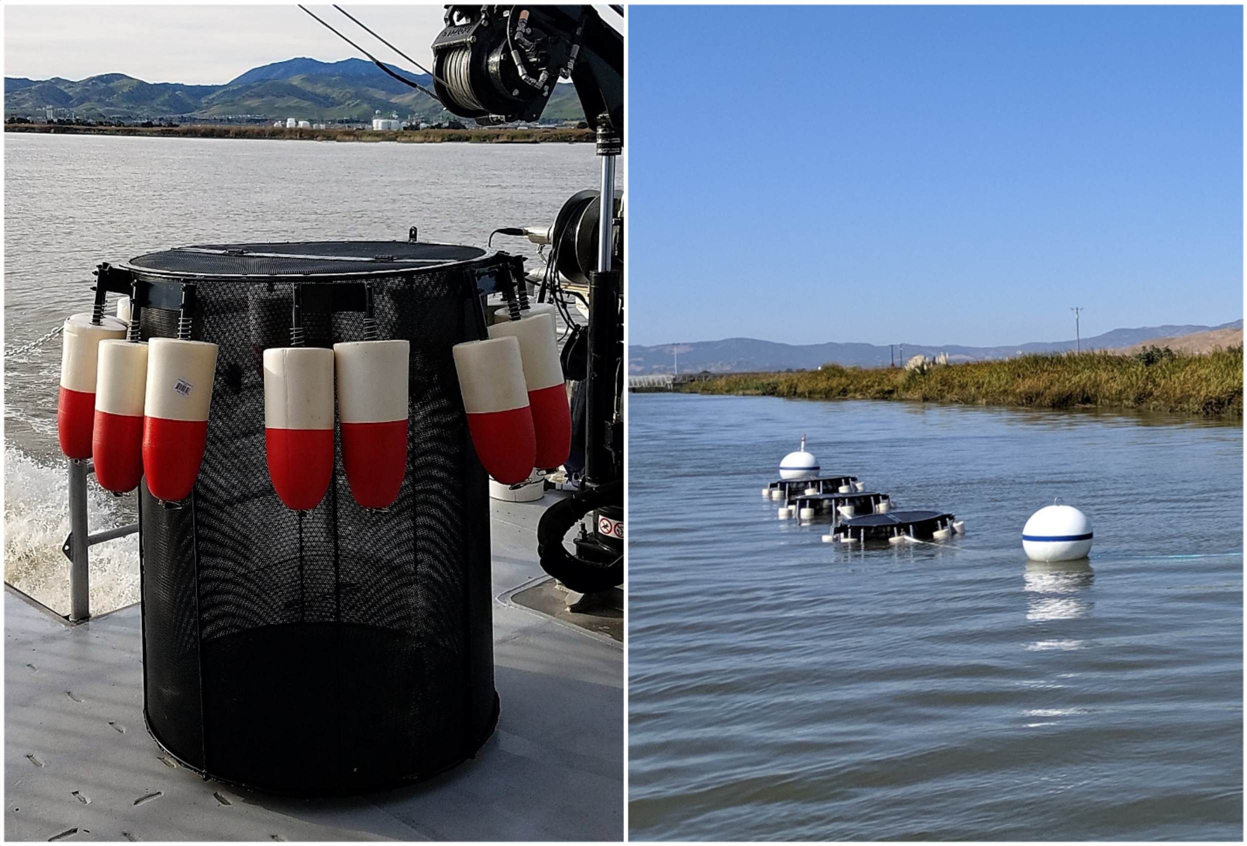
Residual standard error was 0.32 on 5 degrees of freedom. Adjusted R-squared was 0.9411. Overall f-statistic 56.95 on 2 and 5 degrees of freedom, p<0.0001.

Figures



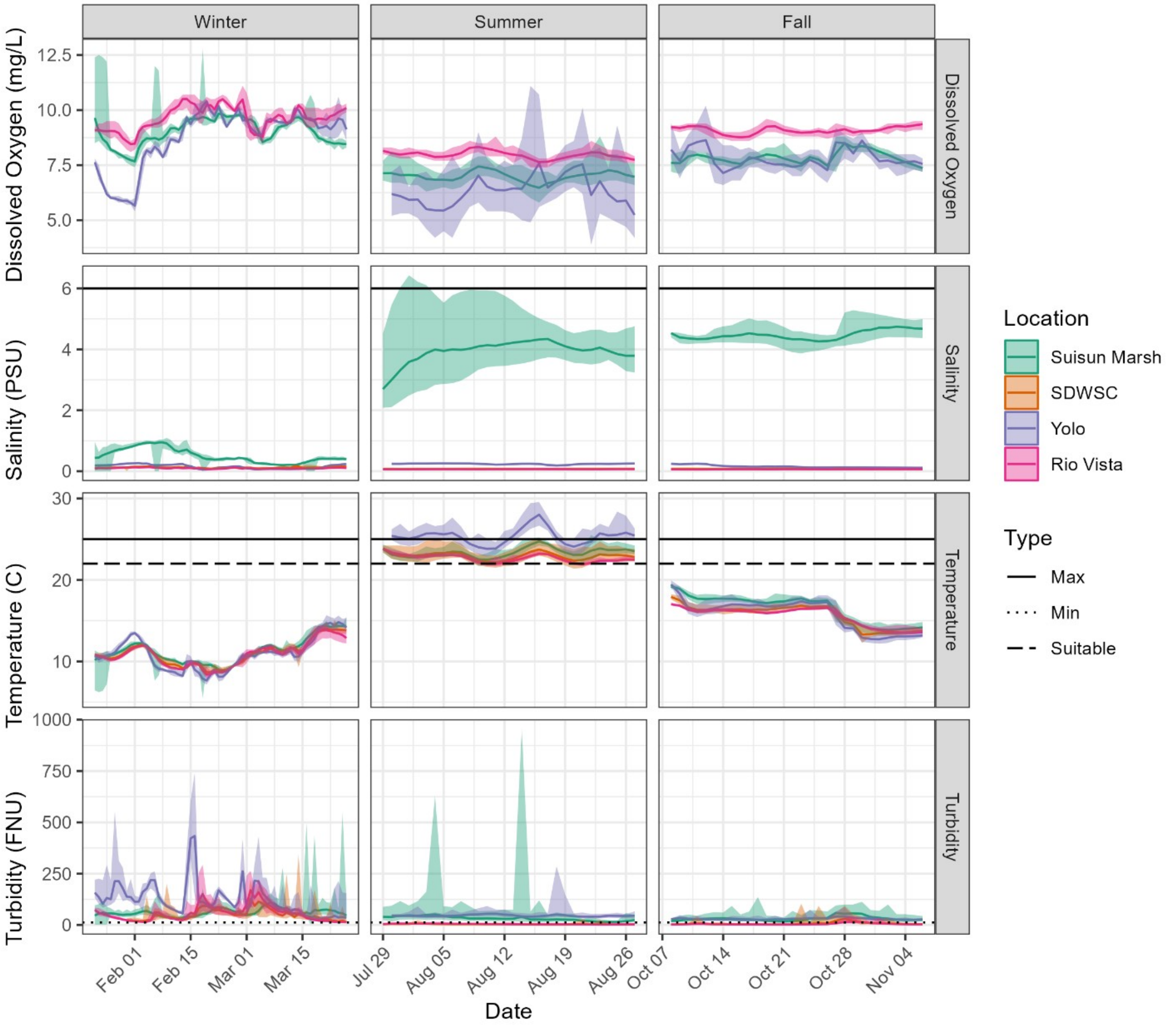
SRDWSC is the Sacramento River Deepwater Shipping Channel and FCCL is the Fish Conservation and Culture Laboratory.

Figure 6-1. Smelt Enclosure Sites in the San Francisco Estuary of California



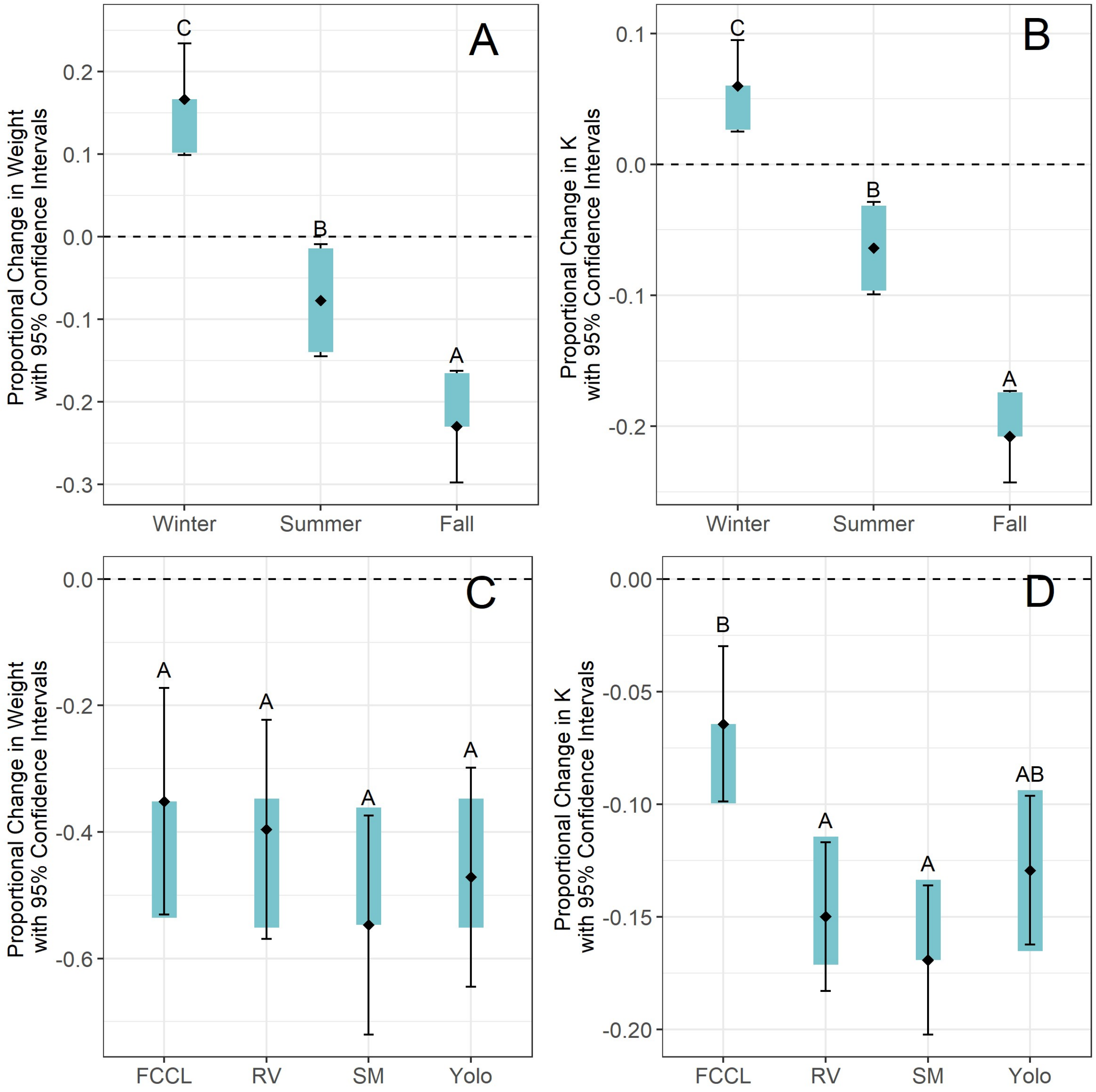
Photographs provided by DWR.

Figure 6-2. Delta Smelt Enclosure Design Used in 2019 Deployments (Photo on Left) and Three Enclosure Replicates in Suisun Marsh (Photo on Right)



Lines indicate daily mean values, bottom and top of colored ribbon are the daily minimum and maximum values. The solid red line idicates the maximum temperature Delta Smelt can survive, the dashed red line indicates the temperature below which smelt can grow, and the dotted red line indicates the minimum turbidity above which Delta Smelt are normally found.

Figure 6-3. Water Quality Parameters Measured Every 15 Minutes Across Seasons and Enclosure Locations in 2019

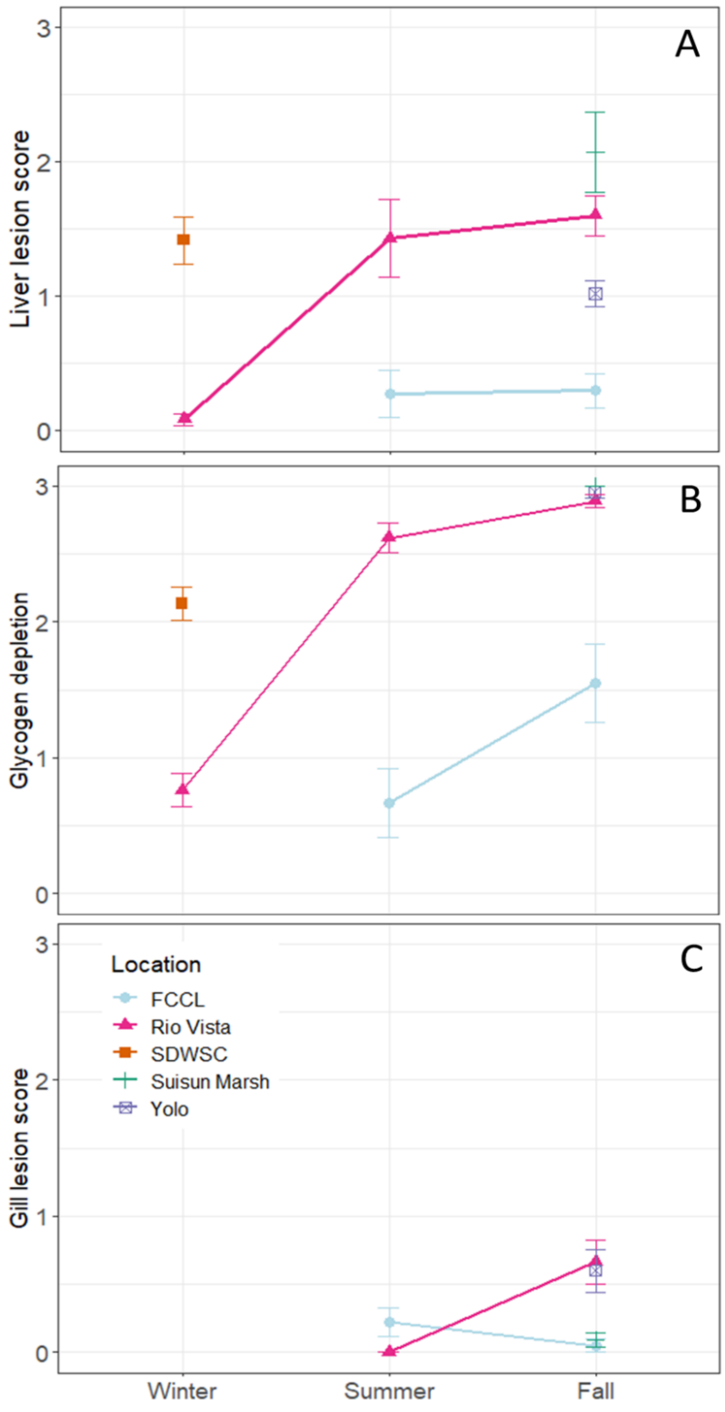


Top panels show mean proportional change in weight (A) and condition factor as K (B) by season at Rio Vista, whereas bottom panels show comparison of delta Weight (C) and delta K (D) means and confidence intervals by site in the fall. Blue bars that overlap indicate lack of significant difference. Pairwise comparisons conducted with the Bonferroni adjustment method. RV is Rio Vista in the Sacramento River, SM is Suisun Marsh, Yolo is the Yolo Bypass, and FCCL is the Fish Conservation and Culture Lab.

Figure 6-4. Modeled Estimate of Proportional Change (with 95% Confidence Intervals) of Delta Smelt Growth Metrics Across Season (A-B) and Sites (C-D)

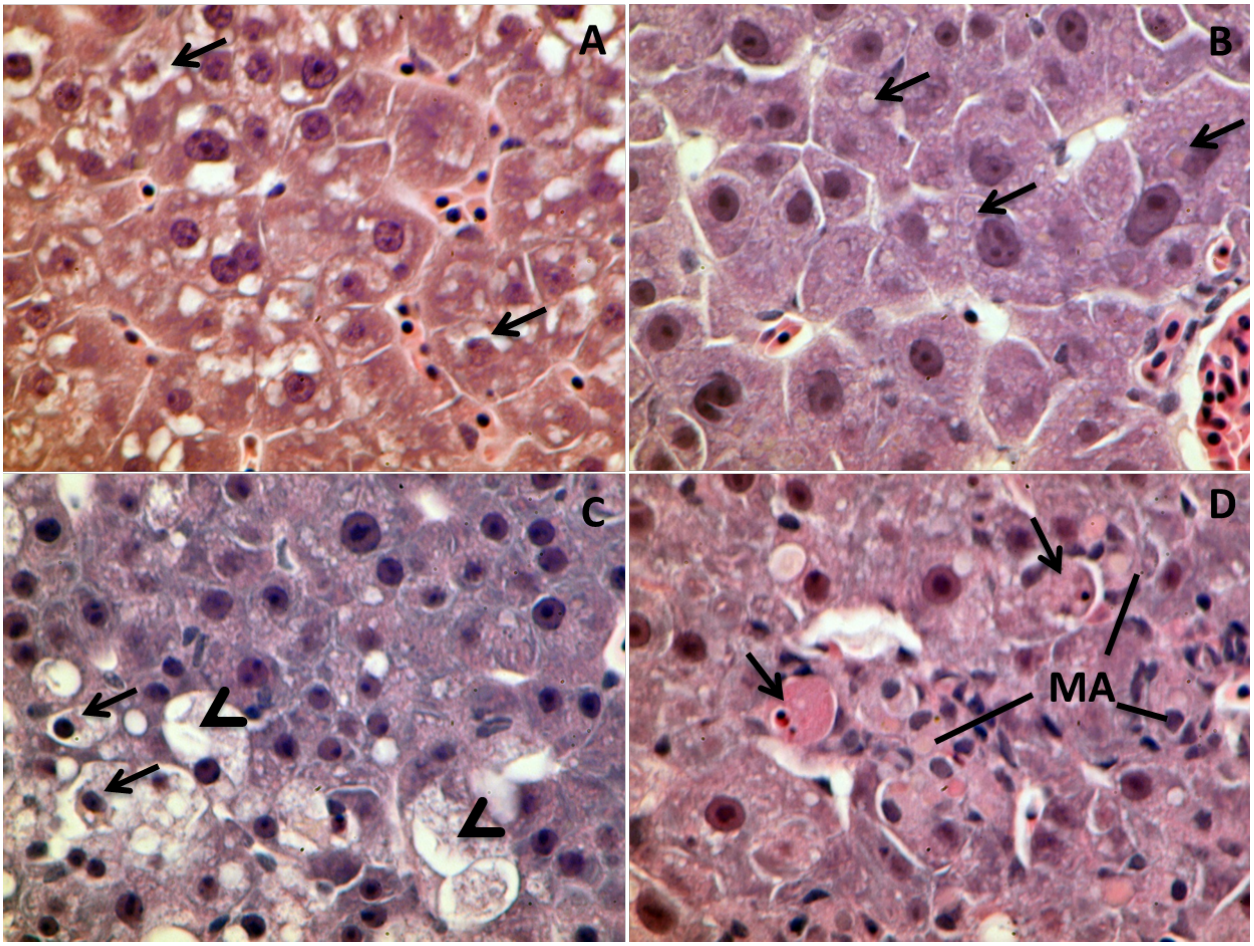
Boxes without data are due to lack of enclosures in those locations in those seasons. Microzooplankton (rotifers and nauplii) were removed in order to focus on larger Delta Smelt prey species. To allow for diets of different sized fish to contribute equally, proportion was calculated by taking the abundance of each taxa divided by the total abundance of all taxa per diet and then averaging across individuals from each enclosure. Number of samples is noted above each bar. Yolo Bypass fish in the summer perished and no diets were recovered.

Figure 6-5. Relative Abundance of All Zooplankton Caught at Each Site During Each Deployment Season and Mean Contribution of Observed Taxa to Delta Smelt Diets at Each Site at the End of Each Deployment



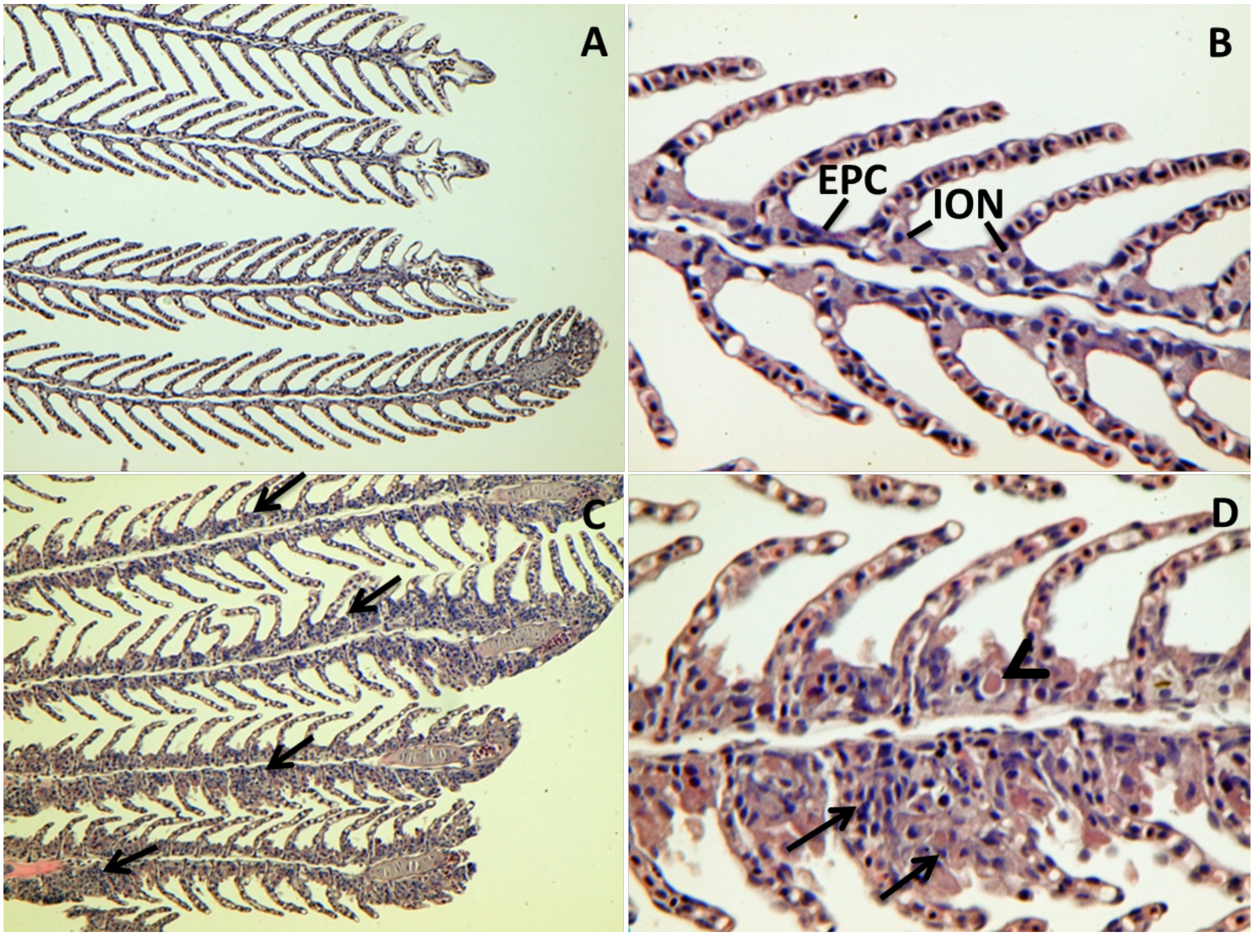
Note that 1) gill histopathology was not performed for the winter deployment, 2) FCCL (control) fish were not collected for the winter deployment, and 3) the Rio Vista winter deployment was 1/23/2019 through 2/25/2019, whereas the SRDWSC winter deployment was 2/27-3/27. The other seasonal deployments occurred concurrently. Error bars are ±SE.

Figure 6-6. Mean Liver Lesion Score (A), Glycogen Depletion (B), and Gill Lesion Score (C) by Season at Each of Five Locations: the Fish Conservation and Culture Laboratory (FCCL), Rio Vista, the Sacramento Deep Water Shipping Channel (SRDWSC), Suisun Marsh, and Yolo Bypass



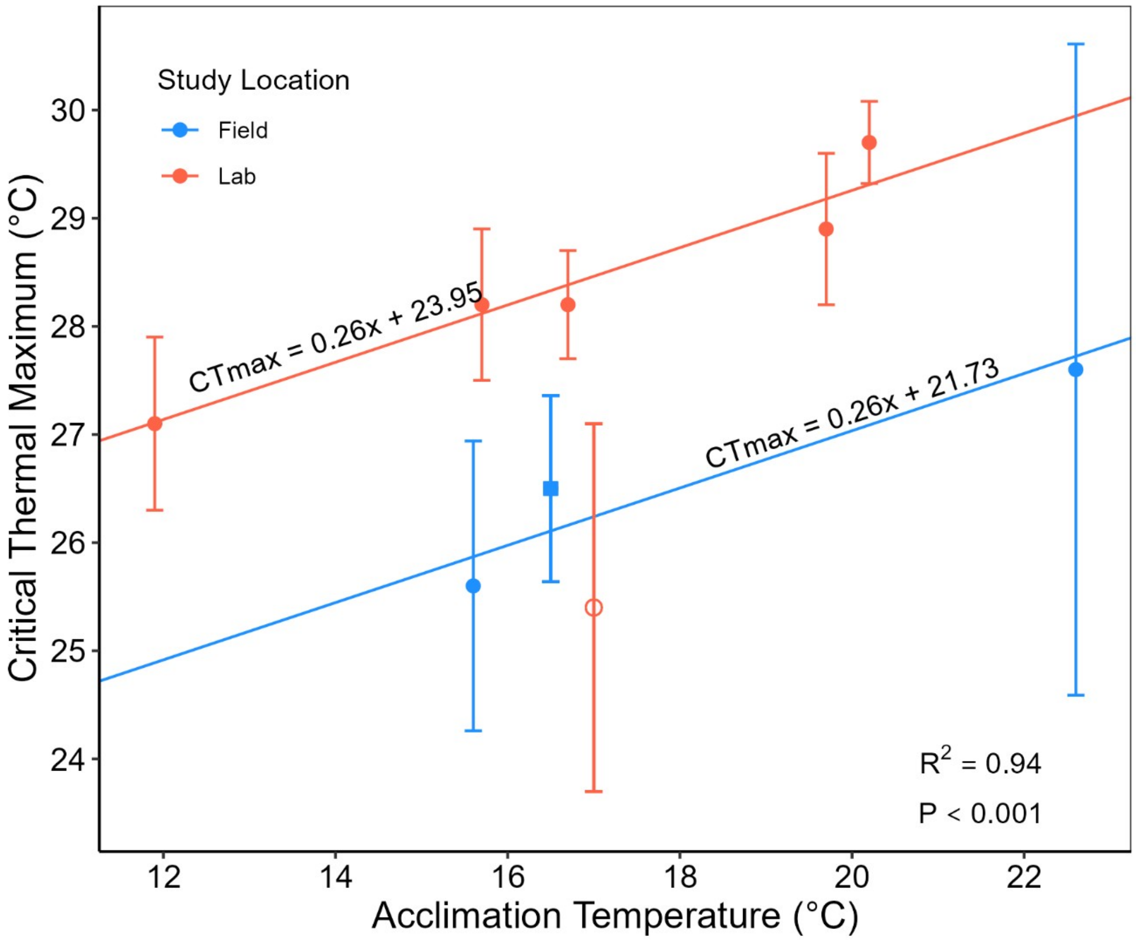
(A) Section of a glycogen-rich liver showing glycogen depots (Arrows) of FCCL control fish in fall. (B) Section of a glycogen-depleted liver of a fish caged in Yolo Bypass for 30 days. Hepatocytes are more basophilic (bluish coloration) and the nutrient stressed cells are packed with cytoplasmic inclusion bodies, presumably autophagosomes (arrows). (C) Section of a glycogen-depleted liver of a fish caged at Rio Vista during fall showing hydropic vacuolar degeneration (arrowheads) and single cell necrosis (arrows). (D) Section of a glycogen-depleted liver of a fish caged in Suisun Marsh for 30 days in fall. Note the infiltration of inflammatory cells forming a macrophage aggregate (MA) surrounding the necrotic cells (arrows).

Figure 6-7. Livers of Delta Smelt H&E 600X



(A) Lower magnification showing normal comb-like architecture of gills in FCCL controls (100X). (B) higher magnification gill epithelial cells (EPC) usually 1-2 cells thick and ionocytes (ION) at junction of primary and secondary lamellae (400X). (C) Lower magnification showing epithelial cell hyperplasia (arrows) in all four primary lamella of fish caged at Yolo Bypass (100X). (D) Higher magnification of a single primary lamellae showing epithelial cell hyperplasia (arrows) and epithelial cell necrosis (arrowhead). Note epithelial layers were approximately 5-10 cells thick and have resulted in distortion and fusion of secondary lamella (400X).

Figure 6-8. Gills of Delta Smelt



Field-acclimatized CTmax data are from the current study summer and fall at Rio Vista (blue circles) and Suisun Marsh in the fall (blue square). Laboratory-acclimated Delta Smelt were reproduced from previous data (Swanson *et al.* 2000; Komoroske *et al.* 2014; Davis *et al.* 2019a). The acclimation-rate of Delta Smelt are represented by a linear regression (slope =0.26, intercept 21.74, p<0.001) with 2.2°C higher CTmax for laboratory-acclimated fish. The laboratory outlier (open red circle at 17, 25.4) represents a single study that used wild-caught Delta Smelt but conducted the CTmax trials in a laboratory (Swanson *et al.* 2000) and was removed from the regression model whereas all other points include hatchery reared Delta Smelt.

Figure 6-9. Critical Thermal Maximum (mean CTmax in °C ± SD) of Delta Smelt by Acclimation Temperature in the Field (blue) or Laboratory (red)

Supplemental Information

Supplemental Tables

Table S6-1. Continuous Water Quality Stations Used in the Study for Year-Round Data

Site of Cages	Yolo Bypass	Suisun Marsh	Rio Vista	North Delta Ship Channel
Station Code - Name	LIS - Lisbon Weir	BDL - Beldens Landing	RVB - Sacramento River at Rio Vista Bridge	DWS - Sacramento Deep Water Shipping Channel
Servicing Agency	DWR	DWR	DWR	USGS
Link	https://cdec.water.ca.gov/dynamicapp/staMeta?station_id=LIS	https://cdec.water.ca.gov/dynamicapp/staMeta?station_id=BDL	https://cdec.water.ca.gov/dynamicapp/staMeta?station_id=RVB	https://cdec.water.ca.gov/dynamicapp/staMeta?station_id=DWS
Coordinates	38.474781°, -121.588226°	38.186900°, -121.970800°	38.159737°, -121.686355°	38.256110°, -121.666670°
Metric	Sensor #	Sensor #	Sensor #	Sensor #
River Stage	1	1	1	1
Temperature	25	25	25	25
Conductivity	100	100	100	100
Turbidity, fnu	221	27	27	221
Chlorophyll	28	28	28	--
Dissolved oxygen	61	61	61	--
pH	62	62	62	--

Table S6-2. Result Summary Growth Metrics of Delta Smelt in Enclosures

Season	Site	Fork Length	Weight	Condition Factor
Winter	RV	Increase	Increase	Increase
Winter	SRDWSC	ns	Decrease	Decrease
Summer	RV	ns	Decrease (marginal) (t = 2.2092, df = 114.81, p = 0.029)	Decreased (t= 3.3645, df = 156.73, p < 0.001)
Summer	FCCL	Increase (t = -3.8593, df = 140.42, p = 0.0001728)	Increase (marginal) (t = -2.1359, df = 145.77, p = 0.034)	Decreased (t = 3.5696, df = 144.76, p < 0.001)
Fall	RV	ns	Decrease (t = 4.4429, df = 04.121, p < 0.001)	Decrease (t = 12.045, df = 106.72, p = 2.2e-16)
Fall	YB	ns	Decrease (t = 5.6128, df = 76.488, p < 0.001)	Decrease (t = 9.3668, df = 145.22, p < 0.001)
Fall	SM	Decrease (marginal) (t = 2.0638, df = 91.708, p = 0.042)	Decrease (t = 6.3606, df = 84.158 p < 0.001)	Decrease (t = 13.041, df = 121.67, p < 0.001)
Fall	FCCL	Decrease (t = 2.3163, df = 112.18, p = 0.022)	Decrease (t = 3.6275, df = 115.53, p < 0.001)	Decrease (t = 4.27, df = 142.68, p < 0.001)

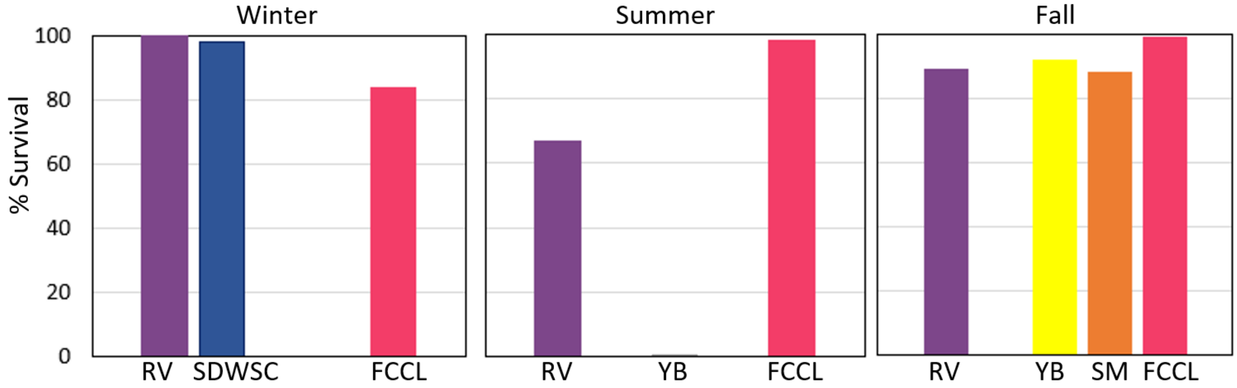
Winter results at Rio Vista (RV) are summarized from Baerwald *et al.* (2023). The directional change in growth (e.g., weight, length, condition) is noted based on the comparative t-test of fish before and after cage deployments; decrease, increase (and if marginal p-value) or no significant difference (ns). Sites include the Sacramento River Deep water Ship Channel (SRDWSC), Suisun Marsh (SM), Yolo Bypass (YB), and the reference fish kept at the Fish Conservation and Culture Laboratory (FCCL).

Table S6-3. Results from Pairwise Comparisons for DeltaK and DeltaWeight by Season (at Rio Vista) and Location (sites in fall)

Model	Contrast	Estimate	SE	df	t ratio	p-value
Seasons at Rio Vista						
Delta K	Winter-Summer	0.124	0.0205	6.32	6.053	0.0019
	Winter-Fall	0.268	0.0197	5.44	13.593	<.0001
	Summer-Fall	0.144	0.0207	6.56	6.964	0.0007
Delta Weight	Winter-Summer	0.244	0.0395	6.3	6.168	0.0017
	Winter-Fall	0.396	0.0381	5.46	10.418	0.0002
	Summer-Fall	0.153	0.0399	6.54	3.838	0.0173
Location within Fall						
Delta K	FCCL-RV	0.0856	0.0211	9.16	4.051	0.0167
	FCCL-SM	0.1049	0.0211	9.09	4.974	0.0045
	FCCL-YB	0.065	0.0211	9.16	3.075	0.0778
	RV-SM	0.0193	0.0199	7.29	0.968	1
	RV-YB	-0.0206	0.02	7.35	-1.031	1
	SM-YB	-0.0399	0.0199	7.29	-2.001	0.5035
Delta Weight	FCCL-RV	0.0444	0.11	8.93	0.404	1
	FCCL-SM	0.1957	0.11	8.87	1.786	0.6495
	FCCL-YB	0.1201	0.11	8.93	1.094	1
	RV-SM	0.1514	0.105	7.38	1.446	1
	RV-YB	0.0758	0.105	7.44	0.722	1
	SM-YB	-0.0756	0.105	7.38	-0.722	1

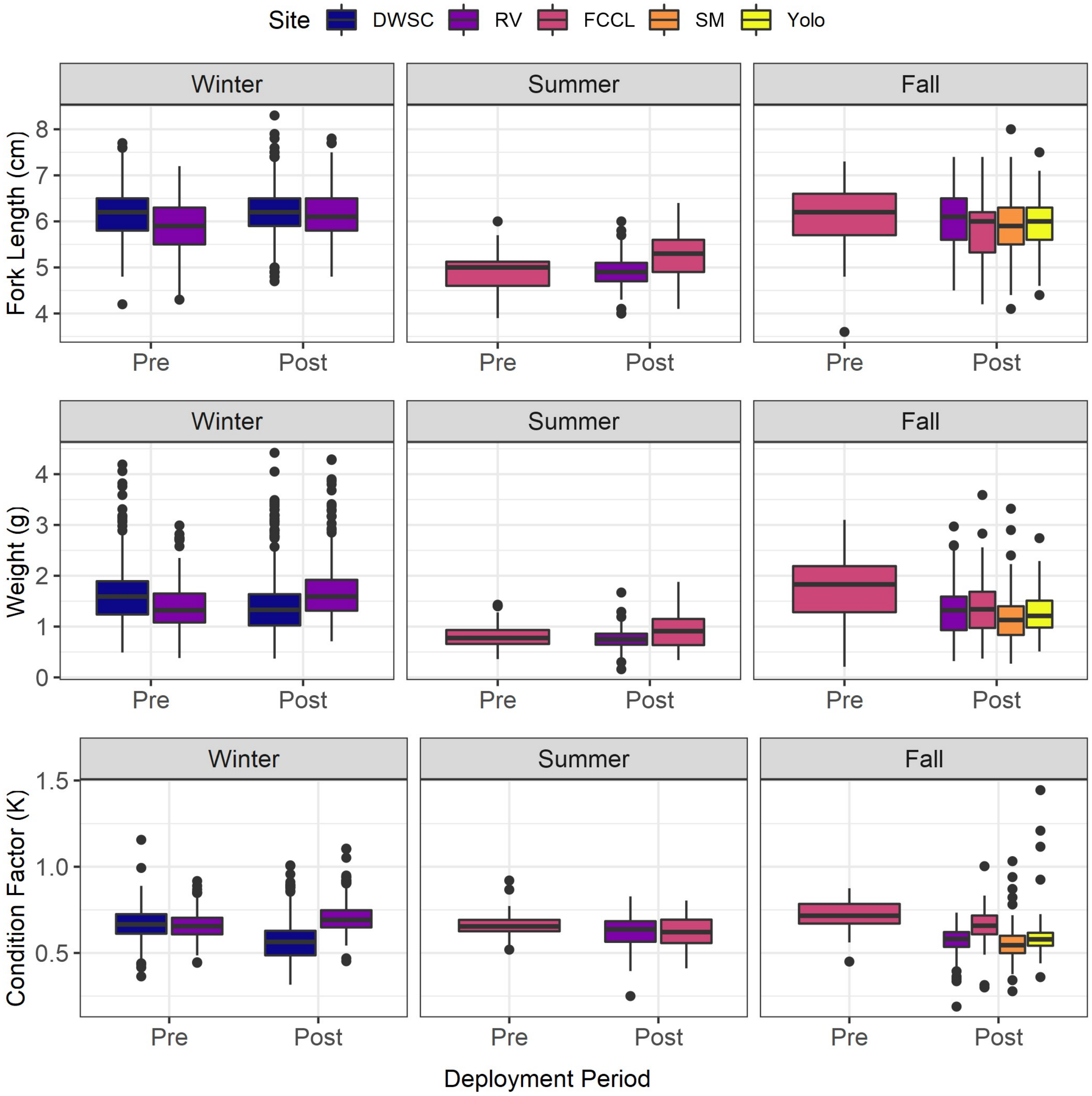
P-values adjusted using the Bonferroni method. Sites include the Sacramento River at Rio Vista (RV), Suisun Marsh (SM), Yolo Bypass (YB), and the reference fish kept at the Fish Conservation and Culture Laboratory (FCCL).

Supplemental Figures



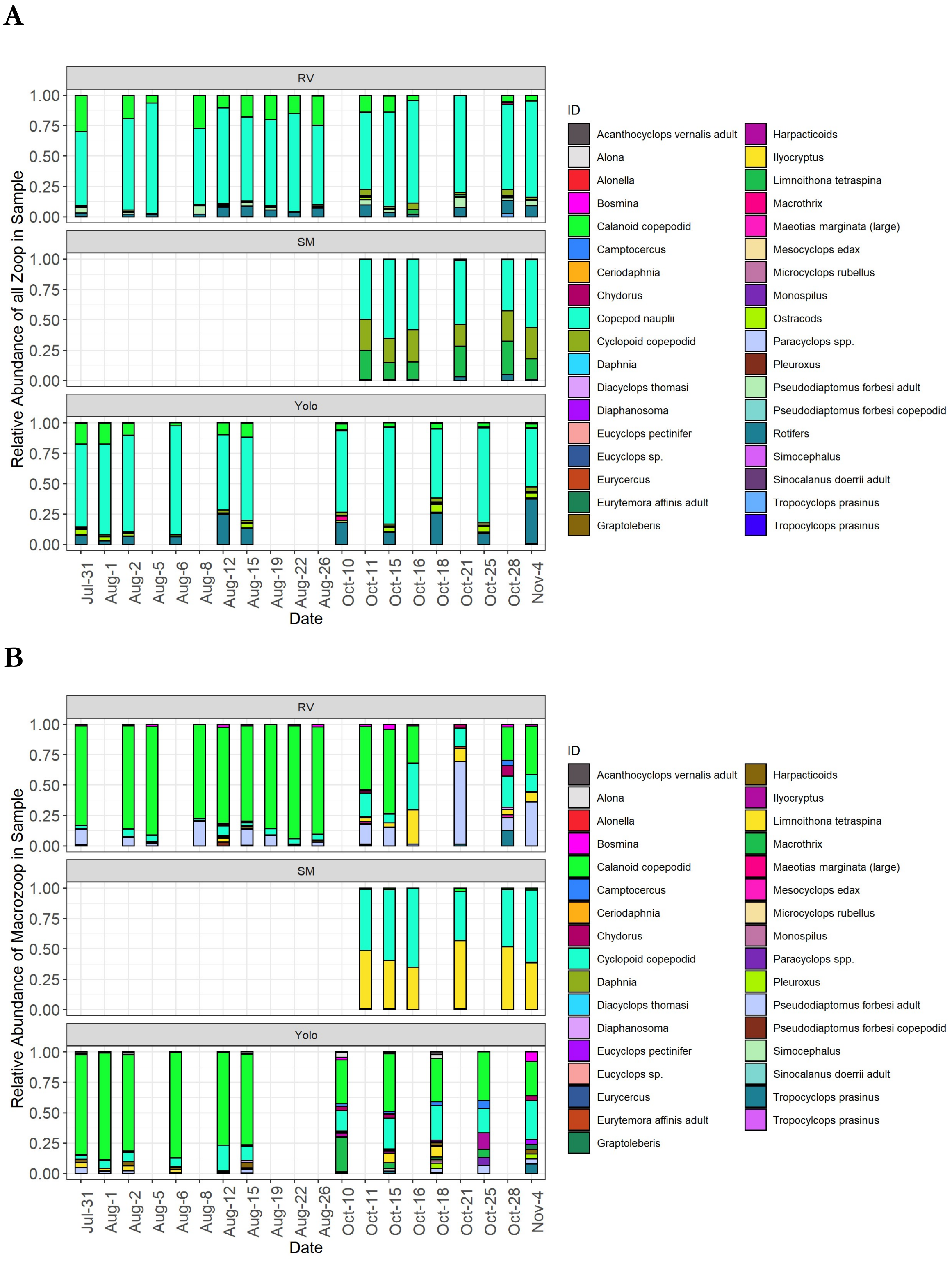
Enclosures at Rio Vista (RV) were deployed each season, Sacramento Deepwater Shipping Channel (SRDWSC) only in winter, Yolo Bypass (YB) in summer and fall, and Suisun Marsh (SM) enclosures only in fall. Control fish at the Fish Conservation and Culture Laboratory (FCCL) were kept each season.

Figure S6-1. Percent Survival of Delta Smelt After Deployments Across Sites and Seasons



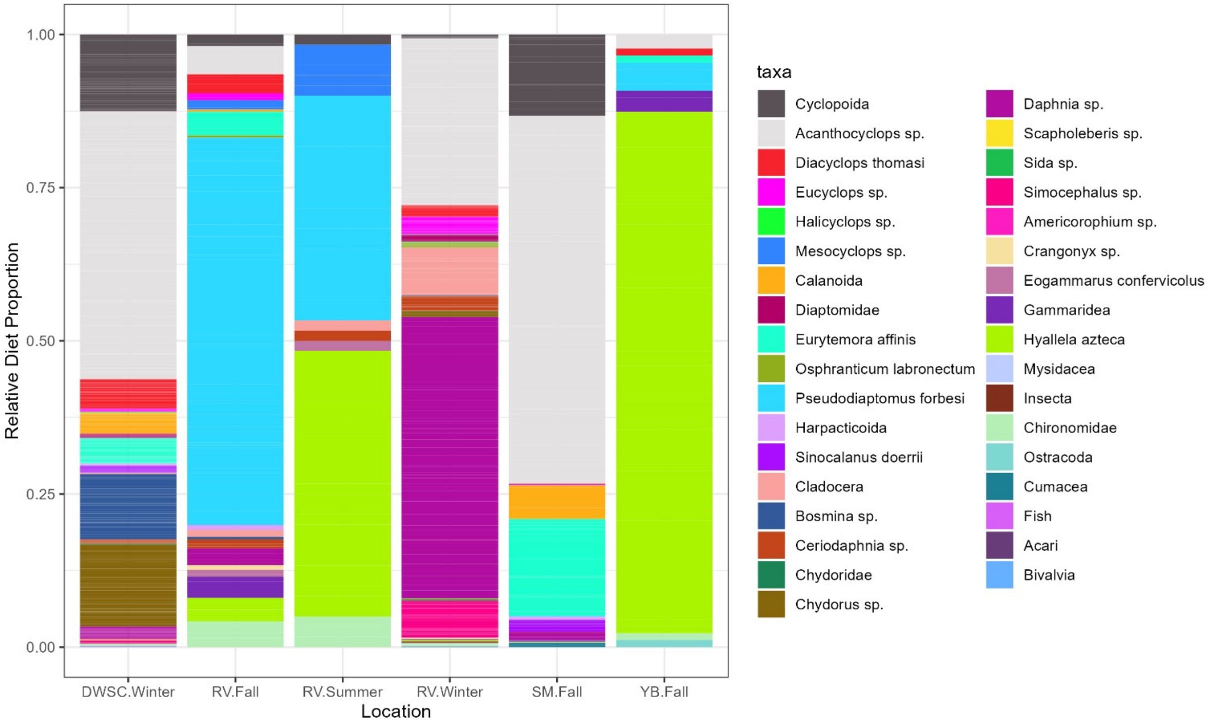
Boxes represent the interquartile range, whiskers indicate 1.5 the interquartile range, the line is the median and points are outliers that exceed 1.5 the interquartile range. RV is Rio Vista in the Sacramento River, SRDWSC is Sacramento River Deepwater Ship Channel, SM is Suisun Marsh, Yolo is the Yolo Bypass, and FCCL the reference fish kept at the Fish Conservation and Culture Lab.

Figure S6-2. Length (cm), Weight (g), and Condition Factor (K) of Delta Smelt Before and After Enclosure Deployments Across Sites and Season



RV is Rio Vista in the Sacramento River, SM is Suisun Marsh, and Yolo is the Yolo Bypass.

Figure S6-3. (A) Relative Abundance of all Zooplankton Caught at Each Site During Each Deployment Season; (B) Relative Abundance of Macrozooplankton Caught at Each Site During Each Deployment Season



SRDWSC is Sacramento River Deep water Ship Channel, RV is Rio Vista in the Sacramento River, SM is Suisun Marsh, YB is the Yolo Bypass.

Figure S6-4. Contribution of Different Taxa to Delta Smelt Diets at Each Location and Season

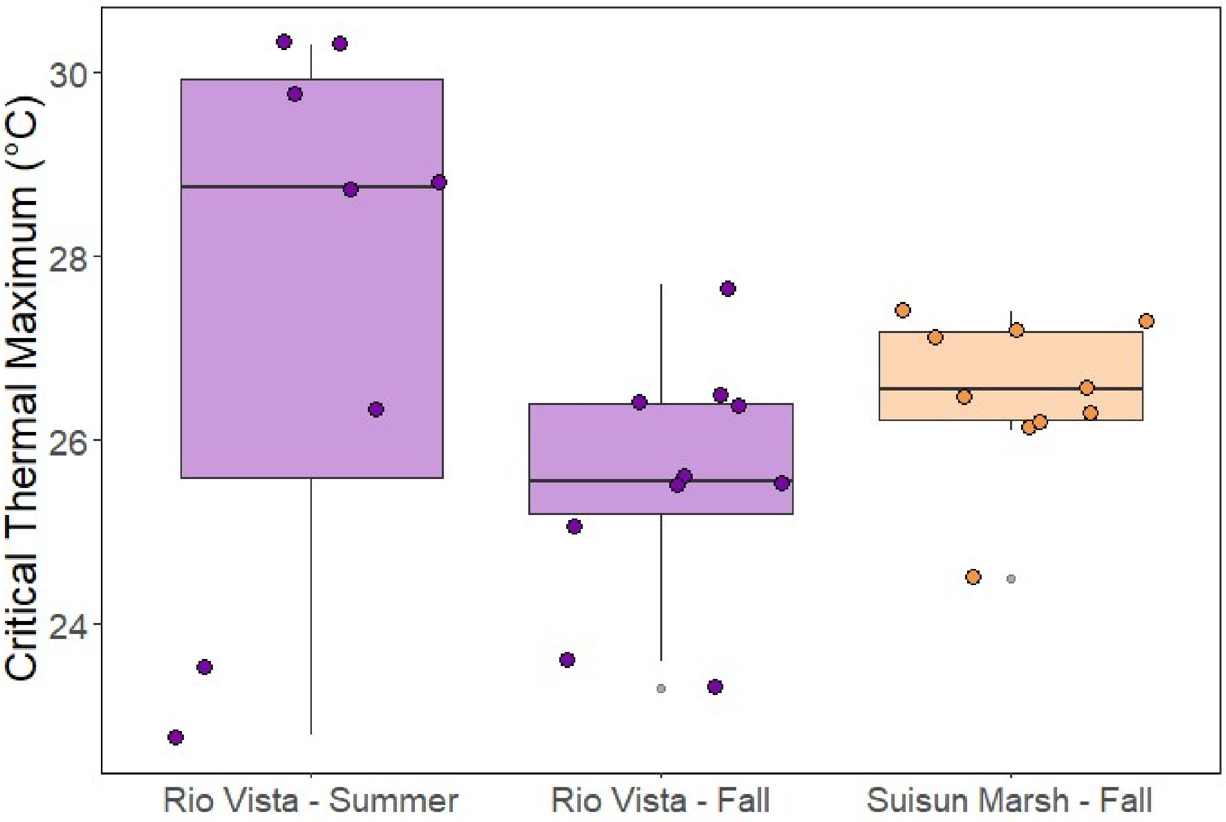


Figure S6-5. Critical Thermal Maxima (CTmax) of all Fish Measured in 2019 Including Cages in the Summer at Rio Vista

Chapter 7. The Influence of Zooplankton Availability on Delta Smelt Condition and Foraging Across Habitat Contexts

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Keywords: fish, San Francisco Estuary, gut fullness, food limitation

Abstract

Strategies for endangered species conservation may have differential outcomes depending on the habitat context in which they are implemented. Understanding these context dependent effects can help optimize and target management efforts. In this analysis, we investigate how environmental and food web conditions interactively affect condition and foraging of Delta Smelt (*Hypomesus transpacificus*), an endangered fish endemic to the San Francisco Estuary (Estuary). Food limitation, in terms of pelagic zooplankton availability, is considered a main contributing factor to the decline in Delta Smelt abundance. Our overarching objective was to examine whether the effect of zooplankton on Delta Smelt depended on habitat context. Specifically, we hypothesized that zooplankton would have a less positive effect on Delta Smelt condition—as measured by hepatosomatic index (HSI)—and foraging success in areas with nearby tidal wetlands, as these adjacent habitats may provide access to prey items from the epibenthos and fringing vegetation. In contrast, in regions with limited proximity to wetlands, we expected that Delta Smelt would be more reliant on pelagic prey, which would manifest as a more positive effect of zooplankton on body condition and foraging success for Delta Smelt. Using models that accounted for habitat in multiple ways, we found little evidence that zooplankton and habitat interactively influenced Delta Smelt condition or gut fullness. Rather, the influence of zooplankton on HSI and gut fullness was generally positive across habitat contexts. Given the well-documented food limitation in the Estuary,

promoting the availability of pelagic zooplankton is a rational, albeit complex, management aim. Furthermore, our results suggest that efforts to increase zooplankton would be broadly beneficial for Delta Smelt across a wide range of habitat contexts.

Introduction

Ecological relationships can vary depending on local environmental conditions, and this context dependence has implications for conservation (Catford et al. 2022). For instance, the net effects of managing local stressors can depend on the context of stressors at larger spatial scales (Brown et al. 2013). As an illustrative example, marine reserves may have the largest conservation impact on sensitive species if they occur in refuges from climate warming (Brown et al. 2013). In a similar vein, the outcomes of habitat restoration for threatened species may depend on other environmental conditions (e.g., physiological stress imposed by poor water quality, competition or predation pressure from invasive species). Moreover, the capacity for management is finite, so resources must be strategically allocated to maximize conservation outcomes (Cote et al. 2016). Understanding how environmental factors interact to influence target species can help optimize conservation and management actions, in addition to better understanding their basic ecology.

The Delta Smelt (*Hypomesus transpacificus*) is a fish endemic to the Sacramento-San Joaquin Delta and San Francisco Estuary (hereafter the Estuary), which is a physically, politically, and hydrodynamically complex ecosystem. The population of Delta Smelt has sharply declined and is nearly extinct in the wild (Moyle et al. 2016), to the point that annual supplementation of the wild population with hatchery-raised fish began in December 2021 (Hung et al. 2022). Population reductions of Delta Smelt and other pelagic fishes in the Estuary are thought to be caused by a variety of proximate factors, including low recruitment, habitat and water quality degradation, increased mortality, and food limitation (Sommer et al. 2007, Moyle et al. 2016). Currently, substantial effort and resources are being invested in conservation and supplementation of Delta Smelt. Understanding the factors that influence Delta Smelt health and condition under a variety of ecological contexts is fundamental to informing which management strategies may have the most positive conservation outcomes.

Here we focus on food limitation as a key factor linked to the imperiled status of Delta Smelt. Pelagic zooplankton are a critical prey resource for Delta Smelt (Slater and Baxter 2014, Slater et al. 2019), but zooplankton abundance has declined across the Estuary (Winder and Jassby 2011, Cloern and Jassby 2012) and has remained consistently low since around 1990 (Hammock et al. 2019b, Bashevkin et al. 2022). Given the uncertainties and expense of management actions, it is critical to understand whether alleviation of food limitation may differentially affect Delta Smelt across habitat contexts. For example, in highly channelized, relatively homogeneous areas such as the Sacramento River Deepwater Ship Channel (SRDWSC), zooplankton may be particularly beneficial to Delta Smelt if the species is highly dependent on prey in the water column. In Suisun Marsh, in contrast, fish may be less dependent on prey within the water column if they forage along adjacent tidal wetlands, the proximity of which correlates with foraging success of Delta Smelt (Hammock et al. 2019a). Understanding the possible context dependence of the relationship between zooplankton abundance and Delta Smelt could guide managers in deciding how, where, and when to focus actions to bolster bottom-up pathways of the pelagic food web.

In this study, we consider whether zooplankton abundance differentially affects Delta Smelt condition and foraging success across habitat contexts. We characterize habitat with three related variables within the Estuary (salinity, tidal wetland proximity, and region), and we hypothesized that there would be an interactive effect between zooplankton biomass and each of these habitat variables on Delta Smelt condition and foraging success. Specifically, we hypothesized that the effect of total zooplankton biomass would be more positive in freshwater habitats (which are generally more channelized), in habitats with limited proximity to tidal wetlands, and that the effect of zooplankton would vary regionally, depending on these characteristics (e.g., in SRDWSC, zooplankton biomass would have a strongly positive influence). In contrast, we hypothesized that zooplankton would have a less positive effect on Delta Smelt in brackish habitats, habitats adjacent to tidal wetlands, and regions more influenced by tidal wetlands (such as Suisun Marsh). The proposed mechanism is the same in each context: in habitats with greater tidal wetland influence, fish foraging may be less coupled to zooplankton availability in the pelagic environment.

Methods

Dataset

We analyzed condition and foraging success of Delta Smelt collected from August 2011 to September 2020 from the Estuary. In considering Delta Smelt condition, we examined hepatosomatic index (HSI):

$$\text{HSI}=\left(\text{liver wt}\right)/\left(\text{total body wt}\right)\times 100$$

HSI reflects short-term energy reserves available to fishes (Mehner and Wieser 1994, Peragón et al. 1999, De Pedro et al. 2003), and is sensitive to food limitation in Delta Smelt, responding to fasting within four days at 16°C (Hammock et al. 2020). To assess Delta Smelt foraging success we examined gut fullness:

$$\text{Gut fullness}=\left(\text{gut content wt}\right)/\left(\text{total body wt}\right)\times 100$$

Gut fullness provides a snapshot of material ingested by fish; however, it does not necessarily correlate with prey quality, nor does it indicate what will be assimilated as fish tissue. Delta Smelt were collected by both the California Department of Fish and Wildlife (CDFW) and United States Fish and Wildlife Service (USFWS). CDFW conducts several long-term fish monitoring fish surveys, namely Summer Townet (STN), Fall Midwater Trawl (FMWT), and Spring Kodiak Trawl (SKT), two of which collect zooplankton concurrently (STN and FMWT). Following a sharp decline in the abundance of Delta Smelt in the wild, more intensive sampling carried out by USFWS was initiated in December 2016 (i.e., Enhanced Delta Smelt Monitoring; EDSM). Beginning in 2017, the consulting company ICF began conducting surveys to complement the EDSM sampling by collecting zooplankton, phytoplankton, and water quality samples. Sampling by ICF does not completely overlap with EDSM surveys; in particular, it occurred from September to November in 2017-2018 and from April to November in 2019-2020 (Schultz and Kalmbach 2023).

Our objective required examining the relationships between Delta Smelt condition and foraging success with zooplankton availability. Thus, we restricted our dataset to Delta Smelt that were collected with a complementary zooplankton tow, such that our observations were coupled in time and space. Accordingly, the dataset included fish from STN and FMWT surveys which collect zooplankton and fish samples concurrently at a subset of survey sites. Similarly, we only included fish collected by EDSM trawls that had an accompanying zooplankton tow from the same date and station. Thus, our analysis focuses on juvenile through sub-adult fish and does not include the more mature fish collected during spring, which lack concurrently collected zooplankton data (e.g., during

SKT). Details on collection methods for Delta Smelt and zooplankton are described elsewhere (Honey et al. 2004, Feyrer et al. 2007, Sommer and Mejia 2013, Damon et al. 2016, Kayfetz et al. 2020, Bashevkin et al. 2022, White and Baxter 2022).

Immediately after collection, fish were wrapped in labeled aluminum foil, flash-frozen in liquid nitrogen kept on each boat, and later transferred to the University of California, Davis. At UC Davis, fork length and body weight were recorded, and specimens were dissected (see Teh et al. 2016 for details). The liver and gut were excised and the liver was weighed. The gut was preserved in ethanol and transferred to the CDFW Diet Study Laboratory where gut contents were identified, enumerated, and gut content weight was estimated (see Slater and Baxter 2014, Slater et al 2019).

Zooplankton data from tows collected during the STN and FMWT surveys were downloaded using the 'zooper' R package (Bashevkin et al. 2022, Bashevkin et al. 2023a, Bashevkin et al. 2023b). We converted the reported units of 'catch per unit effort' (CPUE or catch/m^3) to 'biomass per unit effort' (BPUE; µg C/m^3) using taxon-specific conversion factors (Kayfetz et al. 2020, Burdi et al. 2021). ICF data was also converted to BPUE using conversions from Kayfetz et al. (2020).

Response variables

Our analysis consisted of six linear models with Gaussian distributions, in which either HSI or gut fullness was the response variable (three models for each response variable). We aggregated fish data to the station-date level to match the resolution of the zooplankton data. For HSI, 174 observations from 74 stations (each with observations on 1 to 19 different dates) had corresponding data for zooplankton. However, a small subset of these lacked certain habitat data (e.g., the Estuary region, wetland abundance; n=7), such that sample size varied slightly among our models. Of these 74 stations, 47 were sampled during EDSM, with these stations each being sampled only one time due to EDSM stations being randomly selected.

For gut fullness, data aggregated to the station-date level were available for 136 observations. Gut fullness values were arcsine square root transformed to improve normality. Delta Smelt are visual predators, so gut fullness increases with time of day (Hobbs et al. 2006, Hammock et al. 2017). We corrected for this effect by linearly regressing transformed fullness against time of day; we then subtracted the time-of-day slope coefficient times the time of collection from each fullness observation and added the product of that slope times the average hour of collection across the dataset. Thus, each transformed gut fullness value was corrected for the time of collection. We use these time-corrected arcsine square root transformed gut fullness data in our analysis, but we simply refer to these data as gut fullness. In figures depicting gut fullness, arcsine square root values are plotted, but we back-transform the axis labels so that they show gut fullness percentages.

Predictor Variables

The six models were created using an *a priori* hypothesis testing framework in which 'habitat' was quantified three different ways to assess the hypothesis that zooplankton would interact with habitat to influence HSI and gut fullness. Each model included zooplankton biomass and season, known predictors of HSI and gut fullness for Delta Smelt. Zooplankton biomass had a right-skewed distribution, so we used ln-transformed zooplankton biomass in our models. In terms of season, HSI of wild Delta Smelt is lowest in fall (Hammock et al. 2022). Additionally, seasonal effects can be habitat dependent (e.g., salinity by season interaction), with fuller guts being observed in freshwater habitat in the summer but brackish habitats in the fall (Hammock et al. 2017). Furthermore, zooplankton abundance and composition can shift seasonally throughout the Estuary (Ambler et al. 1985). For example, two calanoid copepod species that are important prey for Delta Smelt (Slater and Baxter 2014) show contrasting seasonal patterns in abundance, with *Pseudodiaptomus forbesi* being abundant in summer/fall and *Eurytemora affinis* being abundant in the winter/spring and rare in summer/fall (Durand 2010, Merz et al. 2016). Thus, seasonal effects on Delta Smelt condition and foraging success may act indirectly through their effects on the zooplankton community. We include season as a categorical variable (summer or fall using a cutoff of 30 August, which corresponds to the transition from STN to FMWT). In supplemental analyses, we examine seasonal patterns in zooplankton biomass, HSI, and gut fullness using the datasets specific to our study and compare the results to previously published studies (Supplemental Information).

In addition to zooplankton and season, each model included one term that characterized habitat; this was either a categorical salinity variable (freshwater or brackish, based on a salinity cutoff of 0.55; Hammock et al. 2017), a categorical wetland abundance variable (low, medium, high; cutoffs were based on the distributions of wetland area within a 2-km radius of the station; Figure 7-1; Hammock et al. 2019a), or a categorical variable for five regions of the Estuary (Cache Slough, Confluence, Suisun Bay, Suisun Marsh, and the SRDWSC; Figure 7-2), which encompass much of the recent distribution of Delta Smelt (Moyle et al. 2016). Categorization of our study sites reveals that our habitat characterizations are interrelated (Figure 7-3); for example, all sites in the Cache Slough and SRDWSC region were freshwater, while all but one site from Suisun Bay and Suisun Marsh were brackish. Additionally, brackish habitats were generally associated with more abundant wetland habitat (Figure 7-3; Hammock et al. 2019a). Thus, although our hypothesis most specifically focused on tidal wetlands, the related habitat variables, salinity and region, were also included to test the hypothesis more broadly. Finally, all models included an interaction term between zooplankton biomass and the habitat variable, the primary parameter of interest in this study. Thus, the six models were in this form:

$$\text{HSI or Gut fullness} \sim \text{zoop} + \text{habitat} + \text{season} + \text{zoop} \times \text{habitat}$$

where ‘habitat’ was ‘salinity,’ ‘region,’ or ‘tidal wetland area.’

Statistical significance was determined based on $\alpha = 0.05$. Results are reported from type-III F-tests unless noted, which were performed with the ‘car’ package (Fox and Weisberg 2019). All analyses were conducted in R v. 4.2.1 (R Core Team 2022).

Results

Similar to previous studies, 1) meso-zooplankton biomass was higher in freshwater than in brackish water (Figure S7-1); 2) HSI declined from summer into fall in both freshwater and brackish water (Figure S7-2); and 3) gut fullness declined in freshwater from summer into fall but showed the opposite trend in brackish water (Figure S7-3). Our analysis of zooplankton biomass showed muted seasonal effects (Figure S7-1) compared to previous studies which analyzed zooplankton abundance (e.g., Hammock et al. 2017). However, a gradual temporal decline in meso-zooplankton biomass generally occurred in fall (Figure S7-4), while HSI declined abruptly from summer into fall in both freshwater and brackish habitat (Figure S7-4; Hammock et al. 2017, 2022). Additional details are in the supplemental results and each of these effects are accounted for in the models below.

HSI Models

We examined the influence of zooplankton biomass on Delta Smelt HSI in different habitat contexts. All HSI models had relatively decent fit to the data based on adjusted R^2 (0.21 to 0.29) (See Table 7-1). In the model that included salinity as the habitat variable, the zooplankton by salinity interaction effect was non-significant (Table 7-1A, Figure 7-4). The main effect of zooplankton biomass on HSI was non-significant (Table 7-1A), but results from a type II F-test, which does not consider (in this case, nonsignificant) interaction effects, supported a positive effect of zooplankton biomass, regardless of salinity ($F = 5.4$, $p = 0.021$; Figure 7-4). HSI values were significantly lower in fall compared to summer (Table 7-1A; Figure 7-4). Overall, we found no support for zooplankton having a more positive effect on HSI in freshwater than in brackish habitat as hypothesized.

In the model that included wetland prevalence as our habitat variable, the zooplankton by wetland interaction was statistically significant (Table 7-1B; Figure 7-5). Specifically, zooplankton biomass had an overall positive effect on HSI, with the effect being most positive in sites in the "medium" wetland category (Figure 7-5). The range of the zooplankton predictor was notably wider for the medium wetland sites, as both the minimum and maximum observed zooplankton biomass values occurred in this category. To evaluate whether these points were unduly influencing the results, we re-ran the model without these two points. While the trend was slightly muted without these points (i.e., higher p-values), the zooplankton by wetland interaction remained statistically significant ($F = 3.89$, $p = 0.022$; Figure S7-5). HSI was lower in fall than summer (Table 7-1B, Figure 7-5). Overall, results suggest that the magnitude of the zooplankton effect on HSI depended on prevalence of wetland, but in a non-linear fashion (i.e., effect was most positive in the 'medium' category).

Lastly, in the model that included region as the habitat variable, the zooplankton by region interaction term was non-significant (Table 7-1C; Figure 7-6). Zooplankton biomass positively affected HSI (Table 7-1C; Figure 7-6). Like the previous two models, season had a significant effect on HSI, with lower HSI in fall (Table 7-1C, Figs 6A and 6B). Overall, we found that the effect of zooplankton on HSI was generally positive and not statistically dependent on region of the Estuary (i.e., overlap in the parameter estimates for each region; Figure 7-6C).

Gut Fullness Models

Models for gut fullness had relatively low fit, with adjusted R^2 ranging from 0.07 to 0.1 (Table 2). In the three gut fullness models across habitat contexts, we did not detect a statistical interaction between zooplankton biomass and habitat (salinity, wetland, or region; Table 2; Figs 7-9). In the model using wetland abundance as the habitat variable, there was a positive effect of zooplankton biomass on gut fullness (Table 2; Figure 7-8). For the models using salinity and region, the main effect of zooplankton was not significant for type-III tests (Table 2C) but was for type-II tests (for salinity: $F = 10.00$, $p = 0.002$, Figure 7-7; for region: $F = 7.23$, $p = 0.008$, Figure 7-9). Thus, gut fullness increased with the biomass of zooplankton in the water column, and this effect was independent of habitat characteristics.

Discussion

We aimed to understand whether local habitat characteristics influence the apparent effect of pelagic zooplankton availability on Delta Smelt condition and foraging success. We found little to no support for our hypothesis that zooplankton would have a more positive effect on Delta Smelt condition and foraging in regions dominated by freshwater habitat and low wetland abundance. Overall, zooplankton biomass showed a positive effect on HSI and gut fullness, nearly regardless of habitat context. These results suggest that foraging and condition for juvenile through sub-adult Delta Smelt would generally benefit from increased zooplankton biomass throughout its range.

The lone interaction detected in our study was between wetland abundance and zooplankton biomass on HSI. This interaction was somewhat counter-intuitive, as zooplankton exerted the most positive influence on HSI at a moderate level of wetlands. Thus, the direction of the interaction was not aligned with our hypothesis, in which we predicted that zooplankton biomass would have the strongest effect on HSI at sites with limited proximity to wetlands. The effect of zooplankton biomass on HSI was nearly indistinguishable between sites with low and high wetland proximity. Perhaps the most plausible explanation is that the interaction is spurious (i.e., unrelated to tidal wetland area). The interaction did not follow the *a priori* hypothesis that higher zooplankton densities would most benefit fish condition in channelized habitat. Moreover, the gut fullness results showed that zooplankton density improved foraging success regardless of tidal wetland influence, so the mechanism by which we expected tidal wetlands to improve juvenile through sub-adult Delta Smelt HSI was not supported.

While the interaction between zooplankton and region was not statistically significant, the region-specific zooplankton effects on HSI were somewhat aligned with our hypothesis. Specifically, Suisun Marsh is relatively rich in tidal wetlands and was the region with the least positive zooplankton influence on HSI (Figure 7-6C). Pelagic prey are particularly scarce in Suisun Marsh but wild-captured Delta Smelt are consistently in relatively good condition (Hammock et al. 2015, 2022). In contrast, Delta Smelt that are experimentally caged in the water column appear starved (Davis et al. *In revision*). Together these studies suggest that Delta Smelt are less dependent on pelagic prey in Suisun Marsh, possibly because they forage along tidal wetlands. However, the region-specific 95% confidence intervals are wide and overlap across regions, and the zooplankton by region interaction is nonsignificant. Nevertheless, managers may consider targeting regions with more positive parameter estimates for actions meant to increase zooplankton abundance, if the goal is to benefit Delta Smelt (i.e., Cache Slough, SRDWSC, and Suisun Bay; Figs 2 and 6C), especially given the reality of operating under resource constraints (e.g., time, costs).

In all HSI models, Delta Smelt condition was lower in fall than summer. This fall decline in HSI is also consistent across a wide range of water-year conditions (Hammock et al. 2022). Moreover, higher HSI values are observed in summer, despite the relative frequency of warm water temperatures. Specifically, water temperatures over 21°C are common, particularly in freshwater habitats, and Delta Smelt tend to experience thermal stress around 20-21°C (Moyle et al. 2016). Thus, higher HSI values in summer occur despite somewhat suboptimal abiotic conditions, and may relate to relatively abundant zooplankton in summer in freshwater (Supplemental Information). Lusardi et al. (2020) described similar results with Coho salmon (*Oncorhynchus kisutch*) growing quickly at water temperatures generally considered suboptimal for the species, showing that abundant prey can offset negative effects of high water temperatures in the wild. That is not to say that prey were sufficiently abundant for Delta Smelt in summer in freshwater. We detected a positive relationship between HSI and gut fullness with increasing zooplankton density in freshwater (Figure 7-7), implying that the population would have eaten more and had better condition had the prey density been higher. The seasonal decline in Delta Smelt HSI may be partly attributed to resource limitation; although, we only observed a decline in zooplankton biomass in freshwater habitats (Supplemental Information, but note that this effect was nonsignificant). Additionally, temporal trends in HSI appear to decline more abruptly across the summer to fall transition compared to zooplankton biomass (which shows a more gradual decline; Figure S7-4). Another possibility is that some amount of survivorship bias may occur in the summer, where only Delta Smelt with high HSI survive through this period of peak water temperature. A recent life cycle analysis for Delta Smelt indicate that summertime survival has a strong influence on the species' population trajectory over the recent decades (Polansky et al. 2024). Our study does not identify the specific mechanism(s) driving the strong seasonal effect on HSI, but identifying this factor should improve understanding of the environmental controls on Delta Smelt condition.

We found that gut fullness was positively related to zooplankton biomass across habitats. The consistent lack of an interaction in our gut fullness models—regardless of the habitat variable—suggests that juvenile through sub-adult Delta Smelt forage on pelagic prey, regardless of habitat context. Interestingly, this positive effect of zooplankton on foraging success was observed despite substantial spatial differences in zooplankton community composition (Winder and Jassby 2011) and resulting composition of prey in the Delta Smelt gut contents across habitats (Slater et al. 2019). Additionally, Delta Smelt consume larger prey items that are not targeted by mesozooplankton sampling (e.g., mysids, amphipods, larval fish) in both brackish and freshwater habitats (Slater et al. 2019). In short, despite the zooplankton tows containing different prey assemblages across habitats, juvenile through sub-adult Delta Smelt seemed to consume prey biomass in relation to the prevalence of zooplankton in the water column.

Previous studies have suggested the importance of non-zooplankton prey items for Delta Smelt, particularly in areas with prevalent tidal wetlands (Hammock et al. 2019a). However, in our study, we did not observe direct benefits of wetland proximity on gut fullness. Hammock et al. (2019a) attribute 60% of the positive effect of tidal wetlands on gut fullness to predation on larval fish. The analysis in Hammock et al. (2019a) included 69 Delta Smelt—mostly collected during winter and spring—with larval fish in their guts; however, in our study, larval fish were found in the gut contents of only five fish (out of approximately 500 individuals). Thus, an important caveat of our study is its exclusion of adult fish collected in January through May (because no concurrent zooplankton tows were collected during the SKT), such that we cannot infer the importance of wetland proximity for foraging of adult fish. Larger bodied adult Delta Smelt are more likely to forage on larval fish compared to juveniles and subadults that dominate in summer and fall (Slater et al. 2019), and many larval fish rear in tidal wetlands (Baltz et al. 1993; Beck et al. 2001; Grimaldo et al. 2017). In addition, Hammock et al. (2019a) conducted their analysis of the effect of tidal wetland area on gut fullness at the individual fish-level. Thus, their sample size was much larger (n = 1380) compared to the current study which was conducted at the station-date level (n = 136). Thus, our study does not refute the association between tidal wetlands and Delta Smelt foraging success and condition but suggests that tidal wetlands may be more important for adult foraging than other life stages. It is also worth noting we found overall low fit for the gut fullness models, which indicate that there may be other factors not considered in this study that play a large role in determining gut fullness of juvenile to sub-adult Delta Smelt.

Overall, zooplankton biomass tended to have positive effects on Delta Smelt HSI and gut fullness across habitats. These results have relatively straightforward—albeit difficult to implement—management implications. That is, any management actions to increase zooplankton biomass should benefit Delta Smelt condition and foraging (Kimmerer and Rose 2018). Accounting for such food web interactions has been recognized as a valuable management approach in fish conservation (Naman et al. 2022). Both HSI and gut fullness eventually correlate with fitness in fishes, so increasing zooplankton abundance is expected to aid the recovery of the population. However, one disconcerting possibility is that increasing zooplankton abundance may also benefit exotic predators (e.g., Striped Bass) and competitors (e.g. Mississippi Silversides, Wakasagi), potentially dampening or offsetting the positive effect of increased zooplankton. In addition, zooplankton taxa vary in nutritional composition (Kratina and Winder 2015) and an increase in total biomass could be from less nutritional or smaller prey (e.g. the cyclopoid *Limnoithona*) as opposed to larger and more nutritious prey (e.g. the calanoid copepod *Pseudodiaptomus* or *Eurytemora*). This could result in different effects on fish condition, despite increase total zooplankton biomass. Thus, management actions more targeted for Delta Smelt might be more beneficial to the species, such as increasing turbidity or population supplementation (Feyrer et al. 2007, Hung et al. 2022).

Despite these straightforward management interpretations for Delta Smelt, manipulating the productivity of the pelagic food web in the Estuary is inherently challenging (Durand 2015). For example, the goal of increasing zooplankton abundance may require different approaches across habitats, even if the net outcome across such actions should broadly benefit Delta Smelt. In some regions, bottom-up pressure from low phytoplankton availability may be the strongest limitation on zooplankton (Kimmerer et al. 2005, Gearty et al. 2021, Rodgers et al. 2024), while zooplankton in other regions may face strong top-down pressure (e.g., invasive clam consumption of nauplii; Kimmerer et al. 1994, Kimmerer and Lougee 2015). A single management action may also have contrasting results in different regions of the Estuary. For example, increased periods of flow may decrease phytoplankton availability for zooplankton in the uppermost areas of the estuary (by reducing residence time), while high flows may increase export of phytoplankton to zooplankton in downstream areas such as Suisun Bay (Jassby 2008, Durand 2015). Increased phytoplankton is associated with greater zooplankton on annual timescales in the estuary (Rogers et al. 2024) but increasing phytoplankton estuary-wide would likely require reducing grazing and increasing residence time (Hammock et al. 2019b). Tidal wetlands are associated with improved foraging success for Delta Smelt, but they are not considered significant sources of zooplankton for nearby channels (i.e., the Outwelling Hypothesis, Herbold et al. 2014), and can even be sinks (Kimmerer et al. 2018, Yelton et al. 2022). Consequently, management of zooplankton—with the ultimate goal of supporting Delta Smelt populations in a bottom-up manner—may require different approaches under different conditions, highlighting the relevance of environmental context dependence for conservation.

Acknowledgments

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Tables

Table 7-1. Effects of Zooplankton Biomass, Habitat, and Seasonality on HSI

Model	Model sample size	Predictor	F	p-value	Adjusted R^2
A.	*n = 174*	Zooplankton	2.15	0.145	0.21
		Salinity	0.11	0.744	
		Season	33.36	<0.001	
		Zooplankton × Salinity	0.14	0.706	
B.	*n = 167*	Zooplankton	0.35	0.553	0.29
		Wetland	4.14	0.018	
		Season	32.21	<0.001	
		Zooplankton × Wetland	4.88	0.009	
C.	*n = 167*	Zooplankton	4.52	0.035	0.23
		Region	1.19	0.316	
		Season	28.79	<0.001	
		Zooplankton × Region	1.21	0.310	

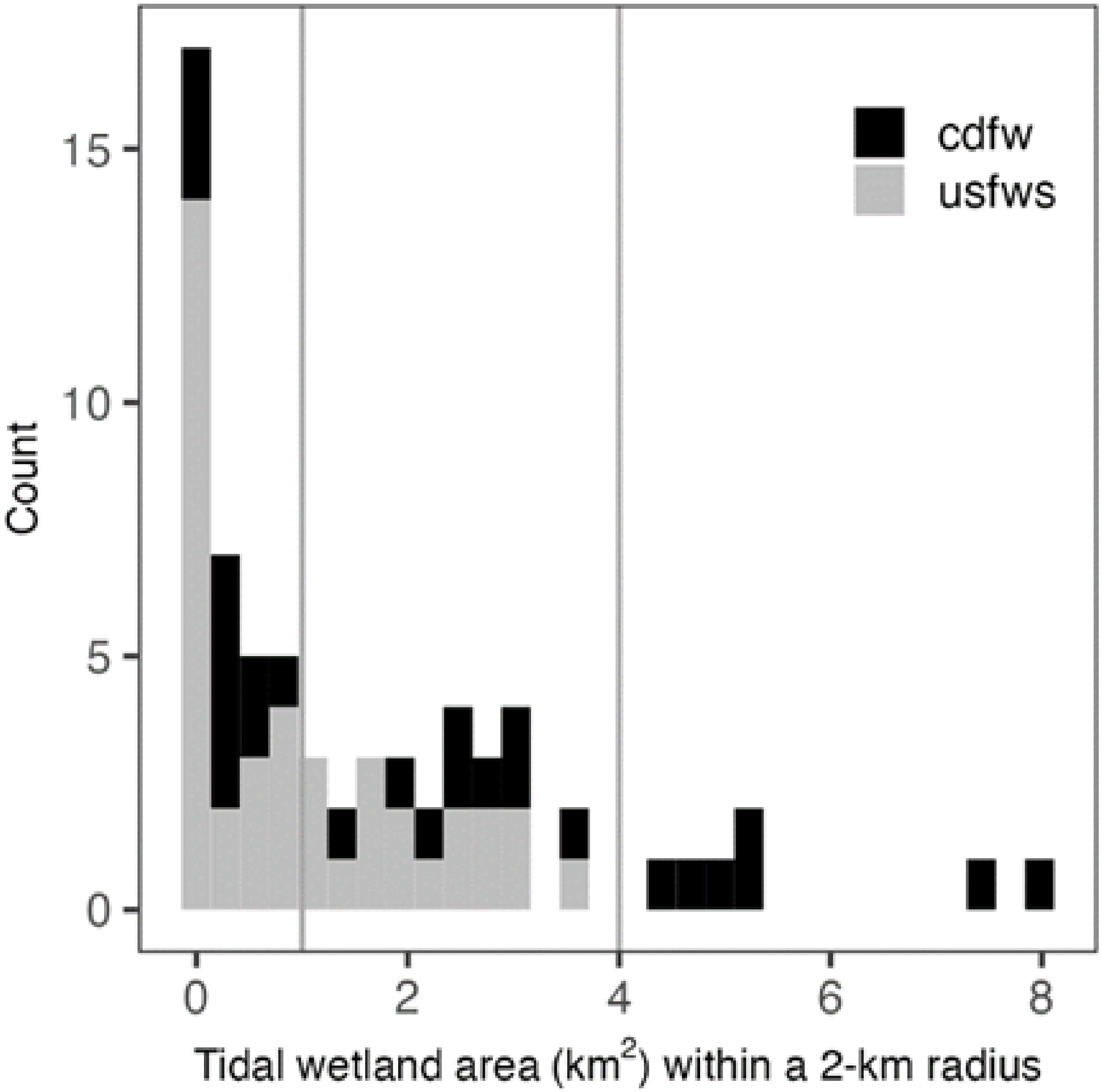
Results are shown for three separate models. Each model had a variable representing habitat: salinity, wetland prevalence, or region—all of which are categorical variables. Results are from type-III F-tests.

Table 7-2. Effects of Zooplankton Biomass, Habitat, and Seasonality on Gut Fullness

Model	Model sample size	Predictor	F	p-value	Adjusted R^2
A.	*n = 136*	Zooplankton	2.45	0.120	0.07
		Salinity	1.22	0.271	
		Season	3.19	0.077	
		Zooplankton × Salinity	1.09	0.297	
B.	*n = 136*	Zooplankton	10.18	0.002	0.10
		Wetland	0.81	0.446	
		Season	3.08	0.082	
		Zooplankton × Wetland	0.49	0.613	
C.	*n = 136*	Zooplankton	0.34	0.559	0.07
		Region	0.52	0.724	
		Season	2.96	0.088	
		Zooplankton × Region	0.55	0.699	

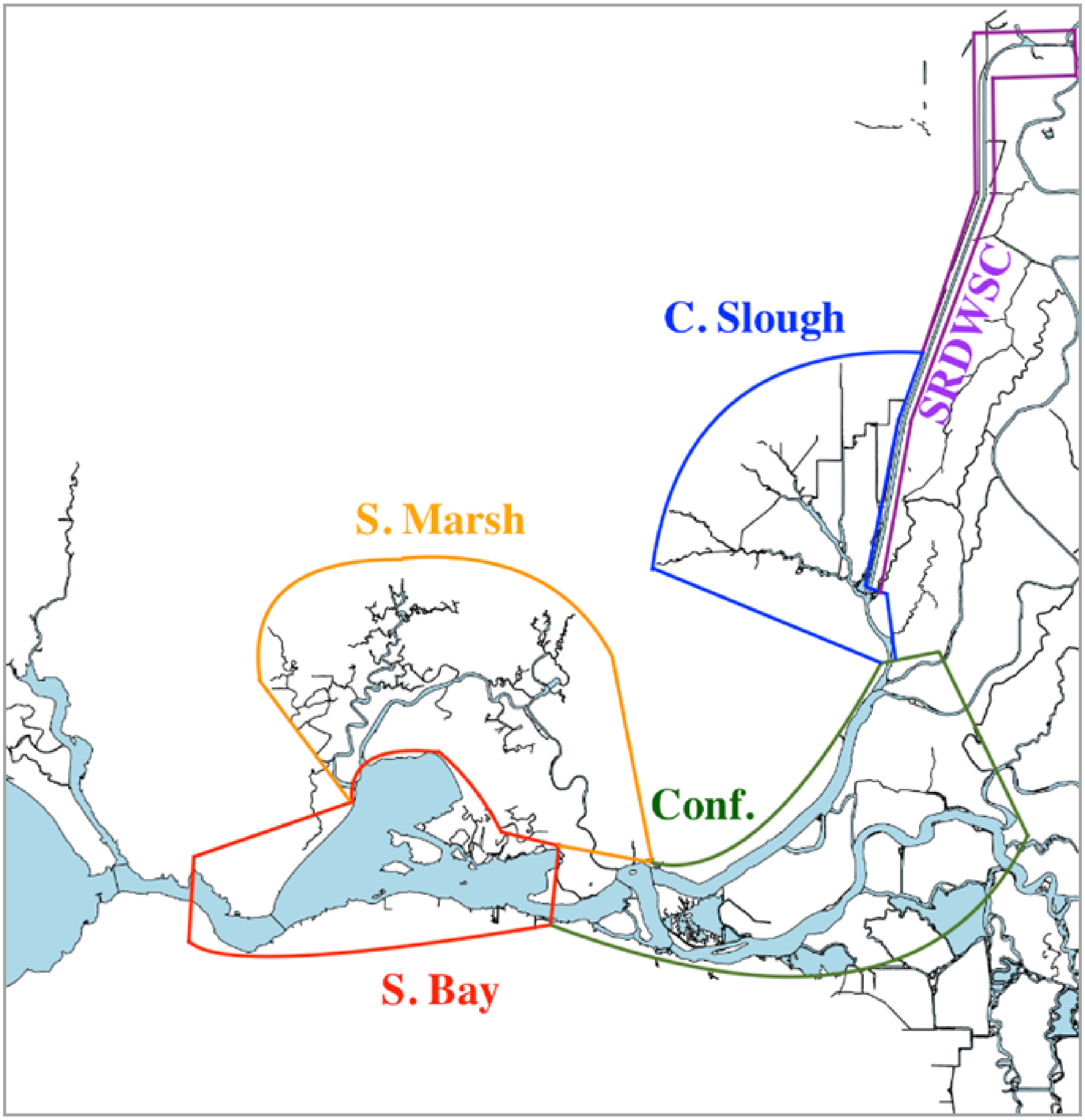
Results are shown for three separate models. Each model had a variable representing habitat: salinity, wetland prevalence, or region—all of which are categorical variables. Results are from type-III F-tests.

Figures



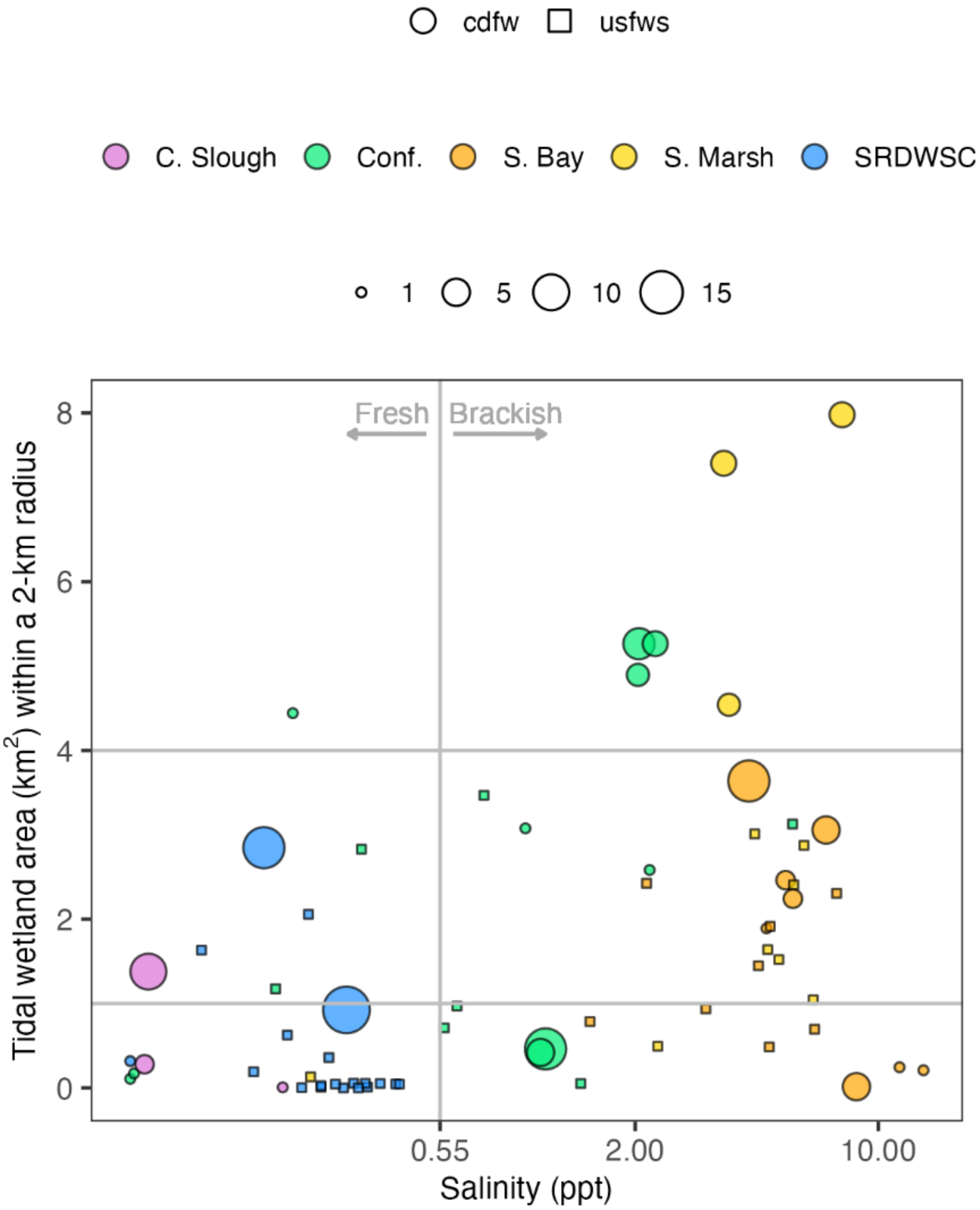
Note: Stations include those surveyed by CDFW (STN and FMWT) and USFWS (EDSM surveys). The vertical lines indicate the thresholds used to categorize stations into 'low', 'medium', and 'high' wetland categories.

Figure 7-1. Distribution of the Abundance of Tidal Wetland Within a 2-km Radius of Each Station Included in our Study (n = 74)



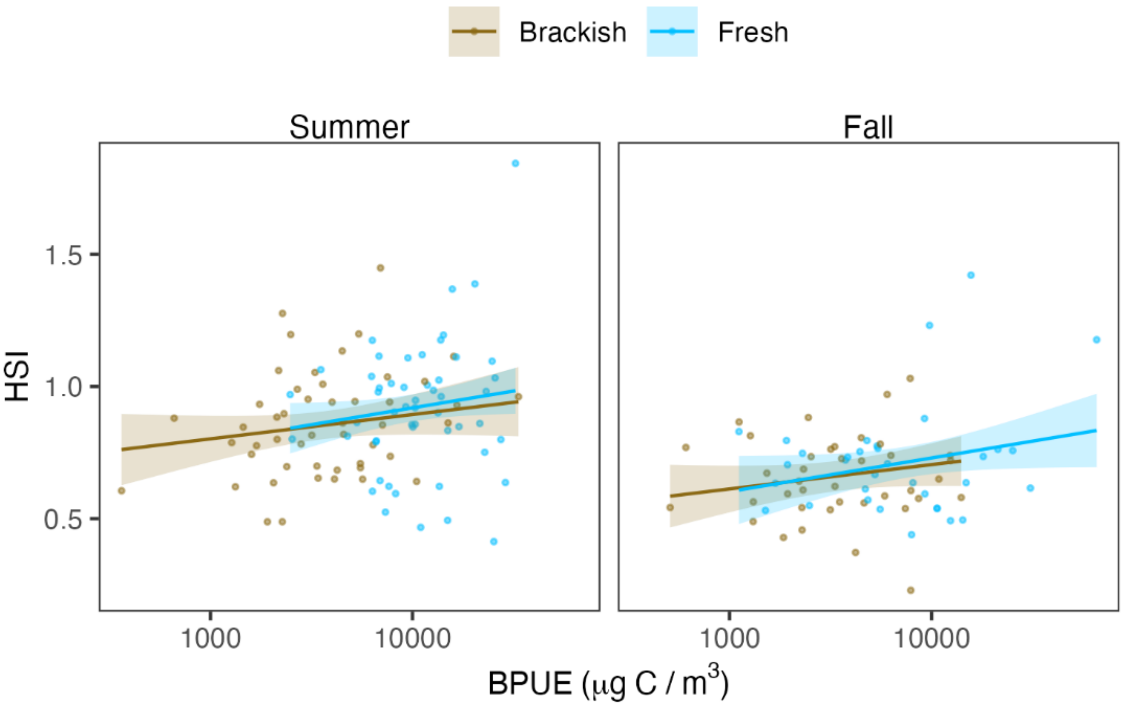
The recent range of Delta Smelt is divided into the five regions depicted, including the Sacramento River Deepwater Ship Channel (SRDWSC), Cache Slough (C. Slough), the Confluence (Conf.), Suisun Bay (S. Bay), and Suisun Marsh (S. Marsh). Note that boundaries are not necessarily geographically accurate and were selected to maximize sample size. For example, several Delta Smelt from the Carquinez Strait (Strait just west of Suisun Bay) were lumped into Suisun Bay.

Figure 7-2. Map of the Sacramento-San Joaquin Delta and San Francisco Estuary (the Estuary)



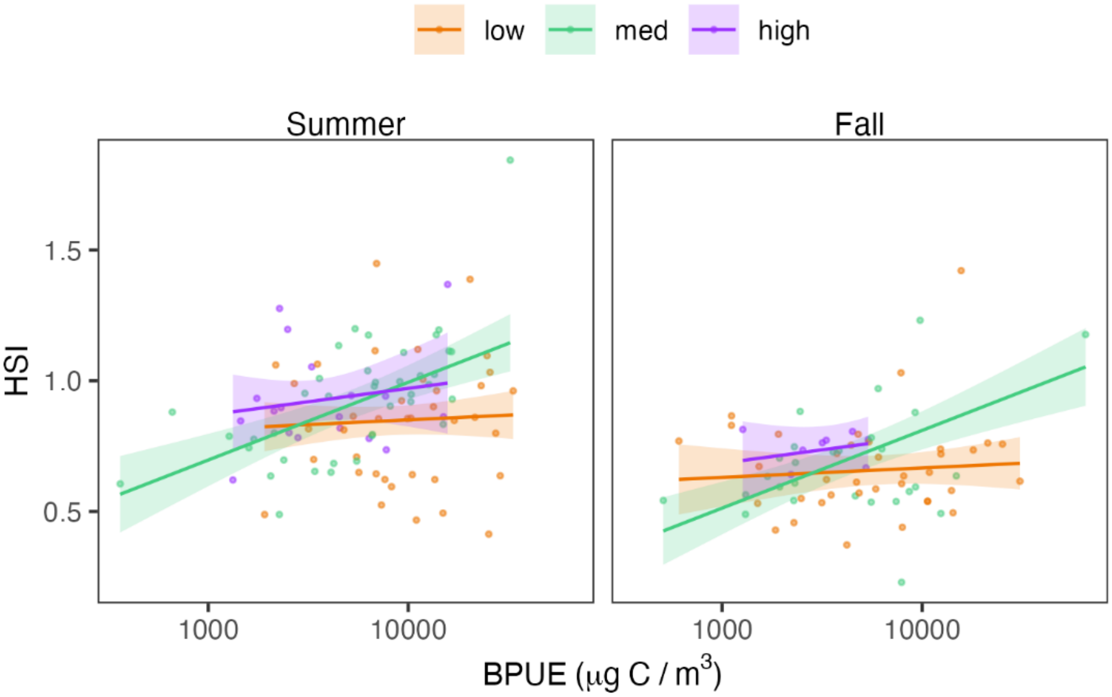
Colors indicate the assigned region for each station. Shapes indicate the agency associated with the station. Size of points indicate the number of dates on which the station was sampled (note: USFWS stations are sampled a single time). Stations to the left of the vertical line (salinity < 0.55ppt) were classified as freshwater and those to the right as brackish; x-axis is shown on a ln-scale to show spread of the freshwater sites. The horizontal lines depict the thresholds used to classify stations as low, medium, or high wetland abundance.

Figure 7-3. Habitat Categorizations for Each Station (n = 74) Included in our Study



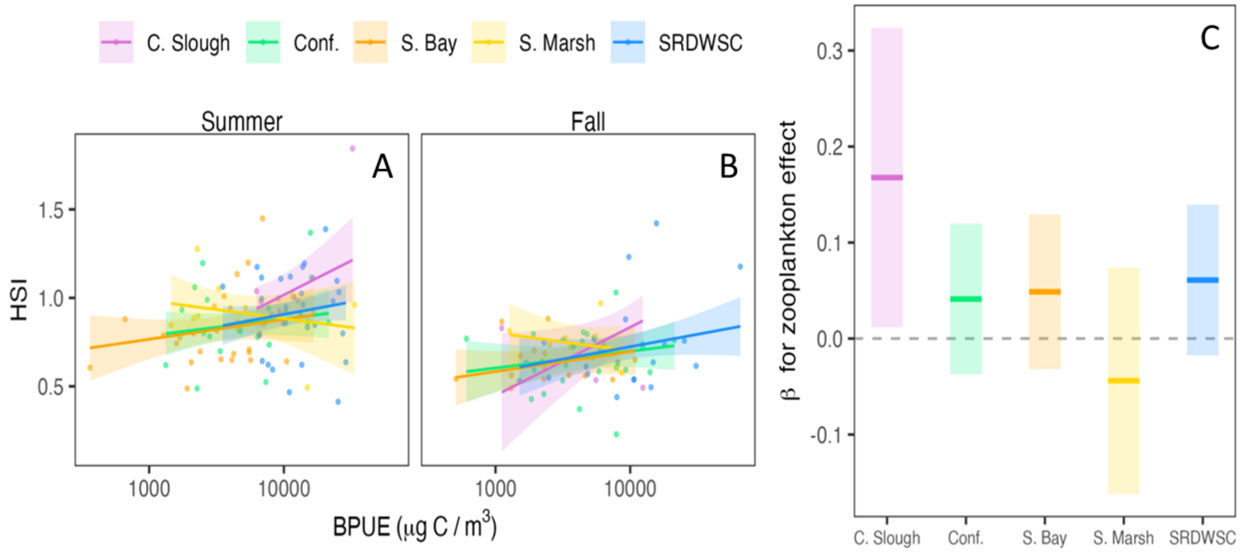
Lines show model fits with shaded regions showing 95% confidence intervals.

Figure 7-4. HSI Shown as a Function of Zooplankton Biomass (BPUE), Salinity, and Season



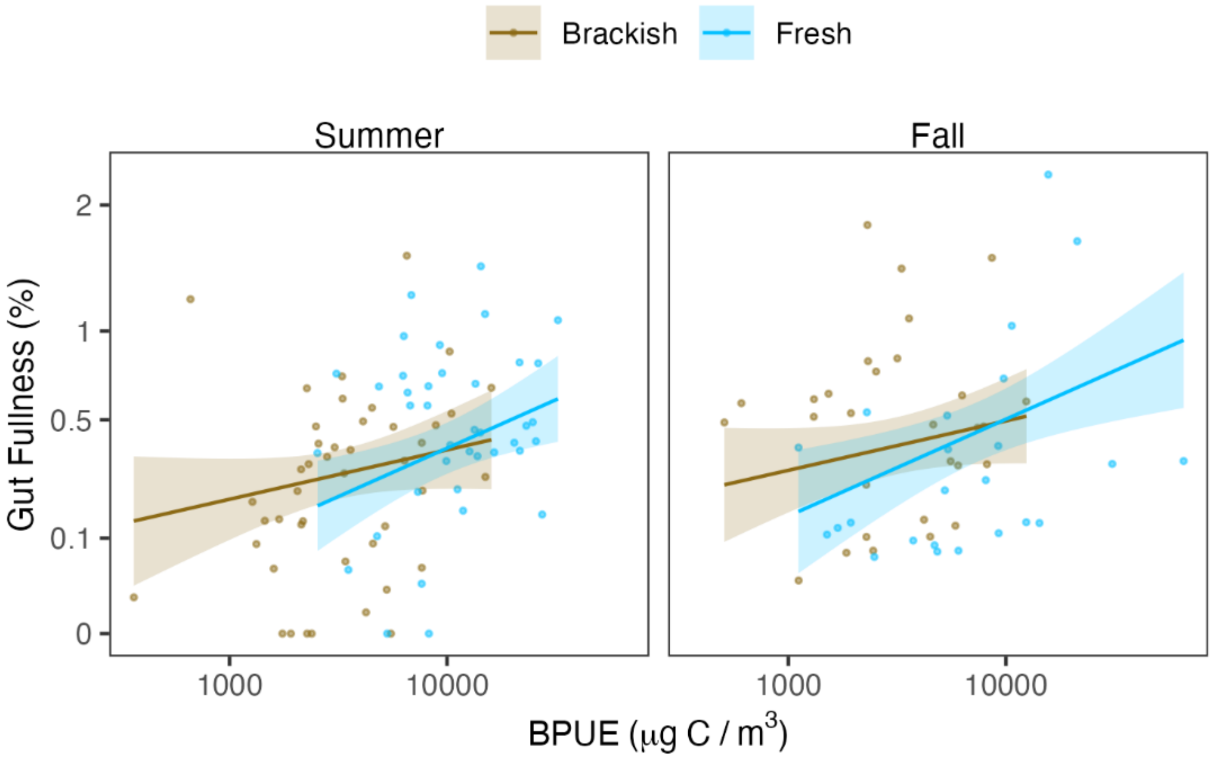
Lines show model fits with shaded regions showing 95% confidence intervals.

Figure 7-5. HSI Shown as a Function of Zooplankton Biomass (BPUE), Season, and Wetland Abundance



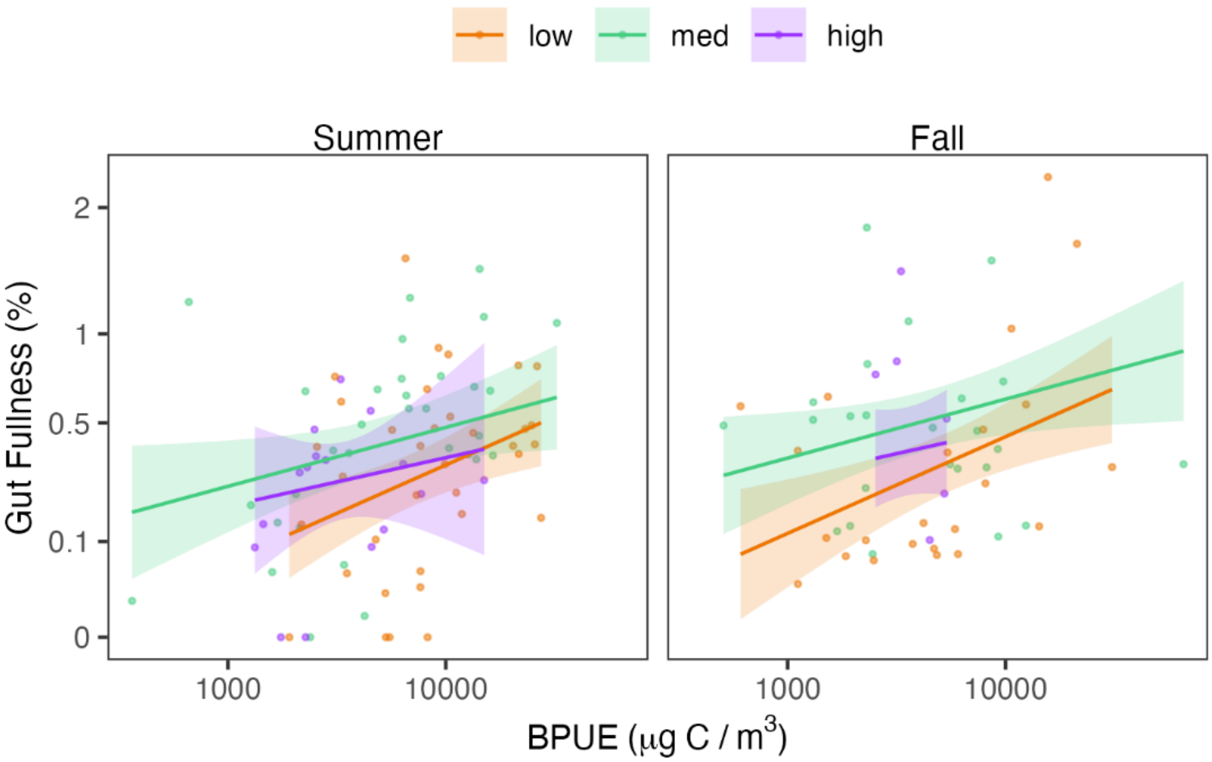
Lines show model fits with shaded regions showing 95% confidence intervals. Panel C shows the parameter estimates from the model for each region.

Figure 7-6. Panels A and B Show HSI as a Function of Zooplankton Biomass (BPUE), Season, and Region



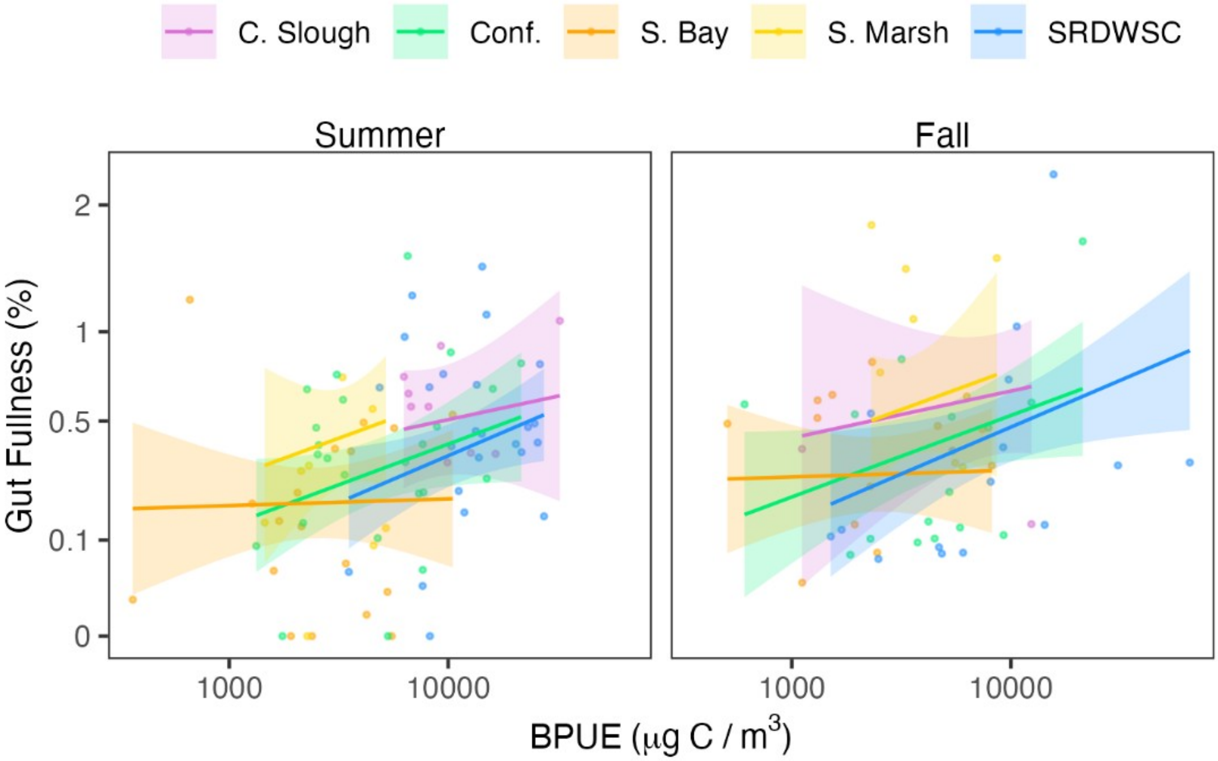
Lines show model fits with shaded regions showing 95% confidence intervals. Gut fullness data are shown as arcsine square-root transformed (as in the analysis), with y-axis ticks showing back-transformed values for reference.

Figure 7-7. Gut Fullness Shown as a Function of Zooplankton Biomass (BPUE), Salinity, and Season



Lines show model fits with shaded regions showing 95% confidence intervals. Gut fullness data are shown as arcsine square-root transformed (as in the analysis), with y-axis ticks showing back-transformed values for reference.

Figure 7-8. Gut Fullness Shown as a Function of Zooplankton Biomass (BPUE), Season, and Wetland Abundance



Lines show model fits with shaded regions showing 95% confidence intervals. Gut fullness data are shown as arcsine square-root transformed (as in the analysis), with y-axis ticks showing back-transformed values for reference.

Figure 7-9. Gut Fullness Shown as a Function of Zooplankton Biomass (BPUE), Season, and Region

Supplemental Information

Statistical Methods

Previous analyses have examined the effects of season and salinity on zooplankton abundance, as well as HSI and gut fullness (Hammock et al. 2017, 2022). Here we conduct similar analyses on the dataset used in our current study and present results that are similar to the aforementioned published results because the way in which the data are analyzed in this study differ from previously published studies. Specifically, 1) our current study focuses on zooplankton biomass, while previous work has considered zooplankton abundance (individuals/m^3; Hammock et al. 2017), 2) our current analysis considers observations at the station-date level (i.e., aggregating data for fish caught from the same survey sample), while previous work on HSI and gut fullness has examined patterns using fish-level data (i.e., treating fish as independent replicates; Hammock et al. 2019a, 2022), and 3) including these results helps illustrate our rationale in including a season term for the HSI and gut fullness models presented in the main text. Given these differences in approach, we first wanted to compare our results to previous findings.

For the models presented in the Supplemental Information, we include zooplankton biomass, HSI or gut fullness as the response variable. Each model included salinity (freshwater, brackish), season (summer, fall), and their interaction as main effects. As described in the main text, we ln-transformed zooplankton biomass and use time-corrected arcsine square root transformed gut fullness values. We present results from linear models using type III F-tests. Effect sizes were calculated by comparing model predictions for a given variable of interest at the two levels of the other predictor. For response variables that were transformed for analysis (zooplankton biomass and gut fullness), we first back-transformed the model predictions and then compared values.

Results

Zooplankton biomass was significantly higher in freshwater than brackish water habitats (Table S7-1; Figure S7-1). The salinity × season interaction had a marginal but non-significant effect on zooplankton biomass (Table S7-1; Figure S7-1). In a previous study, Hammock et al. (2017) observed a significant season by salinity interaction effect on zooplankton abundance (individuals/m^3) from tows collected in 2011-2014. Qualitatively, the trends in zooplankton biomass from our model were somewhat similar to those described by Hammock et al. (2017). Namely, the differences in zooplankton biomass between salinity types was larger during summer than fall (2.9-fold higher in freshwater than brackish water in summer and 1.9-fold higher in fall).

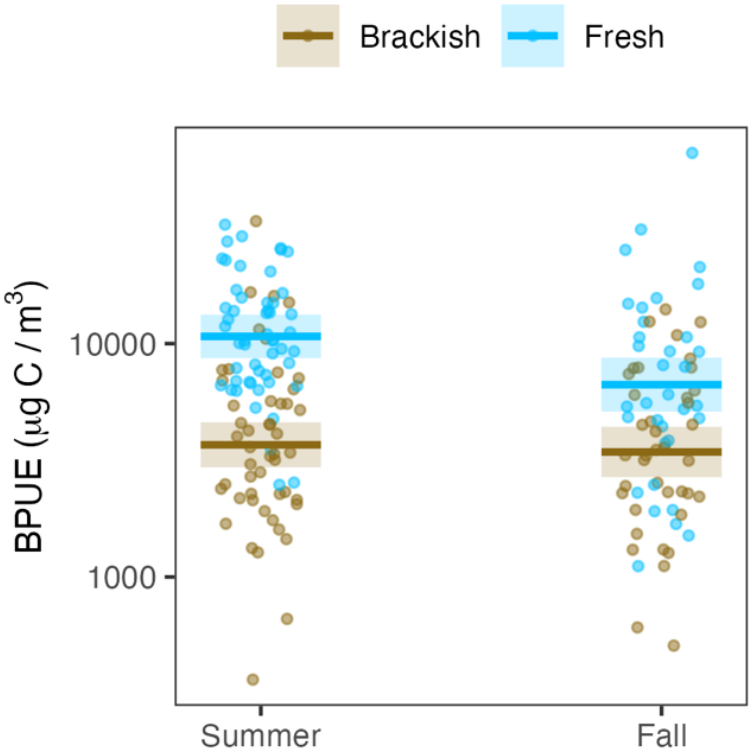
Compared to summer, HSI was lower in fall. This seasonal effect had no statistical interaction with salinity, such that condition declined from summer to fall in both brackish (by 1.3-fold) and freshwater (by 1.3-fold) habitat (Table S7-1; Figure S7-2). Salinity did not statistically affect HSI (Table S7-1; Figure S7-2). Previous results have also documented a decline in HSI during fall (Hammock et al. 2022).

Season and salinity affected gut fullness in an interactive manner (Table S7-1; Figure S7-3). In summer, fish collected in freshwater habitats had 1.9-fold fuller guts than those collected in brackish habitats. This pattern was reversed in the fall, although gut fullness showed more overlap between habitats (i.e., salinity confidence intervals overlap in fall for brackish and freshwater habitats; Figure S7-3). Thus, these results are somewhat similar to those presented in Hammock et al. (2017). However, the interaction in the current analysis is weaker as gut fullness in fall was similar for the two habitats, while the Hammock et al. (2017) analysis using fish-level observations of gut fullness reported a full "crossover interaction."

Table S7-1. Effects of Salinity (brackish and freshwater) and Season (summer and fall) on Zooplankton Biomass, HSI, and Gut Fullness

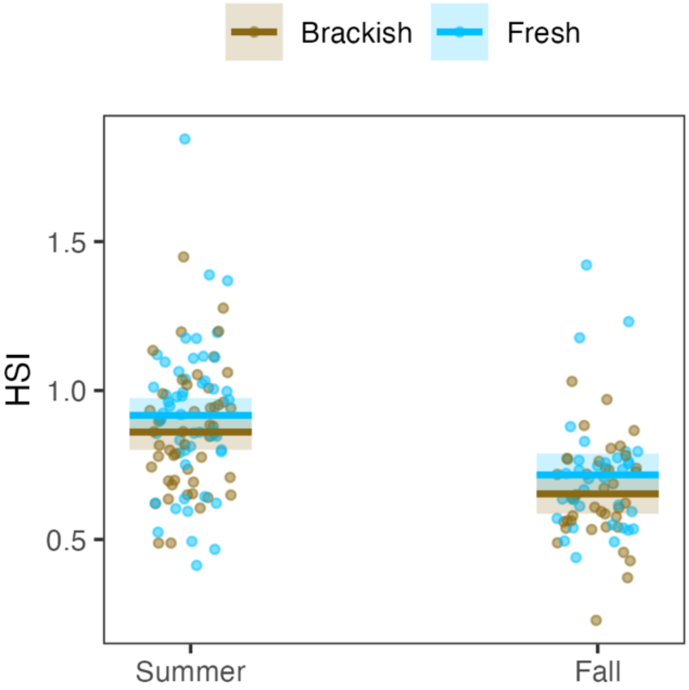
Response variable	Predictor	F	p-value
Zooplankton Biomass	Salinity	46.85	<0.001
	Season	0.17	0.677
	Salinity × Season	2.82	0.095
HIS	Salinity	1.77	0.185
	Season	20.96	<0.001
	Salinity × Season	0.01	0.905
Gut fullness	Salinity	7.71	0.006
	Season	7.69	0.006
	Salinity × Season	8.13	0.005

Results are from type-III F-tests.



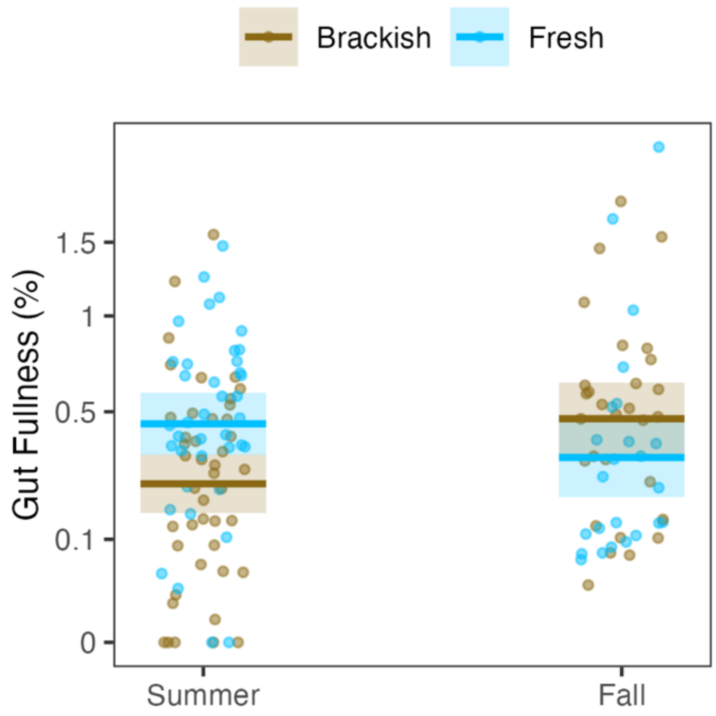
Points show station-date observations. Horizontal lines show model predictions with shaded regions showing 95% confidence intervals.

Figure S7-1. Season and Salinity Effects on Zooplankton Biomass (BPUE)



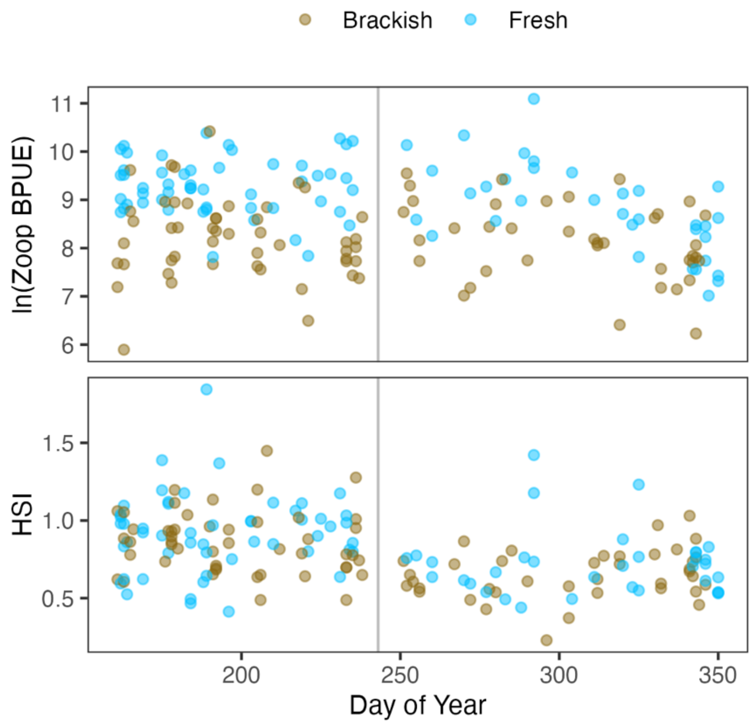
Points show station-date observations. Horizontal lines show model predictions with shaded regions showing 95% confidence intervals.

Figure S7-2. Season and Salinity Effects on HSI



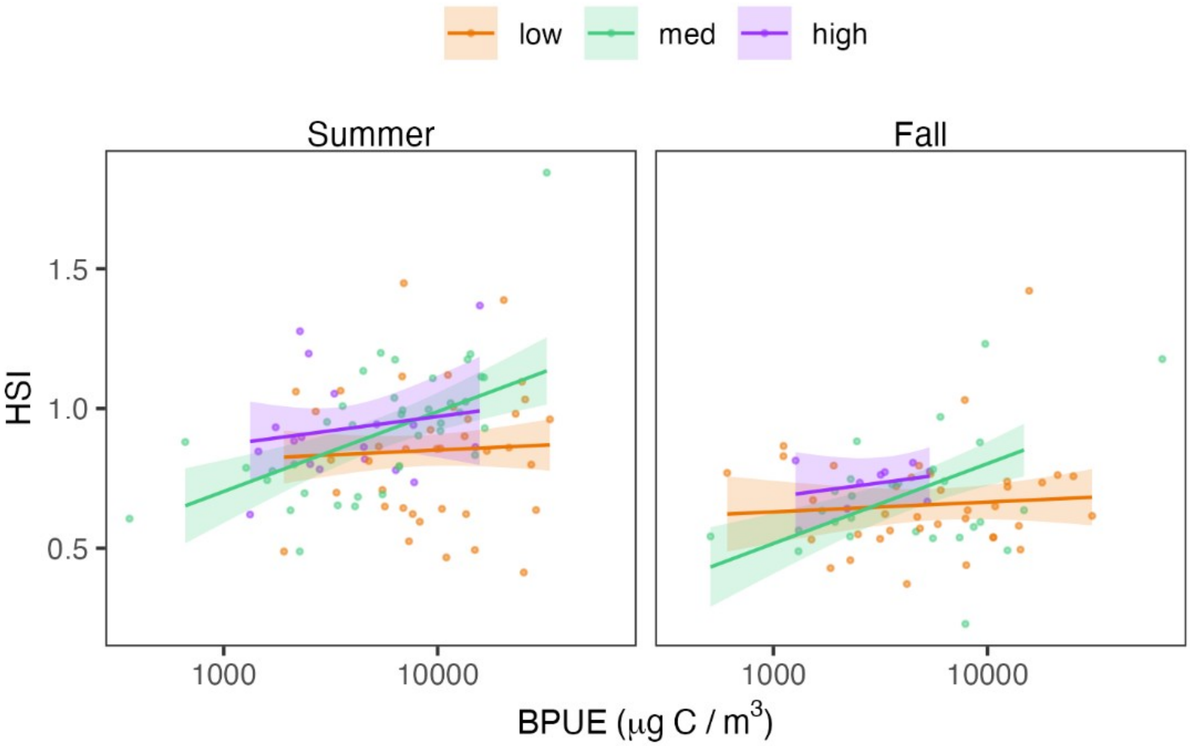
Points show station-date observations. Horizontal lines show model predictions with shaded regions showing 95% confidence intervals. Gut fullness data are shown as arcsine square-root transformed, with y-axis ticks showing back-transformed values for reference.

Figure S7-3. Season and Salinity Effects on Gut Fullness



The vertical lines denote the cutoff (Julian day 243; Aug 31) between summer and fall seasons.

Figure S7-4. Temporal Trends in Biotic Variables from Our Dataset



We conducted this analysis to investigate whether these two points had undue influence on the statistical results of the model presented in the main text. Lines show model fits with shaded regions showing 95% confidence intervals.

Figure S7-5. HSI Shown as a Function of Zooplankton Biomass (BPUE), Season, and Wetland Abundance Modeled from a Dataset that Excluded the Minimum and Maximum Zooplankton Biomass, Both of Which Were Observed in the 'Medium' Wetland Category