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Contaminant Concentrations and Histopathological Effects in Sacramento Splittail (*Pogonichthys macrolepidotus*)

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Abstract Sacramento splittail (*Pogonichthys macrolepidotus*) is a species of special concern in California, due to multiple anthropogenic stressors. To better understand the potential impact of contaminant exposure, adult splittail were captured from the Sacramento-San Joaquin River Delta (California, USA) and analyzed for histopathology and contaminant exposure. Organochlorine contaminants (PCBs, DDTs, dieldrin, chlordanes, and PBDEs) and trace metals (Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se, Sn, V, and Zn) were detected in the tissues of all fish. In many samples, human health screening values were exceeded for PCBs (83 of 90 samples), DDTs (32 samples), and dieldrin (37 samples). In contrast, thresholds for fish effects were rarely exceeded. Histopathological analysis indicated the presence of macrophage aggregates in gonads, kidneys, and liver and a high incidence of liver abnormalities. In the liver, observed effects were often moderate to severe for glycogen depletion (55 of 95 fish), lipidosis (hepatocellular vacuolation; 51 fish), and cytoplasmic inclusion bodies (33 fish). Correlations between histopathology and tissue contaminant concentrations were weak and inconsistent. Significant correlations were observed between histopathology indicators and reductions in fish size, body condition, lipid content, and liver weight. These results suggest that splittail histopathology varies as a function of health and nutritional status, rather than exposure to legacy organic and metal pollutants.

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The Sacramento splittail (*Pogonichthys macrolepidotus*) is classified as a species of special concern in the State of California (U.S. Fish and Wildlife Service 2007). Multiple factors are believed to threaten the species, including predation (by both wildlife and human sport fish consumers), competition, disease, entrainment by water diversions and power plant intakes, alien species, changes to estuarine hydraulics, and exposure to pollutants (Moyle et al. 2004).

All life stages of splittail are potentially exposed to varying amounts and mixtures of chemical contaminants in the Sacramento-San Joaquin River Delta (hereafter, the Delta) and associated water bodies. During the 20th century, agricultural land use activity in the Central Valley of California resulted in extensive application of dichlorodiphenyltrichloroethanes (DDTs), chlordanes, dieldrin, and other agricultural pesticides (Connor et al. 2007), including more than 88,000 metric tons applied statewide in 2005

(California Department of Pesticide Regulation 2006). This application has resulted in elevated concentrations in sediments and in tissues of many local sport fish species (L. Brown 1997; MacCoy and Domagalski 1999; Davis et al. 2000; Greenfield et al. 2005). Elevated concentrations of polychlorinated biphenyls (PCBs) have been detected in fish tissues, particularly in urbanized regions of Stockton and Sacramento (Davis et al. 2000). Although not previously reported in fish from the Delta, concentrations of polybrominated diphenyl ethers (PBDEs) have been increasing in tissues of harbor seals from nearby San Francisco Bay and have been found at high concentrations in San Francisco Bay sport fish (She et al. 2002; F. Brown et al. 2006). Toxic effects to fish and wildlife have been associated with field-measured concentrations of these compounds (e.g., Beyer et al. 1996; Darnerud et al. 2001). As a result of excess PCB, legacy pesticide, and trace metal contamination, the state of California has placed 11 Central Valley water bodies, including the Delta, on the Clean Water Act "303(d) list," a list of waters impaired due to one or more of these contaminants (Central Valley Regional Water Quality Control Board 2003).

Laboratory studies indicate toxicity of current use pesticides to splittail, but field studies to evaluate the linkage of pollutant exposure to effects are lacking. Lethal and sublethal effects of selenium and pesticides to splittail have been documented in laboratory exposure studies (Teh et al. 2002, 2004a, 2005). Diazinon, singly and in concert with the pyrethroid esfenvalerate, causes reduced growth, spinal deformities, liver metabolic dysfunction, and inflammation of the pancreas in splittail larvae (Teh et al. 2004b). Histopathological abnormalities also occur in larvae exposed to orchard runoff containing esfenvalerate or diazinon. These abnormalities include severe glycogen depletion, cytoplasmic protein droplets, vacuolar degeneration, and cell necrosis in the liver (Teh et al. 2005). Selenium exposure (48 h) to splittail embryos results in pericardial edema and deformities of skeletal tissue (Teh et al. 2002). For Komeen (copper ethylenediamine complex), the 96-h LC_{50} (0.51 mg/L) for splittail larvae is lower than the highest measured ambient concentration (0.80 mg/L) in the Delta (Riley 2004).

Histopathological lesions have been used as general field biomarkers of exposure to contaminants (Hinton and Laurén 1990; Hinton et al. 1992; Barron et al. 2000; Anderson et al. 2003; van der Oost et al. 2003) and are a potentially useful tool to evaluate effects of pollutant exposure to splittail. Some tissue lesions, such as macrophage aggregates, hepatic glycogen depletion, and hepatic lipidosis, are general indicators of toxic injury resulting from exposure to contaminants or other stressors. Other lesions indicate response to specific contaminant classes; e.g., cytoplasmic inclusion bodies represent cellular isolation of metals (Hinton and Laurén 1990; Bunton and Frazier 1994). Many comparisons of histopathology versus contamination focus on specific contaminants or tissues (e.g., Bunton and Frazier 1994; Barron et al. 2000; Anderson et al. 2003). Given the wide range of contaminants that biota in the Delta and other human-impacted regions are exposed to (L. Brown 1997; Schrank et al. 1997; Davis et al. 2000; Greenfield et al. 2005; Schmitt et al. 2005), there is a need for comparisons of overall histopathology vs. exposure to multiple contaminants.

We analyzed 95 adult splittail captured from three Delta locations to evaluate histopathology and tissue concentrations of trace metals and organic contaminants. This field study compliments previous laboratory studies in determining the extent of contaminant exposure and histopathological effects in field-captured splittail. Due to the nonspecific nature of many lesions, our evaluation included a multivariate assessment of the relationship between general contaminant burdens and overall lesion scores. Because a number of laboratory studies have documented histopathological effects of individual contaminants (Teh, et al. 2002, 2004a, b, 2005), we hypothesized that there would be significant correlations between contaminant concentrations and histopathology.

Methods

Sacramento splittail were collected from three locations in the Sacramento-San Joaquin River Delta: Big Break (38.021°N, 121.731°W), Nurse Slough (38.179°N, 121.918°W), and Sherman Island (38.052°N, 121.798°W). Supplemental Fig. 1 presents a map of sampling locations on the Delta. These locations were selected because they represent a range of regional water sources and potential pollutant exposure conditions and were expected to have a high splittail abundance during the sampling period. Nurse Slough is located in the marshes and reclaimed pastures north of Suisun Bay, and most likely receives discharges from the Yolo Bypass and runoff from local land uses. Sherman Island is more centrally located and receives flow from the main stem of the Sacramento River. Big Break, the southernmost site, receives Sacramento River water but also may have small inputs from the highly impacted San Joaquin River and local urban and suburban discharges (Randy Baxter, California Department of Fish and Game, pers. commun.). All sampling was performed during June and August of 2001 and July and September of 2002.

Fish were collected using 150-ft paneled nylon gill nets (4- to 9-cm mesh size) placed at nearshore locations with the incoming tide. Fish were transferred live to the laboratory <1 h after removal from the nets. A similar number of fish was captured from each of six year-vs.-site

combinations (mean, 16; median, 15; range, 12–19 fish). Fish were sacrificed with an overdose of 3-aminobenzoic acid ethyl ester (MS-222; Sigma, St. Louis, MO, USA). Clean techniques were used throughout the dissection process. The following physical measurements were performed following Murphy and Willis (1996): weight, length, body condition factor [(body mass, g · 100)/(length, cm)^3], hepatosomatic index (HSI; liver mass/body mass), and gonadosomatic index (GSI; gonad mass/body mass). Visceral mass (liver, intestines, and stomach) and gonads were surgically removed from each fish. Separate portions were stored in liquid nitrogen for chemical analysis and 10% neutral buffered formalin at room temperature for histopathological analysis. The remainder of the fish was wrapped in double-walled heavy-duty aluminum foil (dull side toward fish), placed in a plastic bag, and shipped frozen to the analytical laboratory.

Histopathological analysis was conducted on liver, gonad, and kidney of each sampled fish following the methods of Teh et al. (2004b). After 48 h in 10% neutral buffered formalin, tissues were dehydrated in a graded ethanol series and embedded in paraffin. For each tissue block, serial sections (4 μm thick) were cut and stained with hematoxylin and eosin (H&E). Tissues were screened for a variety of histopathological features and lesions (Table 1) and scored on an ordinal ranking system of 0 = none/minimal, 1 = mild, 2 = moderate, and 3 = severe. General measures of morphology and health also included gonadosomatic and hepatosomatic indexes and muscle and liver tissue lipid content.

Trace organics analysis was performed on whole-body tissue, excluding visceral mass, but including scales and skeleton. A 10-g sample was mixed with ~7 g of pre-extracted Hydromatrix until the mixture was free flowing. The mixture was added to a 33-ml stainless-steel extraction cell and fortified with a solution containing pesticide and PCB surrogate compounds. The mixture was extracted twice using U.S. EPA Method 3545 Pressurized Fluid Extraction (Dionex ASE 200) with acetone:dichloromethane (1:1). All sample extracts were cleaned up using gel permeation chromatography (GPC; J2 Scientific Autoinject 110, AccuPrep 170, DFW-20 Fixed Wavelength Detector, 1-in.-inner diameter glass column with 70 g Bio-Beads SX-3 in 100% dichloromethane). The GPC-purified extracts were then fractionated into four separate fractions by column chromatography using Florisil and petroleum ether (fraction 1), 6% diethyl ether/petroleum ether (fraction 2), 15% diethyl ether/petroleum ether (fraction 3), and 50% diethyl ether/petroleum ether (fraction 4). Organochlorine pesticides, PCBs, and PBDEs in tissue samples were quantified using dual-column high-resolution capillary gas chromatography with dual electron capture detectors (GC/ECD; Agilent 6890 plus GC equipped with two ^{63}Ni micro-electron capture detectors with electronic pressure control and autosampler). Thirty-nine prevalent PCB congeners (listed by Greenfield et al. 2003) were measured and summed to obtain the "sum of PCB congeners." These data were used to estimate the concentration of Aroclors 1248, 1254, and 1260 (following Newman et al. 1998), which were summed to obtain the "sum of Aroclors." Total DDTs included *p*,*p*'-DDT, *o*,*p*'-DDT, *p*,*p*'-DDE, *o*,*p*'-DDE, *p*,*p*'-DDD, and *o*,*p*'-DDD (U.S. EPA 2000). Total PBDEs included BDEs 17, 28, 47, 66, 99, 100, 138, 153, 154, 183, and 190. Total chlordanes included *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, heptachlor, and heptachlor epoxide.

Trace metals analysis was performed on liver, after freeze-drying for 48 h. The dried samples were weighed (5 mg) and allowed to react overnight in 1 ml concentrated nitric acid (trace metal grade). Samples were heated with 10 μl concentrated H_2SO_4 and 50 μl 5% $K_2S_2O_8$ at 110°C for 30 min and then at 130°C until total volume was reduced to <40 μl. Samples were diluted to 5 ml with 3% HNO_3 and then trace metal ion samples were quantified using inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500; Palo Alto, CA, USA) with an autosampler (Cetac ASX 510; Omaha, NE, USA). Matrix spikes were performed for QA/QC with bovine liver as the certified reference material. Three reagent blanks were used with every set of samples that was digested. Three replicates per sample were recorded by the instrument on each analysis to provide a standard deviation for the concentration.

To evaluate whether chemical residues in sampled splittail posed a health risk to human sport fish consumers, or to the fish themselves, tissue concentrations were compared to selected published effects thresholds. Effects thresholds for fish were obtained from Beckvar (2006) for DDTs, Meador (2002) for PCBs, and Teh (2004a) for selenium. Human effects thresholds were the U.S. EPA (2000) screening value concentrations for the general population and sport fish consumers.

Statistical Analysis

For organic contaminants and physical measurements, analysis of variance (ANOVA) on log-transformed data, followed by Tukey's standardized range (HSD) test, was performed to identify significant differences among stations, years, or sex. Linear regression analysis was conducted to characterize the association between tissue lipid content and organic contaminant content. For histopathology scores, a *G*-test was performed to identify significant differences among stations. Unless presented otherwise, all statistical evaluations were conducted at the $\alpha = 0.05$ level.

Table 1 Histopathological indicators

Indicator	Definition
Gonad	
GCN	Primordial germ cell necrosis
GINF	Infiltration of inflammatory cells in gonads
GMA	Macrophage aggregates in gonads
OCN/SPN	Oocyte necrosis (female only); spermatocyte necrosis (male only)
Kidney	
EPD	Eosinophilic protein (hyaline) droplets in cytoplasm of kidney tubules
GN	Glomerulonephritis, increased Bowman's capsule thickness and dilation of Bowman's space
IF	Infectious diseases such as parasite and bacteria infection
KMA	Macrophage aggregates in kidney
TBD	Dilation of tubules, increased lumen diameter
TBI	Basophilic or eosinophilic cast inclusions in lumen of tubules
TBN	Tubular epithelial cell necrosis
Liver	
CIB	Granular cytoplasmic inclusion bodies
FCA	Foci of cellular alteration
GD	Glycogen depletion
LIP	Lipidosis or hepatocellular vacuolation
LMA	Macrophage aggregates
PCVL	Pericholangial and/or perivascular lymphocytes/leukocytes
SCN	Single-cell necrosis or piecemeal necrosis

Spearman rank correlation coefficients (ρ) were used to determine individual associations between contaminant concentrations and histopathology scores. Pearson's correlation coefficients (r) were used to determine associations between contaminant concentrations and growth attributes (i.e., tissue lipid, length, weight, liver weight, HSI, gonad weight, GSI, and body condition factor). These evaluations were interpreted qualitatively, by identifying and describing the coefficients having relatively high magnitudes (>0.4).

Nonmetric multidimensional scaling (NMS) was performed to determine if there was an overall association between contamination (organic and trace metals) and histopathology. NMS was used to conflate the majority of variation in the contamination and histopathology data and determine whether there were readily apparent overall associations. NMS was conducted using PC-ORD Version 4.25 (MjM Software Design, Gleneden Beach, OR, 1999), following the general procedure recommended by McCune and Grace (2002). NMS axes were then compared using Pearson's correlation coefficients (r). Further detail on statistical procedures are given in Supplement 2.

Results

Contaminants

The size range of captured splittail was 19 to 49 cm, averaging 32 cm ($N = 93$); this size range is representative of mature and reproductively active adults (Moyle 2002).

Elevated concentrations of PCBs, pesticides, and PBDEs were detected in sampled fish (whole-body samples, with viscera and gonads removed; Table 2). Trace metals were detected in sampled fish livers (Supplemental Table 3), and liver selenium averaged 11.4 μg/g (SD = 6.3 μg/g; $N = 86$). Tissue concentrations of contaminants also exceeded U.S. EPA (2000) human health screening values. For the sum of PCB congeners, 83 of 90 samples (92%) exceeded the human health screening value of 20 ng/g wet weight. For DDTs, 32 of 90 samples (36%) exceeded the human health screening value of 117 ng/g. No samples exceeded the screening value for chlordanes (114 ng/g), but 37 of the samples (41%) exceeded the screening value for dieldrin (2.5 ng/g). Of 90 samples, 7 (i.e., 8%) exceeded a 2400 ng/g lipid weight tissue threshold for PCBs, above which adverse effects would be expected for salmonid fish (Meador et al. 2002). None of the samples analyzed exceeded a 600 ng/g DDT wet weight tissue residue threshold, above which adverse effects may be expected for fish (Beckvar et al. 2006). Only 2 of 86 samples measured for selenium exceeded liver concentrations at which growth, survival, and histopathology effects are observed in long-term laboratory exposure studies of juvenile splittail (>27 μg/g [Teh et al. 2004a]).

Among the three sample stations, there were significantly higher concentrations of organic compounds at Big Break and Sherman Island than Nurse Slough for all summed organic classes (Tukey's standardized range test, $p < 0.05$; Table 2). This corresponded to the significantly elevated tissue lipid content at those stations, compared to Nurse Slough. Linear regression analysis among individual samples indicated a significant positive relationship ($p < 0.0001$) between tissue lipid content and contaminant concentration for DDTs ($R^2 = 0.39$; $n = 89$), chlordanes ($R^2 = 0.58$; $n = 87$), sum of PCB Aroclors ($R^2 = 0.23$; $n = 87$), sum of PCB congeners ($R^2 = 0.24$; $n = 88$), PBDEs ($R^2 = 0.34$; $n = 88$), and dieldrin ($R^2 = 0.75$; $n = 86$).

For organic contaminants, percentage lipid, and percentage moisture, the best NMS ordination contained two axes. The final stress was 6.4 and the final cumulative R^2 (coefficient of determination between ordination distance and original distance) was 0.98, indicating that the ordination explained almost all of the variation in the original data set. Axis 1 explained the most variance (0.80) and was

strongly positively correlated with every summed organic contaminant in Table 2 (Pearson's r values ranging from 0.71 to 0.93). Axis 2 explained less of the variance (0.19) and was strongly correlated with axis 1 ($r = 0.8$). Because axis 2 did not provide much additional information, it was not used in comparison to histopathology scores.

For metals, length, and percentage moisture, the best NMS ordination contained three axes. The final stress was 10.2, and final R^2 was 0.94, indicating that the ordination explained a majority of the variation in the original data set. Axis 2 explained the most variance (0.37) and was negatively correlated with Ag ($r = -0.86$), Cu (-0.77), and As (-0.47) and positively correlated with length (0.55) and vanadium (V; 0.50). Axis 1 explained 0.33 of the variance and was positively correlated with Cu ($r = 0.69$), Ag (0.66), Zn (0.53), and V (0.53). Axis 3 explained less variance (0.24) and was strongly negatively associated with Pb ($r = -0.84$). These results indicated that variability in a mixture of metals (in particular, Ag, Cu, As, V, and Zn) could be explained by axes 1 and 2, and that individual variability in Pb may also be important for the data set.

Histopathology

In general, histopathological analysis indicated a low to moderate incidence of tissue effects in splittail. Across the three sampling sites, the majority of fish samples showed no or minimal observable tissue effects for most indicators (score of 0; Table 3, Fig. 1A). However, glycogen depletion (Fig. 1B) and lipidosis (Fig. 1C) in the liver exhibited moderate to severe effects (scores of 2 or 3) in the majority of samples, with 55 of 95 fish (58%) exhibiting moderate to severe glycogen depletion (GD) and 51 fish (54%) exhibiting focal and diffuse lipidosis (LIP; i.e., fatty vacuolation of hepatocytes (Table 3). Moderate to severe cytoplasmic inclusion bodies (CIB) were also observed in the livers of 33 fish (35%), and mild effects (score of 1) were common for eosinophilic protein droplets in kidney tubule cytoplasm (EPD; $N = 51$ samples; Fig. 2). Mild macrophage aggregates (Figs. 3 and 4) were also common in the gonads (GMA), kidneys (KMA), and liver (LMA; Table 3).

Spatial comparisons (Table 3) indicated statistically significant differences among the three sites (G test, $p < 0.05$) for six indicators: gonad macrophage aggregates, kidney glomerulonephritis (GN; Fig. 5), kidney infectious diseases (IF), inclusions in lumens of kidney tubules (TBI), liver CIB, and liver GD. For CIB and GD, moderate to severe effects were most common for Nurse Slough and least common for Sherman Island.

For histopathology, the best NMS ordination contained three axes. The final stress was 16.0 and the final cumulative R^2 was 0.83; the higher stress and lower R^2 suggest that caution is warranted in interpreting the NMS results for histopathology (McCune and Grace 2002). Axis 2 explained the most variance (0.33) and was negatively correlated with liver CIB ($r = -0.75$) and GD ($r = -0.42$). Axis 3 explained 0.29 of the variance and was strongly negatively correlated with liver LIP ($r = -0.86$) and GD ($r = -0.83$). Axis 1 explained the least variance (0.21) and was negatively correlated with gonad GMA ($r = -0.74$) and IF ($r = -0.40$), as well as liver CIB ($r = -0.45$). No kidney scores were strongly associated with any axes, suggesting a lack of systematic association among variation in kidney scores or between kidney scores and other histopathology indicators.

Comparison of Histopathology, Growth Attributes, and Contamination

Statistical correlations between individual contaminant residues and histopathology were generally weak and occurred at similar frequencies in positive and negative

Table 2 Summary of growth and organic contaminant attributes for splittail measured in the study

Grouping	N	Length (cm)	Lipid (%)	Σ PCB con-geners (ng/g wet wt)	Σ PCB Aroclors (ng/g wet wt)	Σ PBDEs (ng/g wet wt)	Σ Chlordanes (ng/g wet wt)	Σ DDTs (ng/g wet wt)	Dieldrin (ng/g wet wt)
Big Break	28	30.9[b]	7.8[a]	73[a]	87[a]	29.5[a]	8.9[a]	136[a]	3.2[a]
Nurse Slough	32	34.0[a]	3.7[b]	30[b]	42[b]	9.0[b]	2.6[b]	43[b]	0.9[b]
Sherman Island	33	31.8[ab]	5.9[a]	46[a]	68[a]	33.1[a]	8.7[a]	101[a]	2.7[a]
All females	63	33.5[a]	5.6	46	63	18.0	6.6	90	1.9
All males	26	29.3[b]	5.8	59	77	21.2	7.6	87	2.2
All 2001	44	34.4[a]	4.5[b]	56	76[a]	17.5[b]	6.9	92	1.7
All 2002	44	30.2[b]	6.8[a]	40	57[b]	28.4[a]	7.3	85	2.2
All samples	93	32.3	5.7	49	67	20.4	7.1	88	2.0

Note. Means are presented for length and lipids; medians, for contaminants. Superscript letters a and b indicate statistically significant differences among groups ($p < 0.05$). Statistical comparisons were conducted using ANOVA on log-transformed data, followed by Tukey's standardized range (HSD) test

Table 3 Summary of histopathology scores among the three sites monitored in the study

Indicator	Big Break ($N = 27$)				Nurse Slough ($N = 34$)				Sherman Island ($N = 34$)				p value, G test	Average score
	0	1	2	3	0	1	2	3	0	1	2	3		
Gonad														
GCN	21	6	0	0	27	7	0	0	22	11	1	0	0.450	0.27
GINF	26	0	0	0	32	1	0	1	28	5	1	0	0.077	0.12
GMA*	10	12	3	1	5	14	6	9	17	7	6	4	0.010	1.12
OCN	16	1	0	0	26	0	0	0	17	3	0	1	0.110	0.11
SPN	9	0	0	0	6	0	0	0	11	0	0	0	NA	0.00
Kidney														
EPD	7	18	2	0	10	20	4	0	17	13	3	1	0.243	0.76
GN*	17	7	2	1	31	1	0	2	26	7	0	1	0.027	0.33
IF*	17	10	0	0	24	10	0	0	13	14	6	1	0.005	0.52
KMA	0	26	1	0	1	32	1	0	1	29	4	0	0.412	1.04
TBD	25	2	0	0	32	2	0	0	28	3	2	0	0.331	0.12
TBI*	15	9	3	0	30	3	1	0	28	5	0	1	0.024	0.29
TBN	26	1	0	0	31	2	1	0	31	2	0	1	0.629	0.11
Liver														
CIB*	**15**	**1**	**4**	**7**	**12**	**5**	**12**	**5**	**16**	**13**	**4**	**1**	**0.0007**	**1.03**
FCA	26	1	0	0	34	0	0	0	33	0	1	0	0.331	0.03
GD*	**8**	**0**	**3**	**16**	**7**	**4**	**14**	**9**	**12**	**9**	**6**	**7**	**0.0003**	**1.63**
LIP	5	4	4	14	7	9	11	7	6	13	7	8	0.103	1.65
LMA	2	25	0	0	6	26	2	0	5	28	1	0	0.388	0.89
PCVL	22	5	0	0	29	3	0	0	31	1	0	0	0.133	0.10
SCN	24	2	0	1	32	2	0	0	26	6	1	1	0.324	0.19

Note. NA, not applicable. Results are for indicators monitored in both 2001 and 2002. Scores represent ordinal effects scoring as follows: 0 = none/minimal, 1 = mild, 2 = moderate, and 3 = severe. Bold columns exhibit significant site/frequency associations at a Bonferroni corrected α value of 0.05 (G test). Asterisks indicate significance at an uncorrected α value of 0.05

directions (Table 4). CIB was positively associated (Spearman $\rho > 0.4$) with Ag, Cu, Mo, and Zn in 2001 and negatively associated with liver weight and HSI in 2001 and liver weight in 2002 (Table 4). LMA, KMA, GMA, and GD were not positively associated with any contaminants except for weak associations between GMA and As and between LMA and Cd. GD was negatively associated with a number of growth attributes, including fish length (−0.52), weight (−0.52), liver weight (−0.59), and gonad weight (−0.41). LIP was positively associated with lipids (0.55) and body condition (0.42; Table 4). In 2002, GMA was, in fact, negatively associated with all trace organic contaminants, as well as tissue lipids (−0.68) and body condition (−0.56; Table 4). When fish total length was compared to individual histopathology scores for both years combined ($N = 89$ fish), only GMA was positively associated with length ($\rho = 0.41$). This positive association may account for the higher incidence of GMA in Nurse Slough (Table 3) than Big Break, which had shorter fish (Table 2).

Although general analyses did not indicate strong histopathological effects or correspondence between exposure and effects, one individual 34-mm fish, sampled at Big Break in 2001, did exhibit unusual pathology combined with elevated organic contaminants. The fish appeared to be stressed, with severe glycogen depletion and lipidosis of the liver. Organic contaminants were elevated in this individual, with the second highest tissue PCB concentration of all fish (sum of PCB congeners = 157 ng/g), the sixth highest total DDT concentration (214 ng/g), the third highest chlordane concentration (17.9 ng/g), and the highest PBDE concentration (88.3 ng/g). Similarly, the two individual fish with liver selenium concentrations above the 27 μg/g effects thresholds of Teh et al. (2004a) both had severe glycogen depletion and lipidosis. The highest concentration fish (42.4 μg/g Se) also had a severe incidence of CIB.

NMS axis outputs were compared between histopathology and contaminants to determine whether there was an overall association between tissue contaminant concentrations and histopathology effects. Because it was strongly associated with axis 3 for metals, Pb was also compared individually. For organics, a positive correlation ($r = 0.48$) was observed between organics axis 1 and

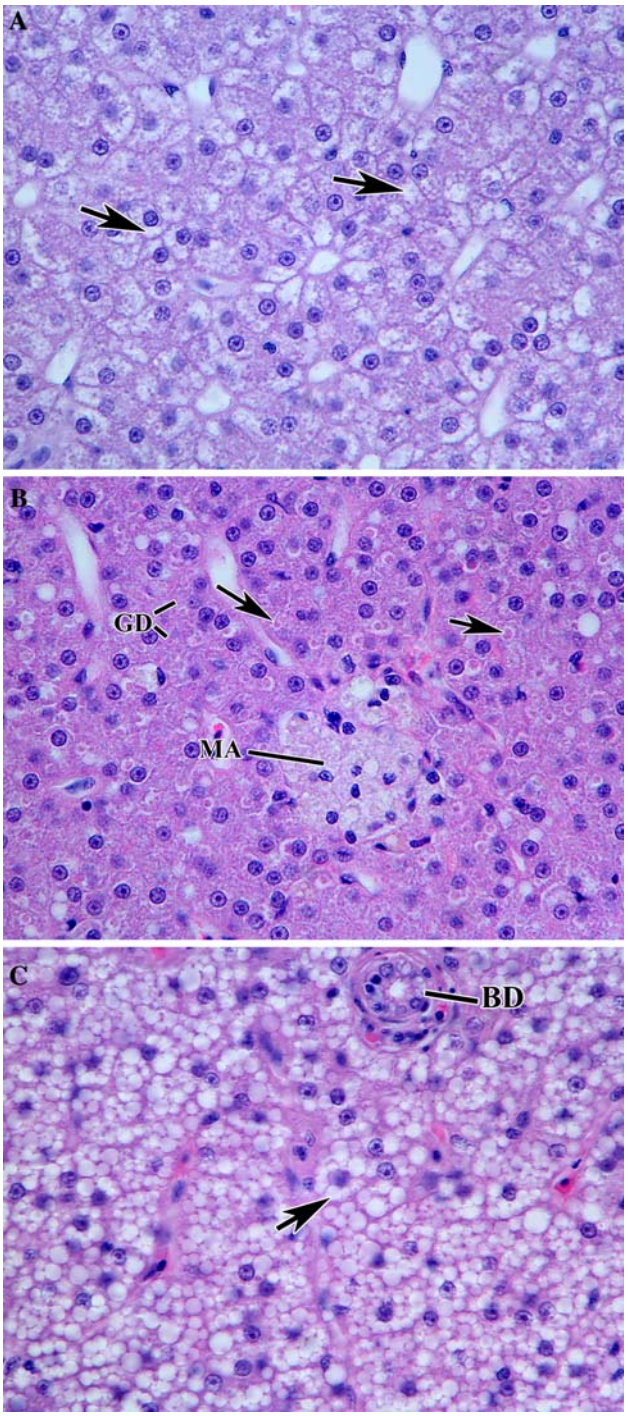

Fig. 1 A Normal liver section in an adult female splittail collected from Sherman Island shows the "lacy," irregular, and poorly demarcated cytoplasmic vacuolation typical of glycogen (arrows). B Liver section of an adult female splittail collected from Big Break shows severe glycogen depletion (GD) and granular cytoplasmic inclusion bodies (arrows). MA, macrophage aggregate. C Liver section of an adult male splittail collected from Nurse Slough shows severe lipidosis or fatty vacuolar degeneration (arrow). BD, small bile duct. (H&E stain.)

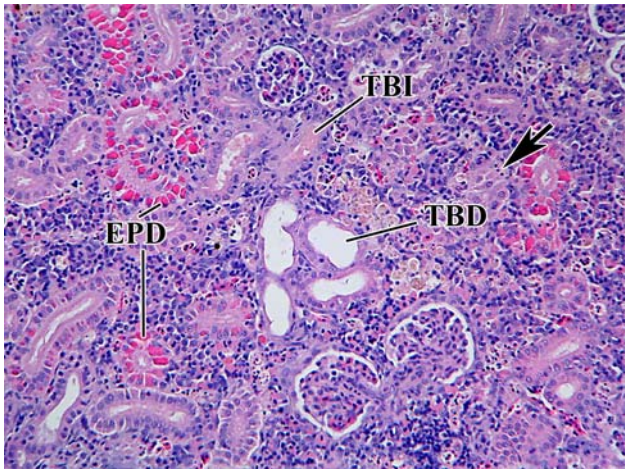

Fig. 2 Moderate eosinophilic protein droplets (EPD) in tubular epithelial cells and mild tubular dilation (TBD) and tubular inclusion (TBI) in kidney of an adult female splittail collected from Sherman Island. Arrow points to mild necrotic tubular epithelial cell

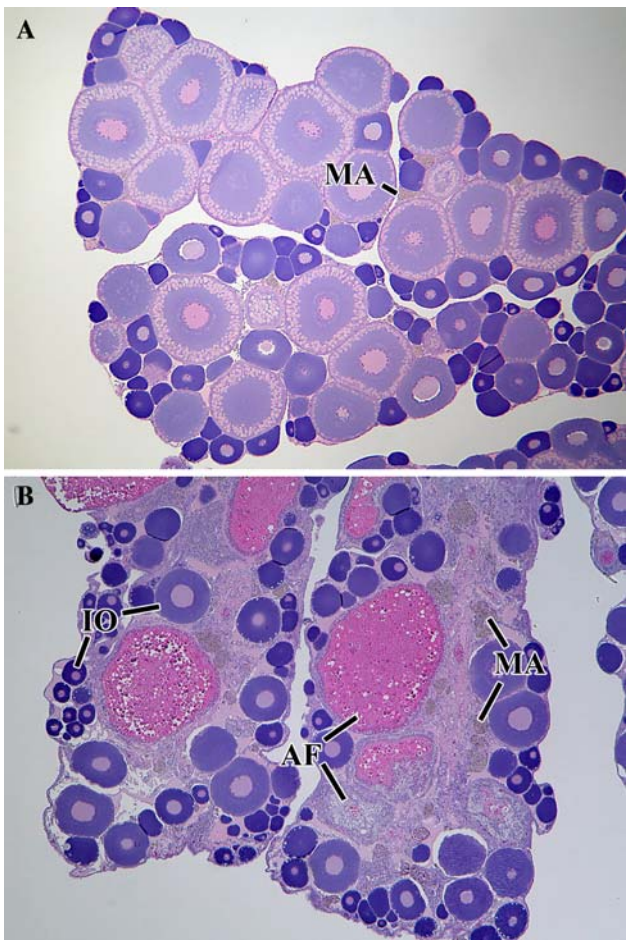

Fig. 3 A Mild macrophage aggregates (MA) in otherwise normal ovary section of adult splittail collected from Big Break. B Severe atretic follicles (AF) and moderate MA in adult splittail collected from Sherman Island. IO, immature oocytes. MA are characterized as a cluster of macrophages packed with coarsely granular yellow-brown pigment

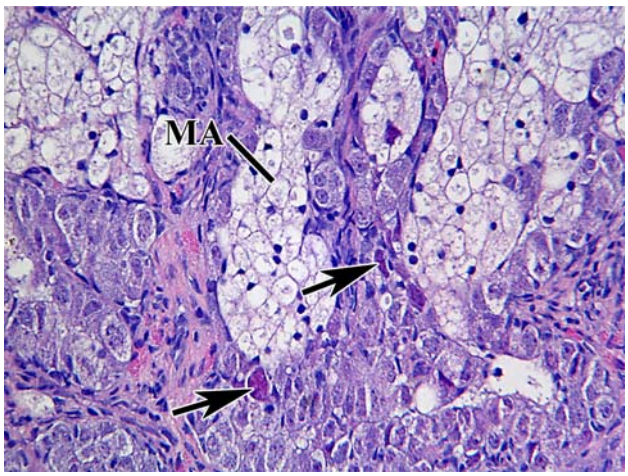

Fig. 4 Testis section of adult splittail collected from Nurse Slough shows severe macrophage aggregates (MA). Arrows point to mild necrosis of the primordial germ cell

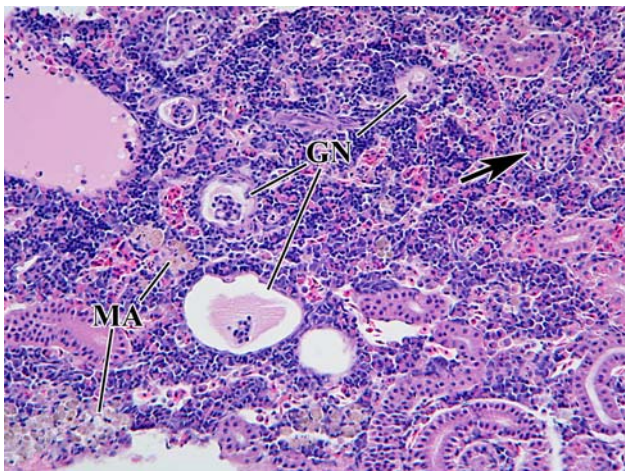

Fig. 5 Glomerulonephritis (GN), characterized by thickening of Bowman's capsule, shrunken glomerular tuft, and edema in glomerular space, was observed in kidney of adult splittail collected from Big Break. Arrow points to a normal glomerulus. (H&E stain.)

histopathology axis 1. This indicated a negative association between organic chemical concentration and GMA, IF, and CIB. This finding was consistent with some of the negative associations observed in individual correlation coefficient comparisons (Table 4). For metals, results indicated no strong associations (Pearson's $r < 0.25$ in all cases) when all histopathology axes were compared to the three metals axes or to Pb.

NMS axes were also separately compared to the sum of histopathology scores for all tissue types combined. This sum of histopathology scores ranged from 2 to 20, with a median value of 10 ($N = 95$). No correlation was observed between sum of histopathology scores and organics axis 1 or any of the three metals axes (Pearson's $r < 0.25$ in all cases).

Discussion

In general, results from this study did not indicate that exposure to the monitored contaminants caused histopathological alterations to adult splittail. Three lines of evidence indicated that most of the splittail captured in the study locations were not severely affected by the measured contaminants: (1) tissue concentrations generally below residue-based effects thresholds, (2) weak statistical associations between tissue residues and histopathology, and (3) observed correlations indicating general nutritional stress or baseline variability within the population. These findings do not preclude the possibility of contaminant effects to splittail, because this study did not monitor additional contaminants that splittail may have been exposed to (e.g., mercury [Beckvar et al. 2006]). Also, some contaminants exhibit toxicity to fish but do not readily bioaccumulate in tissues; these include PAHs (van der Oost et al. 2003) and current-use pesticides such as esfenvalerate, diazinon, and chlorpyrifos (Eder et al. 2004; Teh et al. 2004b). Finally, synergistic effects of contaminant exposure in combination with other stressors (Moyle et al. 2004; Loge et al. 2005) and effects at other levels of organization (e.g., growth, behavior, and subcellular levels) were not evaluated, presenting opportunities for future research.

Contaminants

Tissue concentrations were generally below literature-based thresholds for effects to fish. Between 0% and 8% of samples exceeded tissue effects thresholds for DDTs (Beckvar et al. 2006), selenium (Teh et al. 2004a), and PCBs (Meador et al. 2002). Splittail are relatively insensitive to selenium (Teh et al. 2004a) and multiple aquatic herbicides (Riley 2004). This relative insensitivity to a variety of compounds suggests that splittail may be more resistant to contaminant effects than salmonids and other species.

Splittail may pose a potential health risk to human consumers. The majority of fish exceeded human health screening values for PCBs, many exceeded DDT and dieldrin screening values, and PBDEs were present in all fish sampled. Nevertheless, caution is warranted in using the data from this study for human risk assessment because analyses were performed on whole-body samples (minus viscera), whereas U.S. EPA (2000) recommended screening values are typically applied to muscle fillets, with or without skin. Although federally listed as a species of special concern, splittail are legally fished and are popular

among local Asian communities (Moyle et al. 2004). In a recent survey of low-income women, 69% of Asian respondents reported that they consume sport fish (Silver et al. 2007). The potential risk caused by consuming splittail is consistent with risks documented in other species in the San Francisco Bay-Delta region (Davis et al. 2000; Greenfield et al. 2005).

Histopathology

The most common tissue alterations were macrophage aggregates (MA) of the kidneys, liver, and gonads, and several alterations of liver histology, including CIB, LIP, and GD (Figs. 1, 3, and 4). GD and MA are nonspecific indicators of stress that can reflect poor nutritional status, contaminant exposure, environmental stress, or age of an organism (Myers and Hendricks 1985; Hinton et al. 1992; Teh et al. 1997). GD (Fig. 1B) has also been observed as a precursor to foci of cellular alteration and single-cell necrosis in the pathological development of hepatic neoplasia (Hinton et al. 1992), though these alterations were rare in our study.

Hepatocellular lipidosis (Fig. 1C) and other forms of liver damage have been associated with exposure to chlorinated hydrocarbons and other contaminants (Hendricks

Table 4 Spearman rank correlation coefficients of contaminant, biological, and histopathological measures for 2001 and 2002 splittail samples ($\rho > 0.4$, $p < 0.01$)

Indicator	Contaminant measure		Biological measure	
	2001	2002	2001	2002
Gonad				
GCN	K (−0.418); Zn (−0.413)	ns	Gonad weight (−0.681); GSI (−0.681)	Gonad weight (−0.590); GSI (−0.571)
GINF	ns	ns	ns	ns
GMA	ns	As (0.451); Aroclors (−0.477); PCB (−0.503); chlordanes (−0.644); DDT (−0.520); dieldrin (−0.650); PBDE (−0.552)	Lipids (−0.437)	Lipids (−0.680); body condition (−0.558)
OCN/SPN	Cr (−0.436); Mo (−0.425)	ns	ns	ns
Kidney				
EPD	ns	ns	ns	ns
GN	ns	ns	HSI (0.435)	ns
IF	PBDE (0.427)	ns	ns	ns
KMA	ns	ns	ns	ns
TBD	K (−0.403)	ns	ns	ns
TBI	ns	ns	ns	ns
TBN	ns	ns	ns	ns
Liver				
CIB	Ag (0.407); Cu (0.558); Mo (0.502); Zn (0.518); DDT (−0.423); PBDE (−0.487)	Aroclors (−0.406)	Liver weight (−0.462); HSI (−0.558)	Liver weight (−0.530)
FCA	ns	ns	ns	ns
GD	K (−0.403)	ns	Length (−0.524); weight (−0.515); liver weight (−0.591); gonad weight (−0.412)	ns
LIP	K (−0.737); Na (−0.503); V (−0.556); dieldrin (0.411)	ns	Lipids (0.547); length (−0.422); gonad weight (−0.406); body condition (0.422)	ns
LMA	ns	Cd (0.405)	ns	ns
PCVL	ns	ns	ns	ns
SCN	ns	Ni (0.439)	ns	ns

Note. ns, not significant ($\rho < 0.4$, $p > 0.01$)

et al. 1984; Hinton et al. 1992; Robertson and Bradley 1992; Schrank et al. 1997), including PCBs (Teh et al. 1997; Anderson et al. 2003). Liver effects may also result from age, changing nutritional status, and other environmental factors (Hinton et al. 1992; Robertson and Bradley 1992). Driving mechanisms of lipidosis include toxic injury causing impaired lipid oxidation or protein synthesis, resulting in accumulation of triglycerides in hepatocytes. Alternatively, malnutrition may increase fat mobilization and impair apoprotein synthesis (Hinton and Laurén 1990).

Comparison of Histopathology, Growth Attributes, and Contamination

In this study, NMS was combined with correlation analysis to test for an association between overall variation in tissue contamination and histopathology. This approach can be used to determine whether contaminant mixtures appear to cause a general alteration in histopathology or other nonspecific bioindicators. The approach also reduces reliance on individual comparisons, associated risk of Type I error, and need for conservative corrections (e.g., Bonferroni correction for multiple comparisons). For organic contaminants, the low final stress and high correlation between the NMS axes and individual compound classes indicated that organic pollutants were highly correlated with each other (Huang et al. 2006). Organic compounds were also strongly positively associated with percentage lipids and negatively associated with percentage moisture, reflecting the association between lipid and organic contaminant uptake (Kidd et al. 1998).

Neither NMS axis comparisons nor correlation analysis of individual contaminants and tissue lesions indicated notable correlations between contamination and histopathology. For example, there were no significant relationships between NMS contaminant axes and summed histopathology scores. The only observed correlation was a negative association between organic contaminant axis 1 and a histopathology axis associated with lipid content and three tissue indicators: GMA, IF, and CIB. The association was negative between organic contaminants and the indicators, which was inconsistent with the hypothesis that organic pollutants caused the adverse histological effects. It more likely indicated an association between low lipid mass (i.e., poor nutritional status) and these effects (Hinton et al. 1992). Interestingly, one fish lacking visible gonads also had the highest PBDE concentrations and elevated content of other trace organics; this may have been an individual example of testicular atrophy resulting from contaminant exposure (Hinton et al. 1992).

In general, GMA, GD, and LIP were not positively associated with contaminants but were negatively associated with general indicators of fish health, nutrition, and reproductive status. Smaller fish and fish with lower liver weight had a higher incidence of GD, a nonspecific indicator of stress (Myers and Hendricks 1985; Teh et al. 1997). Fish higher in tissue lipid exhibited higher LIP, presumably due to greater lipid storage (Hinton and Laurén 1990). Also, in 2002 GMA was negatively correlated with organic contaminants, body condition, and tissue lipid content. Lipid and body condition indicate general fish health and nutritional status (Fechhelm et al. 1995). Despite these general trends, three fish with elevated contaminant concentrations did exhibit severe GD and LIP. This association suggests that a small number of individuals within the splittail population may be adversely affected by exposure to the measured contaminants.

Dietary uptakes of hydrophobic organic contaminants and lipids covary (Kidd et al. 1998). Therefore, the negative association between GMA and organic contaminant concentration may have been an artifact of their opposite association with tissue lipids. GMA was also positively associated with fish length, which may indicate the general influence of age on disease development (Hinton and Laurén 1990; Hinton et al. 1992).

In 2001, positive statistical associations were observed between CIB in the liver and liver tissue concentrations of individual metals (Ag, Cu, Mo, and Zn). CIB were also severe in the individual fish with the highest liver Se concentration. CIB result from metallothionein-based hepatic sequestration of tissue metals, and associations between CIB and metal exposure have been observed elsewhere (Hinton et al. 1992; Bunton and Frazier 1994; Peplow and Edmonds 2005). Although CIB do not necessarily indicate toxicity, a high incidence of CIB may correlate with increased metal exposure in other tissues (Bunton and Frazier 1994).

The significantly lower tissue lipid content in fish captured at Nurse Slough may have indicated dietary stress among those fish. Nurse Slough is more isolated from the main-stem water bodies of the Delta than the other sites (Supplemental Fig. 1), and Matern et al. (2002) indicated that splittail spawning and recruitment trends differed among these regions. It is possible that reduced prey availability in Nurse Slough may have resulted in poorer health and nutritional status.

Current data and modeling indicate splittail to be resilient to variations in abundance and recruitment that naturally occur as a result of variable spawning conditions (Moyle et al. 2004). The tissue pathologies observed in this study were only weakly associated with contaminant concentrations. The observations were more consistent with

natural variability in the population or overall nutritional stress. Introduced species such as the overbite clam (*Corbula amurensis*) have reduced availability of pelagic prey items in the Delta, and competition for food resources may have resulted in dietary stress (Moyle et al. 2004). To determine whether the incidence of overall stress may be changing over time, regular histopathology screening could be incorporated into water quality monitoring in the San Francisco Estuary.

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