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Contaminant and food limitation stress in an endangered estuarine fish

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Abstract

The abundance of Delta Smelt (*Hypomesus transpacificus*), a fish species endemic to the upper San Francisco Estuary (SFE), is declining. Several causes for the population decline have been proposed, including food limitation and contaminant effects. Here, using juvenile Delta Smelt collected from throughout their range, we measured a suite of indices across three levels of biological organization (cellular, organ, individual) that reflect fish condition at temporal scales ranging from hours to weeks. Using these indices, the relative conditions of fish collected from five regions in the SFE were compared: Cache Slough, Sacramento River Deep Water Ship Channel, Confluence, Suisun Bay and Suisun Marsh. Fish sampled from Suisun Bay and, to a lesser extent the Confluence, exhibited relatively poor short-term nutritional and growth indices and morphometric condition, while fish from the freshwater regions of the estuary, and Cache Slough in particular, exhibited the most apparent histopathological signs of contaminant exposure. In contrast, fish from the Suisun Marsh region exhibited higher short-term nutrition and growth indices, and better morphometric and histopathological condition. For instance, fish collected from Suisun Marsh had a mean stomach fullness, expressed as a percentage of fish weight, that was 3.4-fold higher than fish collected from Suisun Bay, while also exhibiting an incidence of histopathological lesions that was 11-fold lower than fish collected from Cache Slough. Thus, our findings support the hypothesis that multiple stressors, including food limitation and contaminants, are contributing to the decline of Delta Smelt, and that these stressors influence Delta Smelt heterogeneously across space.

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Keywords

Delta Smelt; San Francisco Estuary; biomarker; *Hypomesus transpacificus*; conservation; partial migration

Introduction

Human activities in estuarine ecosystems are associated with declines in native taxa, but establishing causality is often difficult because estuaries integrate numerous impacts from throughout the watershed (e.g., Hayward et al. 2004, Brooks et al. 2012). This is particularly true in the San Francisco Estuary (SFE), where a large number of co-varying anthropogenic impacts make it perhaps the most altered estuary in the world (Nichols et al. 1986, Cohen and Carlton 1998). It is formed by the interface of inflowing fresh water from the Sacramento and San Joaquin rivers and salt water from the Pacific Ocean (Cloern and Jassby 2012). Its 163,000-km^2 watershed, encompassing 38% of California, is polluted by a range of industrial, urban, natural and agricultural contaminants (e.g., Thompson et al. 2007, Smalling et al. 2013). It is highly channelized by levees, and only 5% of its original wetlands remain (Cohen and Carlton 1998, Sommer et al 2007). A network of pumps remove freshwater from the estuary, with state and federal water exports ranging from 18-62% of annual flow into the SFE between 1990 and 2013 (calculated from Dayflow http://www.water.ca.gov/dayflow). It is highly invaded, with a new species arriving every 14 weeks from 1961 to 1995 (Cohen and Carlton 1998). The influence of the invasive species *Potamocorbula amurensis* is thought to be particularly profound, as its arrival and spread coincided with substantial declines in primary and secondary productivity (Alpine and Cloern 1992, Kimmerer et al. 2005, Greene et al. 2011). Overlaid across these alterations are the influences of climate change (Cloern et al. 2011). Together, these impacts are thought to be causing the long-term decline in the abundance of Delta Smelt (*Hypomesus transpacificus*), a fish endemic to the SFE (Sommer et al. 2007).

The decline of Delta Smelt resulted in its listing under the Federal and California State Endangered Species Acts (USFWS 1993, CDFW 2014) and its international listing as critically endangered (IUCN 2014). It is a small, short-lived (usually one year), pelagic fish (Bennett 2005). Most larval Delta Smelt migrate from freshwater regions downstream to the 'low salinity zone' (defined as the region encompassing salinities from 1-6) in late spring-early summer, where they mature before migrating back upstream to spawn the following late winter and spring (Bennett 2005, Sommer et al. 2011). While researchers agree that many factors are contributing to the decline of Delta Smelt, the relative contribution of these causes remains unclear (Sommer et al. 2007, Feyrer et al. 2011). Hypothesized contributing causes of the decline include habitat loss, entrainment, changes in the physicochemical environment (salinity, turbidity and temperature), declining prey abundance, contaminants and competition and predation by invasive fish (Bennett 2005, Sommer et al. 2007, Feyrer et al. 2011). An additional potential stressor is *Microcystis* sp., a cyanobacteria that blooms during summer months that was first reported in the SFE in 1999 (Lehman et al. 2005). *Microcystis* sp. can be directly toxic to fish and reduce the amount and quality of prey (Lehman et al. 2010, Acuña et al. 2012, Brooks et al. 2012). In general, research into the

causes of the decline has focused on either modeling Delta Smelt abundance data or describing and understanding perturbations to the ecology of the SFE (e.g., Alpine and Cloern 1992, Kuivila and Moon 2004, Miller et al. 2012).

Here, we examined the natural population of the fish itself for evidence of two hypothesized causes of the population decline: contaminants and food limitation. The range of Delta Smelt encompasses a variety of habitat types and potential stressors, making it unlikely that the causes of the population decline are equivalent throughout. We therefore compared the condition of juvenile Delta Smelt (JDS) among five regions encompassing much of their range. Given the substantial evidence for food limitation in Suisun Bay (Alpine and Cloern 1992, Sobczak et al. 2002, Greene et al. 2011) and contaminants in Cache Slough (Werner et al. 2000, Kuivila and Moon 2004, Weston et al. 2014), our hypothesis was that fish from these regions would exhibit respective signs of these stressors. Three strategies were used to increase the likelihood of detecting regional differences, which could be homogenized by movement of fish among regions or be influenced by the chosen endpoints. First, we examined a suite of indices that reflect fish condition ranging from short to long-term (hours to weeks; e.g., Weber et al. 2003). Second, we restricted the study to JDS because we expected the condition of younger fish to better reflect the environment from which they were collected than older fish, which would have had more opportunity to move among regions and be slower to respond to environmental conditions. Finally, following the recommendations of Ham et al. (1997) and Adams (2002), the suite of indices reflects several levels of biological organization (cellular, organ and individual).

Materials and Methods

Indicators of fish condition: rationale

We examined eight indicators of fish condition, which we categorize loosely as follows: nutritional indices (stomach fullness expressed as a percentage of body weight, a histological assessment of liver glycogen, and concentration of triglycerides in muscle [TAG]), growth proxies (RNA-DNA ratio and 10-day otolith increment), morphometric characteristics (Fulton's condition factor and hepatosomatic index), and eleven liver and gill histopathology indices that we summed to produce a general indicator of physiochemical and contaminant stress (summed lesion score). Stomach fullness reflects recent (hours) foraging success and short-term nutritional status (e.g., Bochdansky 2008). Liver glycogen, the major site and form of carbohydrate storage in fish (Heath 1995), is sensitive to short term (24 h) food consumption and physicochemical and contaminant stress (Laidley and Leatherland 1988, Boujard and Leatherland 1992, Heath 1995). Triglycerides, the major energy storage form in fishes, are a longer term measure of nutritional status, particularly in juvenile fishes (Håkanson et al., 1994; Lochmann et al., 1995). RNA-DNA ratio is a widely used indicator of nutritional status and as a proxy for recent growth, as cellular RNA increases with protein synthesis while the quantity of DNA per cell remains relatively constant (Buckley 1979, Buckley 1984; Chicharo et al. 1998). Otolith accretion can be used as a longer-term proxy for growth because in most young fish rings of calcium carbonate and protein are deposited daily in proportion to somatic growth (Campana and Neilson 1985, Feyrer et al. 2004), including in cultured Delta Smelt (Hobbs et al. 2007). Condition factor

and hepatosomatic index are widely used indicators of the general condition of juvenile fish (e.g., Zamal and Ollevier 1995). Histopathology is a sensitive tool for detecting direct toxic effects of contaminant exposure in both the laboratory and in the field (Wester and Canton, 1991; Hinton et al. 1992, Teh et al. 1997).

Study area, sampling and necropsies

Juvenile Delta Smelt (JDS) were collected in 2012 (n=114) and 2013 (n=130) during the Summer Townet (STN) surveys conducted by the California Department of Fish and Wildlife (CDFW; Nobriga et al. 2008). The study area included 40 sampling stations within the San Francisco Estuary (SFE; Fig. 1). Most sampling stations had few fish (Table 1), so we had limited statistical power to examine the influence of individual stations on JDS. In addition, many of the stations are quite similar in terms of habitat. Therefore, the sampling stations were grouped into five geographical regions based on habitat type and proximity (i.e., sites that are nearby one another and had similar habitat types were generally grouped together; Fig. 1, Table 1). The Cache Slough region (C. Slough) is a relatively shallow, freshwater portion of the northern SFE (see Table 1 for depths). The Sacramento River Deep Water Ship Channel (SRDWSC) region has stations along a constructed, freshwater channel with long residence time. The Confluence region contains stations near the confluence of the Sacramento and San Joaquin rivers where there is deep channel habitat adjacent to shoals. The Suisun Bay region contains brackish, open water habitat with a mixture of deep and shallow areas (Table 1). It includes stations in Suisun, Honker and Grizzly bays. The Suisun Marsh region consists of three sampling sites along Montezuma Slough (Fig. 1), a channel through Suisun Marsh, the largest remaining contiguous estuarine wetland on the west coast of the USA (Sommer et al. 2007). Roughly one third of Suisun Marsh is comprised of tidally influenced sloughs, and the remainder is diked wetlands managed for waterfowl (Matern et al. 2002).

Six STN surveys were conducted each year, two each in June, July and August, with surveys occurring on alternate weeks. Each survey took six days and sampling was conducted from sunrise through early afternoon. All 40 stations were sampled during each of the twelve surveys, with the exception of the final survey of 2013 during which stations 323 and 340 were omitted due to vessel breakdown. The net used during STN surveys has an upper and a lower section. The upper net section is made of knotted nylon mesh with 12.7 mm holes. It is 3.35 m long, tapering down to a 0.9 m fyke net. The fyke net fits within the second section, which is 2.4 m of woven mesh with 3 holes per cm. The entire net measures approximately 5.6 m in length. The net was deployed for a stepped oblique retrieval with a target speed, measured at the mouth of the net, of 3.0 kph. A minimum of two ten minute tows were performed at each station, with a third occurring if one or more fish (of any species) were caught in the first two tows. With the exception of the two surveys in July 2012, all JDS caught in the net were individually wrapped in a labeled aluminum foil packet, immediately flash-frozen in liquid nitrogen, and sent to the Aquatic Health Program at UC Davis for use in this study at the end of each sampling day.

Surface water quality measurements were recorded at each station from the boat conducting the trawls (CDFW) and from a nearby boat (UC Davis). CDFW measured surface water

temperature (°C) and specific conductance (μS/cm) using a YSI Model 30 handheld digital meter and a Hach 2100 Handheld Turbidity Meter to measure the turbidity in Nephelometric Turbidity Units (NTU). Specific conductance data were converted to salinity using a regression equation. UC Davis used a YSI 6600 series sonde to measure temperature, salinity and turbidity, which were checked against CDFW data for consistency and then averaged. In addition, CDFW recorded a visual (qualitative) assessment of the density of *Microcystis* sp. on the water surface alongside the research vessel on a scale of 1-5 (1 = absent, 2 = low, 3 = medium, 4 = high, 5 = a contiguous mat).

Fish were kept in liquid nitrogen continuously until processing for the various indices. To begin, fish were removed from liquid nitrogen and fork length and body weight were measured. As each fish thawed it was rapidly necropsied during which the gill, sagittal otolith, stomach, liver and dorsal muscle were removed (5-10 min per fish). The livers were weighed and then liver and gill tissue were preserved in 10% buffered formalin for histological analysis. Stomachs were preserved in 95% ethanol and sent to the CDFW Diet Study laboratory (Stockton, CA) for gut content analysis. Sagittal otoliths were stored dry in tissue culture trays until further processing. Dorsal muscle tissue was stored at −80 °C until it was used to measure RNA-DNA ratio and triglycerides. Due mainly to limitations in tissue mass from smaller fish, not all fish were processed for each biomarker.

Nutritional status

To assess nutritional status, we characterized stomach fullness expressed as a percentage of body weight (n=202), triglyceride concentration (TAG) in muscle (n=152) and a histological measure of glycogen depletion (described in the *Histopathology* methods; n=176). At the CDFW Diet Study laboratory, prey were removed from preserved stomachs, identified and enumerated. Lengths of larger prey were recorded (e.g. mysids, amphipods, larval fish). The wet weight of prey in stomachs was determined by multiplying the count of each prey type by a wet weight estimate or from lengths using length-weight equations for larger zooplankton (Slater and Baxter 2014). We calculated stomach fullness for each fish with the following equation: Stomach fullness = (prey weight in stomach/smelt body weight) × 100 (Carruthers et al. 2005). TAG concentration in muscle was measured as described by Lenz et al. (2011) using the enzymatic Adipogenesis Assay Kit (BioVision, CA) and standardized using the Lowry method on homogenized and digested muscle tissue (Lowry et al. 1951). Samples were analyzed using a Tecan spectrophotometer. TAG concentration is reported in μmoles of triglyceride per mg of muscle protein.

Growth proxies

We measured two proxies for growth, RNA-DNA ratio in muscle (n=222) and ten-day otolith accretion (n=227). RNA-DNA ratio in muscle was measured using the ethidium bromide fluorometric technique (Caldarone et al., 2001) and ten-day otolith accretion was measured following Hobbs et al. (2007).

Morphometric indices of condition

Delta Smelt undergo several allometric changes in their weight-length relationship associated with major lifestage transitions, making the application of a single condition

factor to a population difficult (Hobbs et al. 2007, Slater and Baxter 2014). Here, we used fork length (mm) and fish wet-weight (mg) to calculate Fulton's condition factor for each individual (n=243), as fish were all the same life stage (Froese 2006). We also calculated the hepatosomatic index (n=208) as another indicator of fish condition. We calculated condition factor and hepatosomatic index with the formulas: condition factor = $(W_b/L^3)\cdot 100$ and hepatosomatic index = $(W_l/W_b)\cdot 100$, where W_b is the body weight (mg), L is the fork length (mm) and W_l is liver weight (mg).

Histopathology

We performed histological analysis on gill and liver tissues (n=172) to assess fish for evidence of physiochemical, contaminant and nutritional stress. Tissues were processed according to Teh et al. (1997). Formalin preserved liver and gill tissues were embedded in paraffin, sectioned at 3 μm thickness and stained with hematoxylin and eosin. Slides were assigned a random alpha-numeric identification code to prevent bias. Preneoplastic and neoplastic lesions (tumors) were scored as either present or absent for all tissues. All other lesions were scored semi-quantitatively based on a scale of 0–3 where lesions were 0 = not present, 1 = mild, 2 = moderate, and 3 = severe. Slides were scored for five liver lesions (fatty vacuolar degeneration, single cell necrosis, inflammation, macrophage aggregate, cytoplasmic inclusions or eosinophilic protein droplets) and six gill lesions (lamellar aneurysm, epithelial cell necrosis, ionocyte hyperplasia and hypertrophy, mucus cell hyperplasia, parasitic infestation, and inflammation), plus liver glycogen depletion (ranging from 0 = no depletion to 3 = severe depletion). To obtain a single indicator of contaminant and/or physiochemical stress, the lesion scores for individual fish were summed to obtain a composite score (i.e., fish with a higher score had more lesions). Liver glycogen depletion was not included in the lesion score because we considered it better categorized as a nutritional index (Teh et al. 1997, Adams et al. 1999).

Statistical analyses

The five regions (Cache Slough, SRDWSC, Confluence, Suisun Bay and Suisun Marsh) were compared in terms of eight indices (stomach fullness, TAG, RNA/DNA, 10-day otolith increment, condition factor, hepatosomatic index, summed lesion score and glycogen depletion) using six one-way analysis of variances (ANOVAs) and two one-way analysis of covariances (ANCOVAs; for RNA/DNA and 10-day otolith increment). We created a time interval variable in which sampling times from 6:00-8:00 were 6, 8:01-10:00 were 8, 10:01-12:00 were 10, 12:01-14:00 were 12 and 14:01-16:00 were 14. We accounted for the influence of time of day for the three shorter term indices (stomach fullness, glycogen depletion and RNA/DNA) using this variable, since fish were not sampled at the same time of day at each region (see Results) and these indices exhibit diel periodicity in Delta Smelt, other teleosts, or both. Specifically, Delta Smelt stomach fullness is lower at night and in the early morning (Hobbs et al. 2006), liver glycogen shows a similar pattern in rainbow trout (Boujard and Leatherland 1992), and RNA-DNA ratio increases in the early morning before declining during the day in juvenile red drum (Rooker and Holt 1996). Consistent with Hobbs et al. (2006) and Rooker and Holt (1996), stomach fullness and liver glycogen depletion appeared to vary nonlinerly during the day, reaching a minimum (liver glycogen depletion) or plateauing (stomach fullness) around 10:00, while RNA-DNA declined

throughout the day. Due to the apparent non-linearity for both stomach fullness and liver glycogen we included the time variable as a fixed effect in both ANOVAs. RNA/DNA appeared to decline linearly throughout the day, so we included the time interval variable as a covariable in the ANCOVA. Weight of each fish was included as a second covariable in the RNA/DNA ANCOVA and as the covariable in the 10-day increment ANCOVA to account for any regional differences in the ontogeny of JDS, which influences growth rate (Hobbs et al. 2007). Year class was included as a fixed effect in each model to account and test for possible differences between the two years. To address heterogeneity of variance and non-normality, we used an arcsine square root transformation for stomach fullness (expressed as a proportion of body weight) and a 1/(x+1) transformation for lesion score before performing the ANOVAs. Analyses were run in JMP Pro 11. Following each significant test (i.e., $P < 0.05$), region means were contrasted using Tukey HSD tests. We also report regional means and ranges for salinity, water temperature, and turbidity measurements taken during each trawl in which Delta Smelt were captured. Regional means were weighted based on the number of Delta Smelt captured.

Results

Catches of JDS by region were as follows: 30 fish from the single station representing Cache Slough, 91 fish from the SRDWSC, 46 fish from the Confluence, 37 fish from Suisun Bay and 40 fish from Suisun Marsh (Table 1). Annual and regional means of water quality measurements, weighted by the number of Juvenile Delta Smelt (JDS) collected, are in Table 2. JDS were found in water ranging in salinity from 0.07 ppt (a measurement from Cache Slough) to 10.99 ppt (a measurement from Suisun Bay), at temperatures ranging from 18.7°C in Cache Slough to 24.8°C in the SRDWSC and at turbidities ranging from 10.2 NTU (a measurement from the Confluence) to 208 NTU (a measurement from Suisun Bay; Table 2). *Microcystis* sp. was not observed in Cache Slough and Suisun Marsh but was observed at low levels in the other three regions (Table 2).

Nutritional indices varied substantially by region, particularly for the shorter-term indices. Stomach fullness differed regionally (ANOVA, $F_{[4, 195]} = 12.2113$, $P < 0.0001$), by time of day (ANOVA, $F_{[4, 195]} = 17.4921$, $P < 0.0001$) but not by year class (ANOVA, $F_{[1,195]} = 3.5249$, $P = 0.0619$). The largest regional difference in terms of stomach fullness occurred between the Suisun Marsh and the Suisun Bay regions, with mean stomach fullness 3.4-fold higher in Suisun Marsh (Fig. 2A). Fish collected after 8:00 had a mean stomach fullness that was 4.8 times higher than fish sampled before 8:00 (mean stomach fullness from 6:00-8:00 was 0.112, n = 17, SE=0.05; while from 8:01-15:00 it was 0.538, n=185, SE=0.05). The mean sampling times by region were 9:20 in Cache Slough, 10:20 in the Confluence, 11:30 in the Sacramento River Deep Water Ship Channel (SRDWSC), 11:20 in Suisun Bay and 8:25 in Suisun Marsh. Triglycerides (TAG) in muscle did not differ statistically among regions (ANOVA, $F_{[4, 142]} = 1.5169$, $P = 0.2003$), but it did differ by year class (ANOVA, $F_{[1, 142]} = 4.1419$, $P = 0.0436$). TAG was 1.14-fold higher in 2013 than 2012 (121.7 [SE=8.1] in 2012 and 138.6 [SE=8.0] in 2013). While region did not significantly influence TAG, the spatial pattern was similar to other nutritional metrics, as mean TAG in the Suisun Bay region was lower than in the other regions (Fig. 2B). Histologically, healthy livers with high glycogen in the hepatocytes were characterized by the presence of ‘lacy’, irregular, and

poorly demarcated cytoplasmic vacuolation (Fig. 3A), while glycogen depletion was characterized by decreased cell size and cytoplasmic basophilia (i.e., blue coloration; Fig. 3B). Liver glycogen depletion differed by both region (ANOVA, $F_{[4,166]} = 5.353$, $P < 0.0004$, Fig. 2C) and year class (ANOVA, $F_{[1, 166]} = 17.584$, $P < 0.0001$) but not by time of day (ANOVA, $F_{[4, 166]} = 1.359$, $P = 0.250$). Fish from Suisun Marsh had a significantly lower mean liver glycogen depletion score, with a mean of 0.629 (categorized between 'no' and 'mild' glycogen depletion), while mean glycogen depletion for the other regions combined was 1.48 (between 'moderate' and 'high' glycogen depletion). 18 out of 35 (51.4%) fish in Suisun Marsh had no glycogen depletion, while only 12 out of 142 fish (8.5%) from the other regions combined had no glycogen depletion. Overall, liver glycogen depletion was 1.6-fold higher in 2012 (1.693 [SE=0.085] in 2012 and 1.030 [SE=0.078] in 2013).

RNA/DNA in muscle differed among regions (ANCOVA, $F_{[4, 213]} = 4.4771$, $P=0.0017$; Fig. 2D), by time of day (slope = −0.073, ANCOVA, $F_{[1, 213]} = 13.1440$, $P<0.0004$), but not by year class (ANCOVA, $F_{[1, 213]} = 0.3639$, $P = 0.5470$), and decreased with fish weight (slope = −0.732554, ANCOVA, $F_{[1,213]} = 48.7542$, $P < 0.0001$). The relationship between the Suisun Marsh and Suisun Bay regions was similar to the stomach fullness results, with fish from Suisun Marsh exhibiting RNA/DNA ratios 1.5-fold higher than in the Suisun Bay region (Fig. 2D). The longer-term growth proxy, 10-day otolith increment, did not vary significantly by region (ANCOVA, $F_{[4,219]}=1.0586$, $P = 0.3779$, Fig. 2E) or by cohort (ANCOVA, $F_{[1,219]}=3.0051$, $P = 0.0844$). Consistent with the ontogenic pattern of slower growth in larger (older) individuals, 10-day otolith increment varied significantly by weight of the fish (ANCOVA, $F_{[1,219]}=15.2592$, $P < 0.0001$), with heavier fish having lower 10-day otolith increments (slope = −9.2 μm/g).

Differences in condition factor were significant both regionally (ANOVA, $F_{[4, 237]} = 4.699$, $P = 0.0011$; Fig. 2F) and by year class (ANOVA, $F_{[1, 237]} = 4.3011$, $P = 0.0392$). JDS exhibited the highest mean condition factors in the Suisun Marsh and SRDWSC regions, and the lowest in the Suisun Bay and Confluence regions (Fig. 2F). Mean condition factor was lower in 2012 than in 2013 (0.680 [SE=0.008] in 2012 and 0.697 [SE=0.006] in 2013). Hepatosomatic index also varied by region (ANOVA, $F_{[4, 202]} = 14.1428$, $P < 0.0001$), showing a similar spatial pattern to condition factor, with the Suisun Bay and Confluence regions exhibiting relatively low hepatosomatic indexes (Fig. 2G). There was no significant difference between year classes in terms of hepatosomatic index (ANOVA, $F_{[1, 202]} = 0.3673$, $P = 0.5451$).

Cumulative lesion score also exhibited substantial regional variation (ANOVA, $F_{[4,166]} = 6.7554$, $P<0.0001$; Fig. 2H), and varied by year class (ANOVA, $F_{[1,166]} = 4.1078$, $P = 0.044$). Cache Slough had the highest percentage of fish with lesions, with 65.2% (15 of 23) exhibiting one or more. The SRDWSC had the next highest percentage at 59.3% (35 of 59), followed by Suisun Bay at 57.1% (16 of 28), the Confluence at 48.1% (13 of 27) and Suisun Marsh at 5.7% (2 of 35). Thus, fish from Cache Slough had 11 times the prevalence of lesions as fish from Suisun Marsh. Lesions were nearly twice as prevalent in 2013 as in 2012 (1.27 lesions/fish in 2013 and 0.68 lesions/fish in 2012). Table 3 shows incidence rates of individual lesions in the liver and gill. The most frequently observed lesions were fatty

vacuolar degeneration in the liver and ionocyte hyperplasia and lamellar aneurysm in the gill (Table 3). Fatty vacuolar degeneration (Fig. 3C) was characterized by the presence of clear, round, and well demarcated cytoplasmic vacuoles with nuclei often displaced to the periphery of hepatocytes. Fatty vacuolar degeneration was most prevalent in fish collected from Cache Slough and the SRDWSC (Table 3). In contrast to a healthy gill in Fig. 4A, ionocyte hyperplasia was characterized by increases in ionocyte number and size (Fig. 4B). Ionocyte hyperplasia was most prevalent in fish collected from Cache Slough, SRDWSC, and the Confluence (Table 3). Gill lamellar aneurysm (Fig. 4C) was characterized by focal dilation of lamellar capillaries associated with the disruption of pillar cells and pooling of blood and eventual thrombosis due to stagnation. Gill lamellar aneurysm (Fig. 4C) was most prevalent in fish collected from the SRDWSC and Suisun Bay. Only few fish had gill inflammation, eosinophilic protein droplets, macrophage aggregate, and parasitic infestation. None of the fish had single cell necrosis, gill epithelial cell necrosis, mucus cell hyperplasia, or preneoplastic or neoplastic lesions (tumors).

Discussion

There is a general consensus that multiple stressors have contributed to the decline of Delta Smelt (Sommer et al. 2007, Feyrer et al. 2011). Perhaps the most widely accepted of these is that invasive species have perturbed the SFE foodweb, decreasing the amount and quality of food (Bennett 2005, Sommer et al. 2007, Miller et al. 2012). Contaminants are also considered by some to be an important stressor (Sommer et al. 2007, Kuivila and Moon 2004, Brooks et al. 2012), though to date there is little direct evidence. Here, we compared juvenile Delta Smelt (JDS) in terms of their nutritional and growth status and morphometric and histopathological condition across regions. Responses to the five regions were clearly differentiated by the multiple biomarker approach, providing evidence of both food limitation and contaminant stress in wild JDS. JDS in Suisun Marsh were in better nutritional and histopathological condition than in other regions, while fish in the Suisun Bay and Cache Slough regions exhibited the most severe signs of nutritional and contaminant related stress, respectively.

JDS collected from the Suisun Bay region, and the Confluence to a lesser extent, exhibited relatively poor nutritional status. Fish sampled in Suisun Bay had roughly one third the food in their stomachs, as a percentage of body weight, as fish sampled from Suisun Marsh (Fig. 2A), indicating lower food availability in Suisun Bay. Similarly, liver glycogen, the major short-term energy storage in fish (Heath 1995), was less depleted in Suisun Marsh than the other regions, including the nearby Confluence and Suisun Bay regions, further indicating nutritional stress (Fig. 2C). Surprisingly, while others have found triglyceride concentration (lipids) to be a sensitive biomarker for habitat assessment for fish (e.g., English Sole, Amara et al. 2007), all the regions in our study were statistically equal. One possibility is that regional fidelity was too low for triglycerides to be influenced by region. Nevertheless, given that the regional pattern in triglyceride concentration was consistent with the shorter term nutritional and growth indices (i.e., lowest in Suisun Bay), and since stomachs were significantly fuller and glycogen depletion less severe than in other regions, our results indicate that JDS were under nutritional stress in Suisun Bay, and likely the Confluence as well.

There are several possible explanations for the poor nutritional status we observed in the Suisun Bay and Confluence regions. The influence of *Potamocorbula amurensis* on Suisun Bay is particularly strong, driving down primary and secondary productivity (Alpine and Cloern 1992, Kimmerer et al. 1994, Sobczak et al. 2002, Greene et al. 2011). In addition, there is evidence that high ammonium concentrations prevent spring diatom blooms by inhibiting the uptake of nitrate (Dugdale et al. 2007). Thus, the combined influence of invasive species and contaminants have likely made prey for JDS scarce (Bennett 2005, Kimmerer 2006, Slater and Baxter 2014), leading to the relatively low stomach fullness measurements and high glycogen depletion we observed (Figs. 2A and C). Salinity is another potential cause, as high salinities can increase metabolic demand as fish attempt to maintain homeostasis due to osmotic stress, worsening nutritional status (e.g., De Silva and Perera 1976). Salinities were relatively high in Suisun Bay, averaging 7.25 (Table 2), while juvenile Delta Smelt capture probabilities are highest over a salinity range of 0.6-3 (Nobriga et al. 2008). However, the metabolic demand of many fishes is minimized at intermediate salinities (8-20; Boeuf and Payan 2001), and the metabolic salinity optimum is unknown for Delta Smelt. Thus, it is uncertain whether salinities were high enough to influence nutritional status. Turbidity is another possible contributing factor, as mean turbidity was highest in Suisun Bay (Table 2), and high turbidity (250 NTU) can reduce feeding success of juvenile Delta Smelt (Hasenbein et al. 2013). However, turbidities in our study were generally within the optimal range of for early life stage Delta Smelt feeding in laboratory studies (25-80 NTU, Hasenbein *personal communication*; Table 2). Moreover, we also observed relatively poor nutritional status in the Confluence, where both salinity and turbidity were substantially lower than in Suisun Bay. This suggests that high salinity and turbidity were not strong drivers of the relatively poor nutritional status observed in our study. Finally, although contaminant effects can depress the nutritional condition of fish (Heath 1995), direct effects of contaminants are an unlikely explanation for the poor nutritional status of JDS in Suisun Bay and the Confluence given the relatively good condition of the livers of fish from these regions (Table 3). Thus, we consider prey scarcity to be the most probable explanation for the poor nutritional status observed in Suisun Bay, and the Confluence to a lesser extent.

The relatively poor nutritional status observed in the Suisun Bay and Confluence appeared to have scaled up to limit short-term growth in these regions, a critical determinant of survival in juvenile fish (Post and Parkinson, 2001), and both morphometric indices. Because RNA-DNA ratio is an indicator of protein synthesis rate, it is tightly coupled to nutritional status and an excellent proxy for short-term growth in immature fish (e.g., Buckley 1979, Buckley 1984, Rooker and Holt 1996). Rooker and Holt (1996) detected reduced RNA-DNA ratios in juvenile Red Drum 1-2 days after reductions in feeding, as well as significantly reduced growth, and Buckley (1979) reported reduced RNA-DNA ratios and growth following starvation in Atlantic Cod. In our study, RNA-DNA was significantly depressed in Suisun Bay, the region with the lowest percent stomach fullness and, compared to adjacent Suisun Marsh, a relatively low liver glycogen score. There is also a possibility that toxins could have depressed the RNA-DNA ratios (Acuña et al. 2012, Li et al. 2010), but given the generally good histological condition of the livers in the region (Table 3), food limitation is the more likely cause. However, ten-day otolith increment was not significantly reduced in

Suisun Bay. As with triglycerides, this could indicate that regional fidelity is too short for growth to be influenced by low prey availability at time scales longer than several days, or that otolith increment is not sensitive to growth reductions due to food limitation in Delta Smelt (e.g., Wright et al. 1990). A third possibility is that the fish recently migrated, and were already in poor morphometric condition when they left the freshwater portions of their range (Chapman et al. 2012). Given the otolith increment results, we were somewhat surprised that the relatively poor short-term nutritional and growth indices appeared to scale up to depress the morphometric indices in Suisun Bay and the Confluence regions (Figs. 2F and G). This may indicate that condition factor and hepatosomatic index of JDS respond to poor foraging success more rapidly than ten days.

Given the high proportion of fish collected from Suisun Bay and Confluence (~1/3 of the fish in our study) and clear signs of nutritional stress, our results suggest that food limitation in these regions is contributing to the decline of Delta Smelt. Poor nutrition in juvenile fish leads to slower growth, and slow growth is often tightly correlated with lower survival in juvenile fish and lower adult abundances (Post and Parkinson, 2001). Slower juvenile growth leads to fewer, smaller adults (e.g., Jones 1986) and lower fecundity and recruitment (Bennett 2005, Rose et al. 2013). Thus, given that a substantial proportion of JDS habitat had fish with relatively poor nutritional, growth and condition indices, our results support the hypothesis that poor habitat quality, at least in part due to food limitation, is contributing to the decline of Delta Smelt.

In contrast to the Confluence and Suisun Bay in particular, JDS collected in Suisun Marsh exhibited relatively good nutritional, growth and morphometric status. Despite the presence of numerous invasive species (e.g., Meng et al. 1994, Jones et al. 2009), the nutritional metrics were relatively high in Suisun Marsh, indicating relatively abundant food. Suisun Marsh has complex littoral zones, dead-end sloughs, and connectivity to tidal marsh and wetlands (Matern et al. 2002). These habitat features are generally thought to promote productivity, and likely contribute to the relatively high level of particulate organic matter, food for JDS prey, in Suisun Marsh (Sobczak et al. 2002, Müller-Solger et al. 2002). The relative abundance of food, likely in combination with other habitat traits (e.g., relatively low temperature and moderate salinity; Table 2), likely caused the relatively good short-term growth and morphometric indices we observed. Beck et al. (2001) defined fish nurseries as habitats with relatively high productivity per unit area compared to other juvenile habitats. Our results therefore indicate that Suisun Marsh provided nursery habitat for Delta Smelt.

JDS collected from Suisun Marsh also exhibited relatively few signs of histopathological stress. The only lesion observed in Suisun Marsh was gill lamellar aneurysm, occurring in just two of thirty-five fish (Table 3). The aneurysms could have been caused by handling stress during field sampling as suggested by Wolf et al. (2014), or contaminant effects, as acute exposure to toxicants such as ammonia, crude oil, and pesticides induce gill lamellar aneurysm (Eller 1975; Pickering 1981; Akaishi et al 2004). Whatever the cause, the incidence rate of lesions was dramatically lower in Suisun Marsh than in other regions (Fig. 2H). This indicates that the contaminant load in Suisun Marsh was low, perhaps because it is relatively well connected to wetlands, a habitat type known to remove contaminants from the water (Kivaisi 2001).

JDS collected from the other SFE regions showed significantly higher histopathological lesion scores, although the underlying causes of the lesions likely differed. Many fish in the Confluence region exhibited ionocyte hyperplasia (i.e., proliferation of ionocytes), as did several fish in Suisun Bay (Table 3). Ionocytes are cells that excrete ammonia, regulate pH, and perform ion exchange (ion excretion in hyperosmotic water, absorption in hyposmotic water; Hiroi and McCormick 2012). The proliferation of ionocytes causes gills to thicken, inhibiting gas exchange (Mallatt 1985), and can occur in response to exposure to both salinity changes and contaminants (Perry 1997). For example, exposure of Rainbow Trout to low calcium concentrations caused ionocyte proliferation (e.g., Perry and Wood 1985), Pawert et al. (1998) observed ionocyte proliferation in two riverine teleosts exposed to a heavily polluted stream, and Mazon et al. (2002) reported the same in *Prochilodus scrofa* exposed to copper. While contaminants are a potential cause of the ionocyte hyperplatia observed in the Confluence, we did not find any histopathological lesions in the liver, and the fish may have been exposed to large fluctuations in salinity (during the trawls during which JDS were collected the Confluence varied in salinity by 50-fold; Table 2). Although rare, liver lesions did occur in fish from Suisun Bay, and salinity varied a relatively low 9-fold. Therefore, we suggest that the most likely cause of the ionocyte hyperplasia in the Confluence region was rapid changes in salinity during movement or tidal cycles, whereas the results from Suisun Bay could be related either to movement, tidal cycles or low levels of contaminants.

The histopathology of fish collected from further up the estuary more conclusively indicates exposure to contaminants. The fish with the highest lesion rates, including fish exhibiting the vast majority of liver damage, were sampled from the Sacramento River Deep Water Ship Channel (SRDWSC) and Cache Slough (Table 3). As in the Confluence and Suisun Bay regions, gill lamellar aneurysm and ionocyte hyperplasia contributed substantially to the scores (Table 3). In addition, we observed a relatively high prevalence of fatty vacuolar degeneration in the liver, a common response to chemical exposure in fish (e.g., Metcalfe 1998, Wolf and Wolfe, 2005, Agamy, 2012). The liver damage and ionocyte hyperplasia are unlikely to be related to salinity. Cache Slough and the SRDWSC only varied 1.4 and 6.3-fold in salinity, respectively. Both regions are geographically remote from higher salinity regions, so rapid movement by fish from high to low salinity was unlikely. Moreover, Delta Smelt raised in freshwater at the Fish Culture and Conservation Lab (UC Davis) do not exhibit ionocyte proliferation (S. Teh *personal observation*). Thus, neither salinity changes nor low salinity can easily account for the lesions we observed. Exposure to *Microcystis* sp. is also an unlikely cause because it was not observed during the trawls in Cache Slough, the region with the highest mean lesion score (Table 3; Fig. 2H). The far more likely explanation is that contaminant exposure is damaging the livers and gills of JDS in the freshwater portions of its range, particularly in Cache Slough.

Several studies indicate that Cache Slough has relatively high concentrations of anthropogenic contaminants. The SRDWSC moves urban runoff from West Sacramento to the eastern portion of Cache Slough, and Ulatis Creek, a major freshwater input, carries urban and agricultural runoff and secondary wastewater into western Cache Slough, just upstream of the Cache Slough sampling location in our study (Fig. 1; Weston et al. 2014). Weston et al. (2014) repeatedly detected acute toxicity to *Hyalella azteca* following storms

in both western Cache Slough and Ulatis Creek, and attributed the toxicity largely to pyrethroids in urban runoff. In a two-year study, Werner et al. (2000) found that of the 24 sites sampled throughout the Delta, Ulatis Creek most frequently showed acute toxicity to *Ceriodaphnia dubia* (6 of 21 monthly samples were acutely toxic). Kuivila and Moon (2004) detected high concentrations of the pesticides Molinate and Thiobencarb in Cache Slough, and suggested that mixtures of pesticides could have chronic health effects on Delta Smelt, particularly juveniles. Our results are consistent with this hypothesis, as we observed strong evidence of chronic contaminant effects in a region where Delta Smelt are known to reproduce (Bennett 2005, Sommer et al. 2011). Earlier life stages of animals are more vulnerable to toxins, and the timing of Delta Smelt hatches overlaps with wet weather in late winter and spring when Ulatis Creek exhibits toxicity (Bennett 2005, Brooks et al. 2012, Weston et al. 2014). Therefore, the chronic effects of contaminants observed in this study may be an indicator of acute effects on life stages earlier in the year.

In conclusion, we found evidence of regional variation in nutritional status, growth and morphometric and histopathological condition. Fish collected from Suisun Bay showed the poorest nutritional and short-term growth status, while fish from Cache Slough had the poorest histopathological condition, likely due to contaminant exposure. In contrast, the low incidence of histopathological lesions and relatively excellent nutritional, growth and morphometric status of JDS collected from the region suggests that it provided better habitat than the other SFE regions during summer 2012 and 2013. These results provide insight into regional factors that may affect the success of mitigation and restoration efforts.

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- Condition of juvenile Delta Smelt varied significantly by region
- Fish from Suisun Marsh were in the best condition
- Fish from Suisun Bay exhibited the worst short-term growth and nutritional metrics
- Fish from Cache Slough exhibited the most severe signs of contaminant stress

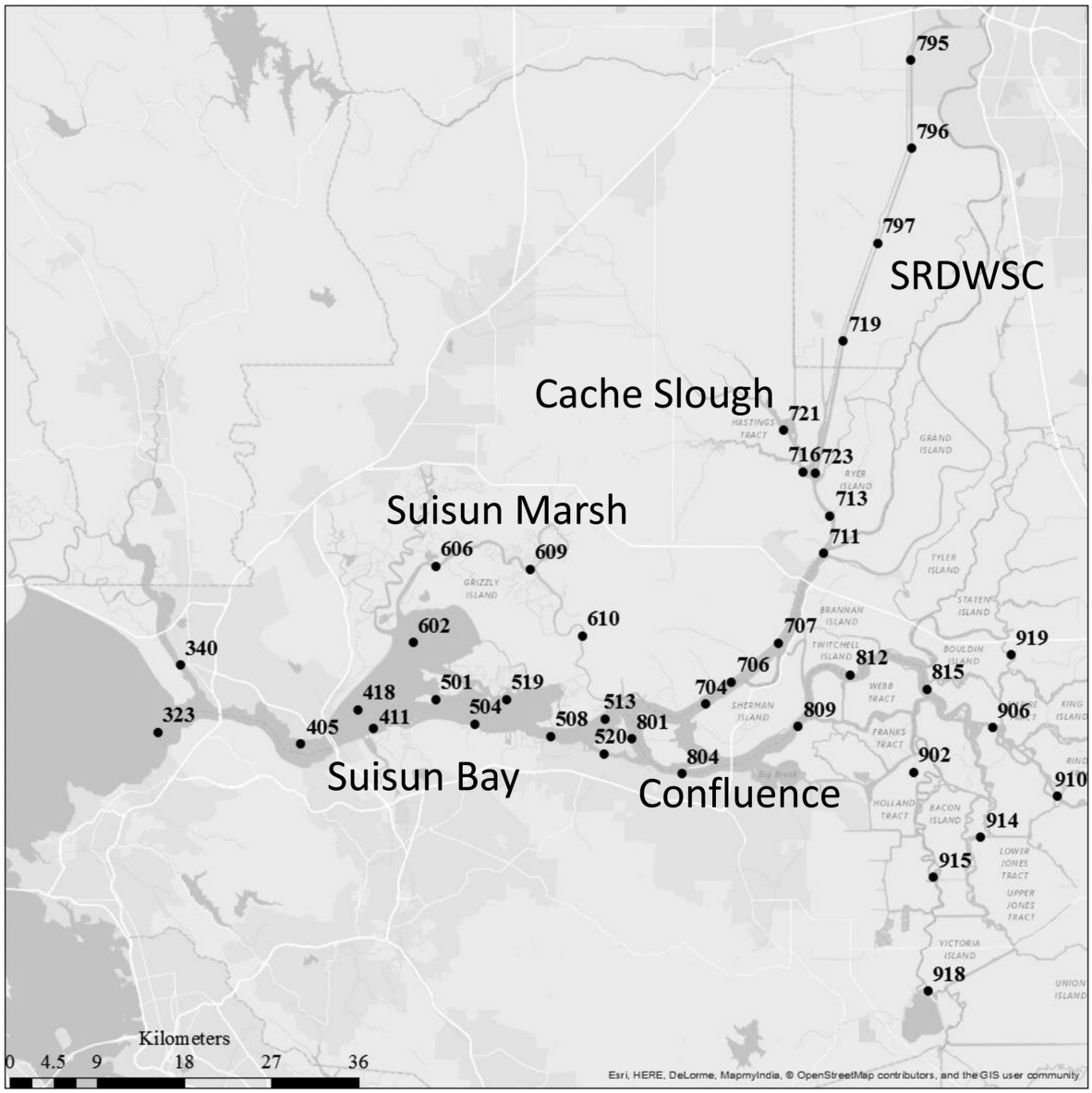

Fig. 1.

Map of stations at which Delta Smelt were surveyed. The stations at which juvenile Delta Smelt were collected were grouped into five regions: Cache Slough, Sacramento River Deep Water Ship Channel (SRDWSC), Confluence (confluence of the Sacramento and San Joaquin rivers), Suisun Bay (including Suisun, Honker and Grizzly Bays), and Suisun Marsh (Table 1).

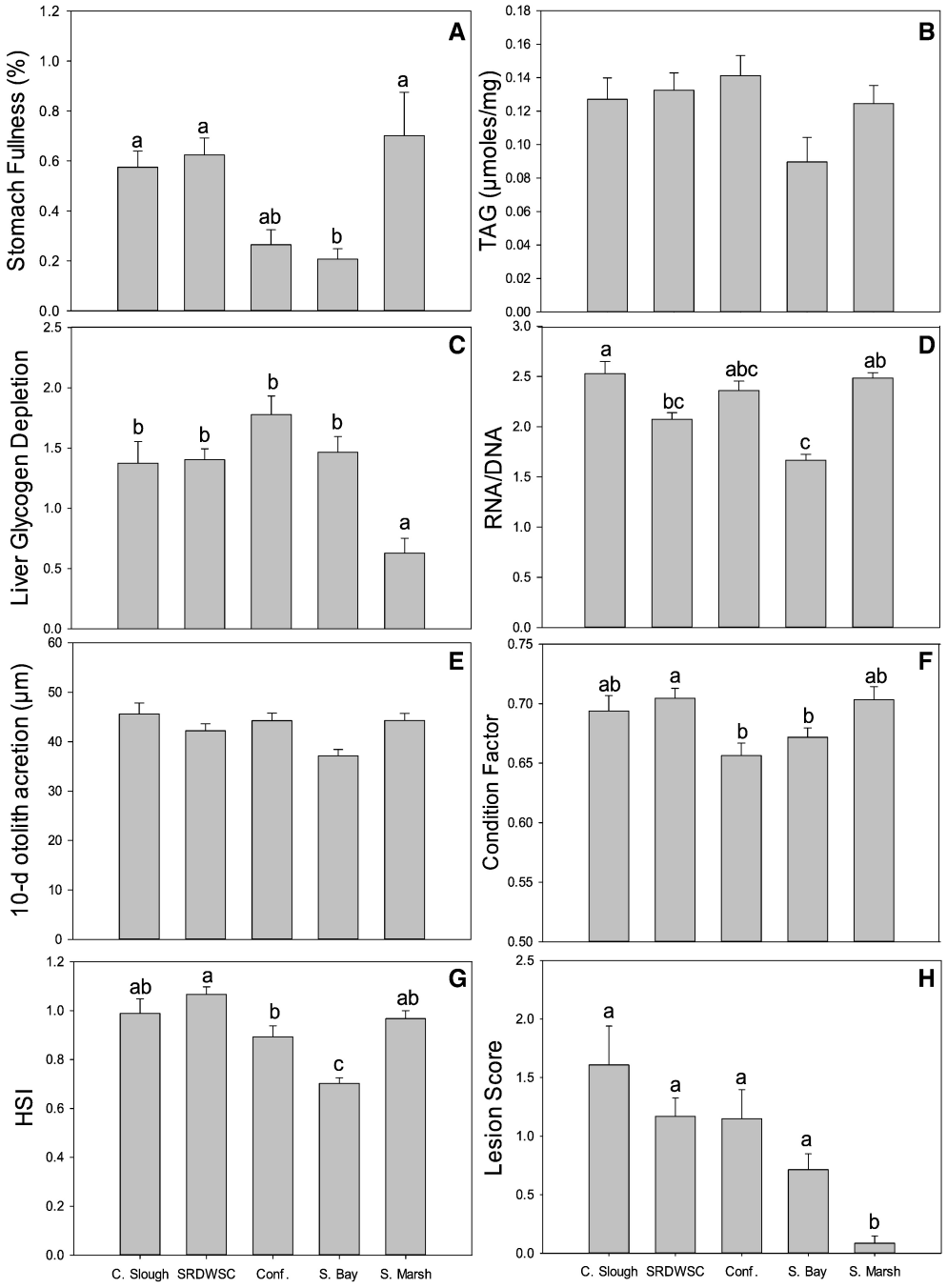

Fig. 2.

Regional comparison of juvenile Delta Smelt mean stomach fullness (%, n=202; A), triglycerides (TAG, µmoles TAG/mg protein in muscle, n=152; B), liver glycogen depletion based on histological assessment (n=176; C), ratio of RNA to DNA in muscle (n=222; D), the length of otolith increment during the 10 days before the fish was caught (n=227; E), condition factor (n=243; F), hepatosomatic index (HSI; n=208; G), and histopathological lesion score (sum of eleven histopathological liver and gill lesion indices; n=176; H). Differing lowercase letters denote significant differences (note: there were no significant

differences in TAG and 10-d otolith increment by region). C. Slough is the Cache Slough region, SRDWSC is the Sacramento Deep Water Ship Channel, and S. Bay and S. Marsh are the Suisun Bay and Suisun Marsh regions. Regional means are averaged across 2012 and 2013. Error bars are ±SE. Note that for panels C and H a lower mean indicates fish in better condition, whereas for the other panels a lower mean indicates fish in worse condition.

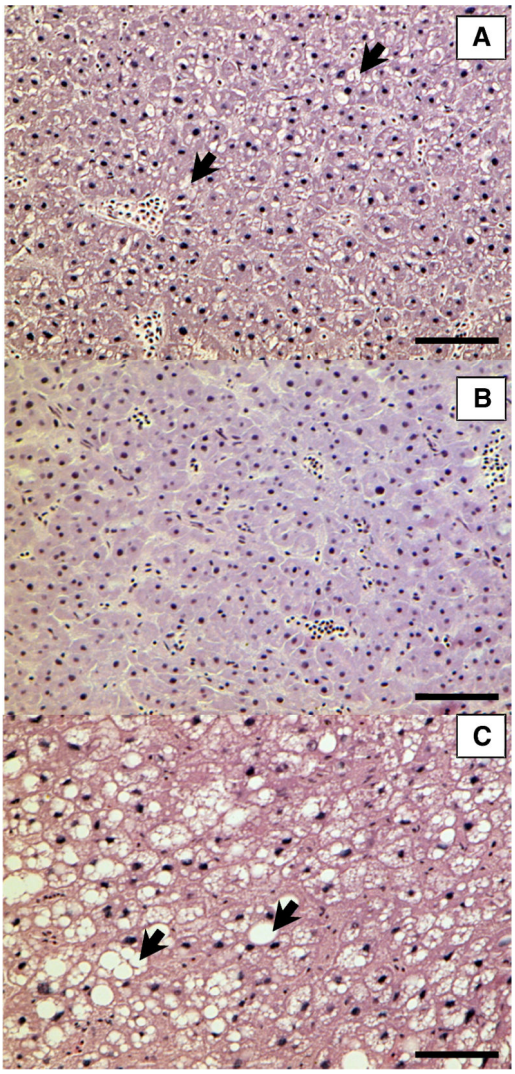

Fig. 3.

A) Normal morphology of glycogen-rich hepatocytes indicated by irregular and poorly demarcated clear cytoplasm (arrows) in juvenile Delta Smelt collected from Suisun Marsh. B) Glycogen depletion characterized by loss of irregular and poorly demarcated clear cytoplasm. C) Fatty vacuolations characterized by excess lipid (arrows) seen in liver of juvenile Delta Smelt collected from Cache Slough. They appear as clear, round and well demarcated cytoplasmic vacuoles. Bars = 500μm.

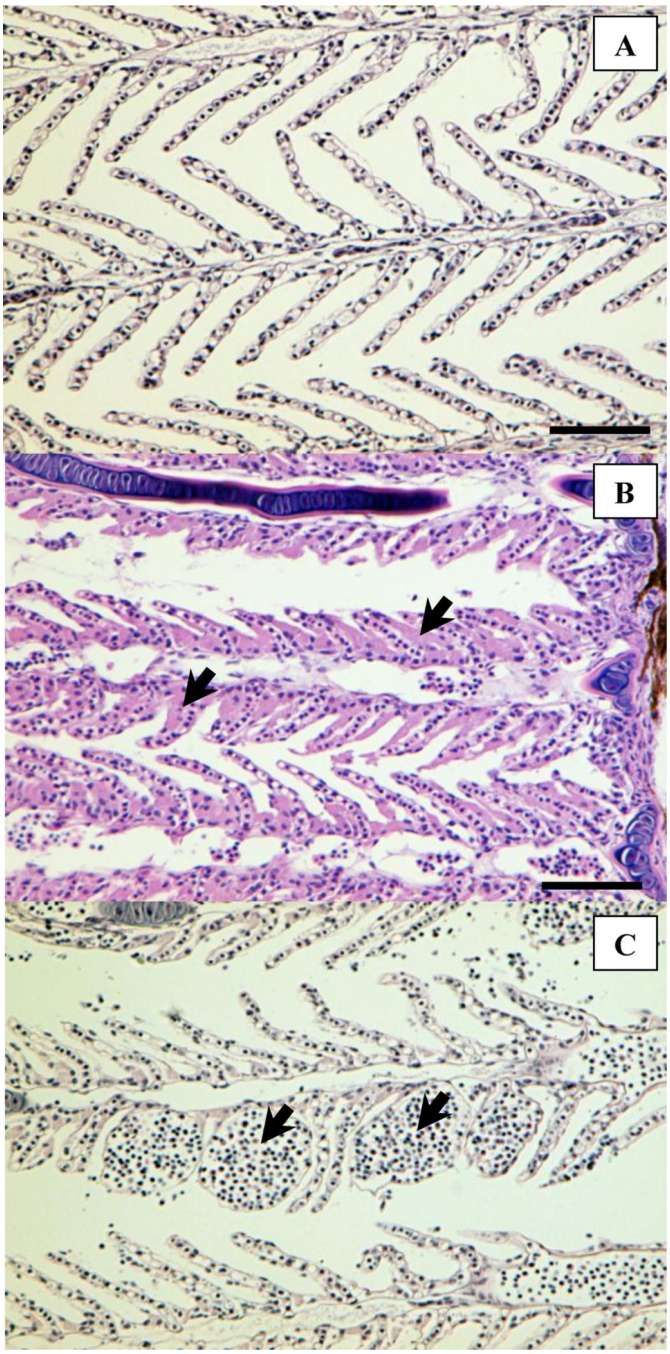

Fig. 4.

A) Normal morphology of primary and secondary lamella in gill of juvenile Delta Smelt, B) ionocyte hyperplasia and hypertrophy (arrows) and C) lamellae aneurysm (arrows) seen in gill of juvenile Delta Smelt collected from Cache Slough. Bars = 500μm.

Table 1

Region names, sampling station, mean depth and the number of juvenile Delta Smelt collected by year and in total. SRDWSC is the Sacramento River Deep Water Ship Channel.

Region	Station	Depth (m)	2012	2013	Total
Cache Slough	721	3	19	11	30
Confluence	508	14	3	2	5
	513	15	14	0	14
	520	12	2	0	2
	704	9	9	9	18
	706	8	1	1	2
	801	7	1	2	3
	804	11	1	1	2
SRDWSC	719	10	20	23	43
	723	11	0	4	4
	796	10	2	0	2
	797	10	31	11	42
Suisun Bay	418	9	0	2	2
	501	10	0	2	2
	519	3	3	23	26
	602	2	0	7	7
Suisun Marsh	606	7	0	7	7
	609	9	7	20	27
	610	4	1	5	6

Table 2

Mean water quality measurements, weighted by the number of juvenile Delta Smelt collected, for regions and years. Ranges are in parentheses. C. Slough is the Cache Slough region, SRDWSC is the Sacramento Deep Water Ship Channel, and S. Bay and S. Marsh are the Suisun Bay and Suisun Marsh regions. *Microcystis* sp. values are based on a visual assessment (1 = absent, 2 = low, 3 = moderate, 4 = high, 5 = contiguous mat).

Region/Year	Salinity (ppt)	Temp (°C)	Turbidity (NTU)	*Microcystis* sp.
C. Slough	0.08 (0.07-0.10)	20.1 (18.7-21.6)	42.0 (17.0-54.3)	1.00 (1-1)
SRDWSC	0.27 (0.08-0.50)	21.9 (20.5-24.8)	34.1 (23.2-50.3)	1.05 (1-2)
Confluence	1.01 (0.09-4.19)	20.4 (19.3-22.6)	26.6 (10.2-45.0)	1.59 (1-2)
S. Bay	7.25 (1.27-10.99)	20.6 (19.1-22.1)	75.3 (23.6-208.0)	1.41 (1-2)
S. Marsh	3.98 (2.79-6.54)	20.4 (19.9-21.4)	53.1 (22.5-64.0)	1.00 (1-1)
2012	0.66 (0.07-5.15)	21.1 (18.7-23.7)	34.1 (12.5-54.3)	1.23 (1-2)
2013	3.27 (0.07-10.99)	20.8 (19.1-24.8)	50.6 (10.2-208.0)	1.16 (1-2)

Table 3

Percent prevalence of histopathological lesions in livers and gills of juvenile Delta Smelt (n= 172 fish). C. Slough is the Cache Slough region, SRDWSC is the Sacramento Deep Water Ship Channel, and S. Bay and S. Marsh are the Suisun Bay and Suisun Marsh regions.

		Liver						Gill				
Region	**n**	**LIP**	**SCN**	**INF**	**LMA**	**EDP**	**ANU**	**GCN**	**IH**	**MCH**	**PAR**	**GINF**
C. Sough	23	22.0	0.0	4.4	0.0	0.0	8.7	0.0	48.0	0.0	0.0	8.7
Confluence	27	0.0	0.0	0.0	0.0	0.0	3.7	0.0	48.0	0.0	0.0	11.1
SRDWSC	59	10.0	0.0	10.2	0.0	1.7	17.0	0.0	31.0	0.0	1.7	3.4
S. Bay	28	3.6	0.0	14.3	3.6	0.0	35.7	0.0	3.6	0.0	7.1	0.0
S. Marsh	35	0.0	0.0	0.0	0.0	0.0	5.7	0.0	0.0	0.0	0.0	0.0

LIP= fatty vacuolar degeneration; SCN= single cell necrosis; INF = inflammation, LMA= macrophage aggregate; EDP= cytoplasmic inclusions or eosinophilic protein droplets, ANU = gill lamellar aneurysm, GCN = gill epithelial cell necrosis, IH = ionocyte hyperplasia and hypertrophy, MCH = mucus cell hyperplasia, PAR = parasitic infestation, GINF = gill inflammation. Preneoplastic or neoplastic lesions (tumors) were not observed in any fish.