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Histopathological assessment of seven year-classes of Delta Smelt

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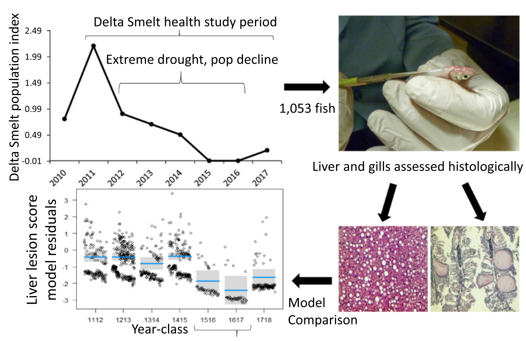
HIGHLIGHTS

- Liver and gill condition of 1053 Delta Smelt collected during a drought was assessed.
- Liver and gill condition was markedly worse in larger fish.
- Fish with the most altered livers were collected from Cache Slough and Suisun Bay.
- Fish with the healthiest livers were collected from Suisun Marsh.
- Liver health improved even as the population crashed during the drought.

GRAPHICAL ABSTRACT



Liver health peaked even as Delta Smelt population reached historical lows

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ABSTRACT

The Delta Smelt (*Hypomesus transpacificus*) is an imperiled, annual fish endemic to the Sacramento-San Joaquin Delta and San Francisco Estuary. This study examined the severity and prevalence of liver and gill lesions of juvenile through adult Delta Smelt from 2011 through 2017 collected from five regions throughout its habitat ($n =$ 1,053). The first and last years of the study were wet, but bracketed an extreme drought in CA (2012–2016), during which the Delta Smelt population reached historical lows. Overall, the three most common lesions were gill ionocyte hyperplasia, liver lipidosis, and gill aneurysm. Individuals with higher fork lengths exhibited increased gill and liver lesion score (summations of the severity scores), suggesting that Delta Smelt accumulate lesions through their lives, and that larger individuals were more tolerant of liver and gill lesions. Liver lesion score showed significant regional differences, while salinity was a better predictor of gill lesions than region, with lower gill lesion scores associated with higher salinities. Largely consistent with previously reported histopathology patterns, Delta Smelt collected from the Confluence and Suisun Marsh had the lowest liver lesion score, while Delta Smelt collected from Cache Slough and Suisun Bay had the highest lesion scores, and Suisun Marsh had the lowest glycogen depletion, suggesting heterogeneous levels of exposure to environmental stressors across regions. Gill and liver lesion score also varied significantly with year-class. The highest gill lesion score occurred in the 2015/16 year-class, and the lowest occurred in the 2017/18 year-class, a 2.8-fold difference. Controlling for size and regional effects, individuals with comparatively high liver lesion scores were prevalent in the

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population until the 2014/15 year-class. In the two subsequent year-classes, Delta Smelt livers were in the best condition, coinciding with peak drought conditions and record low abundances.

1. Introduction

Abundance-based fish monitoring programs, though useful for detecting declining fisheries (Maxwell and Jennings, 2005), provide limited information on the potential influence of sub-lethal stressors on fish populations. Therefore, in recent years, increasing emphasis has been placed on fish health as an indicator of environmental stress since it provides a biological record of previous sub-lethal exposures, rather than the 'snap-shot' in time provided by grab samples (Stentiford et al., 2003; Ruiz-Picos et al., 2015). Assessments of health status using histopathology can detect a variety of stressors, including pathogens, contaminants, or unfavorable nutritional and water quality conditions (Teh et al., 1997; Handy et al., 2003; Stentiford et al., 2003). In addition, histopathology provides an important analytical link between biomolecular or biochemical assays and individual or population relevant endpoints (Adams et al., 1992; Johnson et al., 1993).

The two most widely used organs in fish histopathology studies are the liver and gills (Mallatt, 1985; Hinton et al., 1992; Poleksic and Mitrovic-Tutundzic, 1994; ICES, 1997). In fish, the liver performs metabolic and detoxification functions, stores glycogen to satisfy short-term energy demand, lipid for longer-term energy demand, and is the site of choriogenin and vitellogenin protein production used for egg chorion and yolk development, respectively (Schlenck and Benson, 2003). Gills, in turn, perform gas exchange, regulate internal osmolarity, excrete ammonia, and as such are in constant, direct contact with the water (Mallatt, 1985). Gills therefore respond more rapidly than the liver to stressors and represent a sensitive organ for assessment of water quality and contaminant exposure (Mallatt, 1985; Poleksic and Mitrovic-Tutundzic, 1994; Au, 2004). The degree of morphological alterations in the gills indicates the level of environmental contaminants and physicochemical stressor exposure (Poleksic and Mitrovic-Tutundzic, 1994; Schwaiger et al., 1997; Au, 2004). Thus, the integrated assessment of morphological alterations of the liver and gills can indicate chronic and acute adverse effects of multiple environmental stressors (Adams et al., 1992; Brusle and Anadon, 1996). Moreover, the impairment of these organs reduces growth, survival, and reproductive success (Adams et al., 1992; Teh et al., 1997).

The San Francisco Estuary (SFE) is formed by the convergence of the Sacramento and San Joaquin rivers and the Pacific Ocean. It is the largest estuary on the Pacific coast of the Americas (Moyle, 2002). The geomorphology and hydrodynamics of the SFE are highly altered to accommodate agriculture, urban development and water diversion, and the estuary is also a major drainage for natural and anthropogenic contaminants. Delta Smelt (*Hypomesus transpacificus*) is a small, partially semi-anadromous fish that is endemic to the SFE (Bennett, 2005; Hobbs et al., 2019). It has an annual life cycle, and its population has been in decline for decades (Sommer et al., 2007). This decline led to the listing of the species as threatened and endangered under the California and Federal State Endangered Species Acts, respectively (United States Fish and Wildlife Service (US Fish and Wildlife Service, 1993; CDFW, 2014). One hypothesized cause for the decline in abundance is exports of freshwater from the habitat of Delta Smelt, which provide drinking and irrigation water to much of Southern California (Bennett, 2005; Sommer et al., 2007; Moyle et al., 2016). Other hypotheses include poor water quality, drought, altered habitat, climate change, and food limitation (e.g., Sommer et al., 2007). However, these hypotheses are difficult to assess using only abundance estimates, without data collected at the level of the individual fish.

The present study applies histopathological analysis to wild Delta Smelt collected during abundance monitoring programs over a seven-year period (2011–2017), and uses data collected from individuals to characterize the temporal, spatial and environmental variability of the liver and gill condition of Delta Smelt. This period is ideal for examining the influence of river flow in particular on Delta Smelt condition because it encompasses the most severe drought in modern California history (~2012–2016), bracketed by wet years (2011 and 2017). Our previous work demonstrated that juvenile Delta Smelt collected from Suisun Bay were under apparent nutritional stress during summer, Delta Smelt collected from Cache Slough showed the most liver alterations, and individuals from Suisun Marsh were in relatively good condition overall (Hammock et al., 2015). That a regional pattern in liver and gill alterations was detected implies that stressors can manifest before movement among regions homogenizes the condition of this vagile fish (Sommer et al., 2011; Bennett and Burau, 2015; Hobbs et al., 2019). This study extends this health analysis, both from two to seven years and across juvenile through adult life-stages, and assesses whether previously reported variation in fish condition and nutritional status maintained their regional patterns. In addition to examining the influence of region, we also examined the influence of year-class, salinity, and freshwater outflow on histopathological condition of the liver and gills of Delta Smelt.

2. Methods

2.1. Study area and sampling

Juvenile through adult Delta Smelt were collected from the SFE by the Interagency Ecological Program (IEP) fish monitoring studies conducted by the California Department of Fish and Wildlife (CDFW; $n =$ 961; methods in Honey et al., (2004) and the United States Fish and Wildlife Service (US Fish and Wildlife Service, 1993) Enhanced Delta Smelt Monitoring Program (https://www.fws.gov/lodi/juvenile_fish_monitoring_program/jfmp_index.htm; $n = 92$; see Fig. 1 in Hammock et al., 2015 for a map of the sampling regions). The five regions included the Sacramento River Deep Water Shipping Channel (SRDWSC), a riprapped, constructed, freshwater channel with long residence time; Cache Slough, a freshwater region with known contaminant inputs and tidal wetland remnants; the Confluence, a region with variable salinity and tidal wetland remnants where the Sacramento and San Joaquin rivers converge; Suisun Bay, an open water, generally brackish region heavily invaded by *Potamacorbula amurensis*; and Suisun Marsh, a region with relatively intact tidal wetland habitat that is also heavily invaded by *P. amurensis* (Alpine and Cloern, 1992; Sommer et al., 2007; Hammock et al., 2019; Weston et al., 2019). The CDFW fish were collected from Aug 2011 to Oct 2017 and the USFWS fish were collected from Aug 2017 to Nov 2017. Both agencies collected Delta Smelt in trawls, wrapped each fish live in aluminum foil, and placed the wrapped fish in a dewar of liquid nitrogen kept on each boat (Teh et al., 2016). While fixing the Delta Smelt in formalin would have been more suitable for histopathology, a variety of other endpoints are also measured which precludes the use of formalin (e.g., otolith increment, enzymatic biomarkers, Hammock et al., 2015; Teh et al., 2016). Conductivity [salinity], turbidity, temperature and location data (GPS) were collected at each sampling station. Delta Smelt were transported to our lab at University of California, Davis in dewars, and stored in liquid nitrogen until dissection.

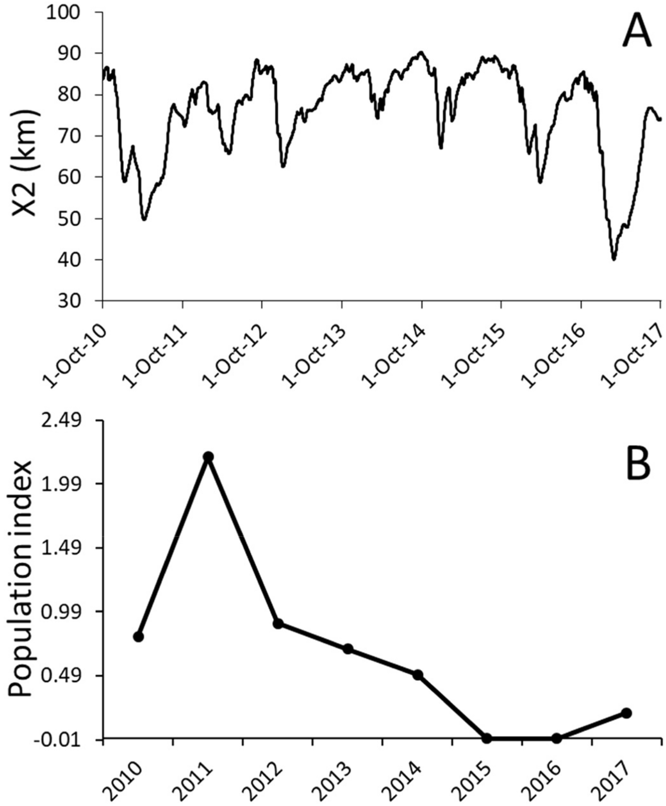

Fig. 1. Panel A is mean daily X2 location over the study period from Oct 1, 2010–Oct 1, 2017. X2 is the tidally averaged distance from the Golden Gate Bridge to the bottom salinity isohaline of two (Kimmerer, 2002). The data reflect an extremely dry period in California bracketed by extremely wet years in 2011 and 2017, and are from the Department of Water Resources Dayflow website (https://data.ca.gov/dataset/dayflow). Panel B is the Delta Smelt population index, estimated from CDFW trawls conducted during fall (https://www.dfg.ca.gov/delta/data/townet/indices.asp?species=3).

3. Sample preparation and histopathology

Each Delta Smelt was removed from liquid nitrogen and rapidly dissected as it thawed (5–10 min per fish; Hammock et al., 2015; Teh et al., 2016). Livers and gills were excised, along with the gonad if the individual was mature enough for excision, preserved in 10% neutral buffered formalin, and processed for histopathology according to Teh et al. (1997, 2016). Other organs were not processed for histopathology because they were used for other purposes, including kidney for *Mycobacterium* infections (Baxa et al., 2015), gut for gut content analysis (Hammock et al., 2015, 2017, 2019), and brain for acetylcholinesterase (Teh et al., 2016). Briefly, liver, gills, and gonad were embedded in paraffin, sectioned (3 μm thickness), and stained with hematoxylin and eosin (H&E stain; Teh et al., 1997). Histopathological analysis was conducted on gills and liver of each sampled fish ($n = 1053$) following the methods of Teh et al. (2004). The gonad (when it could be excised) was sectioned, sexed, and staged following Kurobe et al. (2016). We note that although the quality of tissue sections is reduced somewhat by flash freezing compared to formalin fixation of fresh tissue, the process does not alter the prevalence or severity of liver or gill lesions (Teh et al., 2016). Liver and gill tissues were screened with a compound microscope for a variety of histopathological lesions and scored on an ordinal ranking system of 0 = none/minimal, 1 = mild, 2 = moderate, and 3 = severe. The seven liver lesions and eight gill lesions that were commonly observed are described in Table 1. Both organs were also screened for other tissue alterations, including parasites, bacterial infection, preneoplastic foci and hepatocellular and gill neoplasms, but were not included in analyses because these abnormalities were either extremely rare or absent during the seven-year study, consistent with previous work on Delta Smelt (Foott and Bigelow, 2010). The same histologist (S. Teh) read the liver and gill slides throughout the entire study, maintaining scoring consistency.

Table 1
Descriptions of histopathological lesions observed in Delta Smelt. Liver lesion score included a summation of all liver lesions except glycogen depletion, which was analyzed separately. Gill lesion score included a summation of each of the gill lesions.

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

3.1. Statistical analysis

Three response variables were examined in the study: liver lesion score, liver glycogen depletion, and gill lesion score. Liver and gill lesion scores were analyzed separately due to the rapid response of gills to stressors. Liver lesion score was the summation of each liver lesion besides glycogen depletion in Table 1 (six lesions), gill lesion score was the summation of the eight gill lesion scores in Table 1, and liver glycogen depletion was analyzed separately. We analyzed each of the three variables using model comparison followed by effect size calculations to identify and quantify the drivers of each response.

Six variables were used as predictors in the analysis: year-class, region, fork length, salinity, mean monthly outflow, and X2. X2 is defined as the tidally averaged distance from the Golden Gate Bridge (i.e., Pacific Ocean) to the bottom salinity isohaline of two (Kimmerer, 2002; Fig. 1A). We were interested in year-class because Delta Smelt exhibits substantial variation in interannual abundance, related to environmental variation (e.g., Hamilton and Murphy, 2018; Fig. 1B), which may be reflected in its histological condition. The region variable was included to determine whether the regional pattern detected in our previous work persisted through time (Hammock et al., 2015). Fork length was included because fish can accumulate lesions through their lives (Bernet et al., 1999). We included salinity because we expected that freshwater inputs to the SFE were more contaminated than the Pacific Ocean given our previous results (Hammock et al., 2015). The outflow variable was included because contaminants can both increase or decrease with flow, depending on the contaminant, substrate, and time since the last storm (e.g., Bertrand-Krajewski et al., 1998; Lee et al., 2002). We included X2 as a more stable indicator of water year type (wet vs. dry) than mean monthly outflow. It is akin to the year-class variable in that it groups all fish from the same year class together, but is distinct from year-class because it is a continuous variable describing the hydrodynamic conditions experienced by each year-class. While liver morphology can vary by sex in many adult fishes, it was difficult to include in our analyses because less than half of the fish were sexed histologically (492 of 1053 fish). Using only the fish that were sexed in a preliminary analysis (negative binomial modeling), we found that liver lesion score was not strongly influenced by sex ($P = .08$), while glycogen depletion was affected ($P < .0001$). To determine the influence of sex on glycogen depletion, we ran a separate analysis in which the females were excluded, making the assumption that females that were too immature to be sexed did not differ significantly from males in terms of glycogen depletion (Supplemental Material).

To make the region variable, Delta Smelt were divided among five regions based on collection location. Models included salinity as a continuous variable or as a dummy variable (<0.55 salinity: fresh, >0.55 salinity: brackish). Tidally averaged monthly flow at Chipps Island during the month that each fish was collected was used as the outflow variable (https://water.ca.gov/Programs/Environmental-Services/Compliance-Monitoring-And-Assessment/Dayflow-Data). Finally, each fish in the same year-class was assigned the same X2 variable, consisting of mean daily X2 from June 1 through Dec 31 of each year. This period was meant to encompass the bulk of the maturation period of Delta Smelt, from juvenile to adult.

The same set of 13 models fit to the liver lesion score data were fit to the liver glycogen depletion data. The structures of the first eight models fit to the liver and gill lesion scores were identical. The other three models fit to the gill lesion score results were included based on a divergence in useful predictors of gill and liver lesion score. All liver lesion, gill lesion, and glycogen depletion models had negative binomial distributions to account for over-dispersion and because the response variables were integers from 0 to 10 (McElreath, 2016). The models were fit using the 'glm.nb' command in the program R. The partial residuals for each response variable of highly ranked models were plotted using the package 'visreg' to show the influence of each variable (Breheny and Burchett, 2013). Effect sizes were calculated for selected models using the 'predict.glm' function in R.

4. Results

The study period included an extraordinarily dry period (2012–2015) that was bracketed by wet years in 2011 and 2017 (Fig. 1A). The Delta Smelt population declined as the drought progressed in California, but did not recover in 2017, an extraordinarily wet year (Fig. 1B). Sample size and mean salinity, temperature, and turbidity for each region and year class are in Table 2. A total of 1053 Delta Smelt were examined and 65.6% of the fish had at least one liver or gill lesion (Table 3). Of the fish that were sexed ($n = 492$), mean fork length was 64.3 and 63.1 mm for males and females, respectively.

5. Liver histopathology

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

6. Liver lesions model comparison

The top-ranked liver lesion model included fork length ($P < .0001$), outflow ($P = .09$), year-class ($P < .0001$), and region ($P = .002$; Table 4). However, because the second-ranked model had a very similar AIC_c score to the top-ranked model (i.e., a ΔAIC_c less than two, Bolker, 2008) but was more parsimonious, and outflow was non-significant, we selected the second-ranked model (although we note that the parameter estimate for outflow was negative [−0.33], Table 4). Based on the second-ranked model, liver lesion score increased with increasing fork length (Fig. 3A), was highest in C. Slough and S. Bay and lowest in S. Marsh and the Confluence (Fig. 3B). Liver lesion score peaked during the 2014/15 year-class and was lowest during the 2016/17 year-class (Fig. 3C). Based on model predictions, as fork length increased from the minimum (24 mm) to the maximum (88 mm), liver lesion score increased 15-fold, from 0.181 to 2.636. Also based on model predictions, the highest mean year-class liver lesion score was 0.742 and occurred in the 2014/15 year-class; the lowest was 0.097 and occurred in the 2016/17 year-class, a 7.6-fold difference.

The top-ranked liver glycogen depletion model included fork length ($P = .022$), X2 ($P < .0001$), and region ($P = .039$; Table 5). Like the liver lesions models, the two top-ranked models had similar AIC_c weights. However, the top-ranked glycogen depletion model was also the most parisomonious, so it was selected (we note that the X2 and year-class variables are similar in that both reflect hydrologic conditions during the year-class, so the distinction may be unimportant). In the top-

Table 2
Sample size and mean salinity (Sal), temperature (Temp), and turbidity (Turb) for each region and year class. SRDWSC is Sacramento River Deep Water Shipping Channel. Water quality measurements were made during trawls and the means are calculated measurements coinciding with Delta Smelt catch.

Region/Year class	n	Sal	Temp (°C)	Turb (NTU)
Cache Slough	84	0.10	16.87	40.25
Confluence	340	0.73	16.04	36.31
Suisun Bay	167	4.26	18.66	63.72
Suisun Marsh	192	3.35	13.40	53.71
SRDWSC	270	0.25	15.70	36.02
2011/12	165	2.16	16.12	36.34
2012/13	312	0.94	13.83	40.83
2013/14	142	2.70	18.61	38.66
2014/15	218	1.43	16.02	48.78
2015/16	55	1.00	16.64	41.14
2016/17	41	0.49	12.40	67.75
2017/18	120	2.15	18.99	55.79

Table 3
Prevalence (%) and mean score of Delta Smelt lesions overall, by region, and by year-class. The first column is prevalence; all other columns are mean scores. Prevalence was calculated as the number of fish with scores >1 divided by the total number of fish for glycogen depletion and gill ionocyte hyperplasia (which can occur in response to very minor stress), all other lesions were calculated as >0.

		Region					Year-class						
Lesion	Prevalence (%)	C. Slough	SRDWSC	Conf.	S. Bay	S. Marsh	2011/12	2012/13	2013/14	2014/15	2015/16	2016/17	2017/18
Liver glycogen depletion	66.29	2.02	1.88	1.84	2.11	1.61	2.24	1.81	1.46	1.71	1.85	1.68	2.33
Gill ionocyte hyperplasia	31.72	0.83	0.99	0.68	0.22	0.63	0.42	0.95	0.47	0.77	1.09	1.49	0.03
Liver lipidosis	31.62	0.54	0.52	0.36	0.34	0.58	0.43	0.75	0.27	0.3	0.4	0.61	0.16
Gill aneurysm	18.71	0.19	0.23	0.25	0.26	0.19	0.28	0.29	0.16	0.22	0.22	0.17	0.15
Liver inflammation	6.74	0.13	0.07	0.09	0.14	0.02	0.14	0.02	0.09	0.15	0.09	0.02	0.03
Gill epithelial cell hyperplasia/hypertrophy	4.75	0.01	0.05	0.12	0.01	0.03	0.02	0.02	0.00	0.05	0.29	0.00	0.23
Liver macrophage aggregate	3.61	0.07	0.04	0.04	0.06	0.04	0.07	0.03	0.04	0.06	0.05	0.00	0.03
Liver sinusoid congestion	3.23	0.06	0.05	0.05	0.07	0.04	0.05	0.08	0.02	0.05	0.00	0.02	0.04
Liver single cell necrosis	3.04	0.06	0.04	0.04	0.03	0.01	0.07	0.04	0.01	0.01	0.04	0.00	0.04
Liver cytoplasmic inclusions or eosinophilic protein droplets	2.47	0.01	0.02	0.04	0.07	0.02	0.04	0.01	0.01	0.09	0.00	0.10	0.01
Gill fusion	0.95	0.02	0.01	0.00	0.01	0.02	0.02	0.03	0.00	0.00	0.00	0.00	0.00
Gill mucous cell hyperplasia	0.85	0.02	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.02	0.07	0.00	0.00
Gill secondary lamella edema	0.85	0.00	0.01	0.02	0.01	0.00	0.05	0.01	0.00	0.00	0.00	0.00	0.00
Gill inflammation	0.76	0.05	0.01	0.01	0.00	0.00	0.01	0.02	0.02	0.00	0.00	0.00	0.00
Gill epithelial cell necrosis	0.19	0.00	0.00	0.01	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.00

ranked model, glycogen depletion increased with increasing fork length (Fig. 4A), was lowest in S. Marsh (Fig. 4B), and decreased with increasing X2 (Fig. 4C). Based on model predictions, as fork length increased from the minimum to the maximum, glycogen depletion increased 1.29-fold, from 1.72 to 2.22. The lowest model estimated glycogen depletion was 1.58 in S. Marsh, while all the other regions had higher estimates of glycogen depletion (i.e., Fig. 4). As X2 increased from the minimum to the maximum, model estimated glycogen depletion decreased 1.43-fold, from 1.67 to 2.39 (i.e., Delta Smelt had more glycogen rich livers under drought conditions).

The models fit to the dataset without the 163 female fish showed similar results in terms of region, with Suisun Marsh still having fish with less glycogen depleted livers (Fig. S1). However, fork length was no longer an important predictor of glycogen depletion with the females excluded, indicating that mature, glycogen depleted females were driving the relationship with fork length in the analysis of the full dataset (Fig. 4A; Supplemental Material, Table 1). More simply, mean glycogen depletion was 1.5 and 2.1 for males and females, respectively. In addition, year class replaced X2 in the selected model. With the females excluded, year classes during the drought generally showed less glycogen depletion than during wet years (Fig. S2).

7. Gill histopathology

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

8. Gill model comparison

The top-ranked gill lesion model included fork length ($P < .0001$), salinity ($P < .0001$), and year-class ($P < .0001$; Table 6). Gill lesion score increased with increasing fork length (Fig. 6A) and decreased with increasing salinity (Fig. 6B). The lowest lesion score, after accounting

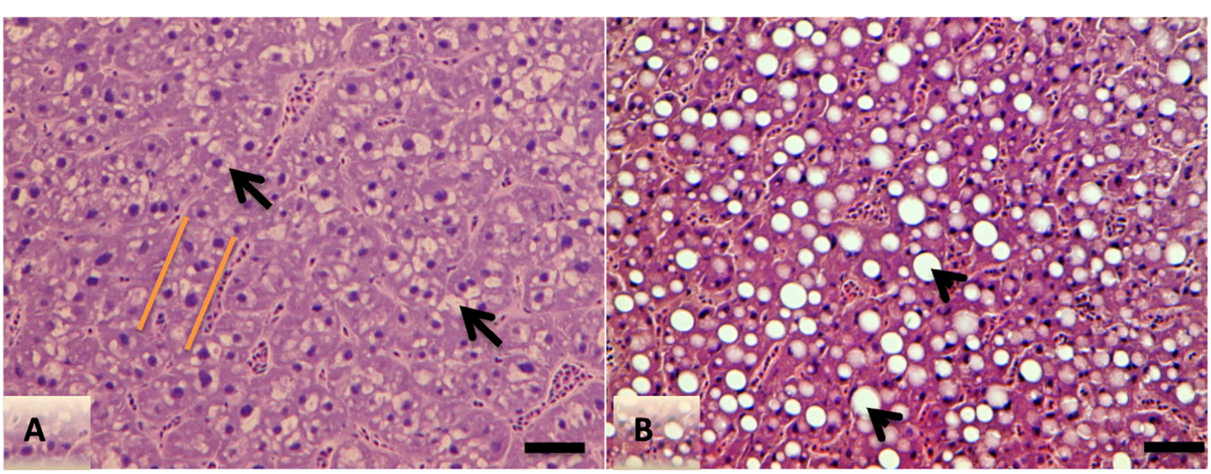

Fig. 2. Panel A shows a normal glycogen-rich liver (arrows) from a 2017 subadult male wild Delta Smelt. Liver morphology is lined with rows of organized tubular liver structure (orange outlines). Bar 50 μm. Panel B shows a glycogen depleted liver with severe lipidosis from a 2017 subadult male wild Delta Smelt. The well-organized tubular liver structures are distorted. Hepatocytes are smaller, more basophilic (bluish coloration) and often consist of single large cytoplasmic fatty vacuoles displacing the nucleus to the cell's periphery (arrowheads). H&E stain. Bar = 50 μm.

Table 4
Model comparison for liver lesion score. FL is fork length, Out is mean monthly outflow at Chipps Island (log_{10}-transformed), Reg is region (C. Slough, SRDWSC, Conf, S. Bay, S. Marsh), SalDum is salinity as a dummy variable (fresh/brackish), Sal is salinity as a continuous variable, YC is year-class as a factor, and X2 is mean X2 from June 1 to Dec 31 for each year class (continuous).

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

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

for fork length and salinity, occurred in the 2017/18 year class (Fig. 6C). Based on model predictions, increasing fork length from the minimum to the maximum increased gill lesion score 3.4-fold, from 0.56 to 1.94. Increasing salinity from the minimum to the maximum decreased gill lesion score 6.5-fold, from 1.32 to 0.20. After accounting for the influence of fork length and salinity, the highest gill lesion score (1.28) occurred in the 2015/16 year-class, and the lowest (0.46) occurred in the 2017/18 year-class, a 2.8-fold difference.

9. Discussion

Our previous histological assessment of Delta Smelt included only juveniles, and encompassed just two year-classes (Hammock et al., 2015). This study is therefore the longest and most comprehensive health assessment of the species, covering seven year-classes and the juvenile through adult life-stages, before, during and after a severe drought in California. Overall, the majority of Delta Smelt in the study exhibited at least one gill or liver lesion. This suggests that altered liver and gill condition affect Delta Smelt throughout their distribution and across multiple life-stages.

Indicator species can be divided into two types, I and II. Type I indicator species have narrow tolerances to most stressors, and their abundances rapidly decline with relatively minor environmental perturbations (Ryder and Edwards, 1985). Type II indicator species are highly tolerant of a range of stressors, and tend to increase in abundance as levels of human perturbation increase (Ryder and Edwards, 1985). In consequence, the abundances of Type I species serve as an early warning to environmental perturbations, while Type II species are more likely to record evidence of stressors in their tissues rather than dying, causing the information to be lost (e.g., contaminants [Ryder and Edwards, 1985]). Ideally, Type II indicator species are widespread, abundant, occupy high trophic levels, and are relatively sessile, so that an individual's health reflects local conditions (Goede and Barton, 1990; Adams et al., 1990; Teh et al., 1997; Schwaiger et al., 1997). The Delta Smelt can therefore be categorized as a Type I indicator species. Typically, histopathology is used on Type II indicator species, where the goal is to identify environmental stressors and to quantify the spatial and temporal extent of their effects (Schwaiger et al., 1997; Teh et al., 1997). However, the Delta Smelt was selected for this study to inform conservation and recovery efforts of the species itself, rather than for its traits as an indicator species of water quality. In addition to being sensitive, Delta Smelt has limited abundance and a complex, vagile life-history (Hobbs et al., 2019). Therefore, the histological data are

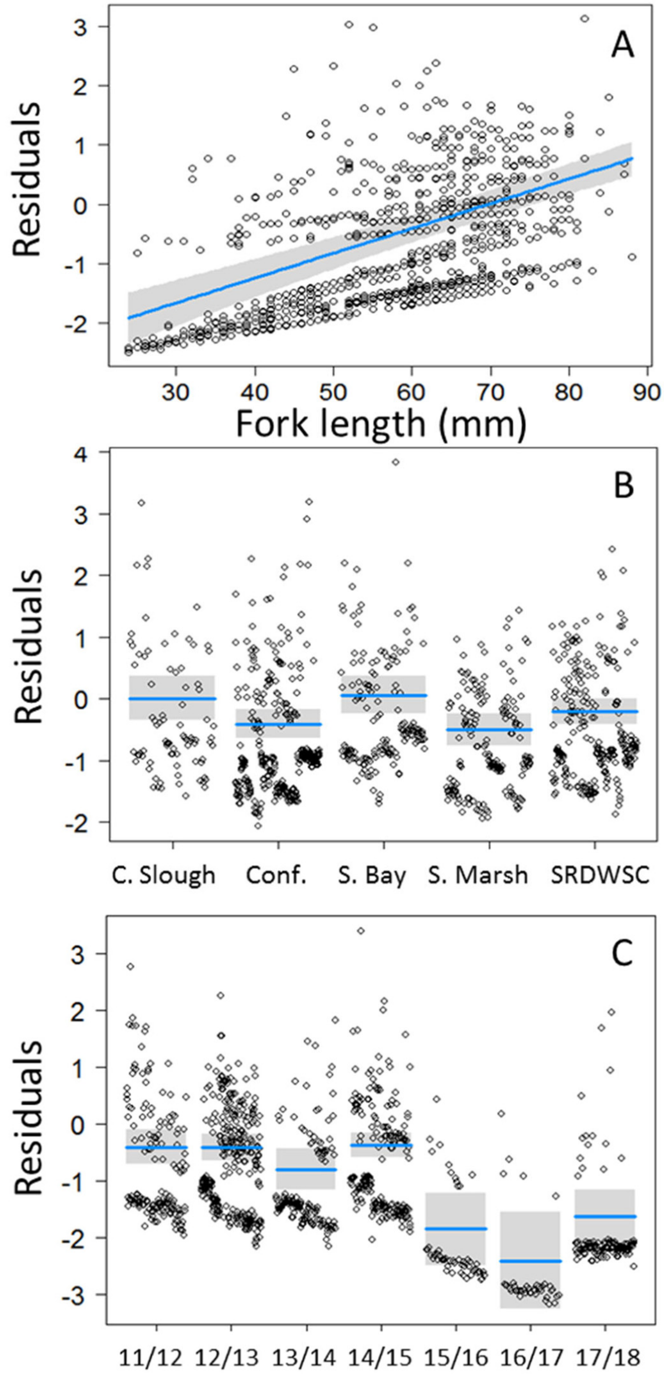

Fig. 3. The partial residuals from the second-ranked liver lesion model by fork length (A), region (B), and year class (C; Table 4). For panel C, the x-axis is year class (e.g., 11/12 refers to the 2011–12 year-class). The grey bands show the 95% confidence interval.

relatively difficult to interpret, and the traits of the species must be considered when interpreting its histopathology.

Fork length was the most important predictor of gill and liver condition (Tables 4-6), with larger individuals exhibiting gills in the poorest condition, and the most severely altered and glycogen-depleted livers. In contrast, the positive relationship between glycogen depletion and fork length in the analysis with all 1053 fish was caused by sub-adult and adult females having more glycogen depleted livers on average (Supplemental Material). The relationships between liver and gill lesions and fork length are consistent with previous work showing that larger fish exhibit a higher prevalence and severity of lesions (Bernet et al., 1999), and we suggest two possible explanations for this pattern.

Table 5
Model comparison for liver glycogen depletion. FL is fork length, Out is mean monthly outflow at Chipps Island ($\log_{10}$-transformed), Reg is region (C. Slough, SRDWSC, Conf, S. Bay, S. Marsh), SalDum is salinity as a dummy variable (fresh/brackish), Sal is salinity as a continuous variable, YC is year-class as a factor, and X2 is mean X2 from June 1 to Dec 31 for each year class (continuous).

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

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

Larger individuals may be less likely to succumb to poor health, and therefore better able to persist in sub-optimal condition. For example, Capkin et al. (2006) found that the mortality of juvenile Rainbow Trout declined with increasing size when exposed to endosulfan. A second possibility is that larger, presumably older individuals have had a longer period of exposure and therefore longer times to develop more severe lesions than smaller, younger individuals. These possibilities could be addressed empirically by exposing a cohort of Delta Smelt to stressors and recording size-class specific mortality and lesion rates through time.

Delta Smelt showed a marked improvement in liver health as a severe drought progressed in California. The drought began in 2012 and peaked in 2015 and 2016, the same period when liver lesion score reached its lowest levels (Figs. 1A, B, and 3C). The improvement in liver condition appeared to occur both because individuals with unhealthy livers were less prevalent than during previous years (i.e., absence of fish with high lesion scores during 2015/16 and 2016/17 in Fig. 3C), and because individuals with livers in the best condition exhibited improved liver condition compared to previous years (Fig. 3C). We propose two interpretations. As a Type 1 indicator species, Delta Smelt have narrow tolerances for most environmental stressors, some of which may have worsened during the drought (e.g., temperature, prey availability, ammonia; Ryder and Edwards, 1985). Therefore, individuals with high liver lesion scores may have been absent during the latter years of the drought because the least healthy individuals were not able to persist under the stressful conditions, thereby improving mean liver condition for the population. Another possibility is that liver health improved simply due to reduced loading of contaminants during low flow years (e.g., Sansalone and Buchberger, 1997). Whatever its cause, improving liver health was not a positive sign for the species, as the population reached historical lows during the worst of the drought, even while exhibiting the least altered livers. Our results therefore suggest that the decline in Delta Smelt population during the drought was not due to poor liver condition, because Delta Smelt livers were in relatively good condition when the population reached its lowest point (2015–16; Figs. 1A, B, 3C).

Salinity was a better predictor of gill lesions than region, with higher gill lesion scores associated with less saline habitat. The gill lesions with the highest incidence were ionocyte hyperplasia (32% of fish had moderate or severe ionocyte hyperplasia) and gill aneurysm (19% exhibited mild to severe lesions, Table 3). The major function of fish gill ionocytes (also known as chloride or mitochondria-rich cells), is ionic regulation and ammonia excretion (Perry and Laurent, 1993). Ionocyte hyperplasia has been reported in fish migrating from saltwater to freshwater (Hirai

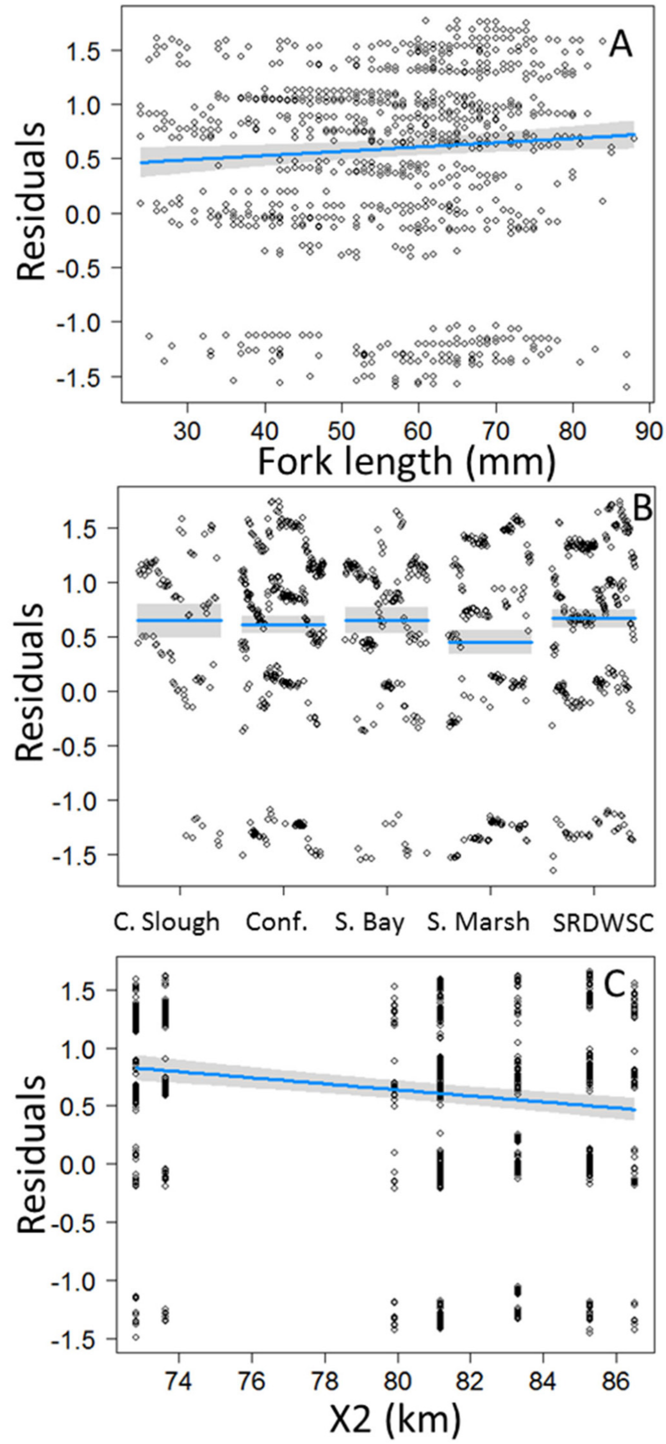

Fig. 4. The partial residuals from the top-ranked liver glycogen depletion model by fork length (A), region (B), and X2 (C; Table 5). X2 is the tidally averaged distance from the Golden Gate Bridge to the bottom salinity of two isohaline (Kimmerer, 2002). The grey bands show the 95% confidence interval.

et al., 1999), freshwater to saltwater (Evans, 1984) and following exposure to pollutants (Evans, 1987). A gill aneurysm (Fig. 5C) is formed due to weakening or necrosis of pillar cells resulting in blockage and excessive stagnation of blood, and is a common response of fish exposed to pollutants (Meyers and Hendricks, 1985; Evans, 1987). The relationship between salinity and gill lesion score (increase in gill lesion score in freshwater) suggests a possible natural response of the fish to the salinity of its environment as it attempts to maintain its internal osmolarity (e.g., Hirai et al., 1999), or possibly increased exposure to contaminants

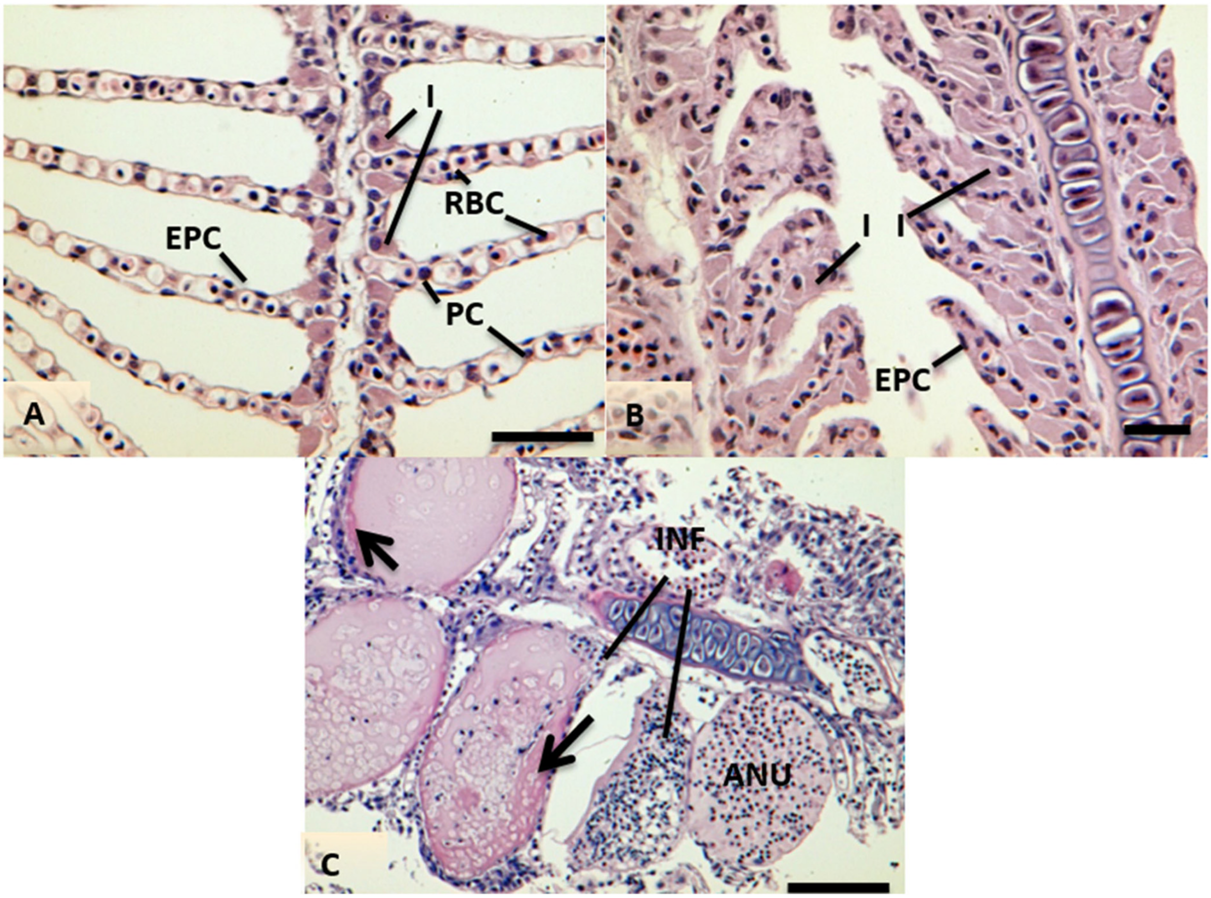

Fig. 5. Panel A shows a normal gill morphology of a Delta Smelt collected from the 2017–18 year-class. One or two ionocytes (I) are usually located at the junction between the filament and lamella. The secondary lamellae of the gill filaments are the sites of gas exchange and are mainly composed of two epithelial layers joined together by pillar cells (PC). Red blood cells (RBC) flow through lamellar capillary (LC) formed by walls of pillar and specialized endothelial cells where gas exchange occurs. H&E stain, bar = 30 μm. Panel B shows severe ionocyte hyperplasia in a Delta Smelt collected in 2017. Note that the entire secondary lamella is covered by proliferated ionocytes. EPC = epithelial cells. H&E stain, bar = 30 μm. Panel C shows severe aneurysm (ANU) in the secondary lamellae from a 2012 juvenile wild Delta Smelt. Aneurysm is caused by pillar cell necrosis resulting in excessive localization of blood cells in the secondary lamallae. Note the formation of fibrin thrombi (arrows) within the aneurysms. Repair of lamella aneurysm is shown by the infiltration of inflammatory cells (INF) to remove autolyzed RBC. H&E stain, bar = 60 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in fresher water. Ionocyte hyperplasia does not appear to be an entirely normal response of movement into freshwater (e.g., Hirai et al., 1999), as most instances in this study resulted in the gill structure appearing abnormal, suggesting impairment of the organ. Both gill ionocyte proliferation and aneurysm can result in hypoxia, respiratory failure, and

Table 6
Model comparison for gill lesion score. FL is fork length, Out is mean monthly outflow at Chipps Island ($\log_{10}$-transformed), Reg is region (C. Slough, SRDWSC, Conf, S. Bay, S. Marsh), SalDum is salinity as a dummy variable (fresh/brackish), Sal is salinity as a continuous variable, YC is year-class as a factor, and X2 is mean X2 from June 1 to Dec 31 for each year class (continuous).

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

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

problems with ionic and acid-base balance and thus affect the general health of fish (Adams et al., 1992).

Our previous work on juvenile Delta Smelt demonstrated regional differences in lesion score (Hammock et al., 2015). Individuals collected from C. Slough had significantly more liver and gill alterations than fish collected from other parts of the Delta and SFE, especially S. Marsh (Hammock et al., 2015). In the present study, which included 809 more individuals, sub-adults, adults, and five more year-classes, a similar pattern was found (Fig. 3). Accounting for the strong influence of fork length and year-class, Delta Smelt with the most altered livers occurred in C. Slough (as before) and S. Bay (Fig. 3), while the healthiest fish occurred in S. Marsh (as before) and the Confluence (Hammock et al., 2015). The simplest explanation is that S. Marsh and the Confluence had better water quality than S. Bay and C. Slough. However, another possibility is that conditions were too stressful for individuals with even moderate cellular alterations to persist, leading to better average health. The latter explanation seems less likely however, given that the Confluence and Suisun Marsh regions are relatively good habitat for Delta Smelt (Feyrer et al., 2011), and Delta Smelt exhibit relatively good nutritional condition in these regions (Hammock et al., 2015, 2019). Regardless of the interpretation, lesions are likely impacting Delta Smelt throughout their distribution and across multiple life-stages, likely reducing survival.

Lipidosis or fatty vacuolar degeneration was one of the recurrent alterations found in the livers of Delta Smelt (Table 3). While lipid vacuoles can be found in otherwise healthy wild fishes or present normally

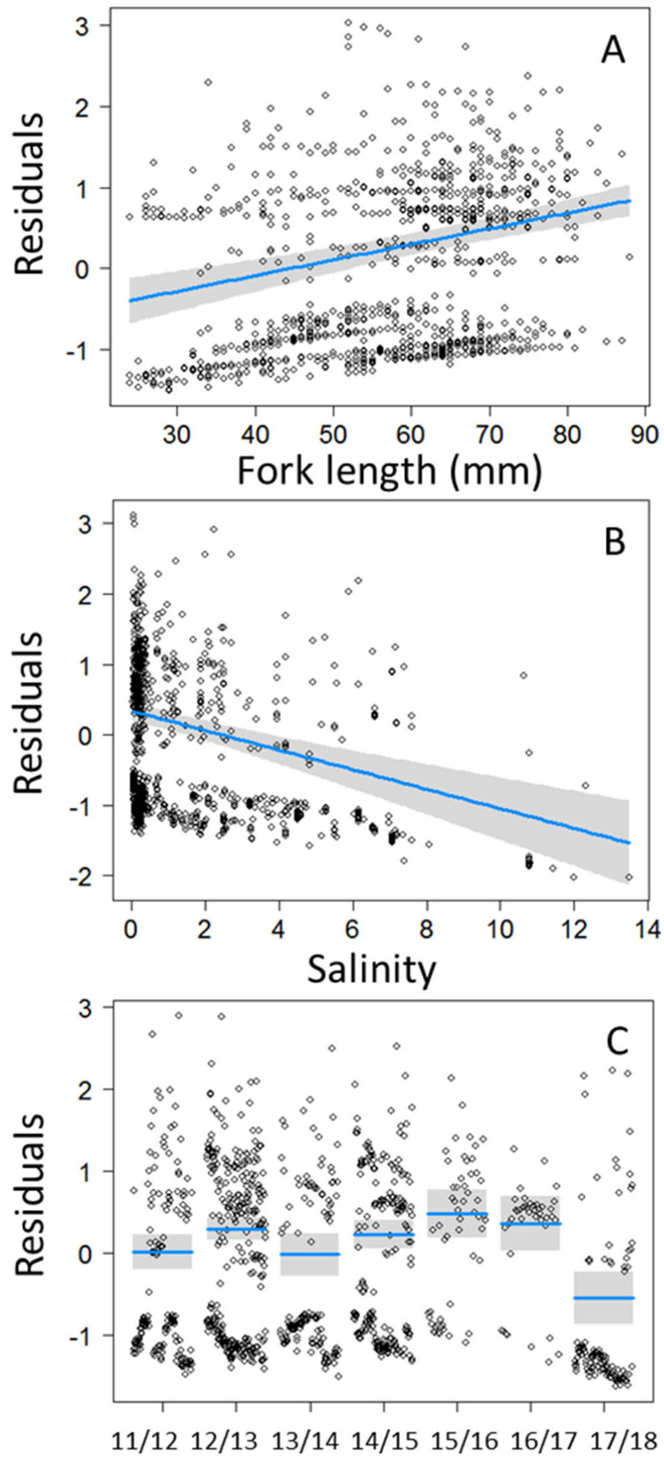

Fig. 6. The partial residuals for the top-ranked gill lesion score model by fork length (A), salinity (B), and year class (C [e.g., 11/12 refers to the 2011–12 year-class]; Table 6). The grey bands show the 95% confidence interval.

in hepatocytes of reproductively active females (Wolf and Wolfe, 2005; Wolf et al., 2015), they can also be associated with exposure to chlorinated hydrocarbons and other contaminants (Hinton et al., 1992), including PCBs (Teh et al., 1997; Anderson et al., 2003), crude oil extracts (Solangi and Overstreet, 1982), metals (Arellano et al., 1999; Giari et al., 2007) and in feral fish from sites contaminated by mixtures of xenobiotics (Greenfield et al., 2008; Triebskorn et al., 2008). Driving mechanisms of lipidosis include toxic injury causing impaired lipid oxidation or protein synthesis, resulting in accumulation of triglycerides in hepatocytes. Although malnutrition may increase fat mobilization and impair apoprotein synthesis (Hinton and Laurén, 1990), increased hepatocellular vacuolation is more commonly associated with overnutrition or toxicity (Wolf and Wolfe, 2005). Since obesity is uncommon in wild Delta Smelt because fish generally live in food limited environments, the present study suggests that the contaminants in C. Slough are affecting Delta Smelt (Hammock et al., 2015), though the habitat may provide mitigating benefits allowing the Delta Smelt population to persist there despite the contaminant inputs (Werner et al., 2000; Kuivila and Moon, 2004; Weston et al., 2014, 2019).

In addition to having fish with the lowest liver lesion score, fish collected from S. Marsh showed the most glycogen rich livers. A common response of fish to contaminants is a loss of hepatic glycogen (Hinton and Laurén, 1990; Schwaiger et al., 1997; Wolf and Wolfe, 2005), but glycogen depletion can also be indicative of food limitation or physicochemical stress (Adams et al., 1992). Thus, the presence of fish with glycogen rich livers in S. Marsh suggests some combination of the following: relatively low metabolic rates (likely given that S. Marsh had the lowest temperature of the five regions; Table 2), low contaminant exposure, low environmental stress, and abundant food. The latter point is possibly related to the relative abundance of tidal wetlands in the region (Matern et al., 2002), which are generally productive habitats (Shaffer and Sullivan, 1988; Beck et al., 2001; Müller-Solger et al., 2002), and were recently associated with improved foraging success of Delta Smelt (Hammock et al., 2019). Given the relatively good liver condition of fish collected from S. Marsh, and population collapse during the drought when the salinity of the region would have increased (Fig. 1A, B), access to the S. Marsh region still appears to be important for the persistence of Delta Smelt (Feyrer et al., 2011).

We conclude that histopathology is a useful tool for assessing the health of the Delta Smelt, but that complementary use of a Type II indicator species is warranted. Despite the intense interest in conserving the indicator species, the traits of Delta Smelt (lower trophic level, short-lived and narrow tolerances to most cultural stressors) are not ideal for its use as a sole indicator species for monitoring water quality in the highly altered SFE. Nevertheless, we can draw some conclusions from these results regarding Delta Smelt and the water quality of its habitat. Larger individuals will likely have increased prevalence and severity of lesions suggesting increased tolerance and/or exposure to contaminants. Consistent with our previous work, Suisun Marsh continues to appear to be favorable habitat when available to Delta Smelt (i.e., not too saline), as fish show relatively low liver lesion scores and rich liver glycogen, combined with relatively full stomachs (Hammock et al., 2015). The livers of fish in C. Slough and S. Bay were altered, and gills were in worse condition in fresher water, possibly suggesting contaminant exposure. Surprisingly, liver condition improved as a historic drought progressed in California, possibly because the least healthy fish have lower tolerance to cultural stress so could not survive the harsh conditions, or because of decreased loading of contaminants during low flow conditions. Given the difficulties of interpreting histopathology of Delta Smelt, multiple variables should therefore be considered, including the population dynamics of the species, additional complementary indicator species if possible, and the ambient and antecedent environmental conditions.

CRediT authorship contribution statement

Swee J. Teh: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing - original draft, Visualization. **Andrew A. Schultz:** Conceptualization, Investigation, Writing - review & editing. **Wilson Ramírez Duarte:** Conceptualization, Investigation, Writing - review & editing. **Shawn Acuña:** Conceptualization, Investigation, Writing - review & editing. **Denise M. Barnard:** Investigation, Writing - review & editing, Data curation, Supervision. **Randall D. Baxter:** Conceptualization, Investigation, Supervision. **Pedro Alejandro Triana Garcia:** Investigation, Writing - review & editing.

Bruce G. Hammock: Conceptualization, Funding acquisition, Investigation, Methodology, Writing - original draft, Writing - review & editing, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.138333.

References

Adams, S.M., Shugart, L.R., Southworth, G.R., Hinton, D.E., 1990. Application of bioindicators in assessing the health of fish populations experiencing contaminant stress. In: McCarthy, J.F., Shugart, L.R. (Eds.), Biomarkers of Environmental Contamination. 19. Lewis Publishers, Boca Raton, pp. 333–353 cap.

Adams, S.M., Crumby, W.D., Greeley, M.S., Ryon, M.G., Schilling, E.M., 1992. Relationships between physiological and fish population responses in a contaminated stream. Environ. Toxicol. Chem. 11, 1549–1557.

Alpine, A.E., Cloern, J.E., 1992. Trophic interactions and direct physical effects control phytoplankton biomass and production in an estuary. Limnol. Oceanogr. 37, 946–955.

Anderson, M.J., Cacela, D., Beltman, D., Teh, S.J., Okihiro, M.S., Hinton, D.E., Denslow, N., Zelikoff, J.T., 2003. Biochemical and toxicopathic biomarkers assessed in smallmouth bass recovered from a polychlorinated biphenyl-contaminated river. Biomarkers 8, 371–393.

Arellano, J.M., Storch, V., Sarasquete, C., 1999. Histological changes and copper accumulation in liver and gills of the Senegalese sole, *Solea senegalensis*. Ecotoxicol. Environ. Safe. 44, 62–72.

Au, D.W.T., 2004. The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. Mar. Pollut. Bull. 48, 817–834.

Baxa, D., Javidmehr, A., Mapes, S., Teh, S., 2015. Subclinical Mycobacterium infections in wild delta smelt. Austin J. Vet. Sci. Anim. Husb 2, 1004.

Beck, M.W., Heck Jr., K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello, T.J., 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates: a better understanding of the habitats that serve as nurseries for marine species and the factors that create site-specific variability in nursery quality will improve conservation and management of these areas. Bioscience 51, 633–641.

Bennett, W., Burau, J.R., 2015. Riders on the storm: selective tidal movements facilitate the spawning migration of threatened Delta Smelt in the San Francisco Estuary. Estuar. Coasts 38, 826–835.

Bennett, W.A., 2005. Critical assessment of the delta smelt population in the San Francisco Estuary, California. San Francisco Estuary and Watershed Science 3.

Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., Wahli, T., 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. J. Fish Dis. 22, 25–34.

Bertrand-Krajewski, J.-L., Chebbo, G., Saget, A., 1998. Distribution of pollutant mass vs volume in stormwater discharges and the first flush phenomenon. Water Res. 32, 2341–2356.

Bolker, B.M., 2008. Ecological Models and Data in R. Princeton Univ Pr.

Breheny, P., Burchett, W., 2013. Visualization of Regression Models Using Visreg. R Package. pp. 1–15.

Brusle, J., Anadon, G.G., 1996. The structure and function of fish liver. In: Munshi, J.S.D., Dutta, H.M. (Eds.), Fish Morphology, pp. 77–93 Boston: Massachusetts.

Capkin, E., Altinok, I., Karahan, S., 2006. Water quality and fish size affect toxicity of endosulfan, an organochlorine pesticide, to rainbow trout. Chemosphere 64, 1793–1800.

CDFW, 2014. State & Federally Listed Endangered & Threatened Animals of California. California Department of Fish and Wildlife. pp. 1–14.

Evans, D.H. 1984. The role of gill permeability and transport mechanisms in euryhalinity. In "Fish Physiology Vol XB" Ed by WS Hoar, DJ Randall, Academic Press, New York, pp 239–283.

Evans, D.H., 1987. The fish gill: site of action and model for toxic effects of environmental pollutants. Env Hlth Persp 71, 47–58.

Feyrer, F., Newman, K., Nobriga, M., Sommer, T., 2011. Modeling the effects of future outflow on the abiotic habitat of an imperiled estuarine fish. Estuar. Coasts 34, 120–128.

Foott, J.S., Bigelow, J., 2010. Pathogen survey, gill Na-K-ATPase activity, and leukocyte profile of adult Delta Smelt. Condition and disease of Delta Smelt. California Fish and Game 96 (4), 223–231.

Giari, L., Manera, M., Simoni, E., Dezfuli, B.S., 2007. Cellular alterations in different organs of European sea bass *Dicentrarchus labrax* (L.) exposed to cadmium. Chemosphere 67, 1171–1181.

Goede, R.W., Barton, B.A., 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition in fish. In: Adam, S.M. (Ed.), Biological Indicators of Stress in Fish, American Fisheries Society, Symposium 8, Bethesda, pp. 93–108.

Greenfield, B.K. Teh, S.J. Ross, J.R.M. Hunt, J. Zhang, G.H Davis, J.A. Ichikawa, G. Crane, D. Hung, S.S.O. Deng, D.F. Teh, F.-C. and P.G. Green. 2008. Contaminant concentrations and histopathological effects in Sacramento splittail (*Pogonichthys macrolepidotus*) Arch. Environ. Contam. Toxicol., 55, pp. 270–281.

Hamilton, S.A., Murphy, D.D., 2018. Analysis of limiting factors across the life cycle of Delta smelt (*Hypomesus transpacificus*). Environ. Manag. 1–18.

Hammock, B.G., Hobbs, J.A., Slater, S.B., Acuña, S., Teh, S.J., 2015. Contaminant and food limitation stress in an endangered estuarine fish. Sci. Total Environ. 532, 316–326.

Hammock, B.G., Slater, S.B., Baxter, R.D., Fangue, N.A., Cocherell, D., Hennessy, A., Kurobe, T., Tai, C.Y., Teh, S.J., 2017. Foraging and metabolic consequences of semi-anadromy for an endangered estuarine fish. PLoS One 12, e0173497.

Hammock, B.G., Hartman, R., Slater, S.B., Hennessy, A., Teh, S.J., 2019. Tidal wetlands associated with foraging success of Delta Smelt. Estuar. Coasts, 1–11 https://doi.org/10.1007/s12237-019-00521-5.

Handy, R.D., Galloway, T.S., Depledge, M.H., 2003. A proposal for the use of biomarkers for the assessment of chronic pollution and in regulatory toxicology. Ecotoxicology 12, 331–343.

Hinton, D.E., Laurén, D.J., 1990. Liver structural alterations accompanying chronic toxicity in fishes: Potential biomarkers of exposure. In: McCarthy, J.F., Shugart, L.R. (Eds.), Biomarkers of Environmental Contamination. Lewis, Boca Raton, FL, pp. 17–57.

Hinton, D.E., Baumann, P.C., Gardner, G.R., Hawkins, W.E., Hendricks, J.D., Murchelano, R.A., Okihiro, M.S., 1992. Histopathologic biomarkers. In: Hugget, R.J., Kimerle, R.A., Mehrle, P.M., Bergman, H.L. (Eds.), Biomarkers, Biochemical, Physiological, and Histologic Markers of Anthropogenic Stress. Lewis, Boca Raton, FL, pp. 155–209.

Hirai, N., Tagawa, M., Kaneko, T., Seikai, T., Tanaka, M., 1999. Distributional changes in branchial chloride cells during freshwater adaptation in Japanese sea bass *Lateolabrax japonicus*. Zool. Sci. 16, 43–49.

Hobbs, J.A., Lewis, L.S., Willmes, M., Denney, C., Bush, E., 2019. Complex life histories discovered in a critically endangered fish. Sci. Rep. 9, 1–12.

Honey, K., Baxter, R., Hymanson, Z., Sommer, T., Gingras, M., Cadrett, P., 2004. IEP Long-Term Fish Monitoring Program Element Review. State of California, the Resources Agency, Department of Water Resources, Interagency Ecological Program.

ICES, 1997. ICES review of the status of biological effects techniques relative to their potential application programmes. ICES Cooperative Research Report, No. 222, pp. 12–20.

Johnson, L.L., Stehr, C.M., Olson, O.P., Myers, M.S., Pierce, S.M., Wigren, C.A., McCain, B.B., Varanasi, U., 1993. Chemical contaminants and hepatic lesions in winter flounder (*Pleuronectes americanus*) from the northeast coast of the United States. Environ. Sci. Technol. 27, 2759–2771.

Kimmerer, W., 2002. Effects of freshwater flow on abundance of estuarine organisms: physical effects or trophic linkages? Mar. Ecol. Prog. Ser. 243, 39–55.

Kuivila, K.M., Moon, G.E., 2004. Potential exposure of larval and juvenile delta smelt to dissolved pesticides in the Sacramento–San Joaquin Delta, California. American Fisheries Society Symposium. American Fisheries Society, pp. 229–242.

Kurobe, T., M, P., Javidmehr, A., Teh, F.C., Acuña, S.C., Corbin, C.J., Conley, A., Bennett, W.A., Teh, S.J., 2016. Assessing oocyte development and maturation in the threatened Delta smelt, *Hypomesus transpacificus*. Environ. Biol. Fish 99, 423–432.

Lee, J., Bang, K., Ketchum Jr., L., Choe, J., Yu, M., 2002. First flush analysis of urban storm runoff. Sci. Total Environ. 293, 163–175.

Mallatt, J., 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Can. J. Fish. Aquat. Sci. 42, 630–648.

Matern, S.A., Moyle, P.B., Pierce, L.C., 2002. Native and alien fishes in a California estuarine marsh: twenty-one years of changing assemblages. Trans. Am. Fish. Soc. 131, 797–816.

Maxwell, D., Jennings, S., 2005. Power of monitoring programmes to detect decline and recovery of rare and vulnerable fish. J. Appl. Ecol. 42, 25–37.

McElreath, R., 2016. Statistical Rethinking: A Bayesian Course with Examples in R and Stan. CRC Press.

Meyers, T.R., Hendricks, J.D., 1985. Histopathology. In: Rand, G.M., Petrocelli, S.R. (Eds.), Fundamentals of Aquatic Toxicology. Methods and Applications. Hemisphere Publishing Corp, Washington, DC, pp. 283–331.

Moyle, P.B., 2002. Inland Fishes of California. University of California Press, Berkeley, USA.

Moyle, P.B., Brown, L.R., Durand, J.R., Hobbs, J.A., 2016. Delta smelt: life history and decline of a once-abundant species in the San Francisco estuary. San Francisco Estuary and Watershed Science 14.

Müller-Solger, A.B., Jassby, A.D., Müller-Navarra, D.C., 2002. Nutritional quality of food resources for zooplankton (daphnia) in a tidal freshwater system (Sacramento-San Joaquin River Delta). Limnol. Oceanogr. 47, 1468–1476.

Perry, S.F., Laurent, P., 1993. Environmental effects on fish gill structure and function. In: Rankim, J.C., Jensen, F.B. (Eds.), Fish Ecophysiology. Chapman and Hall, London, pp. 231–264.

Poleksic, V., Mitrovic-Tutundzic, V., 1994. Fish gills as a monitor of sublethal and chronic effects of pollution. In: MuÈller, by R., Lloyd, R. (Eds.), Sublethal and Chronic Effects of Pollutants on Freshwater Fish. FAO, Fishing News Books, Oxford, pp. 339–352.

Ruiz-Picos, R.A., Sedeño-Díaz, J.E., López-López, E., 2015. Histopathological indicators in fish for assessing environmental stress. In: Armon, R., Hänninen, O. (Eds.), Environmental Indicators. Springer, Dordrecht.

Ryder, R.A., Edwards, C.J., 1985. International Joint Commission. Great Lakes Regional Office, Ontario. Ministry of Natural Resources. Fisheries Branch, Great Lakes Fishery Commission, Conceptual Approach for the Application of Biological Indicators of Ecosystem Quality in the Great Lakes Basin. International Joint Commission (IJC) Digital Archive https://scholar.uwindsor.ca/ijcarchive/348.

Sansalone, J.J., Buchberger, S.G., 1997. Partitioning and first flush of metals in urban roadway storm water. J. Environ. Eng. 123, 134–143.

Schlenk, D., Benson, W.H., 2003. Target Organ Toxicity in Marine and Freshwater Teleosts: Organs. CRC press.

Schwaiger, J., Wanke, R., Adam, S., Pawert, M., Honnen, W., Triebskorn, R., 1997. The use of histopathological indicators to evaluate contaminant-related stress in fish. J. Aquat. Ecosyst. Stress. Recover. 6, 75–86.

Shaffer, G.P., Sullivan, M.J., 1988. Water column productivity attributable to displaced benthic diatoms in well-mixed shallow estuaries. J. Phycol. 24, 132–140.

Solangi, M.A., Overstreet, R.M., 1982. Histopathological changes in two estuarine fishes, *Menidia beryllina* (cope) and *Trinectes maculatus* (Bloch and Schneider), exposed to crude oil and its water-soluble fractions. J. Fish Dis. 5, 13–35.

Sommer, T., Armor, C., Baxter, R., Breuer, R., Brown, L., Chotkowski, M., Culberson, S., Feyrer, F., Gingras, M., Herbold, B., 2007. The collapse of pelagic fishes in the upper San Francisco estuary. Fisheries 32, 270–277.

Sommer, T., Mejia, F.H., Nobriga, M.L., Feyrer, F., Grimaldo, L., 2011. The spawning migration of delta smelt in the upper San Francisco estuary. San Francisco Estuary and Watershed Science 9.

Stentiford, G.D., Longshaw, M., Lyons, B.P., Jones, G., Green, M., Feist, S.W., 2003. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Mar. Environ. Res. 55, 137–159.

Teh, S., Adams, S., Hinton, D., 1997. Histopathologic biomarkers of anthropogenic stress in resident redbreast sunfish (*Lepomis auritus*) and largemouth bass (*Micropterus salmoides* lacèpède) from contaminant impacted sites. Aquat. Toxicol. 37, 51–70.

Teh, S.J., Zhang, G.h., Kimball, T, and F.C. Teh. 2004. Lethal and sublethal effects of esfenvalerate and diazinon on splittail larvae. In Early Life History of Fishes in the San Francisco Estuary and Watershed; Feyrer, F., Brown, L. R., Brown, R., Orsi, J. J., Eds.; American Fisheries Society: Bethesda, MD, 2004.

Teh, S.J., Baxa, D.V., Hammock, B.G., Gandhi, S.A., Kurobe, T., 2016. A novel and versatile flash-freezing approach for evaluating the health of Delta Smelt. Aquat. Toxicol. 170, 152–161.

Triebskorn, R., Telcean, I., Casper, H., Farkas, A., Sandu, C., Stan, G., Colărescu, O., Dori, T., Köhler, H.-R., 2008. Monitoring pollution in River Mureş, Romania, part II: metal accumulation and histopathology in fish. Environ. Monit. Assess. 141 (2008), 177–188.

US Fish and Wildlife Service, 1993. Endangered and threatened wildlife and plants; determination of threatened status for the delta smelt. Fed. Regist. 58, 12854–12864.

Werner, I., Deanovic, L.A., Connor, V., de Vlaming, V., Bailey, H.C., Hinton, D.E., 2000. Insecticide-caused toxicity to *Ceriodaphnia dubia* (CLADOCERA) in the Sacramento–San Joaquin River delta, California, USA. Environ. Toxicol. Chem. 19, 215–227.

Weston, D.P., Asbell, A.M., Lesmeister, S.A., Teh, S.J., Lydy, M.J., 2014. Urban and agricultural pesticide inputs to a critical habitat for the threatened delta smelt (*Hypomesus transpacificus*). Environ. Toxicol. Chem. 33, 920–929.

Weston, D.P., Moschet, C., Young, T.M., 2019. Chemical and toxicological effects on Cache Slough after storm-driven contaminant inputs. San Francisco Estuary and Watershed Science 17, 3.

Wolf, J.C., Wolfe, M.J., 2005. A brief overview of nonneoplastic hepatic toxicity in fish. Toxicol. Pathol. 33, 75–85.

Wolf, J.C., Baumgartner, W.A., Blazer, V.S., Camus, A.C., Engelhardt, J.A., Fournie, J.W., Frasca Jr., S., Groman, D.B., Kent, M.L., Khoo, L.H., 2015. Nonlesions, misdiagnoses, missed diagnoses, and other interpretive challenges in fish histopathology studies: a guide for investigators, authors, reviewers, and readers. Toxicol. Pathol. 43, 297–325.