Final Report of Histopathological Evaluation of Starvation and/or Toxic Effects on Pelagic Fishes

Title: Pilot Study of the Health Status of 2005 Adult Delta Smelt in the Upper San Francisco Estuary

Submitted To:

Randall Baxter
Senior Biologist Supervisor
California Dept of Fish and Game
Bay-Delta Region
4001 N. Wilson Way
Stockton, California 95205-2486
Phone (209) 942-6081

By

Swee J. Teh, Ph.D.
Department of Anatomy, Physiology, and Cell Biology
School of Veterinary Medicine,
University of California -Davis
Davis, California 95616
Phone (530) 754-8183
Fax (530) 752- 7690
Email: sjteh@ucdavis.edu

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Abstract

As part of a large multidisciplinary Interagency Ecological Program (IEP) to estimate pelagic organism decline, histopathology was employed as an initial weight-of-evidence approach to assess food limitation, disease, and health status on 385 adult delta smelt (*Hypomesus transpacificus*) in the upper San Francisco Estuary. Females were significantly larger than males. No evidence of food limitation was observed except for those collected from Sacramento Deep Water Channel (SDWC) in North Delta. In addition, there was no evidence of significant diseases and parasitic infestations. Liver lesions detected in smelt included glycogen depletion (GD), inflammation, cytoplasm eosinophilic inclusion, focal and diffuse fatty vacuolar degeneration (LIP), and single cell necrosis (SCN). Smelt collected from Suisun Bay, Suisun Marsh, and South Delta had higher GD, LIP, and SCN while those collected from North Delta had higher GD and SCN when compared to Central Delta. The sum of mean lesion scores was lowest in smelt collected from the lower Sacramento River near Sherman and Decker Islands indicating that smelt collected from Central Delta were in better health and that food limitation, disease, and contaminant stressors were unlikely factors in affecting the survival of smelts. Kidney and gonads lesions were minimal and do not seem to affect the survival and reproduction of smelt. In summary, histopathological biomarkers appear to be a valuable tool to detect starvation and to differentiate between contaminant and physicochemical stressors. Higher prevalence and severity of GD, LIP, and SCN could indicate contaminant exposure while higher prevalence and severity of GD and SCN with low LIP could indicate exposure to physicochemical stressors. It is suggested that restoration efforts should focus on Suisun Bay, Suisun Marsh and South Delta for contaminant effect and SDWC in North Delta for

physicochemical effects on smelt. In addition, the highest priority of the IEP management team should be continuous monitoring and protection of delta smelts and their habitat in the Central Delta, where delta smelt are thriving to help support and restore these fish populations.

1. Introduction

The San Francisco Estuary is a complex ecosystem formed by the convergence of water from the Sacramento and San Joaquin Rivers (SSJR) with the Pacific Ocean (Figure 1). Over the last 150 years, the Estuary has been markedly altered by human activities that in turn, have caused dramatic declines in the abundance of many species of fish. These declines have been attributed to habitat degradation and loss, reductions in freshwater outflow, entrainment losses to water diversions, entrainment at power plant intakes, food limitation, competition and predation from exotic invasive aquatic species, and to the toxicity of both legacy and contemporary environmental contaminants. Declines in both estuarine zooplankton and native fish suggest a potential trophic linkage.

The delta smelt (*Hypomesus transpacificus*), a small (60-80 mm SL) nearly translucent native fish with steel-blue lateral stripe is endemic to the upper San Francisco Estuary. Smelt is a short-lived (1-2 years) pelagic fish inhabiting low salinity habitat at the interface of inflowing fresh water from the Sacramento and San Joaquin Rivers and salt water from the Pacific Ocean (Moyle et al., 1992; Moyle, 2002). Adult Delta smelt mature by the end of their first year of life, and spawn in freshwater sloughs during late winter and spring. In moderate to high flow years, prespawning adults gather in the lower Delta and upper Suisun Bay, and disperse into the Delta for spawning (reviewed in Wang, 1991). The yolk-sac larvae once hatched from adherent eggs in fresh water will swim near the surface during the first few days after hatching toward the brackish water in Suisun Bay. The timing of the hatch relative to the amount of freshwater flowing into and through the Delta influences the transport and distribution of larval smelt. In wet years (e.g., 1995-1999, and 2003) post-larval/juvenile delta smelt were abundant in Suisun Marsh and Bay. Larval smelt grow significantly during the first seven to nine months of its life in Suisun Bay until the adult stages in the following year when they make their return journey upstream to spawn.

The delta smelt is a planktivorous species whose prey is selected depending on the age (and size) of the smelt and by the availability of zooplankton. The composition and abundance of zooplankton in the Delta have changed dramatically over the last 20 to 25 years, with populations experiencing declines in abundance and changes in the relative importance of certain species (Nobriga, 1998; Lott, 1998). These shifts in zooplankton communities have primarily been attributed to the effects of competition from exotic species, particularly the filter-feeding clam *Potamocorbula amurensis*. Although the introduction of *P. amurensis* is undoubtedly a significant factor in zooplankton declines (Alpine and Cloern, 1992), aquatic contaminants can adversely affect grazing rates, reproduction, sex ratios and survival of zooplankton (Medina et al., 2004; Sibley et al., 2004; Bengtsson et al., 2004; Ramirez-Perez et al., 2004). Contaminants have also been tied to decreased zooplankton diversity (Richards et al., 2004), suggesting that in the Delta and Estuary, direct toxicity to zooplankton may also be an important factor in population declines (Kuivila and Foe, 1995).

Histopathologic biomarkers of toxic injury, dysfunction and carcinogenesis are now well established and have been proven to be sensitive and valuable tools to detect direct toxic effects of metals and pesticides within target organs of fish in laboratory experiments (Wester and

Canton, 1991; Schwaiger et al., 1992; Teh et al., 2004a; Teh et al., 2004b; Teh et al., 2005) and as a sensitive and reliable indicators for the health assessment of wild fish populations responses to a variety of natural and anthropogenic environmental stressors such as contaminants, food quality and quantity, salinity, temperature, and pathogens in field investigations (Adams et al., 1999; Hinton et al., 1992; Teh et al., 1997; Schwaiger et al., 1997; Feist et al., 2004; Zimmerli et al., 2007). Adult fish health is a resultant of cumulative insults from prior acute and chronic adverse environmental conditions. Histopathological biomarkers differentiate between organ lesions induced by diseases and other environmental factors from those lesions due to contaminant exposure, and help differentiate acute and sublethal lesions which occurred within weeks as a result of contaminant exposure from chronic lesions induced by contaminant exposure that occurred months and years ago (Teh et al., 1997; Schwaiger et al., 1997; Teh et al 2004b; Teh et al 2005).

Historically, the San Francisco Delta and Estuary supported well over 100 species of fish. Since the 1970's, fish populations have been declining, with particularly dramatic declines in delta smelt populations. By 1993, the delta smelt was listed as a threatened species (USFWS, 1993) with both food availability and contaminant-induced stress as possible factors in their decline (Moyle et al., 1992; Bennett and Moyle, 1996; Bennett et al., 1998). In 2005, abundance of delta smelt plummeted to the lowest levels ever recorded, i.e., approximately 2.4 percent of the abundance measured when the species was listed in 1993 (Greiner et al., 2006). The reports of decline in multiple species of fish populations and Delta food web stimulated an initial examination of the health of adult smelt in the San Francisco Estuary. The objective of this study is to use histopathology as a screening tool to establish an initial weight of evidence of food limitation and contaminant effects in adult smelt. In addition, this study seeks to identify histopathological biomarkers which may occur from environmental stressors other than contaminants (Schwaiger et al., 1997, Teh et al., 1997).

2. Materials and methods

2.1 Sampling locations and smelt collection

The Spring Kodiak Trawl Survey of California Department of Fish and Game monitors yearly adult delta smelt distribution and relative abundance and runs every other week beginning January to the end of February. Each survey takes 4-5 days and samples 39 stations from the Napa River to Stockton on the San Joaquin River, and up to Walnut Grove on the Sacramento River (Figure 1). In this study, 385 delta smelt (n= 1 to 48 per station) were selected from 22 stations (340, 418, 501, 519, 606, 609, 610, 699, 704, 706, 716, 719, 799A, 799B, 801, 809, 812, 815, 902, 906 and 920) from the Spring Kodiak Trawl Survey of California Department of Fish and Game between January and February in 2005 (Figure 1, Table 1). These stations were collectively assigned to five subjective geographic regions i.e., Suisun Bay, Suisun Marsh, Central Delta, South Delta, and Northern Delta including Sacramento Deep Water Channel in the upper San Francisco Estuary. Since only three fish were collected from Station 340, Napa River data will be shown but will not be discussed in this study.

2.2 Sample preparation, processing, and histopathology

Upon being captured and sorted from other fish species on the boat, adult smelts were measured (total length) and decapitated immediately; Heads were fixed in 100% ethanol for otolith microchemistry as described elsewhere. Bodies were immediately fixed in 10% neutral buffered formalin before shipment to University of California, Davis. Upon receipt, decapitated fish were trimmed to remove anal fins then longitudinally divided into two identical sections with a surgical blade, dehydrated in a graded ethanol series and embedded with both surgical cut sections face down in paraffin. Serial longitudinal sections (2-3μm) were stained with hematoxylin and eosin (H&E), and lateral views of gonads, livers, and kidney were examined under a light microscope for lesions.

Histopathological analyses were conducted to evaluate the health status of individual smelt. Stomach was screened for the presence or absence of food. Liver, kidney and gonad alterations were qualitatively scored on a scale of (0 = not present, 1= mild, 2= moderate, and 3 = extensive severe pathological alterations). Lesion scores were further semi-quantitatively evaluated by statistical analysis. Prevalence of lesion scores was used to establish percentage of fish with significant organ damage. Mean of individual organ lesion scores was used to compare severity of lesions between stations. Mean of all organ lesion scores was used to establish health status of smelt for each station. Finally, mean lesion of stations within each region were combined and processed to assess regional differences in health status of adult smelt. Regions with higher total mean lesion score were considered to have poorer health than regions with lower mean lesion score.

2.3 Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) to determine station and regional health effects of smelt using STATISTICA6.0 software. We used the $p < 0.05$ level of significance. Results are expressed as 'mean ± standard error'.

3. Results

3.1 Growth

The average length of adult smelt collected in 2005 was 68.23 ± 0.22 mm (n = 385). Females (69.18 ± 0.27 mm; n=241) were significantly larger ($p<0.0001$) than males (66.80 ± 0.33; n=144). Furthermore, smelt collected from stations 704 and 706 were significantly smaller than fish collected from the rest of the stations ($p < 0.05$) (Table 1).

3.2 Food Limitation and Diseases

Figures 2A and 2B show the prey items in stomach of male and female smelts. There was no evidence of food limitation in adult smelt collected from all stations except for those collected from Stations 799A and 799B. Nor was there any evidence of severe parasitic and disease infestations (Table 1). Of those with parasitic infections (6 of 385 fish) and disease (14 of 385 fish), only focal and mild inflammatory reactions were observed (Table 1).

3.3 Liver Histopathology

Typical healthy and reproductive-active adult female and male smelt are shown in figures 2 C-2F. Smelt liver was characterized by the presence of 'lacy', irregular, and poorly demarcated cytoplasmic vacuolation typical of glycogen in the hepatocytes (Figures 2C and 2D). Figures 2E and 2F show various stages of ovarian and testicular development in the reproductive-active prespawning female and male smelts. Mature and active female livers had predominantly smaller and higher number of hepatocytes (Figure 2C) whereas mature and active males tend to have larger but less numerous number of hepatocytes in the parenchyma (Figure 2D). Several types of histopathological changes in liver were observed in smelt including cytoplamic glycogen depletion (GD), lipid or fatty vacuolar degeneration (LIP), cytoplasmic inclusions or eosinophilic protein droplets (CI/EDP), and single cell necrosis (SCN) (Table 2). No preneoplastic and neoplastic lesions were found. None of the liver examined had moderate to severe macrophage aggregate and only few fish collected from Central Delta (Station 716, n= 1 of 42 fish) and North Delta (Station 719, n= 1 of 32 fish; Station 799A (n= 2 of 29 fish; Station 799B, n= 2 of 62 fish) had moderate to severe cytoplasmic inclusions or eosinophilic protein droplets (Table 2). Qualitative analysis indicated higher percentages of fish collected from Suisun Bay (Stations 501 and 519), Suisun Marsh (Stations 606, 609, 610, and 699), South Delta (station 812), and North Delta (Station 719, 799A, and 799B) had significant reduction in liver glycogen (Figure 3A) when compare to Central Delta (Stations 704, 706, and 716) (Table 2). In addition, prevalence of fatty vacuolar degeneration (Figures 3B and 3C) characterized by the presence of clear, round, and well demarcated cytoplasmic vacuoles with nuclei often displaced to the periphery of hepatocytes were higher in smelt collected from Suisun Bay (Stations 501), Suisun Marsh (Stations 606, 609, 610, and 699), Central Delta (Stations 704 and 706), and South Delta (station 812) (Table 2). While single cell necrosis (Figure 3B) which is characterized by cells having eosinophilic (i.e., pink coloration) cytoplasm with nuclear pyknosis and karyorrhexis were higher in fish collected from Suisun Marsh (Station 609), North Delta (Station 799A), and South Delta (Station 812).

Figure 4 shows the individual mean liver lesion score of smelt from each station. Glycogen depletion were significantly higher ($p<0.05$) in smelt collected from Suisun Marsh (Station 606) and North Delta (Stations 799B) than in smelt collected from Central Delta (Station 706). Fatty vacuolar degeneration which is uncommon in smelt liver was significantly higher ($p<0.05$) in Suisun Marsh (Station 699) and South Delta (Station 812) when compared to Central Delta

(Station 716). Single cell necrosis was significantly higher ($p<0.05$) in smelt colleted from Suisun Marsh (Station 609) and North Delta (Station 799A) than smelt collected from Central Delta (station 706). To assess health status of smelt, we compared mean of the combined individual liver lesions (GD, LIP, SCN, and CI/EDP) among stations (Figure 5). Smelts collected from Suisun Marsh (Station 609) and South Delta (Station 812) had significantly higher ($p<0.05$) combined mean lesion score than smelt collected from all three Central Delta stations (Stations 704, 706, and 716). North Delta (Stations 799A and 799B) and Suisun marsh (Station 606) had significantly higher mean lesion score when compared to Central Delta (Station 706). Since smelt has wide-ranging movement patterns and may incur stress at other areas in the San Francisco Estuary, stations within a geographical region were combined and health statuses among these regions were compared as shown in Figure 6. Smelt collected from Central Delta were significantly ($p<0.05$) healthier than those collected from Suisun Marsh, North and South Delta. There were no significant differences in health status of smelt among Suisun Bay, Suisun Marsh, North Delta, and South Delta.

3.4 Kidney and Gonads Histopathology

Kidney and gonads lesions of 2005 smelt were low and were not found in most of the sampling stations (Table 2). Therefore, only qualitative data will be presented below. Kidney lesions such as glomerular nephritis and tubular cell necrosis were more prevalent in smelt collected from station 799A of North Delta (Table 2, Figure 7). Ovarian cell necroses (OCN), characterized by the presence of necrotic oocytes and atretic follicular cells (Figure 8), were found in 5 of 31 females from station 716 and 3 of 44 females from station 799B. Intersex, characterized by the presence of oocytes in testis (Figure 9), were observed in males collected from stations 609 (2 of 17 males), 704 (1 of 8 males), 716 (2 of 11 males), 799A (1 of 11 males), and 799B (3 of 18 males).

4. Discussion

In 2005, Fall Midwater Trawl Survey performed by the California Department of Fish and Game (CDFG) indicated the lowest delta smelt abundance index on record (Greiner et al., 2006). However, the causes and evidences for the widespread delta smelt's decline are apparently unknown due to the complex and variable natural and anthropogenic activities in the San Francisco Estuary. The purpose of this study was to use histopathology as screening tool to establish an initial weight-of-evidence of food limitation, disease, and contaminant effects in adult delta smelt. We conclude that the overall health status of adult smelt is not affected by food limitation (88.8% have prey items in gut) and diseases (1.3% have internal parasite). Similarly, CDFG studies on 2005 age-0 delta smelt condition and health indices indicated that 100% of smelt examined had prey items in gut, 0% had external parasite and 2% had internal parasite (Unpublished report). The most prominent findings in this study were liver histopathology which clearly differentiates among smelts from different stations and geographical regions. Smelt collected from Central Delta were healthier than those collected from North Delta, South Delta, and Suisun Marsh (Figure 6). Kidney, testis and ovarian lesions were clearly mild injuries in terms of immediate survival, therefore are unlikely causes of a low smelt abundance index.

During the course of migration to fresh water for spawning in late winter/early spring, pre-spawning adult smelts usually gather in the lower Delta and upper Suisun Bay before disperses into the upper Delta for spawning. Therefore, dependent on their migration routes and stations where pre-spawn smelts resided, fish may experience unfavorable environmental conditions (e.g., starvation, temperature, and entrainment by water diversion) and/or contaminant exposures which affect their health status and eventually their spawning success. Fish liver is a well known target organ in toxicopathic investigation because of its function in food conversion, storage, biotransformation and excretion of xenobiotics, and protein productions (e.g., chorogenesis and vitellogenesis) for reproduction. Thus, fish livers have been extensively used for the health assessment of wild fish populations (Teh et al., 1997, Feist et al., 2004). Metabolic disorders indicated by increase of cell death, fatty vacuolar degeneration and/or glycogen depletion have been suggested as early liver injury in response to toxic chemicals exposure as documented in other studies of fish in marine and freshwater habitats (Rhodes et al., 1987; Hinton and Lauren, 1990; Schwaiger et al., 1997; Teh et al., 1997; Adams et al., 1999; Teh et al., 2005). It is well-known that the disruption of liver functions will result in significant negative consequences for growth, health and reproductive success of individuals and their progeny and eventually lead to adverse effects to whole populations (Adams et al., 1990; Hinton, 1993). The types of liver histopathological lesions observed in this study indicate that smelt are responding to both contaminant and physicochemical stressors such as low dissolved oxygen, starvation, temperature, and entrainment in water diversions. We are aware that other unknown sampling station differences that were not mentioned herein may have contributed to the results of this study. Our results strongly suggest that the effects of stress on pre-spawning smelt residing at different locations are different. While we cannot conclusively state that the differences are due to contaminants, certainly the high prevalence of glycogen depletion, fatty vacuolar degeneration and single cell necrosis strongly suggest that this is indeed the case for smelt collected from Suisun Marsh and South Delta (Table 2). A substantial amount of laboratory and field information has been accumulated associating fatty vacuolar degeneration or lipidosis with exposure to anthropogenic toxicants in fish (Meyers et al., 1985; Adams, 1990; Hinton and Lauren, 1990; Teh et al 1997; Adams et al 1999; Teh et al., 2005). Therefore, fish collected from North Delta (SDWC) that have a high prevalence of glycogen depletion and single cell necrosis but low in fatty vacuolar degeneration suggest the likely effects of physicochemical stressors (e,g., low dissolved O_2 or high CO_2 levels) instead of contaminant exposure. Furthermore, severe macrophage aggregates which were observed in 2003 smelt (data not shown) were not seen in 2005 smelt. The absence of macrophage aggregates in liver of 2005 smelt suggests that the histological alterations are recent and probably occurred within a few weeks before sampling. Macrophage aggregates have been suggested as indicators of contaminant exposure and more often, as a generalized nonspecific response to several stressful stimuli (including starvation, heat stress, bacterial infections, parasitic infestation and age) (Wolke et al., 1985: Herraez, 1986; Blazer et al., 1987; Couillard et al., 1999), but was not a significant lesion in current study. Therefore, prior acute and chronic exposure, e.g., during their larval and juvenile stages, to the above stressful stimuli is unlikely the cause for the significant liver lesions seen in the current study. In fact, histopathology results strongly suggest that contaminant and physicochemical effects are current and are correlated to the locations or regions at which the smelt were collected. Conservatively, we are convinced that there are regional differences in health status of smelt in the San Francisco Estuary. Further study investigating the smelt larvae, which have smaller distribution range, is warranted.

Although kidney and gonad lesions were observed, these lesions were limited to small percentages of fish (Table 2). Kidney is a well known target organ for diseases, heavy metals and organic xenobiotics studies. Glomerular nephritis (GN), tubular cell necrosis (TBN), cellular debris and proteinaceous casts within the dilated tubular lumina (TBI/D) have been reported as a consequence due to contaminant exposure and physicochemical condition such as high CO_2 levels (Harrison and Richards, 1979; Gill et al., 1989; Goering et al., 1993; Fischer-Scherl et al., 1991). It is unclear whether contaminant and/or physicochemical stressors are the consequence for the higher prevalence of glomerular nephritis and tubular cell necrosis seen in smelt collected from Sacramento Deep Water Channel (Station 799A) of North Delta. Based on our liver histopathology results, we believe that food depletion (24% of fish have food in stomach; Table 1) and physicochemical factors such as salinity fluctuations, low dissolved oxygen, and temperature are likely the results of the higher prevalence of kidney lesions in Station 799A.

There are relatively few histopathologic lesions of gonads in fish exposed to a variety of toxicants and physiochemical stressors (Glasier, 1986). Oocytes or ovarian cell necrosis is a normal occurrence in the ovaries of all fish species. These changes can be exacerbated by disease, malnutrition or exposure to xenobiotic compounds (Reviewed in Hinton et al., 1993). In addition, OCN can also be observed in mature females as a consequence of mature egg re-absorption due to failing to mate. In this study, relatively few adult female smelts have severe OCN which indicates that the reproduction of smelt is not impaired. The presence of intersex (i.e., ovatestis) in smelt can result from exposure to endocrine disrupting chemicals or occurred spontaneously. Since there is little information on endocrine disruption effects on delta smelt, additional laboratory evaluation is needed to identify the cause.

In summary, histopathological biomarkers appear to be a valuable tool to detect starvation and to differentiate between contaminant and physicochemical stressors effects in adult delta smelt. In addition, it indicates that the stressor effects were current and there are regional differences on fish health. Contaminant and physicochemical stressors can weaken fish rendering them more susceptible to mortality from numerous causes such as predation and diseases. A long-term depletion of energy reserved in liver can threaten the survival and reproduction of adult smelt. The histopathology results suggest restoration efforts should focus on affected regions to mitigate contaminant and physicochemical effects on pre-spawning adult delta smelt. The likely explanation for the lack of interaction between contaminant/physicochemical and disease effects in smelt of this study is in part due to the bias of sampling adult fish. Smelt are known to be extremely sensitive to environmental stressors (Moyle et al., 1992). As a matter of fact, there is a common phrase used to describe smelt by many research scientists: " You can kill a smelt just by looking at it". Therefore, it is likely that we are examining smelt that have survived from the environmental hardship and omitted those that did not survive to the spawning stage. Finally, adult smelt movements in the San Francisco estuary are influence by many factors including tidal fluxes, food, environmental barriers, temperature, and salinity making it extremely difficult to attribute cause-and-effect of deleterious health of smelt. Future studies should focus on larval and juvenile smelts which are more sensitive to environmental stressors than adult smelts.

5. Acknowledgements

This study was funded by IEP POD at Department of Water Resources.

Table 1 Collection and survey sites of delta smelt between January and March 2005

Location (Site no.)	Location description	N	SEX F/M	Length F	Length M	%FS	ND/P
340	Napa River, along Vallejo seawall and park	3	3/0	70.67±2.29	NA	67	0/0
418	SB, Mothball fleet opposite Avon oil dock	3	3/0	70.67±2.29	NA	100	0/0
501	SB, between Roe and Ryer Islands	14	8/6	67.44±1.32	64.83±1.62	100	1/0
519	SB, west end of Honker Bay	9	5/4	69.40±1.77	67.00±1.98	89	0/0
606	SM, Montezuma slough off Joice Island	40	28/12	71.44±0.99	66.08±1.10	87	1/0
609	SM, Montezuma slough in front of mouth of Nurse slough	48	31/17	70.29±0.62	67.68±0.79	98	2/1
610	SM, Montezuma slough at Birds landing	11	7/4	70.43±1.50	63.50±1.98	100	0/1
699	SM, from first bend toward mouth of Nurse slough	29	12/17	70.54±1.10	68.71±0.96	100	0/0
704	CD, Sacramento River, Sherman island lake at lights 11 & 12	20	12/8	63.80±1.25	63.89±1.32	100	1/0
706	CD, Sacramento River below southern tip of Decker island	29	15/14	66.00±1.06	63.93±1.02	84	0/0
716	CD, Cache slough north of cable ferry 1 & 51 near Boat sheds	42	31/11	67.71±0.71	67.20±1.25	94	2/0
719	ND, in SDWC, between lights 59 & 60	32	16/16	69.25±0.81	66.91±0.83	81	1/1
799A	ND, in SDWC, between lights 63 & 64	29	18/11	70.13±0.99	68.62±1.10	24	2/2
799B	ND, from SDWC, Cache Slough, & Steamboat Slough	62	44/18	69.54±0.58	67.78±0.93	77	4/1
812	SD, San Joaquin River at middle of Broad Slough (801;n = 1), in north channel opposite to Jersey point (809;n=2), lower San Joaquin River (812; n=5), at mouth of Little Potato slough (815;n=1), Old River at boat dock on Holland tract levee (902; n=1), at Medford Island (906;n=1), and South fork of Mokelumne River in Sycamore slough (920;n=2)	14	8/6	70.43±1.50	67.00±1.77	93	0/0

SB= Suisun Bay
SM= Suisun Marsh
CD= Central Delta
ND= North Delta
SDWC = Sacramento Deep Water Channel
SD= South Delta
%FS = Percentage of smelt with food in stomach
ND/P = Number of smelt with diseases and/or parasites. Disease is characterized by the influx of mononuclear inflammatory cells (macrophages and lymphocytes) and parasite is characterized by trematode presence in kidney, liver and gonads.
F= 241 females (average length = 69.18±0.27 mm), M= 144 males (average length = 66.80±0.33mm)

Table 2 Prevalence and severity of histopathological alterations in liver, kidney, ovary and testis of smelt

Location (Site no.)	n	Liver Prevalence of Lesion (%)*					Kidney Prevalence of Lesion (%)*				Gonads Prevalence of Lesion (%)*	
		GD	LMA	LIP	CI/EPD	SCN	GN	TBI/D	TBN	EPD	OCN/AF	INT
340	3	100	0	100	0	0	0	0	0	0	0	0
418	3	100	0	0	0	0	0	0	33	0	0	0
501	14	43	0	21	0	7	0	7	0	0	0	0
519	9	78	0	11	0	11	0	0	0	11	20	0
606	40	60	0	15	0	10	0	0	0	3	4	0
609	48	58	0	27	0	23	0	0	0	2	0	12
610	11	45	0	27	0	9	0	0	0	0	14	0
699	29	41	0	41	0	10	0	7	7	0	0	0
704	20	30	0	15	0	10	0	0	0	0	0	13
706	29	31	0	17	0	10	3	0	0	0	0	0
716	42	36	0	7	2	12	0	2	0	5	16	18
719	32	53	0	9	3	3	0	9	0	0	0	0
799A	29	59	0	3	7	34	17	7	14	0	17	9
799B	62	63	0	8	3	10	0	0	2	3	5	17
812	14	57	0	36	0	21	0	7	0	0	0	0

*For qualitative purpose, only pathological alterations of moderate (rank 2) and severe (rank 3) were considered as significant lesions.
GD= glycogen depletion; LMA= macrophage aggregate; LIP= lipidosis or fatty vacuolar degeneration; CI/EDP= cytoplasmic inclusions or eosinophilic protein droplets, SCN= single cell necrosis; GN= glomerular nephritis is characterized by fibrosis of glomerular tuft, shrunken glomerulus and cellular debris within the dilated glomerular space; TBI/D= cellular debris and bluish cast materials within the dilated tubular lumina; TBN= tubular cell necrosis; EPD= eosinophilic protein droplets within tubular epithelial cells; OCN/AF= Oocyte necrosis or atretic follicle in ovary; INT (intersex) is characterized as the presence of oocytes in testis (Ovatestis).

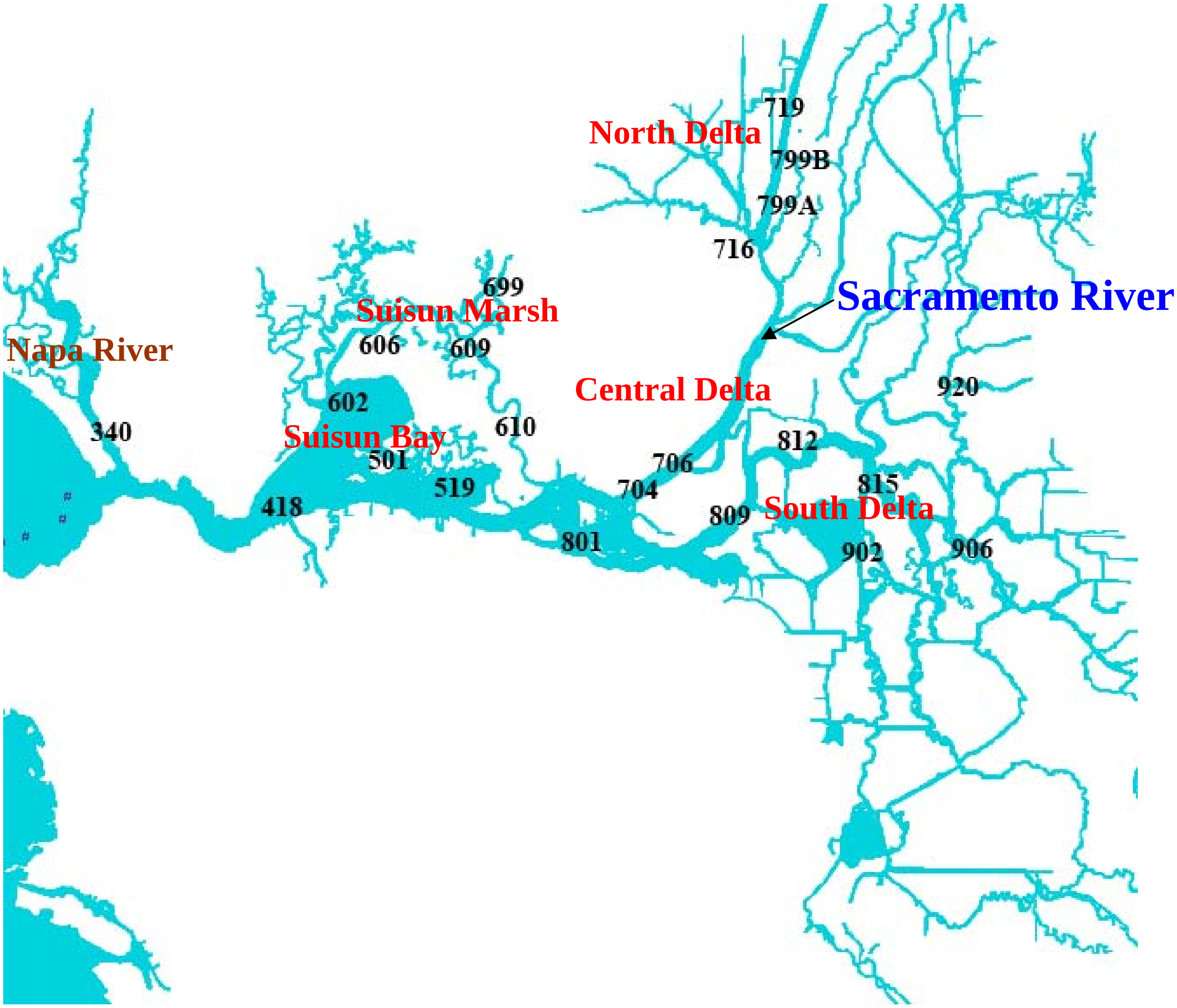

Figure 1 Map of the San Francisco Estuary showing sampling locations

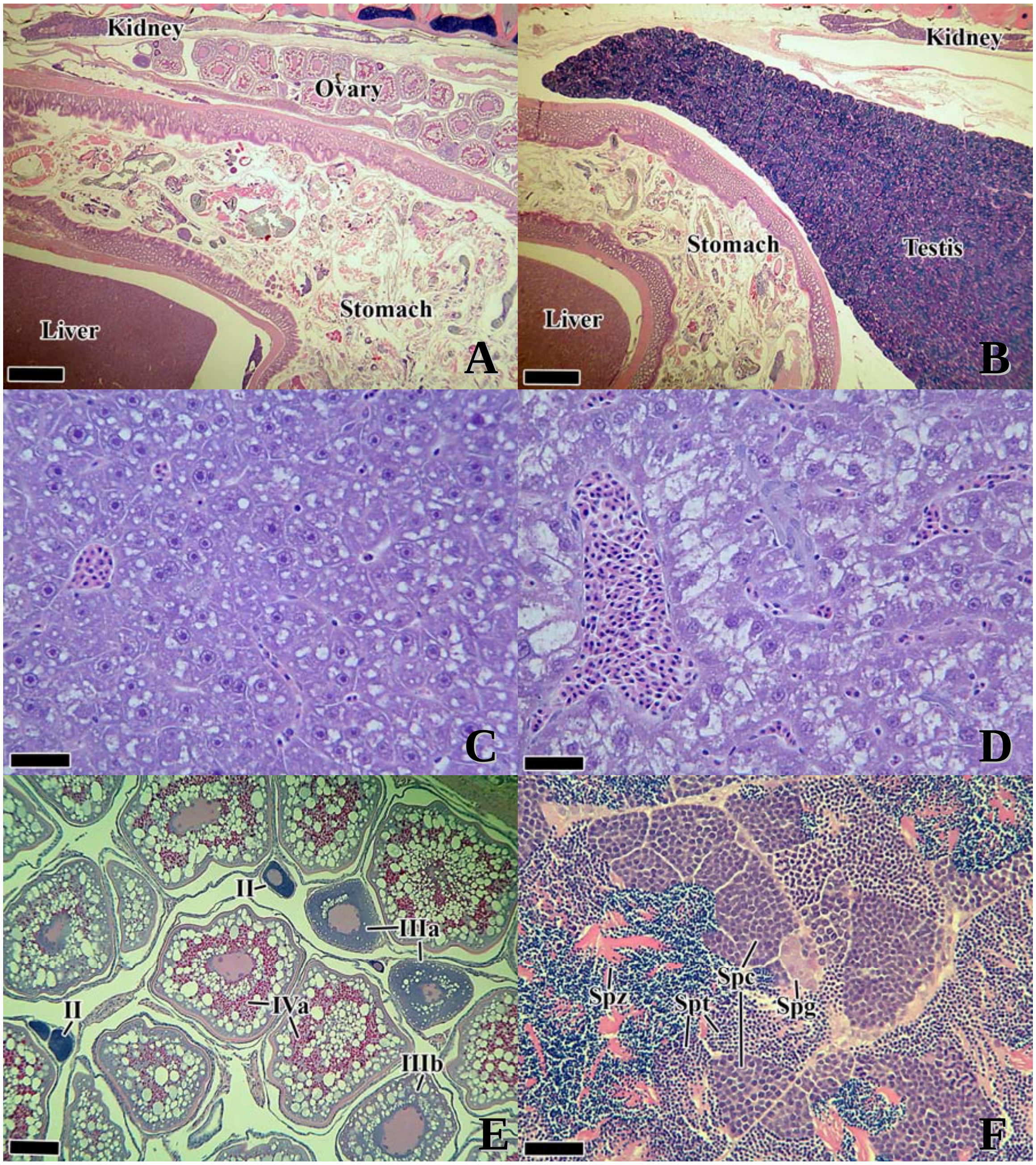

Figure 2. Hematoxylin and eosin (H&E) stained histological sections showing abundant prey items in stomach of normal adult female (A) and male (B) delta smelts collected from Station 716 in Central Delta. Bars = 500 µm. Higher magnifications of A and B showing 1) normal morphology of female (C) and male (D) glycogen-rich hepatocytes. Note the smaller and more abundant hepatocytes in female liver when compared to the male liver. Bars = 30µm; and 2) various stages of normal oocytes (E, Bar = 100µm) and sperm cells development (F, Bar=30 µm). II = perinucleolar stage, IIIa = Early corticle aveoli stage, IIIb = late corticle aveoli stage, IVa = early vitellogenic stage with small yolk spheres; Spg = spermatogonia; Spc = spermatocytes; Spt = spermatids; Spz = spermatozoa.

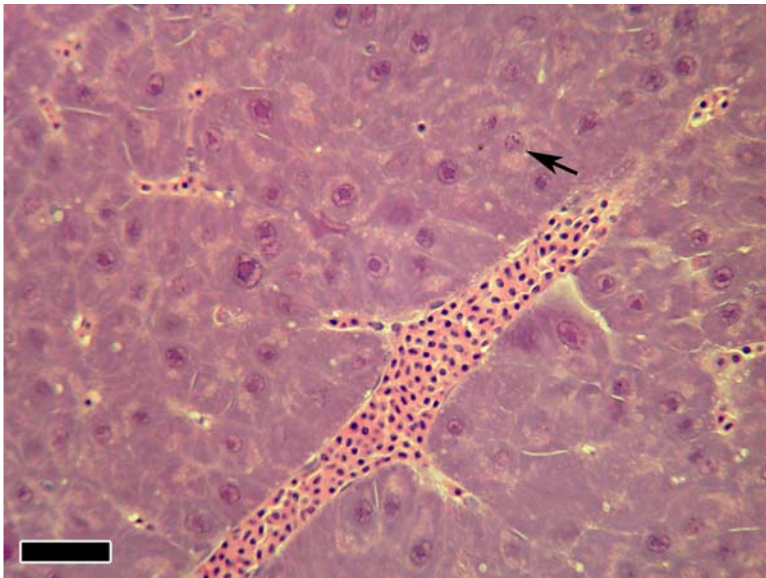

Figure 3A. Liver section of adult smelt collected from Sacramento Deep Water Channel (Station 799A) in North Delta showing severe glycogen depletion. Arrow is pointing to a necrotic cell. Bar = 30 µm. H&E.

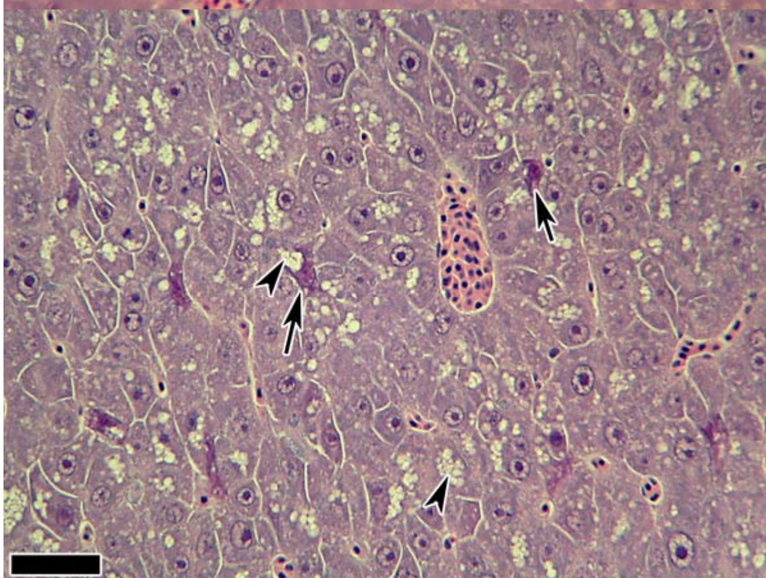

Figure 3B. Liver section of adult smelt collected from Station 609 in Suisun Marsh showing severe single cell necrosis (arrows) and mild lipidosis (arrowheads). Bar = 30 µm. H&E.

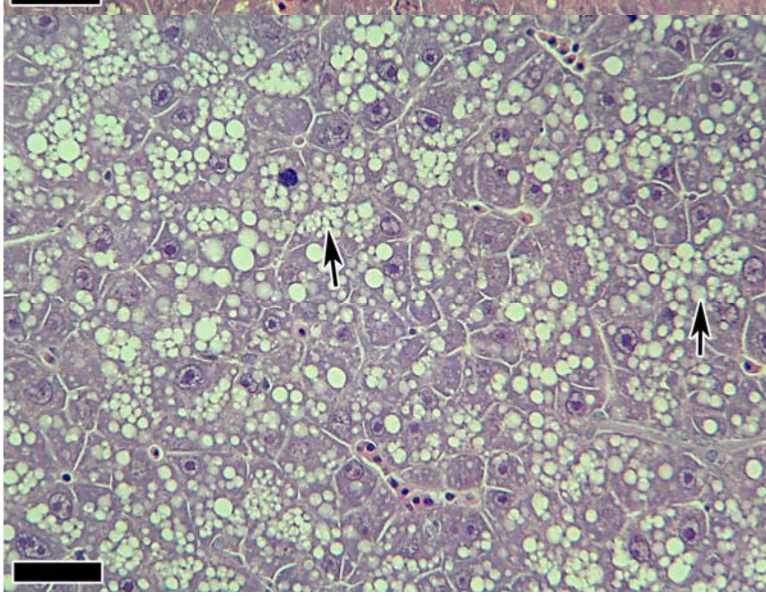

Figure 3C. Liver section of adult smelt collected from Station 609 in Suisun Marsh showing severe fatty vacuolar degeneration or lipidosis (arrows). Bar = 30 µm. H&E.

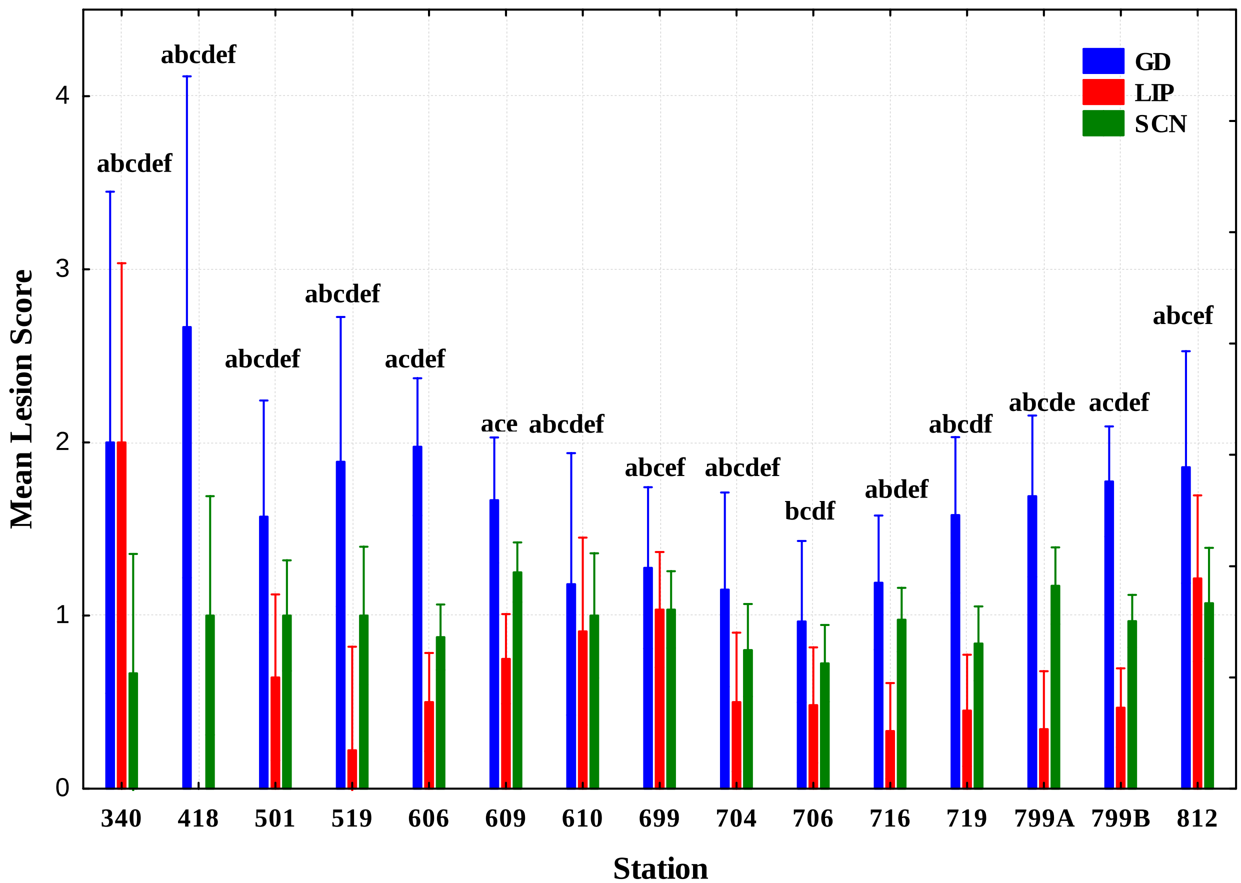

Figure 4. Mean ± standard errors of individual semi-quantitative score lesion in liver of smelts from each station. GD= glycogen depletion; LIP= lipidosis or fatty vacuolar degeneration; and SCN= single cell necrosis. Significant differences (P <0.05) between stations for GD were indicated by a and b, LIP by c and d, and SCN by e and f.

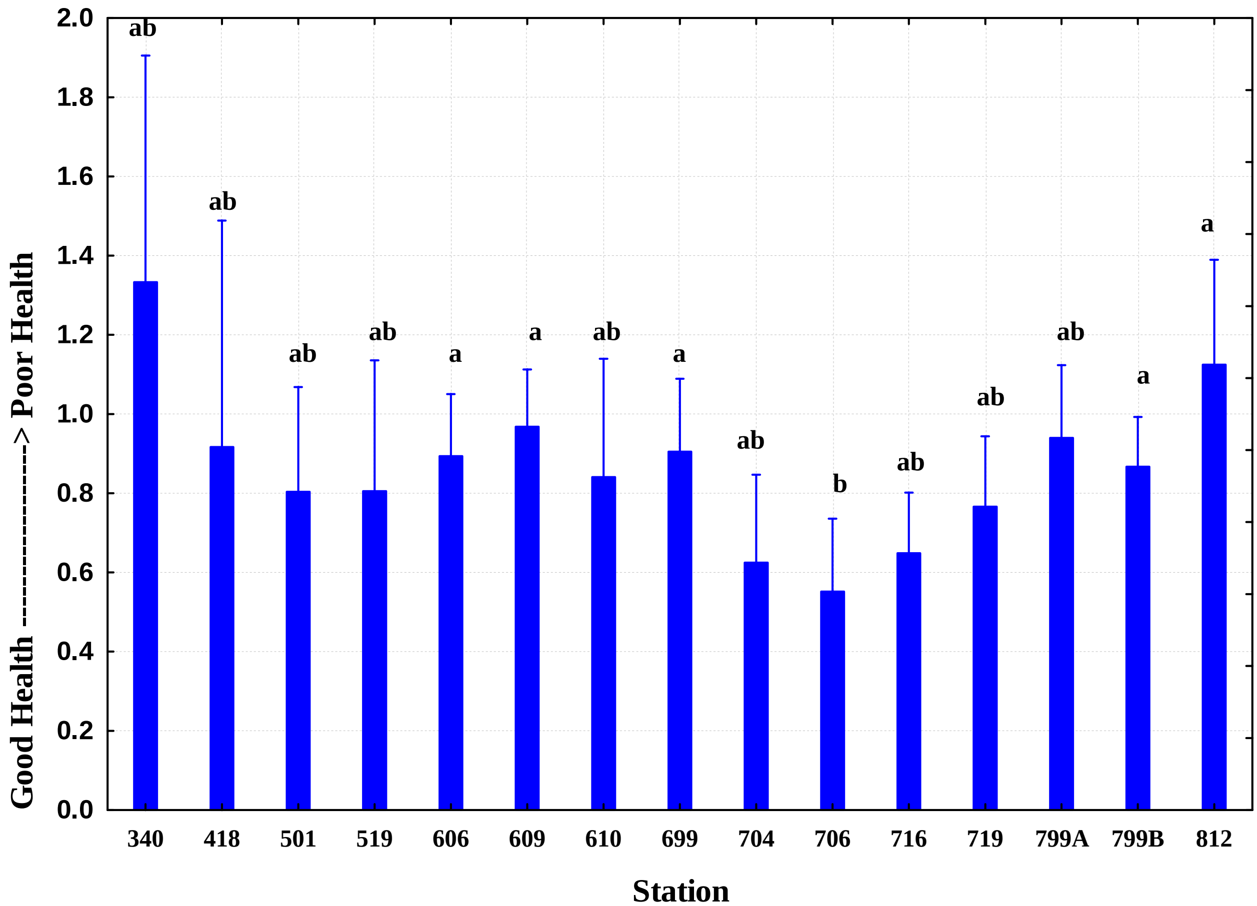

Figure 5. Mean ± standard errors of all lesion scores (GD, LIP, SCN, and CI/EDP) within each station. Higher mean signified poorer health of fish in each station. Significant differences (P <0.05) between stations were indicated by a and b.

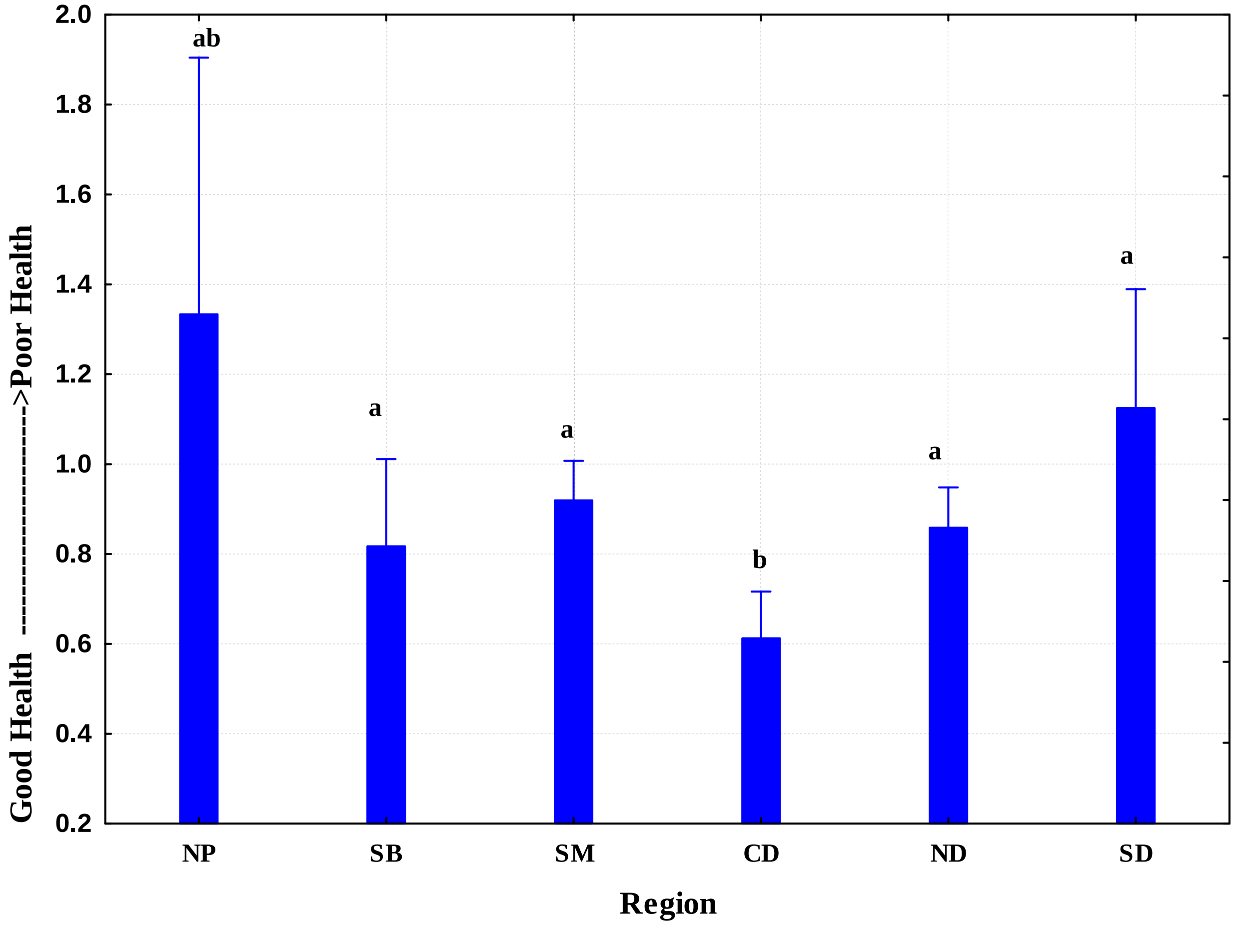

Figure 6. Mean ± standard errors of all station lesions within each region. Higher mean signified poorer health of fish in each station. Significant differences (P <0.05) between stations were indicated by a and b.

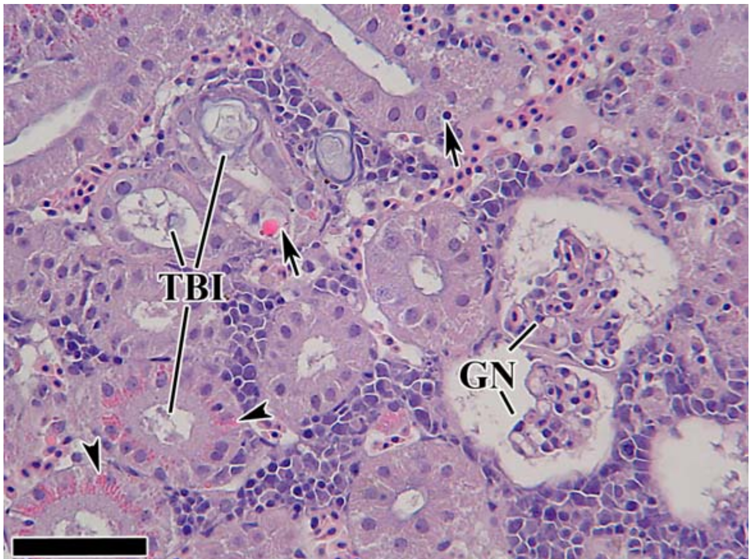

Figure 7. Kidney section of adult smelt collected from Sacramento Deep Water Channel (Station 799A) in North Delta showing the presence of eosinophilic or protein droplets within single tubular cells (arrowheads), cellular debris and bluish cast materials within the dilated tubular lumina (TBI), and cellular debris within the dilated glomerular space (GN). Arrows are pointing to tubular epithelial cells necrosis, Bar = 50 µm.

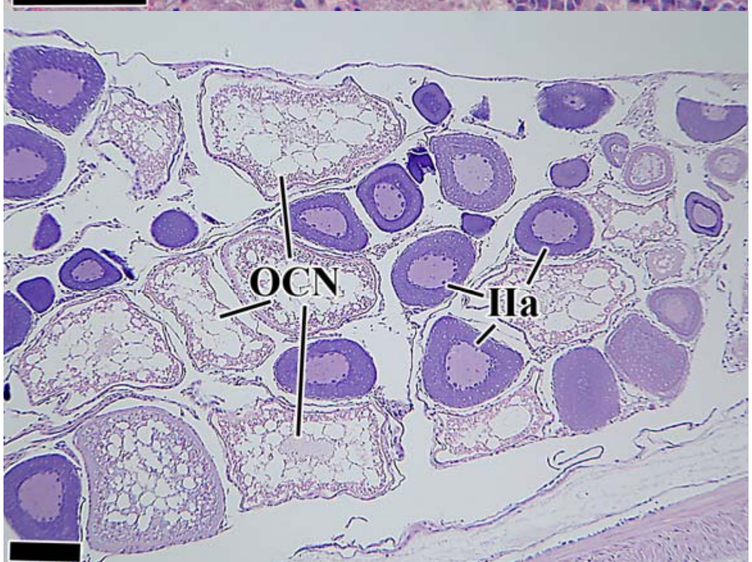

Figure 8. Severe ovarian cell atresia or necrosis (OCN) in adult smelt collected from Station 519 in Suisun Bay. IIa = immature oocytes at perinucleolar stage. Bar = 100 µm. H&E.

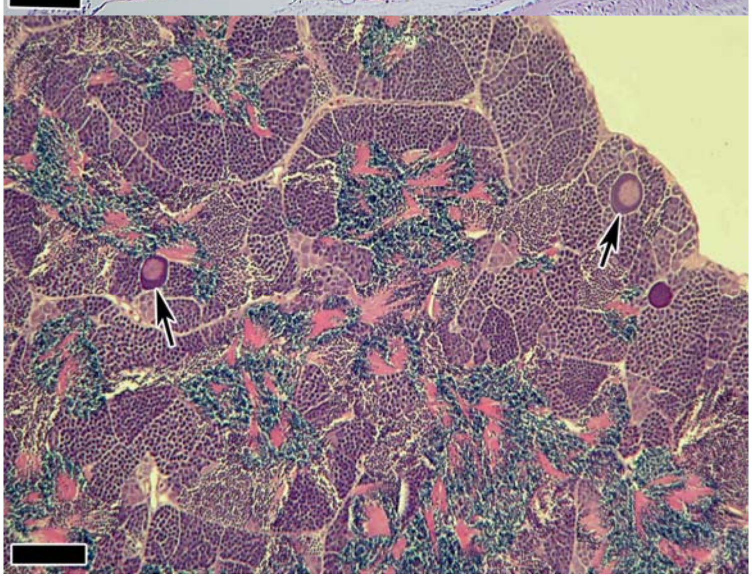

Figure 9. Intersex in adult smelt collected from Station 609 in Suisun Marsh. Arrows point to the immature oocytes. Bar = 50 µm. H&E.

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