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### Authors

Tempel, Trishelle  
Hartman, Rosemary  
Meyer, Scott  
[et al.](#)

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## RESEARCH

# Responses of Delta Smelt in Experimental Enclosures to Different Habitats and Biofouling Mitigation Methods During the Critical Summer-Fall Period

Trishelle Tempel<sup>\*1</sup>, Rosemary Hartman<sup>1</sup>, Scott Meyer<sup>1</sup>, Christina E. Burdi<sup>1</sup>, Jesse Adams<sup>1</sup>, Bruce G. Hammock<sup>2</sup>, Brittany E. Davis<sup>1</sup>

## ABSTRACT

In 2023 we placed Delta Smelt (*Hypomesus transpacificus*) into field enclosures for 6 weeks to assess whether an enclosure study could effectively detect Delta Smelt responses to habitat differences during the summer-fall period. Prior enclosure studies have noted high biofouling accumulation during warm months, so we simultaneously tested two biofouling mitigation techniques (cleaning vs. replacement) to better understand their efficacy and potential effects on the fish inside. Fish were held at two sites in the San Francisco Estuary: the Sacramento River near Rio Vista, and Montezuma Slough near Belden's Landing. Both biofouling mitigation measures were employed at each site. We evaluated water quality and zooplankton inside and outside of the enclosures as well as algal and macroinvertebrate accumulation on the enclosures, and assessed

fish survival, health, and diets. We observed significantly improved fish health metrics at Rio Vista compared to Belden's Landing, potentially associated with higher-quality food, lower water temperatures, and lower water velocities; however, it is unclear whether these differences were in response to the geographic location or to localized enclosure effects, because the fish were unable to seek refuge or forage. We also observed differences in enclosure biofouling and associated macroinvertebrates between the two mitigation measures and saw higher mortality in replaced enclosures but no differences in fish health among survivors. Macroinvertebrates were detected on nearly all enclosures and often made a sizeable proportion of smelt diet biomass, regardless of location or mitigation method, indicating that the enclosures may provide an altered food environment. This study demonstrates the complexity of implementing and interpreting Delta Smelt enclosure studies, and highlights limitations of this tool. Our data offers physiological clues that enhance our understanding of the response of cultured Delta Smelt to differing habitats, but uncertainties highlight that enclosure studies should not be used in isolation to address large-scale management questions.

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\* Corresponding author: [Trishelle.Tempel@water.ca.gov](mailto:Trishelle.Tempel@water.ca.gov)

1 California Department of Water Resources  
Division of Integrated Science and Engineering  
West Sacramento, CA 95691 USA

2 University of California-Davis  
Department of Anatomy, Physiology, and Cell Biology  
Davis, CA 95616 USA

## KEY WORDS

habitat, biofouling, Delta Smelt, physiology, zooplankton, temperature, summer–fall

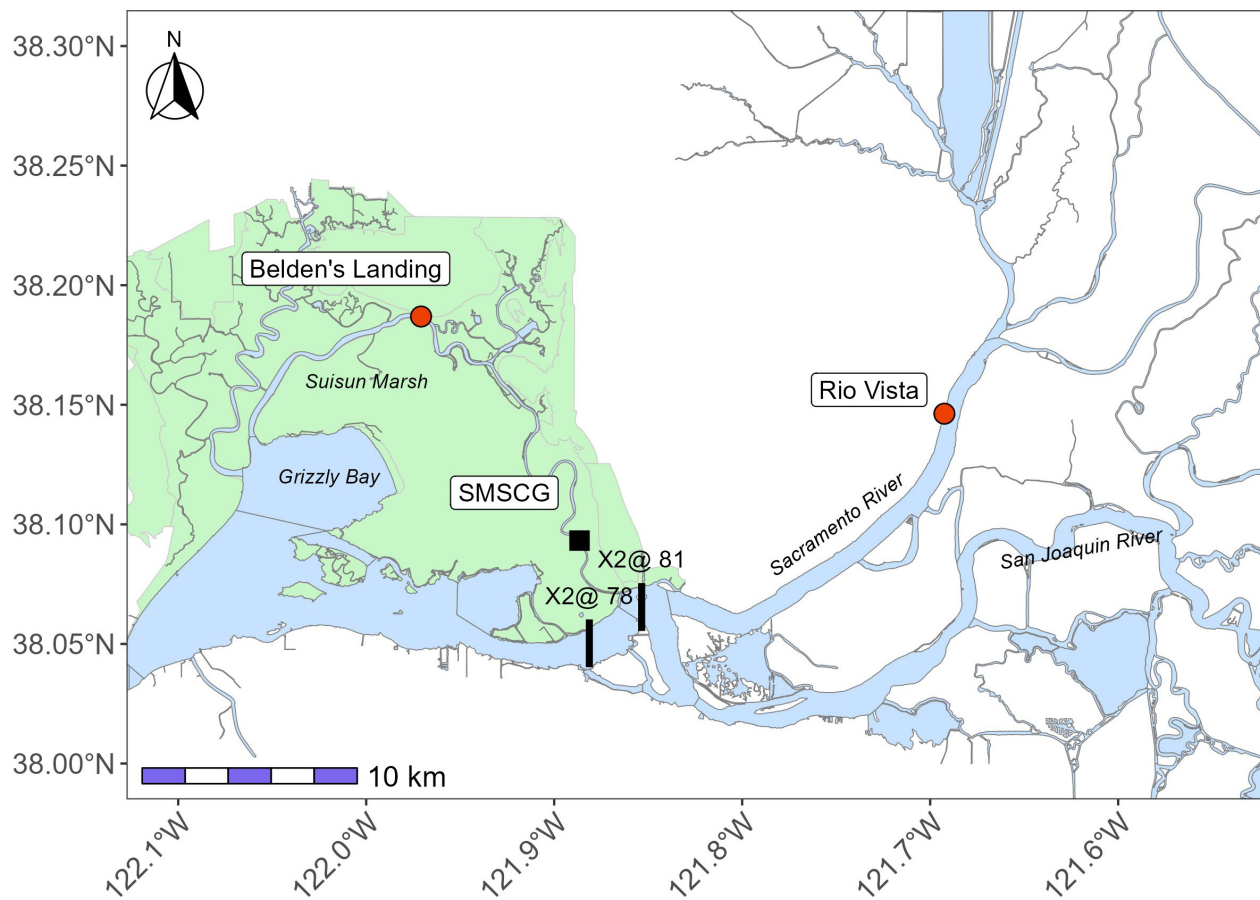
## INTRODUCTION

Delta Smelt (*Hypomesus transpacificus*) are small, pelagic fish that are endemic to the upper San Francisco Estuary (hereafter “the estuary”) and reside in low-salinity or freshwater habitats. The species has suffered precipitous declines in abundance in recent decades, and within the last decade their abundance has dropped below the detection limits of the monitoring gear designed to survey them (Tempel et al. 2021). The species is currently listed as endangered under the California Endangered Species Act and threatened under the federal Endangered Species Act, and these listings have resulted in management actions to, in part, improve Delta Smelt habitat (e.g., Sommer et al. 2020; Lee et al. 2023). However, low detections in the wild impede the ability of resource managers to verify how these actions affect the very species they are designed to benefit. As a result, in 2019 the California Department of Water Resources (CDWR), in conjunction with the University of California–Davis and multiple state and federal agencies, began exploring the feasibility of using field enclosures to deploy captive-reared Delta Smelt into semi-controlled field environments within their native range (Lessard et al. 2018; Baerwald et al. 2023). These initial deployments indicated that captive-reared Delta Smelt were able to survive in a field-like environment, and, in the years since, this tool has been used to explore survival and growth across varying seasons and geographic regions (Davis et al. 2024).

In the wild population, long-term monitoring and life cycle modeling have identified the juvenile Delta Smelt life stage, which occurs during summer and fall, as a key bottleneck (Bennett 2005; Polansky et al. 2021; Smith and Nobriga 2023). To bolster survival during this period, the CDWR and the US Bureau of Reclamation (Reclamation) implement a summer–fall habitat action, a management tool intended to increase the area of suitable habitat for Delta Smelt and

bolster food supply during this critical season. Implementation of the summer–fall action varies but could include increasing outflow to push the position of the 2 practical salinity unit (psu) isohaline (X2) to less than 80 km from the mouth of the San Francisco Estuary (the Golden Gate Bridge) during fall of wetter years, and/or operating the Suisun Marsh Salinity Control Gates (SMSCG) during summer or fall of moderate years to prevent salinity intrusion and maintain freshwater habitat. These actions are designed to increase suitable habitat for Delta Smelt in Suisun Marsh and Grizzly Bay by decreasing salinity below 6 psu, allowing Delta Smelt access to water with potentially lower temperatures, lower contaminant levels, higher turbidities, and improved foraging opportunities, which are all beneficial to their growth and survival (Sommer and Meija 2013; Hammock et al. 2015; Hammock et al. 2019; Lewis et al. 2021). Together, the intent of these actions is to contribute to the recruitment, growth, and survival of Delta Smelt (CDFW 2020; Sommer et al. 2020).

In 2023, the summer–fall habitat action included operation of the SMSCG from August 15 through October 17, which maintained salinity at <4 psu near Belden’s Landing, and increased outflow to push the location of X2 to 78 km from the Golden Gate Bridge for the month of September and 80.6 km from the Golden Gate Bridge for the month of October, on a monthly average basis (Figure 1; USBR Reclamation and CDWR 2024). Before 2023, enclosures had not been used to explicitly assess the effectiveness of a Delta Smelt-targeted management action such as this one; however, the successes of past deployments indicate that they may be a useful tool for this purpose (Baerwald et al. 2023; Davis et al. 2024). Therefore, we conducted the study described here to test whether we could detect differences in the health of fish after their exposure to two differing environments: one within the 2023 action area and one outside of it. To do so, we placed cultured Delta Smelt into enclosures at two field locations: the Sacramento River near Rio Vista, which is located outside of the direct influence of the action area, and Montezuma Slough near Belden’s Landing, which is located in the center of the



**Figure 1** Location of enclosures (Belden's Landing and Rio Vista, red circles), and the location of the Suisun Marsh Salinity Control Gates (SMSCG, black square). The vertical lines crossing the Sacramento River indicate the location of X2 during September (78 km) and October (80.6 km) of 2023 during the deployment.

action area (Figure 1). We hypothesized that Delta Smelt positioned in the center of the action area would display measurably improved health metrics than those positioned outside of the action area.

Previous enclosure studies conducted in summer showed that the enclosures were susceptible to dense biofouling during the warm summer months (Davis et al. 2024), and we were concerned that these biofouling issues might mask any effects of habitat. In aquaculture systems, biofouling can be a serious threat to fish health: blocking mesh openings, reducing dissolved oxygen, and increasing waste products inside the enclosure (Bannister et al. 2019). If this was to occur in our field enclosures, it could limit our ability to use the field enclosures to test

regional differences in habitat suitability and potentially confound measures of fish responses. To explore the validity of these concerns, we expanded our study design to test two biofouling mitigation techniques and compared their effects on the health of fish, the composition of their diet, and the composition of the zooplankton community. We hypothesized that higher levels of algal accumulation on the enclosures would reduce water flow and limit entry of zooplankton to the interior while increasing the density of amphipods inside—thereby increasing amphipods in diets—and would generally result in habitat conditions that did not reflect conditions outside the enclosures.

To test our hypotheses, we evaluated water quality and zooplankton inside and outside of

the enclosures at both sites and at multiple times throughout the study. At the end of the study period, we assessed fish health and survival, fish diets, biofouling biomass, macroinvertebrate accumulation, and zooplankton biomass inside and outside the enclosures. We used these markers to assess the following study questions:

1. Can enclosure studies be used to detect the physiological responses of Delta Smelt to differing environmental conditions?
2. Do different biofouling mitigation methods result in differences in enclosure conditions and/or fish health?

## METHODS

### Study Location and Enclosure Installation

The study was conducted at two field locations in the upper estuary (Figure 1). The upstream location was in the lower Sacramento River near the city of Rio Vista where the river is approximately 700 m wide. Enclosures were placed approximately 10 m from the west bank, where the water was approximately 3 m deep. This location is not directly altered by SMSGC management actions but is in an area of high historic Delta Smelt presence. The downstream location was in Montezuma Slough near the Belden's Landing boat launch, where the channel is approximately 200 m wide. The enclosures were placed approximately 3 m from the north bank, where the water was approximately 2.5 m deep. This location is approximately 18 km downstream of the SMSGC and within the target action area, where salinity would decrease during the action period (< 6 psu) and turbidity would remain high (> 12 formazin nephelometric units [FNU]). This region is also in an area of high historic Delta Smelt abundance (Bennett 2005). We deployed four enclosures at each of the two locations and held fish inside for 6 weeks.

The enclosures used were specifically designed to house Delta Smelt and have an internal volume of approximately 720 L (Baerwald et al. 2023; Gille et al. 2026). At each location, four enclosures were connected in a line, with a buoy connected

on either end. At the Rio Vista location, each buoy was connected to two existing pilings; at the Montezuma Slough location, the upstream buoy was connected to the existing piling of a water-quality station, and the downstream buoy was secured to two 61-kg pyramid anchors. The resulting systems were able to move vertically with the tides while maintaining the same relative distance between enclosures.

### Delta Smelt Deployment

Delta Smelt were obtained from the UC–Davis Fish Conservation and Culture Laboratory (FCCL) at approximately 200 days post-hatch. On August 18, 2023, all fish were marked with an adipose fin clip ( $n = 660$ ) and beginning on August 23 they were slowly acclimated from 12°C to 18.2°C  $\pm$  1.6 (mean  $\pm$  SD) to prepare them for warmer field temperatures. During this time, all fish were held in a single 400-L tank at a density of 1.7 fish per liter. A subset of 32 fish was weighed and measured to fork length immediately before they were transported to the field locations. These fish ranged from 46 to 61 mm in length, with an average of 50.6  $\pm$  6.74 mm (SD). Weights ranged from 0.33 to 1.93 g, with an average of 1.07  $\pm$  0.45 g (SD). We transported fish on August 30 (Belden's Landing) and August 31 (Rio Vista) in insulated 19-L black buckets. Each bucket was salted to 5 psu, treated with API stress coat aquarium water conditioner (MARS Fishcare North America, Chalfont, Pennsylvania), supersaturated to approximately 180% oxygen, then loaded with 35 fish, resulting in a transport density of 1.8 fish per liter. We transported eight buckets to each field location, and deployed two buckets into each field enclosure, for a total of 70 fish per enclosure at a density of 0.08 fish per liter. A total of 24 Delta Smelt remained in a single 400-L tank at FCCL for the duration of the study (0.06 fish per liter) and were used to compare health metrics between fish in the hatchery environment and fish in the two field environments. The FCCL group was kept at a temperature of 18.5°C, salinity of 0.1 ps, flows of 6 L min<sup>-1</sup>, and 0 nephelometric turbidity units (NTU). They were fed a satiating ration of BioOregon-MASH fish food. Fish remained in the holding tank and field enclosures for a total of 6 weeks during the SMSGC management action

and we removed them on October 10 (Belden's Landing and FCCL) and October 12 (Rio Vista).

In the field enclosures, Delta Smelt were exposed to one of two biofouling mitigation treatments:

1. **Enclosure cleaning ("Scrubbed"):** Fish remained in a single enclosure throughout the deployment period. Once per week, field crews scrubbed these enclosures from the outside using hand-held scrub brushes. The length of the brushes allowed crews to scrub the enclosures to approximately 0.5 m depth. This process occurred for two enclosures per location (four enclosures total). This was a low-effort process, requiring just one vessel, one crew member, and an additional 10 to 15 minutes of labor per enclosure, and mimicked biofouling mitigation methods employed in previous Delta Smelt enclosure studies (Baerwald et al. 2023; Davis et al. 2024).
2. **Enclosure replacement ("Exchanged"):** Fish were transferred from a fouled enclosure and into a clean enclosure at 2 and 4 weeks after deployment. This was accomplished by removing the lid of the fouled enclosure and securing an unlidded, inverted, clean enclosure to the top of the fouled enclosure. Three clamps secured the enclosures together to prevent fish from escaping. Once attached, the conjoined enclosures were inverted 180°, thus completing the fish transfer without removing the fish from the water. The clean enclosure was secured to the buoy system and re-fitted with a lid, and the fouled enclosure was removed from the field. This process occurred for two enclosures per location (four enclosures total). This was a high-effort process that was selected to represent the maximum practical effort to mitigate biofouling concerns. It required a minimum of two vessels (one with specialized equipment to lift an enclosure), five crew members, and an additional 20 to 40 minutes of labor per enclosure.

The FCCL fish were not exposed to either of these biofouling mitigation treatments,

because these fish were intended to provide a baseline comparison of fish health in a hatchery environment.

## Fish Metrics

### **Survival and Condition**

At the end of the experiment, we collected all surviving fish from each enclosure and transported them to shore in sealed, black, insulated buckets for a survival count. We also weighed (g) and measured them to fork length (mm). We dissected twenty-five fish from each field enclosure and 11 FCCL fish in the lab to assess fish health; however, one enclosure from Belden's Landing experienced high mortality, and in that instance we dissected only eight fish. For each dissected fish, we calculated:

1. Fulton's condition factor (K), using the formula  $K = 100 * [\text{Fish Weight (g)} / \text{Fish Length (mm)}^3]$ . This can be used to estimate changes in nutritional condition.
2. Hepatosomatic index (HSI) using the formula  $\text{HSI} = [\text{Liver Weight (mg)} / \text{Fish Weight (mg)}] * 100$ . This can be used as an indicator of energy reserves in the liver.
3. Liver glycogen, using a colorimetric spectrophotometry assay described in Dhayalan et al. (2023). This is a biochemical measure of metabolic responses and a potential biomarker of food limitation. In brief, livers were homogenized, boiled to denature enzymes and preserve glycogen, and centrifuged. The resulting supernatants were collected to determine glycogen concentrations (as  $\mu\text{g}$  of glycogen per mg of tissue).

### **Temperature Tolerance**

Six fish from each field enclosure and 10 fish from the FCCL underwent upper temperature tolerance testing on-site using Critical Thermal Maximum (CT<sub>max</sub>) methodology, as described in Davis et al. (2024) with modifications from Beiting et al. (2000) for field measurements. These fish were removed from the enclosures, placed into

5-gallon buckets of ambient field water, brought to shore, and then were immediately placed into individual chambers filled with 1.5 L of ambient field water (~18 to 19°C). Fish were provided a 30-min acclimation period in the chamber, after which the water temperature was increased at  $0.3^{\circ}\text{C min}^{-1}$  until fish experienced loss of equilibrium and were unable to right themselves. When this occurred, the temperature was recorded as the CT<sub>max</sub> (in °C), and fish were rapidly transferred to a recovery bath of cooler ambient field water. Fish were observed in the recovery bath for 3.5 hrs on shore, after which survival was determined, and length and weight were recorded. Fish that did not survive the recovery bath were not included in the dataset.

### **Diets**

We removed the stomachs of five fish per enclosure (40 fish total), dissected them, and sent them to the Wetland Ecosystem Team laboratory (Seattle, Washington USA) for diet analysis. The total contents of each stomach were weighed when practicable, and all prey items were identified and enumerated. For analysis, we combined prey into higher taxonomic groups (e.g., calanoid copepods) to match the taxonomic resolution of the zooplankton and macroinvertebrate samples. We evaluated diets by diet abundance (which emphasizes smaller, more numerous prey) and diet biomass (which emphasizes larger, heavier prey). Refer to the “[Biomass Conversions](#)” section for specifics on biomass conversions and caveats. We calculated gut fullness (%) using a gut content weight based on calculated total diet biomass converted to wet weight (g) and adjusted for fish size (g). Although the contents of each stomach were weighed, accurate weights were not always possible because of the small size of the prey or damage to the stomach. We therefore used only calculated gut values in our analyses.

### **Habitat Metrics**

#### **Water Quality**

We collected water-quality data weekly from the interior of each enclosure at three points adjacent to the enclosures using a YSI Pro DSS

(digital sampling system), and included water temperature (°C), dissolved oxygen ( $\text{mg L}^{-1}$ ), specific conductivity ( $\mu\text{Siemens cm}^{-1}$ ), and turbidity (FNU) data. To estimate the relative difference in current speed between treatments and study locations, we attached a pre-weighed clod card (per Jokiel and Morrissey 1993) to the interior and exterior of each enclosure 1 week before the enclosure was removed. (Clod cards are bricks of gypsum that dissolve in water at a rate linearly related to water movement and can be used to compare relative rates of water movement between locations.) To construct the clod cards, we mixed 1,000 g of Presto-Patch drywall patching compound with 100 g of Cascamite powdered resin glue and 500 g of water. We placed approximately 100 g of mixture ( $\pm 5$  g) into each of 16 8-oz paper cups and left it to cure for 72 hrs. We then removed the clod cards from the cup, glued them to a 10-cm square of corrugated plastic using 100% silicone adhesive, and dried them in a drying oven at 60°C until weights stabilized (final weights between 65 and 75 g). We attached the clod cards to the field enclosures and removed them after 1 week. After removal, we placed them into a drying oven for 72 hrs and re-weighed to calculate the loss in weight over the 1-week period.

#### **Zooplankton**

We collected zooplankton samples from both field locations at 2, 4, and 6 weeks after deployment. At each time and field location, we collected one sample from inside each enclosure and three samples outside the enclosures (within 10 m). We collected zooplankton samples by filtering 10 gallons of water through a 60- $\mu\text{m}$  mesh using Geopump Easy-Load II peristaltic pumps with a combined flow rate of approximately  $2 \text{ gal min}^{-1}$  (the maximum pump velocity). To do so, we set up three Geopumps in parallel and connected them to a total of five 3/8-inch-internal-diameter intake hoses. We carefully set pump intake hoses at 0.15, 0.36, 0.57, 0.79, and 1 m below the water's surface, to ensure no intakes were in direct contact with the surface of the enclosures. We preserved samples in 10% formalin, dyed with rose Bengal dye, and transported to ICF International, Inc. (Richmond, California) for processing. All

invertebrate samples were enumerated and identified to the lowest practicable level of taxonomic resolution.

## Biofouling

### Algal Growth

To measure algal growth (biofouling) within the enclosures, we attached a 15-cm × 15-cm-square of painted steel mesh (“coupon”) to the inside of each field enclosure, far enough below the waterline to ensure it remained submerged during wave action. For all scrubbed enclosures, we attached coupons at the beginning of the deployment. For all exchanged enclosures, we installed coupons 4 weeks after deployment, immediately following the final enclosure replacement. We retrieved all coupons on the final day of the study, placed them on ice, then placed them in a freezer. To process the samples, we thawed each coupon at room temperature and then rinsed or lightly scraped into a fine sieve to separate debris and macroinvertebrates from algae. We placed each algae sample into a drying oven at 60°C and held for 11 days to evaporate all moisture, then weighed for total mass (Lipps et al. 2023).

### Macroinvertebrates

To quantify macroinvertebrate colonization on the enclosures after 6 weeks, we attached a 0.25-m<sup>2</sup> quadrat 10 cm below the water line inside each enclosure immediately before we removed that enclosure, and attached 0.5-mm mesh aquarium net to the base of the quadrat. Once we removed the enclosure, we scraped all macroinvertebrates within the quadrat off the enclosure and into the aquarium net. (Here we define “macroinvertebrates” as all invertebrates captured by a 0.5-mm mesh net, which chiefly comprised aquatic insects, amphipods, and isopods.) We preserved these samples in 10% formalin dyed with Rose Bengal dye and transported to ICF for analysis where they were identified, enumerated, and measured.

### Biomass Conversions

We calculated biomass (carbon weight, µg) for meso- and micro-zooplankton samples and

in the diets using the conversions in Kayfet et al. (2020), modified with more recent data where needed (see Appendix A, Table A1). We calculated biomass for macroinvertebrates using length–weight equations (Burdi et al. 2021, 2026). We used the equation for the amphipod *Americorophium spinicorne* for tanaid biomass conversions based on similar size and shape, and the lack of an available length–weight equation for tanaids. Munnidae isopods also lacked a length–weight equation, so we used the equation for *Gnorimosphaeroma* isopods and adjusted to 50% of the weight, based on the smaller and thinner body shape of Munnidae compared to *Gnorimosphaeroma*. We used a standard weight for chironomid larvae based on previous diet data (Slater et al. 2019). Since prey lengths in diets were not measured, we used the lengths from amphipods in the macroinvertebrate samples to represent those in the diets and limited to < 2 mm based on previous diet data for fish caught in August to October (Slater et al. 2019). We removed amphipods in the diets that had only part of an organism (e.g., head only) from biomass calculations.

## DATA ANALYSIS

### Fish Metrics

The statistical design for condition factor, HSI, liver glycogen, and CTmax was unbalanced, with only a single source population at FCCL, and varied sample sizes between field locations and treatments; therefore, we took a two-step approach to analyze the effects of location and biofouling mitigation method. We first ran a series of univariate linear models—one for each response metric—with location, biofouling mitigation method, fish body weight, and enclosure replicate as predictor variables, including only the field-deployed enclosures (Table A2). Then we ran a series of reduced models on the full dataset, including the FCCL fish, to test only for the effect of location on condition factor, HSI (log transformed), liver glycogen, and CTmax, followed by post-hoc tests using the ‘emmeans’ package in R (Lenth et al. 2024; Table A3). We included fish weight as a predictor variable in the liver glycogen and

CTmax models to account for differences in glycogen concentrations and thermal tolerance with size (Figure A1). We removed one outlier (20.6 °C) in the CTmax dataset, because it was lower than any minimum measures previously reported, and was most likely the result of handling stress rather than temperature (Swanson et al. 2000; Davis et al. 2024). We used a binomial mixed-effect regression model (the *glmer* function from the ‘lme4’ package) to quantify how location and biofouling mitigation method affected survival. We did not include FCCL fish in the survival analysis, because there was no replication at that site. We conducted all analyses using R (v.4.4.1; R Core Team 2024).

## HABITAT METRICS

### Water Quality

To test the effects of enclosure location and biofouling mitigation method on water quality, we fit separate linear mixed-effects models with the discrete water-quality value (pH, DO, temperature, turbidity and salinity) collected inside and outside the enclosures at each time using the following model structure for each water-quality parameter, where *Enclosure Effect* indicates whether the sample was taken inside or outside the enclosure:

$$Value \sim EnclosureEffect + (1 | Date) \quad \text{Eq 1}$$

Because preliminary models indicated no difference between the inside and the outside of the enclosures for any of the water-quality parameters, we used data from the nearest continuous water-quality stations to visualize the differences between locations and over time (CDEC station BDL for Belden’s Landing and RVB for Rio Vista) (California Data Exchange Network, <https://cdec.water.ca.gov>). Data were provided by the CDWR and underwent quality-control procedures.

We used data from clod cards to assess the difference in current speed between locations and treatments. We calculated the percentage of weight lost per day over the course of the

week-long clod card deployment, then modeled the change in weight per day using an Analysis of Variance (ANOVA) with the following model structure:

$$\Delta Weight \sim Location + Treatment \quad \text{Eq 2}$$

where *Treatment* indicates whether the card came from inside a scrubbed enclosure, inside an exchanged enclosure, or outside the enclosures. This was followed by a Tukey post-hoc test to see which treatments differed from one another.

### Algal Growth, Macroinvertebrates, Zooplankton, and Diets

We assessed differences in algal biomass, macroinvertebrate biomass, macroinvertebrate abundance, zooplankton catch per unit effort (CPUE), zooplankton biomass per unit effort (BPUE), and Delta Smelt gut fullness using linear models with the *lm* function in R. All values were log-transformed to meet assumptions of normality of residuals. We then conducted a Type 2 ANOVA using the *Anova* function from the ‘car’ package in R (Fox et. al. 2020), with the following model structure:

$$\text{Log}(x) \sim Treatment + Location \quad \text{Eq 3}$$

where *x* is biomass or abundance, and treatment and location are fixed effects. For algae and macroinvertebrates, *Treatment* indicates whether the sample came from a scrubbed or exchanged enclosure. For zooplankton, an additional treatment (pump samples from outside the enclosures) was also included. To further investigate differences in zooplankton, catch between sampling methods and locations, we conducted an additional ANOVA on CPUE of adults and copepodites of the cyclopoid copepod *Limnoithona* spp., which is thought to be poor smelt food because of its small size and low nutritional value (Gould and Kimmerer 2010; Kratina and Winder 2015). We also ran an ANOVA on the CPUE of adults and copepodites of the calanoid copepod *Pseudodiaptomus* spp., which is the most frequently consumed diet item by Delta

Smelt in the summer (Slater et al. 2019), is selected for over other calanoid and cyclopoid copepods (Slater and Baxter 2014) and is thought to be of high nutritional value because of its large size and high nitrogen content (Kratina and Winder 2015). We believe *Pseudodiaptomus* spp. may be under-sampled in pump samples because of its larger size and ability to escape pump velocities (Sameoto et al. 2000; Kiørboe et al. 2010).

To test whether zooplankton communities varied between locations, biofouling mitigation method, and sample type (inside vs. outside), we calculated the Bray–Curtis dissimilarity of all samples collected, and ran a permutational multivariate analysis of variance (PERMANOVA) test using the `adonis2` function in the ‘vegan’ package in R (Oksanen et al. 2022), with the following model structure:

$$\text{Distance Matrix} \sim \text{Treatment} + \text{Location} \quad \text{Eq 4}$$

For these data, zooplankton were identified to species and, to reduce noise, we combined taxa into larger taxonomic groups based on similarity of species and importance in Delta Smelt diets. We divided the count of each species by volume of the sample to calculate CPUE (catch  $\text{m}^{-3}$ ), then converted into biomass (see “[Biomass Conversions](#)”) to calculate BPUE (biomass  $\text{m}^{-3}$ ).

To determine whether biofouling affected fish diets through changes in zooplankton consumption or community composition, we compared invertebrate communities in Delta Smelt diets to zooplankton and macroinvertebrate samples collected inside the enclosures via a second PERMANOVA that used the same methods as above, with the model structure:

$$\text{Distance Matrix} \sim \text{Data Source} + \text{Treatment} + \text{Location} \quad \text{Eq 5}$$

where *Data Source* indicates whether the sample was from a Delta Smelt diet or from a zooplankton or macroinvertebrate sample. This was done for both diet abundance and diet biomass. We checked all PERMANOVA models for homogeneity

of dispersion using the `betadisp` function in the ‘vegan’ package in R (Oksanen et al. 2022). We compared data visually using stacked bar plots and Principal Components Analysis (PCA) biplots (in R) biplots from the `betadisp` outputs.

## RESULTS

### Fish Metrics

Delta Smelt survival and other physiological metrics—including condition factor, HSI, liver glycogen, and CTmax—differed by location (ANOVA,  $P < 0.0001$ , [Table 1](#)). In general, the highest physiological markers were observed in the fish held at FCCL, followed by the field fish at Rio Vista, while markers were lowest for fish held at Belden’s Landing. Fish held at FCCL had a CTmax 1–2 °C higher than fish in the field enclosures, and liver glycogen and HSI measurements that were almost two times higher than the field fish. The FCCL fish had a similar condition factor to fish held at Rio Vista, which was about 15% higher than fish held at Belden’s Landing ([Table A3](#)). Between the two field sites, Delta Smelt deployed at Rio Vista had roughly: 25% higher survival (logistic regression,  $P < 0.05$ ), slightly higher HSI ( $< 10\%$ ), 25% higher liver glycogen, and 1 °C higher CTmax than fish deployed at Belden’s Landing (*emmeans* ‘Tukey’,  $P < 0.05$ ; [Figure 2](#), [Table A3](#)).

Between biofouling mitigation methods, we observed roughly 20% higher survival in the scrubbed enclosures compared to the exchanged enclosures (logistic regression,  $P < 0.05$ ). We did not detect differences in any other physiological metrics, including condition factor, HSI, liver glycogen, or CTmax. ([Figure 3](#)).

### Habitat Metrics

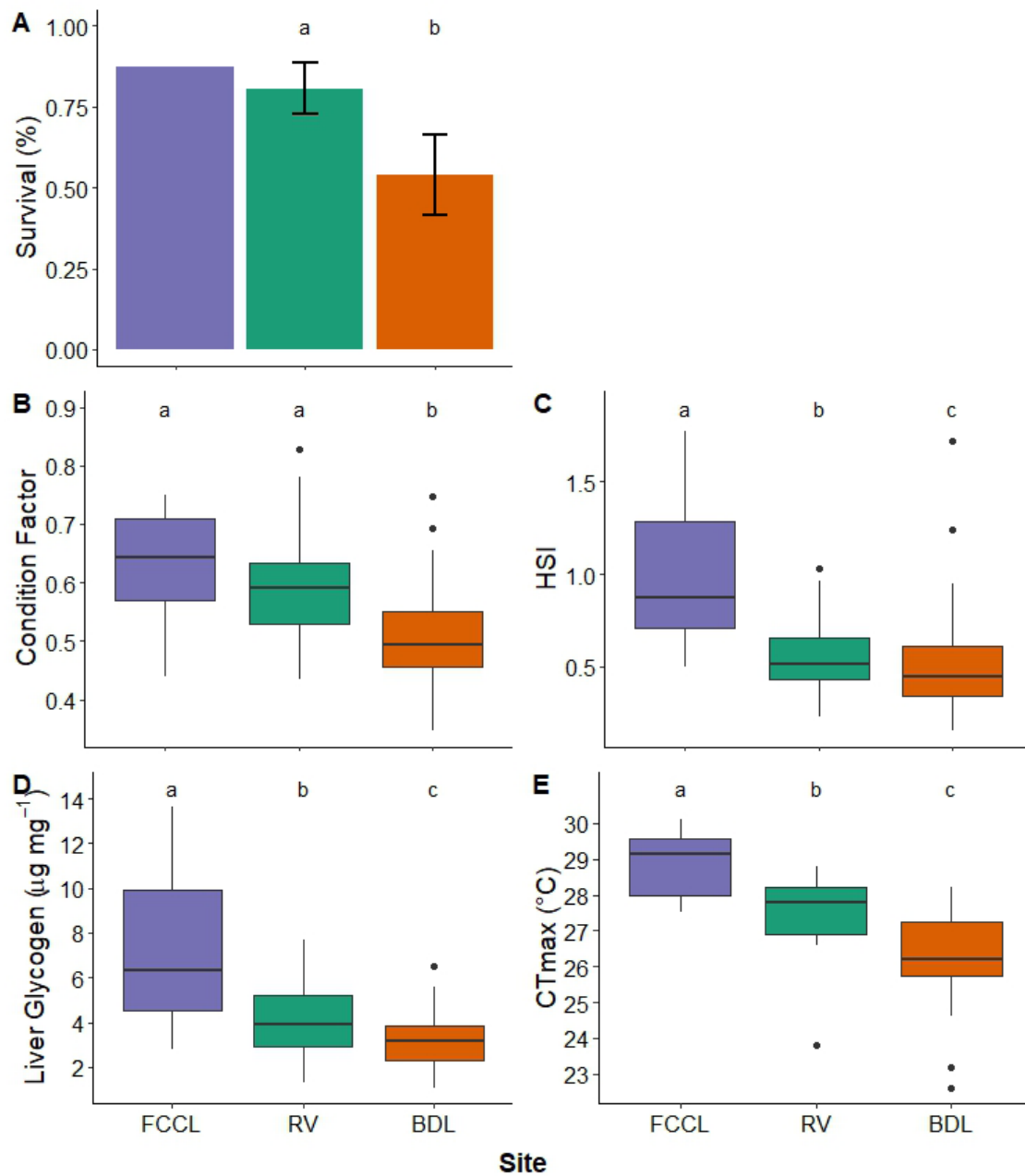
#### Water Quality

Water-quality data at nearby continuous sondes showed that Belden’s Landing tended to have higher chlorophyll, salinity, water temperature, and turbidity than Rio Vista during the study period ([Figure 4](#)).

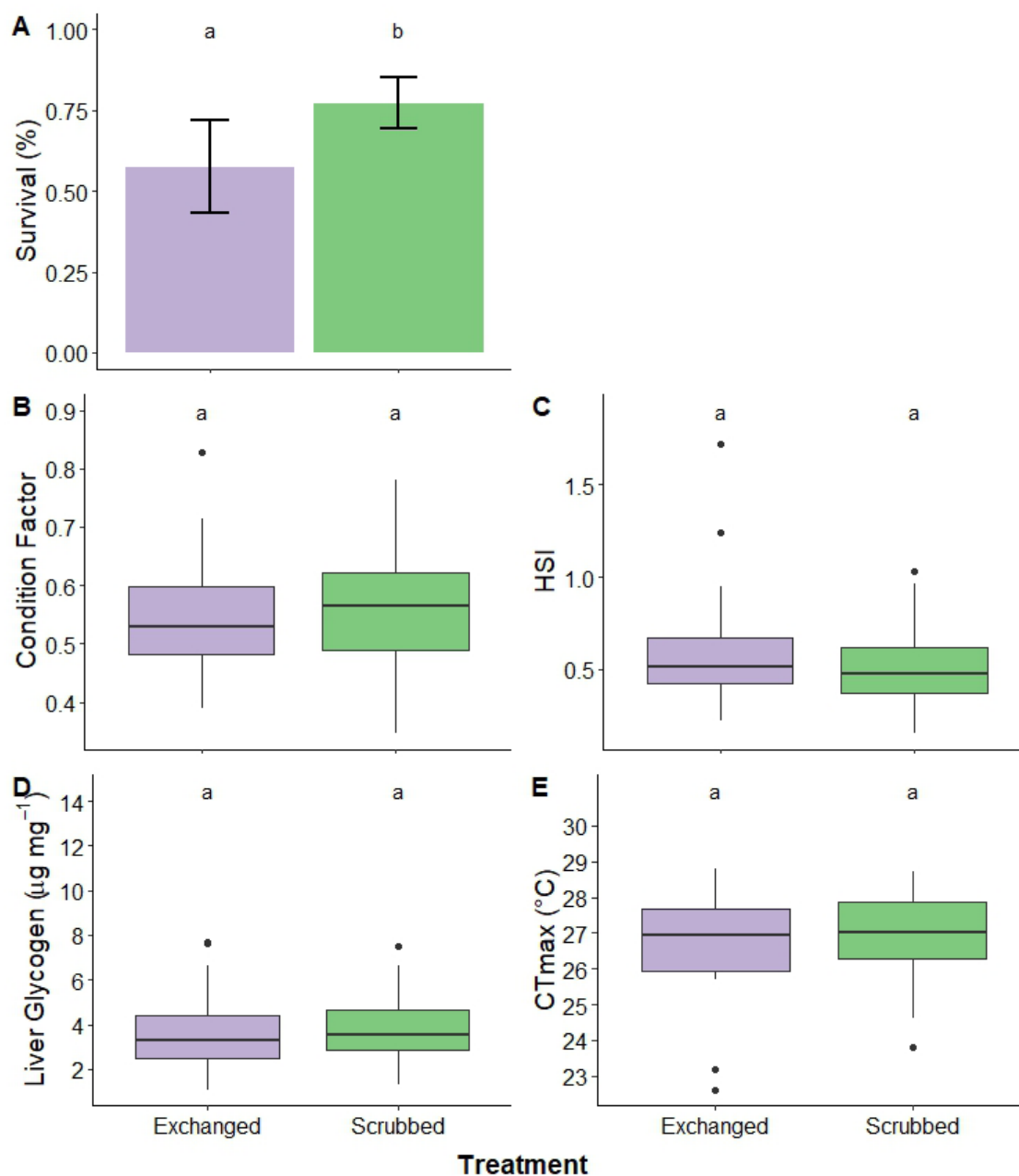
**Table 1** Results of generalized linear mixed model on survival and two-way ANOVAs on Delta Smelt responses, clod card loss of weight per day, algal biomass, biofouling macroinvertebrate abundance, biofouling macroinvertebrate biomass, zooplankton catch per cubic meter (CPUE), zooplankton biomass per cubic meter (BPUE), CPUE of *Limnoithona* spp. in zooplankton samples, CPUE of *Pseudodiaptomus* juveniles and adults in zooplankton samples, and smelt gut fullness (%)

| Metric      | Response                               | Predictor   | SumSq  | DF  | F Value | P-value  | Sig <sup>a</sup> |
|-------------|----------------------------------------|-------------|--------|-----|---------|----------|------------------|
| Fish        | Survival                               | Location    |        |     |         | 0.0050   | **               |
|             |                                        | Treatment   |        |     |         | 0.0455   | *                |
|             | Condition Factor                       | Location    | 0.41   | 2   | 31.472  | < 0.0001 | ***              |
|             |                                        | Residuals   | 1.24   | 192 |         |          |                  |
|             | HSI                                    | Location    | 0.97   | 2   | 18.291  | < 0.0001 | ***              |
|             |                                        | Residuals   | 5.06   | 191 |         |          |                  |
|             | Liver Glycogen                         | Location    | 127.52 | 2   | 31.454  | < 0.0001 | ***              |
|             |                                        | Fish Weight | 64.37  | 1   | 31.755  | < 0.0001 | ***              |
|             |                                        | Residuals   | 381.10 | 188 |         |          |                  |
|             | CTmax                                  | Location    | 50.51  | 2   | 15.5341 | < 0.0001 | ***              |
|             |                                        | Fish Weight | 0.07   | 1   | 0.0415  | 0.8396   |                  |
|             |                                        | Residuals   | 68.28  | 42  |         |          |                  |
| Biofouling  | Clod Cards                             | Treatment   | 12.77  | 1   | 14.28   | 0.0006   | ***              |
|             |                                        | Location    | 2.75   | 2   | 6.16    | 0.0280   | *                |
|             |                                        | Residuals   | 5.36   | 12  |         |          |                  |
|             | Algal Biomass                          | Treatment   | 10.17  | 1   | 9.41    | 0.0107   | *                |
|             |                                        | Location    | 2.66   | 1   | 2.46    | 0.1450   |                  |
|             |                                        | Residuals   | 11.89  | 11  |         |          |                  |
|             | Biofouling Macroinvertebrate Biomass   | Treatment   | 36.55  | 1   | 6.35    | 0.05319  |                  |
|             |                                        | Location    | 10.58  | 1   | 1.84    | 0.23321  |                  |
|             |                                        | Residuals   | 28.79  | 5   |         |          |                  |
|             | Biofouling Macroinvertebrate Abundance | Treatment   | 17.97  | 1   | 15.21   | 0.0114   | *                |
|             |                                        | Location    | 5.40   | 1   | 4.57    | 0.0856   |                  |
|             |                                        | Residuals   | 5.91   | 5   |         |          |                  |
| Zooplankton | Zooplankton CPUE                       | Treatment   | 94.85  | 3   | 51.05   | < 0.0001 | ***              |
|             |                                        | Location    | 27.33  | 1   | 44.12   | < 0.0001 | ***              |
|             |                                        | Residuals   | 34.69  | 56  |         |          |                  |
|             | Zooplankton BPUE                       | Treatment   | 57.33  | 3   | 87.01   | < 0.0001 | ***              |
|             |                                        | Location    | 6.69   | 1   | 30.47   | < 0.0001 | ***              |
|             |                                        | Residuals   | 12.30  | 56  |         |          |                  |
|             | <i>Limnoithona</i> spp. CPUE           | Treatment   | 423.75 | 3   | 148.77  | < 0.0001 | ***              |
|             |                                        | Location    | 164.35 | 1   | 173.10  | < 0.0001 | ***              |
|             |                                        | Residuals   | 53.17  | 56  |         |          |                  |
|             | <i>Pseudodiaptomus</i> spp. CPUE       | Treatment   | 16.33  | 3   | 4.47    | 0.0078   | **               |
|             |                                        | Location    | 1.86   | 1   | 1.52    | 0.2233   |                  |
|             |                                        | Residuals   | 56.02  | 46  |         |          |                  |
|             | Gut Fullness (%)                       | Treatment   | 0.005  | 1   | 0.4047  | 0.5288   |                  |
|             |                                        | Location    | 0.01   | 1   | 0.7227  | 0.4010   |                  |
|             |                                        | Residuals   | 0.46   | 35  |         |          |                  |

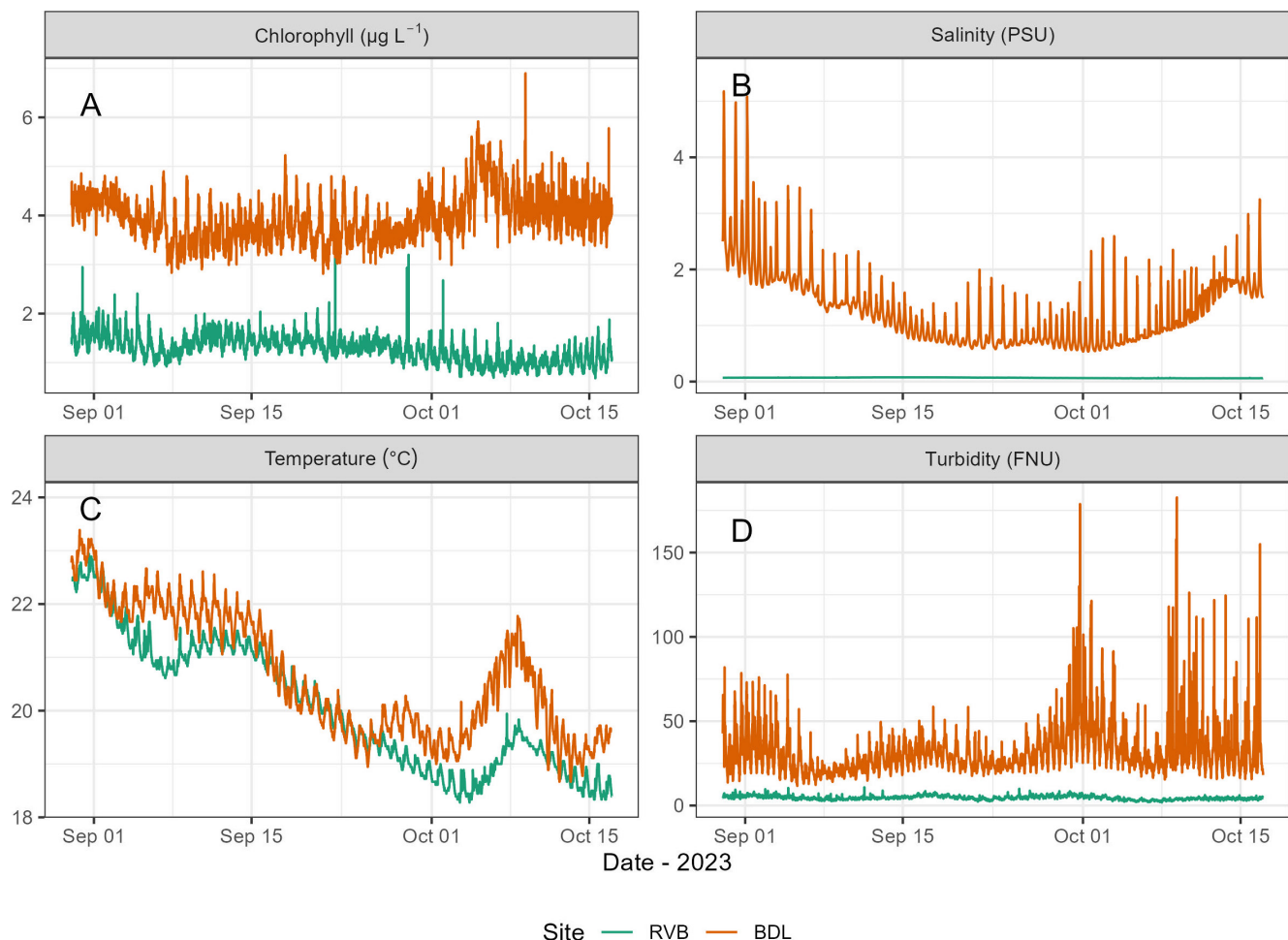
a. Sig = Significance. \* =  $P$ -value < 0.05, \*\* =  $P$ -value < 0.01, \*\*\* =  $P$ -value < 0.001.



**Figure 2** Delta Smelt survival and health metrics by location after the 6-week deployment. Survival is shown as a *bar graph with standard error bars*. Condition factor, HSI, liver glycogen, and CTmax are shown as *box and whisker plots*, with outliers included. FCCL are fish that remained in the hatchery. RV = Rio Vista and BDL = Belden's Landing are deployed fish. Different letters (*a, b, c*) indicate significant differences ( $P < 0.05$ ).



**Figure 3** Delta Smelt health metrics by biofouling mitigation treatment after the 6-week deployment. Survival is shown as a *bar graph with standard error bars*. Condition factor, HSI, liver glycogen, and CTmax are shown as *box and whisker plots*, with outliers included. Different letters (*a*, *b*) indicate significant differences ( $P < 0.05$ ).



**Figure 4** Water-quality data from nearby continuous sondes during the study period. Data were collected at CDEC stations BDL (Belden's Landing) and RVB (Rio Vista Bridge) and underwent quality-control checks.

### Water Movement

Clod card data showed a 120% greater loss of weight outside the enclosures than inside, indicating that water velocities were higher outside than inside (Figure 5, ANOVA,  $P < 0.05$ ; Tukey post-hoc,  $P < 0.05$ ). Inside the enclosures, there was a 34% greater loss of clod card weight at Belden's Landing than at Rio Vista, indicating higher water velocities at Belden's Landing (ANOVA,  $P < 0.05$ ). However, there were no differences between loss of weight (and therefore, no differences in velocities) inside scrubbed vs. exchanged enclosures within each location (Tukey post-hoc,  $P > 0.05$ ).

### Zooplankton

Total zooplankton biomass was twice as high at Belden's Landing than at Rio Vista, and total zooplankton abundance was eight times as high (Figure 6C, Table 1). There was no difference in CPUE or BPUE between biofouling mitigation methods or between pump samples taken inside and outside of the enclosures (Tukey,  $P > 0.05$ ). Catch per unit effort of the cyclopoid copepod *Limnoithona* spp. was 46 times higher at Belden's Landing than at Rio Vista (Table 1). Catch per unit effort of the calanoid copepod *Pseudodiaptomus* spp. was 13 times higher at Rio Vista than at Belden's landing (Table 1) but there was no difference between biofouling mitigation methods or the inside and outside of the enclosures (Tukey,  $P > 0.05$ ).

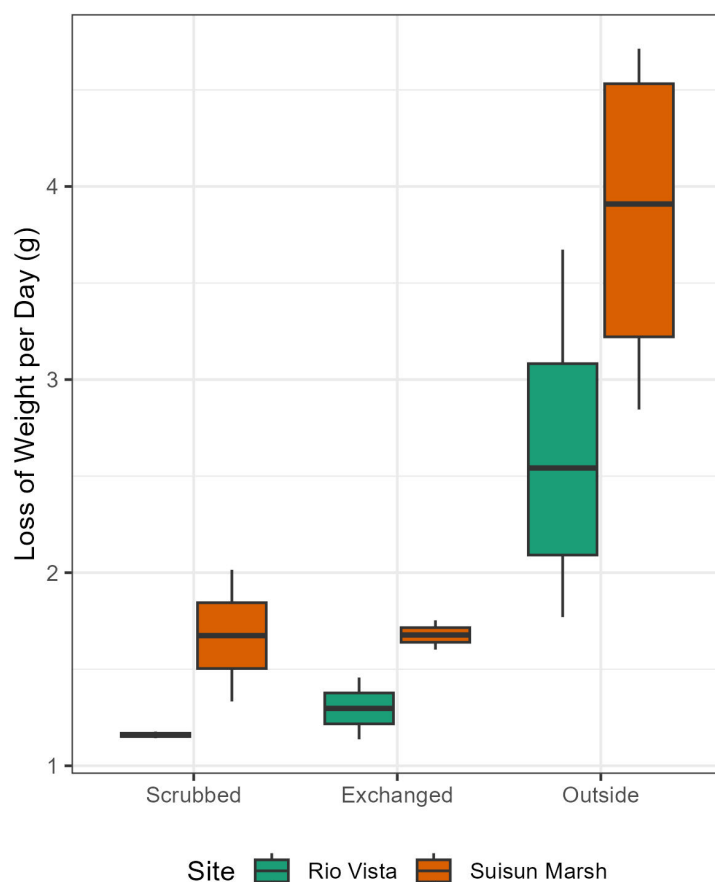


Figure 5 Loss of weight of clod cards per day placed inside (scrubbed/exchange) or outside each enclosure during the final 7 days of the experiment

The composition of the zooplankton community differed between locations (Figure 7C; PERMANOVA,  $P < 0.001$ ,  $R^2 = 0.61$ ; Table 2), but there was no difference between biofouling mitigation methods within locations (Figure 7C;  $P = 0.101$ ,  $R^2 = 0.058$ ). Data met the assumption of homogeneous dispersion across both treatments (ANOVA of beta dispersion results,  $P = 0.91$ ). When comparing locations, dispersion at Belden's Landing was significantly greater than dispersion at Rio Vista ( $P < 0.05$ ); however, examination of biplots found no overlap in PCA hulls (Figure A2).

### Biofouling Measurements

Algal biomass was about six times higher in scrubbed enclosures than in exchanged ones (ANOVA,  $P = 0.01$ ; Figure 6A). We found no statistical differences in algal biomass between locations (ANOVA,  $P > 0.05$ ), though this may have been the result of the low sample size. We were unable to include an interaction term, because

we had only two samples for each treatment and location combination.

Total biofouling-associated macroinvertebrate abundance differed between enclosure treatments (ANOVA,  $P = 0.01$ ; Table 1) with fewer macroinvertebrates on exchanged enclosures (Figure 6B), and lower biomass as well ( $P = 0.01$ ; Table 1). We observed a trend of higher macroinvertebrate abundance at Rio Vista compared to Belden's Landing, though it did not meet the threshold of statistical significance (ANOVA,  $P = 0.08$ ). There was also a significant difference in community composition of biofouling macroinvertebrates between locations (PERMANOVA,  $P = 0.03$ ,  $R^2 = 0.46$ ), but not between biofouling mitigation methods ( $P > 0.05$ ). Data met the assumption of homogeneous dispersion across both treatments and locations (ANOVA of beta dispersion results  $P = 0.73$  and  $0.25$ , respectively; biplots available in Figure A2).

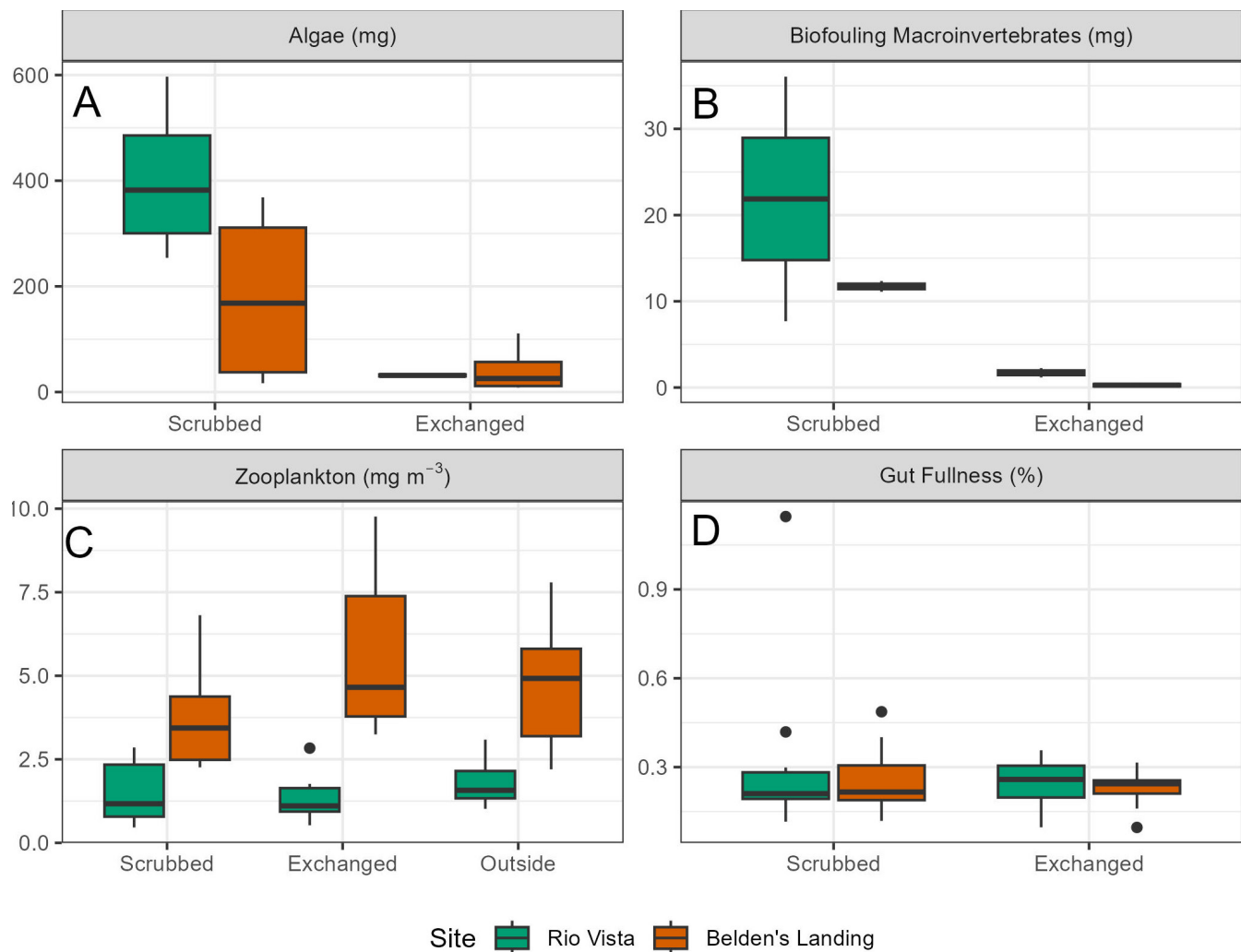


Figure 6 Box and whisker plots of biofouling algae biomass, biofouling-associated macroinvertebrate biomass per sample, total zooplankton BPUE, and smelt gut fullness accounting for body weight, for scrubbed and exchanged treatments at Belden's Landing and Rio Vista locations

### Diets

No differences in gut fullness (%) were detected between biofouling mitigation method ( $P = 0.39$ ) or location ( $P = 0.53$ ). No differences in diet composition were detected between mitigation method; however, differences were detected between sites ( $P = 0.003$ ,  $R^2 = 0.13$ ; Table 2). Data met the assumptions of homogeneity of dispersion by treatment ( $P = 0.62$ ), but dispersion was significantly lower at Rio Vista than at Belden's Landing ( $P = 0.03$ ; Figure A2). Delta Smelt at Rio Vista consumed higher levels of amphipods and *Pseudodiaptomus* spp., whereas Delta Smelt

at Belden's Landing consumed higher levels of *Limnoithona* spp. and cumaceans (Figures 7A, 7B).

When comparing Delta Smelt diets to invertebrates collected in the zooplankton and biofouling samples, we found that fish were consuming invertebrates present in both the zooplankton and biofouling communities (Figure 7, Table 2), with differences between locations ( $R^2$  of 0.14) and between data sources ( $R^2 = 0.28$ ), but no difference between biofouling mitigation method ( $P > 0.05$ ,  $R^2 = 0.01$ ). Treatments and locations had equal dispersions,

**Table 2** Results of two permutational multivariate analysis of variance (PERMANOVA) comparing the relative abundance and relative biomass of biofouling communities, diet samples, and zooplankton communities. *Data Source* refers to whether data were from biofouling invertebrates, Delta Smelt diet, or zooplankton samples. *Treatment* refers to whether the enclosure was scrubbed or exchanged, and *Enclosure Effect* refers to whether samples were taken from inside or outside the enclosure (zooplankton only). The results for zooplankton CPUE and BPUE are similar; therefore, only the BPUE results are shown here.

| Model                                 | Term             | Df | SumOfSqs | R <sup>2</sup> | F      | Pr(>F) | Sig <sup>a</sup> |
|---------------------------------------|------------------|----|----------|----------------|--------|--------|------------------|
| Biofouling macroinvertebrate biomass  | Treatment        | 1  | 0.000    | 0.008          | 0.101  | 0.572  |                  |
|                                       | Location         | 1  | 0.026    | 0.663          | 8.083  | 0.023  | *                |
|                                       | Residual         | 4  | 0.013    | 0.328          |        |        |                  |
|                                       | Total            | 6  | 0.038    | 1.000          |        |        |                  |
| Zooplankton biomass - inside          | Treatment        | 1  | 0.039    | 0.024          | 0.602  | 0.659  |                  |
|                                       | Location         | 1  | 0.990    | 0.617          | 15.460 | 0.003  | **               |
|                                       | Residual         | 9  | 0.576    | 0.359          |        |        |                  |
|                                       | Total            | 11 | 1.605    | 1.000          |        |        |                  |
| Zooplankton BPUE - inside vs. outside | Enclosure effect | 1  | 0.036    | 0.015          | 0.667  | 0.554  |                  |
|                                       | Location         | 1  | 1.476    | 0.638          | 27.584 | 0.001  | **               |
|                                       | Residual         | 15 | 0.802    | 0.347          |        |        |                  |
|                                       | Total            | 17 | 2.314    | 1.000          |        |        |                  |
| Diet biomass                          | Treatment        | 1  | 0.211    | 0.024          | 0.947  | 0.415  |                  |
|                                       | Location         | 1  | 1.175    | 0.131          | 5.265  | 0.003  | **               |
|                                       | Residual         | 34 | 7.587    | 0.846          |        |        |                  |
|                                       | Total            | 36 | 8.973    | 1.000          |        |        |                  |
| Data source - abundance               | Treatment        | 1  | 0.126    | 0.005          | 0.691  | 0.662  |                  |
|                                       | Location         | 1  | 3.081    | 0.124          | 16.916 | 0.001  | **               |
|                                       | Data source      | 2  | 10.240   | 0.411          | 28.108 | 0.001  | **               |
|                                       | Residual         | 63 | 11.475   | 0.460          |        |        |                  |
|                                       | Total            | 67 | 24.922   | 1.000          |        |        |                  |
| Data source- biomass                  | Treatment        | 1  | 0.085    | 0.004          | 0.401  | 0.903  |                  |
|                                       | Location         | 1  | 3.002    | 0.145          | 14.187 | 0.001  | **               |
|                                       | Data source      | 2  | 5.626    | 0.271          | 13.294 | 0.001  | **               |
|                                       | Residual         | 57 | 12.062   | 0.581          |        |        |                  |
|                                       | Total            | 61 | 20.776   | 1.000          |        |        |                  |

a. Sig = Significance. \* =  $P$ -value < 0.05, \*\* =  $P$ -value < 0.01, \*\*\* =  $P$ -value < 0.001.

but the data sources had unequal dispersions ( $P < 0.05$ ), with much lower dispersion (average distance to the median) in the biofouling (0.0001) data than in the zooplankton (0.536) or diet data (0.380). However, examining the PCA biplots shows that zooplankton and diet hulls did not overlap, while the hull for biofouling invertebrates was completely contained within the hull for diet invertebrates (Figure A3).

## DISCUSSION

After 6 weeks in experimental enclosures, we found that Delta Smelt at Belden's Landing had lower survival, condition factor, hepatosomatic index (HSI, indicating lower energy reserves) liver glycogen (indicating nutritional limitations), and CTmax (suggesting overall lower health or capacity to deal with stressors) than Delta Smelt at Rio Vista. These findings were opposite to our hypothesis that Delta Smelt positioned in Suisun Marsh (center of the summer-fall

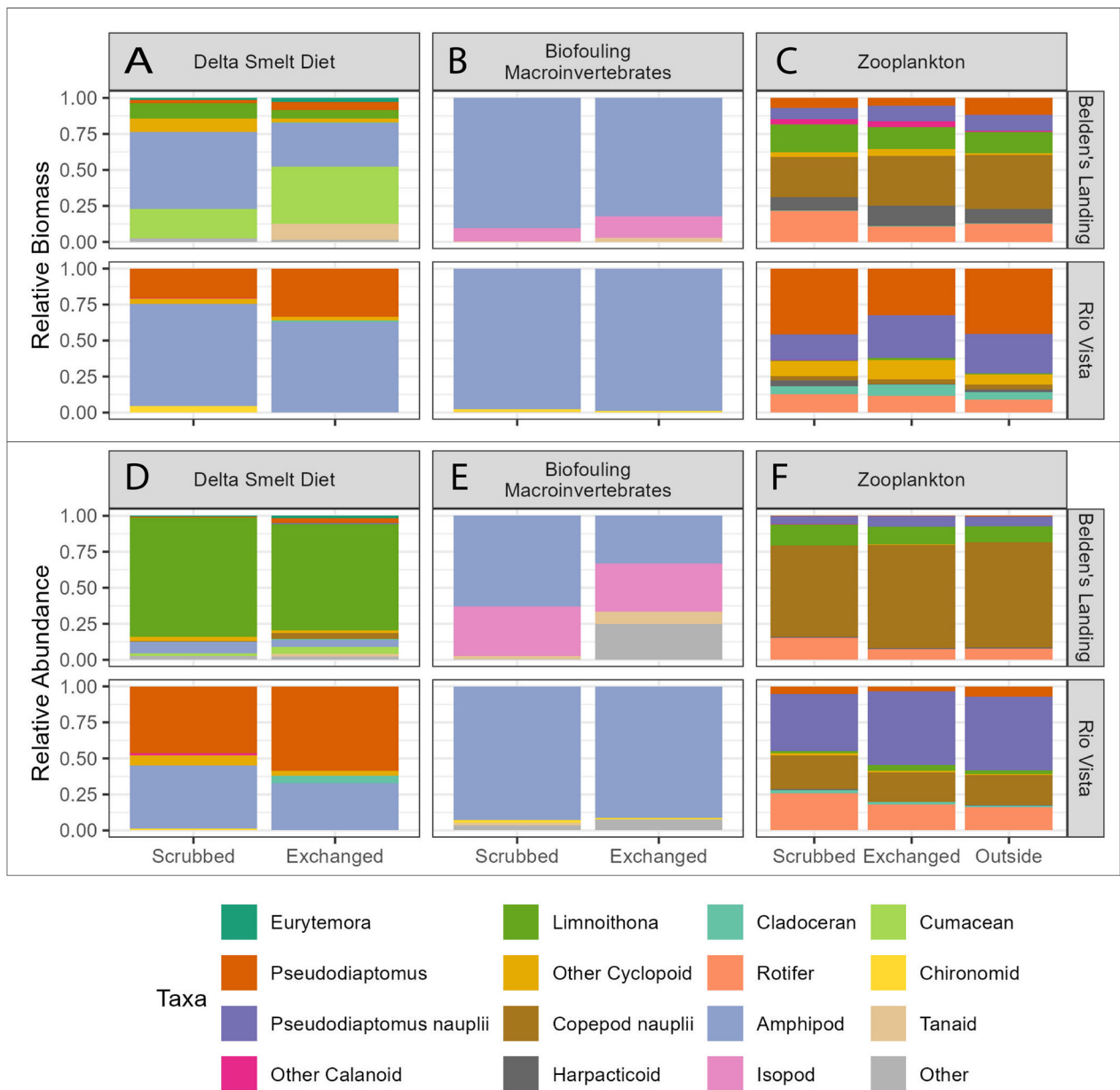


Figure 7 Stacked bar plot showing community composition in relative biomass and relative abundance for biofouling, diet samples, and zooplankton samples by location and biofouling mitigation method

management action area) would display measurably improved health metrics than those positioned in the lower Sacramento River (outside of the action area). This may suggest that habitat conditions in Suisun Marsh were not beneficial to Delta Smelt compared to conditions in the Sacramento River; however, it is important to note that localized enclosure effects may have affected our results. Fish in enclosures are unable to seek preferred habitats or forage for food outside of the enclosures, which may have had a pronounced effect on fish health at Belden's Landing, where we observed higher water velocities, warmer water temperatures, and poorer food quality inside the enclosures compared to Rio Vista. Furthermore, because the enclosures were installed in a single location within each geographic region, it's possible that our results do not reflect the region as a whole, given the wide variability in micro-habitats across Suisun Marsh and the lower Sacramento River. We did successfully reduce algae biomass and macroinvertebrate on the enclosure walls by exchanging fouled enclosures rather than scrubbing them, but these biofouling reductions did not translate to differences in Delta Smelt feeding patterns or health metrics, and the exchange process did appear to have a negative effect on fish survival. Furthermore, amphipods were detected on nearly all enclosures and were commonly consumed by the Delta Smelt inside, regardless of the biofouling mitigation method employed, suggesting that biofouling may create a food environment that does not reflect what a wild Delta Smelt would encounter, even when significant efforts—such as enclosure replacement—are employed.

### Habitat Differences

Overall, Delta Smelt in the Belden's Landing enclosures likely experienced increased energetic demands compared to Delta Smelt in the Rio Vista enclosures. These demands came from multiple sources including increased temperatures, increased water velocities, and the need to consume more prey because of the lower quality of prey at Belden's Landing. This combination may explain the overall poorer fish health

observed at Belden's Landing during the study period.

We predicted that cooler water temperatures and higher turbidity in Suisun Marsh would lead to increased health and survival for Delta Smelt in enclosures at Belden's Landing compared to Rio Vista. In line with our predictions, we observed higher turbidity at Belden's Landing (Figure 4D), which can increase smelt foraging success (Hasenbein et al. 2013, 2016) and reduce stress levels (Pasparakis et al. 2023). Contrary to our predictions, water temperatures were slightly higher at Belden's Landing than Rio Vista for most of the study period (Figure 4C). This difference likely contributed to poorer overall health at Belden's Landing. At the onset of the study, the temperature at both locations exceeded 22°C, and remained near or above 20°C for the first 3 weeks the fish were in the field. During the second 3 weeks of the study, temperatures at Rio Vista were consistently below 20°C, but temperatures at Belden's Landing again rose over 21°C. Several prior studies have found that Delta Smelt experience stress in water temperatures over 20°C, as indicated by changes in gene expression and reduced consumption rates of prey above 22°C (Komoroske et al. 2015, 2021; Smith and Nobriga 2023), behavioral changes and increased energy demand above 21°C (Davis, Hansen, et al. 2019), and lower growth rates in wild Delta Smelt above 20°C (Lewis et al. 2021). In the wild, food limitation may exacerbate thermal stressors, as noted by Hammock et al. (2022) where they observed increased stress in wild Delta Smelt, as indicated by condition factor and reduced HSI, starting at temperatures as low as 13°C. A similar combination of factors likely affected fish in our study. Hammock et al. (2020) tracked differences in HSI for fed and fasted Delta Smelt in a laboratory setting; in our study, fish that were held in the field enclosures had HSI values similar to those reported for fasted fish, while our FCCL fish had HSI values similar to those reported for fed fish, suggesting food limitation was likely an issue at both of our selected field sites. This tracks with historic comparisons for wild Delta Smelt, which also have shown HSI scores similar to those of fasted fish (Hammock 2020).

While food was likely limiting at both sites, this may have been more pronounced at Belden's Landing, because the enclosures themselves contained lower-quality prey. We did not see a difference in gut fullness between field sites, but we did see regional differences in diet composition. Diets at Belden's Landing contained higher levels of small prey such as copepod nauplii and the small cyclopoid copepod *Limnoithona tetraspina*, which is low in nutritional value (Gould and Kimmerer 2010; Kratina and Winder 2015) and is negatively selected for by Delta Smelt (Slater and Baxter 2014). In comparison, Rio Vista diets contained higher levels of larger prey such as the calanoid copepod *Pseudodiaptomus forbesi*, which is an order of magnitude larger in weight than *L. tetraspina* (Gould and Kimmerer 2010), and one of the most important food resources for Delta Smelt in summer and fall (Hammock et al. 2017; Slater et al. 2019). As a result of the differences in size, fish need to consume large amounts of *L. tetraspina* to achieve the same gut fullness as those eating larger prey such as *P. forbesi*. This may have translated to higher energy expenditures necessary to capture prey, contributing to the lower condition of Delta Smelt observed at Belden's Landing. Notably, diets were only sampled at the end of the study and likely varied over time; however, previous studies have shown that Delta Smelt diets closely resemble that of the available zooplankton in the environment, which was sampled throughout (Burdi et al. 2023). The community composition of zooplankton samples followed a similar trend, with Belden's Landing having a lower CPUE of *Pseudodiaptomus* spp., and a higher CPUE of *L. tetraspina* compared to Rio Vista (Figure 7F). However, the pumps that were necessary to sample inside the cages may under-sample the large, mobile copepods, such as *Pseudodiaptomus* spp. adults that are the basis of sub-adult Delta Smelt diets (Kayfetz et al. 2020), so we may have an incomplete picture of food availability. Previous work has indicated that Delta Smelt have greater foraging success in Suisun Bay and Suisun Marsh than upstream near Rio Vista, despite lower densities of zooplankton caught in open-water trawls (Hammock et al. 2017). One hypothesized explanation for this

pattern is that smelt are taking advantage of tidal wetlands and other off-channel habitat where zooplankton and larval fish are more abundant (Hammock et al. 2019). This would have been a limitation for fish held in field enclosures in the marsh, because they would not be able to naturally forage across the landscape. Furthermore, enclosure fouling appears to have provided an additional food source for the fish inside. Rio Vista had higher levels of macroinvertebrates found on the enclosures and in the diets compared to Belden's Landing (Figures 6, 7D, 7E), but amphipod consumption at both locations was higher in the enclosures than previously shown in wild populations (Slater et al. 2019). This further signifies that habitat conditions in the enclosures may not have accurately reflected those in the outside environment.

Differential water velocities between habitats may also have played an important role in Delta Smelt responses. Clod card data revealed higher velocities inside Belden's Landing enclosures compared to Rio Vista's. This suggests increased energy expenditure to maintain position for fish at Belden's Landing, likely contributing to lower growth, condition, and liver glycogen levels (Swanson et al. 1998; Hammock et al. 2020; Dhayalan et al. 2023). Wild Delta Smelt are typically captured more frequently in water with lower current speeds, though this relationship is weaker than the relationship between catch and salinity, turbidity, or temperature (Bever et al. 2016); and higher water velocities have been shown to activate stress responses of Delta Smelt (Young et al. 2010). Unlike fish kept in the enclosures, wild Delta Smelt would have had the ability to choose their preferred micro-habitats within Suisun Marsh, potentially targeting areas with lower velocities.

### Biofouling Mitigation Methods

Enclosure biofouling was successfully reduced across the study period by exchanging enclosures every 2 weeks compared to weekly scrubbing, but fish survival was higher in enclosures that were scrubbed compared with enclosures that were exchanged. The process of transferring fish

from one enclosure to another was likely stressful for the fish inside. Each exchange took 20 to 40 minutes to complete, and the enclosures tended to knock against one another and onto the two vessels required to complete the exchange. Delta Smelt have particularly low tolerance for handling and transport (Afentoulis et al. 2013), and this added source of stress may have led to reduced survival (Pasparakis et al. 2022; Hudson et al. 2026).

When examining fish that survived to the end of the trial, no measurable differences in smelt health, diet, or water quality inside the enclosures were detected between the two biofouling mitigation methods, suggesting that the differing levels of algal and macroinvertebrate accumulation did not affect the experience of the fish inside. These results tell only part of the story, because they provide data for relatively “low” and relatively “high” levels of biofouling, but do not account for the extremes of either “zero” biofouling or “maximum” (unrestricted) biofouling. The biofouling component of this study stemmed from concerns that biofouling may result in an enclosure environment that does not represent the wild, and in this way similarities in fish metrics between relatively “high” and “low” biofouling levels—coupled with the absence of a “zero” biofouling comparison—make interpretation of the data difficult. Algal accumulation on the enclosures attracts higher rates of macroinvertebrate settling (Young et al. 2018; Hartman et al. 2019), and in our study macroinvertebrates were detected on almost all enclosures that were deployed—regardless of mitigation technique—although scrubbed enclosures did house far greater than exchanged enclosures. Amphipods are a common prey for Delta Smelt in the wild, while other macroinvertebrate taxa (e.g., isopods) are not (Slater et al. 2019); however, despite the increased number of macroinvertebrates on scrubbed enclosures, we did not detect any significant differences in macroinvertebrate consumption between treatments.

There are various explanations for this result. One potential explanation is that enclosure

biofouling level (low vs. high) may have had no effect on the Delta Smelt inside. This could explain why reductions in algal accumulation and macroinvertebrate densities did not affect fish health. In addition to not detecting any physiological differences in the smelt, we also did not detect any differences in water quality (temperature, dissolved oxygen, turbidity, salinity) or zooplankton densities between the interior of the enclosures and the ambient environment, regardless of biofouling mitigation method. Alternatively, it is also possible that all enclosures housed enough macroinvertebrates to support fish health regardless of biofouling mitigation technique. This may be a more plausible explanation. While physical-habitat parameters and zooplankton concentrations were similar between biofouling mitigation methods, abundance of macroinvertebrates living on the enclosure walls was significantly lower in the exchanged enclosures than in the scrubbed enclosures. (Table 1, Figure 6B), and macroinvertebrates observed in Delta Smelt diets tended to be higher in the scrubbed enclosures than in the exchanged ones (though not significantly so). Even the enclosures that were exchanged every 2 weeks contained observable numbers of amphipods living in the mesh, and diet data confirms that they were being eaten (Figure 7A). Given the small size of a Delta Smelt, it might not take many amphipods to make a difference. Thus, it is possible that amphipod abundance in all deployed enclosures was sufficient to produce an enclosure effect, limiting the comparability of our study to what we would expect to see in free-swimming fish.

Another possible explanation is that other factors—such as habitat differences between field sites or high temperatures throughout the study period—may have had an outsized effect on Delta Smelt, masking any biofouling-related differences. Our study design consisted of two enclosures per biofouling mitigation method at each field location. This means that our observed differences in fish health between field sites may have reduced the statistical power of any potential effects from biofouling. In the same vein, the high temperatures at both locations during this study,

particularly in the beginning of the study, may have pushed the Delta Smelt to their physiological limits, masking the effect of different amounts of biofouling and macroinvertebrate densities. Delta Smelt can tolerate high temperatures of 25–28 °C (depending on prior acclimation; Swanson et al. 2000. Komoroske et al. 2014; Davis, Cocherell, et al. 2019), though they prefer cooler temperatures (Hung et al. 2022). Recent enclosure studies suggest that Delta Smelt, when acclimated to variable field conditions (Davis et al. 2024), have lower thermal tolerances that correlated with reduced weight, liver glycogen, and increased liver lesions.

To test the assertions listed above, Delta Smelt in enclosures could be compared to wild-caught Delta Smelt. However, only four wild-caught Delta Smelt (one being a Delta Smelt–Wakasagi hybrid) were collected by monitoring program surveys during our study period (USFWS et al. 2024). With such low catch, there are not enough data to effectively compare the health of wild Delta Smelt at the time of our study to those in the enclosures, further limiting our assessment of enclosure effects (biofouling-related or otherwise) on the Delta Smelt inside.

Exchanging the enclosures was a high-effort pilot, designed to test an aggressive, yet feasible, mitigation tactic. Unfortunately, the method lowered survival and did not definitively negate the potential effects of biofouling. Similarly, it is possible that scrubbing biofouling material from the outside may even be detrimental to the fish, because it could increase particulate matter inside the enclosures. In salmon aquaculture, where pens are cleaned with mechanical scrubbers or water jets as frequently as once every 5 days (Bannister et al. 2019), particulate matter from pen cleaning remains in suspension for up to 30 hours, posing risks to gill health (Bloecher et al. 2024). Other potential problems include spreading pathogens between enclosures and “seeding” biofouling organisms on adjacent enclosures (Floerl et al. 2016). As such, we suggest utilizing Delta Smelt enclosures with caution when designing studies where differential biofouling rates and related macroinvertebrate food

availability could interfere with interpretation of the results. If biofouling is a concern, future studies could avoid conducting enclosure studies during summer and fall months, when water temperatures and biofouling loads are high.

### Management Implications

In our study, Delta Smelt held in Suisun Marsh for 6 weeks during late summer and early fall showed poorer health and condition markers compared to Delta Smelt held in the lower Sacramento River. This could indicate that habitat in Suisun Marsh during this time-period was not as beneficial in 2023 as originally hypothesized. However, conditions in the enclosures at the chosen sites may or may not reflect conditions elsewhere in the larger geographical regions they were intended to represent (Suisun Marsh and the lower Sacramento River, as a whole). Furthermore, it is likely that high water temperatures influenced our results; and fish exposed to the highest temperatures likely fared worse. In 2023, late-summer to early-fall temperatures tended to be higher in the marsh than at Rio Vista, but this pattern is not consistent across years, with some summers having warmer temperatures at Rio Vista (e.g., 2022, see Reclamation USBR and CDWR 2023). Ambient water temperatures should be an important consideration when habitat improvements for Delta Smelt are being implemented. Lastly, this study highlights critical enclosure effects that complicate the interpretation of our results, specifically regarding altered food availability and the inability of Delta Smelt to escape higher flows. The source and magnitude of these effects remain unclear but highlight that Delta Smelt enclosure studies can be used only with the knowledge that they cannot replicate the experience of a wild Delta Smelt, and data likely cannot be extrapolated to a broad geographic area—though future studies could improve on this by exploring whether deploying multiple enclosures within micro-habitats of a given area produce a more robust regional picture. Retuning to our initial study question, we found that yes, enclosures can be used to detect Delta Smelt physiological responses to differing

environmental conditions, but it is difficult to understand to which conditions the fish are responding, and whether those responses are a reflection of the larger environment or an artifact of the enclosure itself.

## DATA ACCESSIBILITY STATEMENT

The data generated for this study are publicly available in the Environmental Data Initiative (EDI) data portal. The dataset can be accessed via the following DOI: <https://doi.org/10.6073/pasta/3e168eaf6b33a4e3e276b8d28d29d2a5>. Continuous water-quality data is available on the California Data Exchange Center (<https://cdec.water.ca.gov/>).

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