U.S. Fish & Wildlife Service

California Nevada Fish Health Center
FY2007 Investigational Report:

Histological Evaluation and Viral Survey of Juvenile Longfin Smelt (*Spirinchus thaleichthys*) and Threadfin Shad (*Dorosoma petenense*) collected from the Sacramento – San Joaquin River Delta, April – November 2007.

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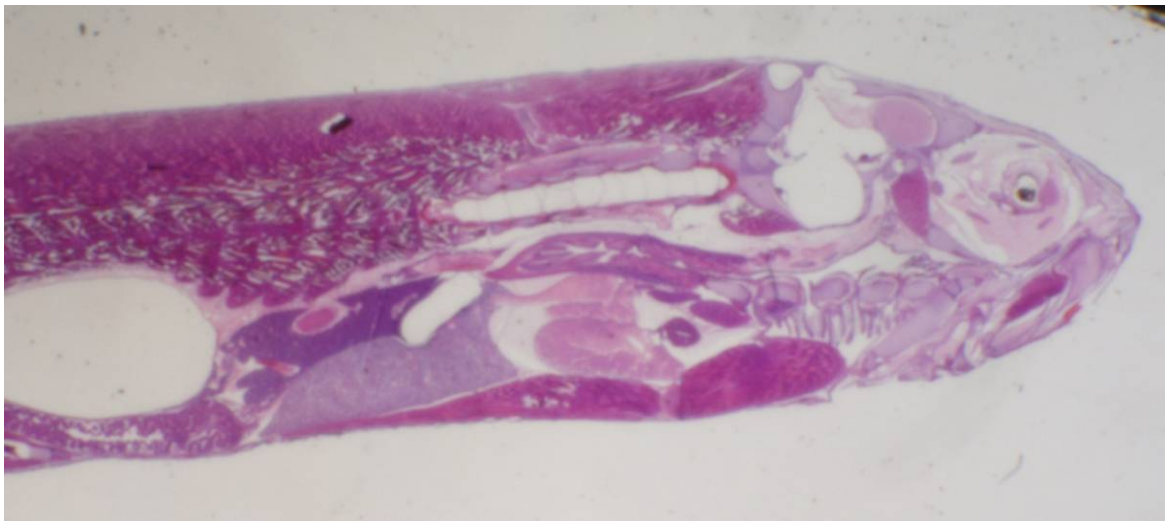

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Introduction:

Significant declines in pelagic fish abundance in the Sacramento – San Joaquin River Delta over the last decade has prompted the Interagency Ecological Program to augment its monitoring program with a disease screening effort for juvenile Longfin smelt (**LFS**, *Spirinchus thaleichthys)* and Threadfin shad (**TFS**, *Dorosoma petenense*). In 2007, the California-Nevada Fish Health Center and our cooperators focused on virus isolation and histological evaluation of juvenile LFS and TFS captured between April and November.

Methods:

Fish were collected by the Department of Fish and Game's Bay-Delta monitoring program using sampling gear and methods specific for the spring 20mm, Summer Townet, Bay Study and Fall Midwater Trawl surveys. Over half of the TFS were collected by the U.S. Fish and Wildlife Service (USFWS) Stockton Fish and Wildlife Office (FWO) beach seine program in the lower Sacramento and San Joaquin rivers. Locations sampled are referenced to sampling gear in use at the time; Summer Townet stations represent a subset of those for the 20mm Survey, as such Tow net stations are not referenced in the text or documented on separate maps (Tables 1 and 2 , Figures 1 - 3). For LFS, we used the term "larvae" to denote fish < 40 mm in fork length (FL) and juveniles for fish > 41 mm. Longfin smelt remain very slim and semi-transparent until about 40 mm, at which point pigment and body mass increases (R. Baxter, CDFG, pers. comm. 2007).

Fish selected for histological examination were placed in Davidson's fixative immediately after being identified. Nonetheless, larvae and older fish spent an estimated 35 min in stressful and hypoxic conditions prior to fixation due to tow times (10-12 min), net cleaning and identification time. Preserved fish were transferred to 70% ethanol after 24 – 48 h, processed for 6 μm paraffin sections (sagittal whole body or dissected organs in fish >75mm) and stained with hematoxylin and eosin (Humason 1979). The general sectioning protocol was to place a "shallow" (~ 50 - 100μm from the epidermis) and "deeper" (midline of fish) section onto each sagittal slide. Some duplicate sections were also stained with Periodic Acid – Schiff stain to document the quantity of glycogen in liver tissue or Jenner-Giemsa stain for parasites (Humason 1979).

Fish selected for viral assays were either immediately frozen on dry ice or held on 5°C blue ice if they were to be processed within 24 h. In the laboratory, samples were placed into cold antibiotic-mycotic solution in pools of 5 or fewer fish. Both a 20 and 100x dilution of whole body homogenate was inoculated onto 5 piscine cell lines: Epithelioma Papulosum Cyprini (EPC), Chinook Salmon Embryo (CHSE214), Bluegill fibroblast (BF-2), Koi fin (KF), and Striped Snakehead (SSN-1). The EPC and CHSE214 cultures were incubated at 15°C while BF-2, KF and SSN-1 cultures were held at 20°C. Cultures were maintained for 18 – 21 d and examined for cytopathic effects. Suspect cultures were subsequently filtered (0.2μm) and re-inoculated on new cell cultures.

Table 1. Longfin smelt (*Spirinchus thaleichthys*) collection dates in 2007, monitoring survey station numbers and their referenced survey and estuary location, sample designation (larvae = 18-40 mm fork length, or juvenile >41 mm FL), and number of fish assayed for virus and histological sections examined.

Date	Station No.	Lifestage	Virus no.	Histology no.
24April	519, 602, 20mm Survey, Suisun Bay	Larvae	12	16
25April	513,706 20mm Survey, West Delta	Larvae	87	13
26April	609 20mm Survey, Montezuma Slough	Larvae	ND	1
08May	508, 513, 606 20mm Survey, West Delta, Suisun Slough	Larvae	ND	24
23May	519,520, 606 20mm Survey, Suisun Bay, Suisun Slough	Larvae	7	8
18June	418 20mm Survey, Suisun Bay	Larvae	ND	1
		Total Larvae	106	63
11Sept	428 Bay Study Survey, Suisun Bay	Juvenile	ND	1
19Sept	534 Bay Study Survey, Suisun Bay	Juvenile	ND	1
04Oct	427 Bay Study Survey, Suisun Bay	Juvenile	ND	1
10Oct	211, 215 Bay Study Survey, Central Bay	Juvenile	ND	6
07Nov	325, 345 Bay Study Survey, San Pablo Bay	Juvenile	ND	9
08Nov	213 Bay Study Survey, Central San Francisco Bay	Juvenile	ND	1
13Nov	108 Bay Study Survey, South San Francisco Bay	Juvenile	ND	1
15Nov	534 , 750 Bay Study Survey, Suisun Bay, Sacramento River	Juvenile	ND	3
		Total Juvenile	0	23

ND Not done

Table 2. Threadfin shad (*Dorosoma petenense*) collection dates in 2007, monitoring survey station numbers and their referenced survey location, sample designation as larvae (18-40 mm fork length) or juveniles (age 0+, 41 -100 mm FL), and number of fish assayed for virus and examined by histology.

Date	Station No.	Lifestage	Viral No.	Histology No.
17July	906, 912 Directed sampling see 20mm Survey, Stockton Deepwater Channel	Larvae	58	15
01Aug	865, 912 Townet Survey and Bay Study, San Joaquin River, Stockton Deepwater Channel	Larvae	7	10
		Total Larvae	65	25
16Aug	707townet site FWS beach seine, Brannon island 38°11'49.69"N, 121°68'27.46'W	0+Juvenile	12	10
11Sept	703 FMWT site FWS beach seine, Sherman island 38°54'12.10"N, 121°78'48.66"W	0+Juvenile	54	10
12Sept	707townet site FWS beach seine, Brannon island	0+Juvenile	40	20
19Sept	912 Fall Midwater Trawl Survey, Stockton Deepwater Channel	0+Juvenile	60	12
21Sept	904, 912 Fall Midwater Trawl Survey, Venice Island, Stockton Deepwater Channel	0+Juvenile 1+ **	55	16
24Sept	706 Fall Midwater Trawl Survey, Sherman island	0+Juvenile	16	10
23Oct	912 FMWT site FWS beach seine, Dad's Point 37°95'57.21"N, 121°34'92.66"W	0+Juvenile	60	15
		Total Juvenile	297	93

** Two TFS captured on 21Sept were considered 1+ age (FL 151 and 119)

Figure 1. Map of 20mm Survey sample station numbers.

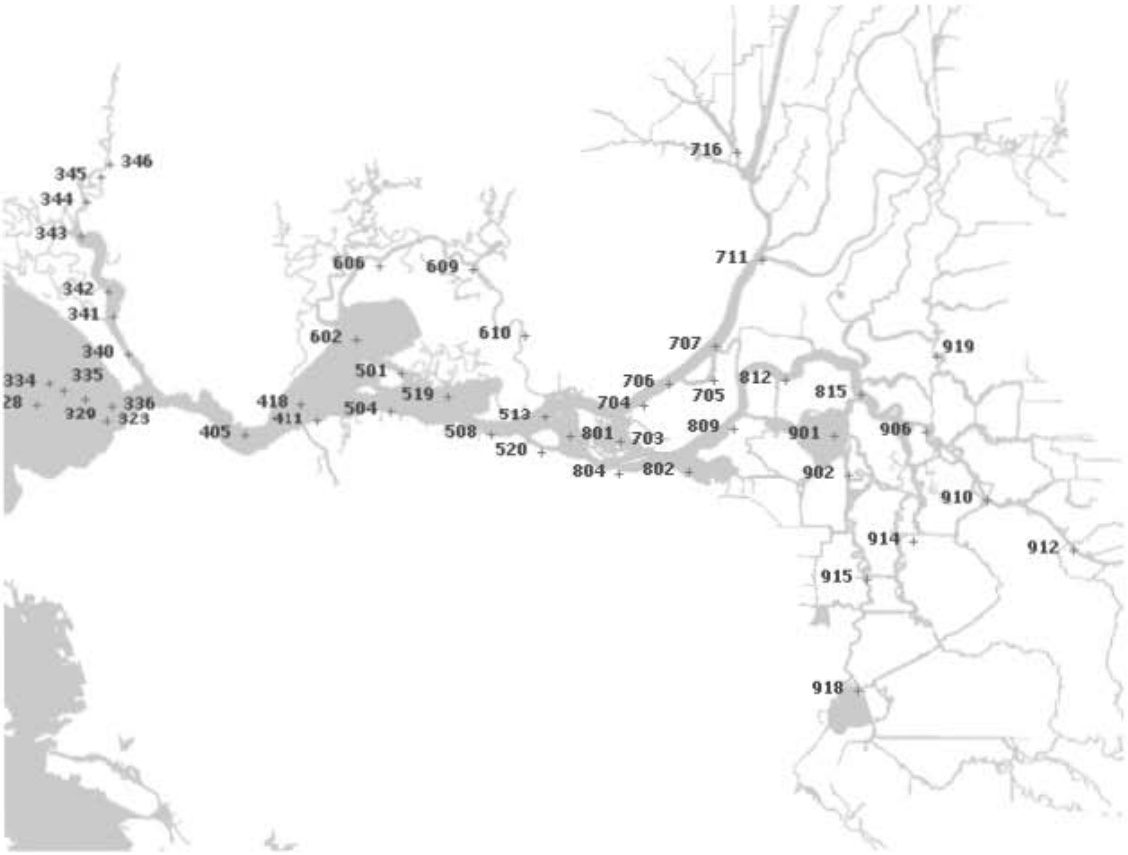


Figure 2. Fall Midwater Trawl Survey Stations.

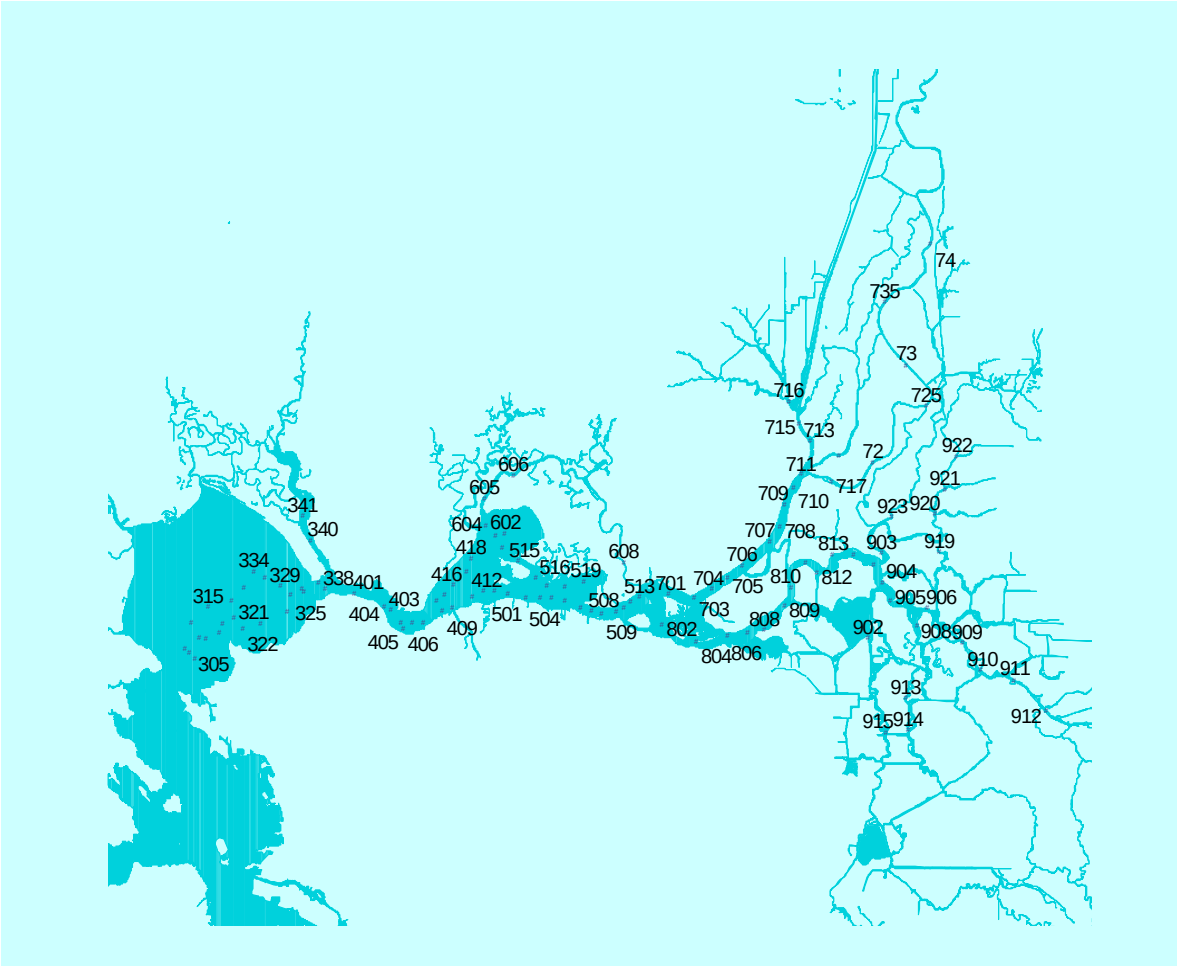
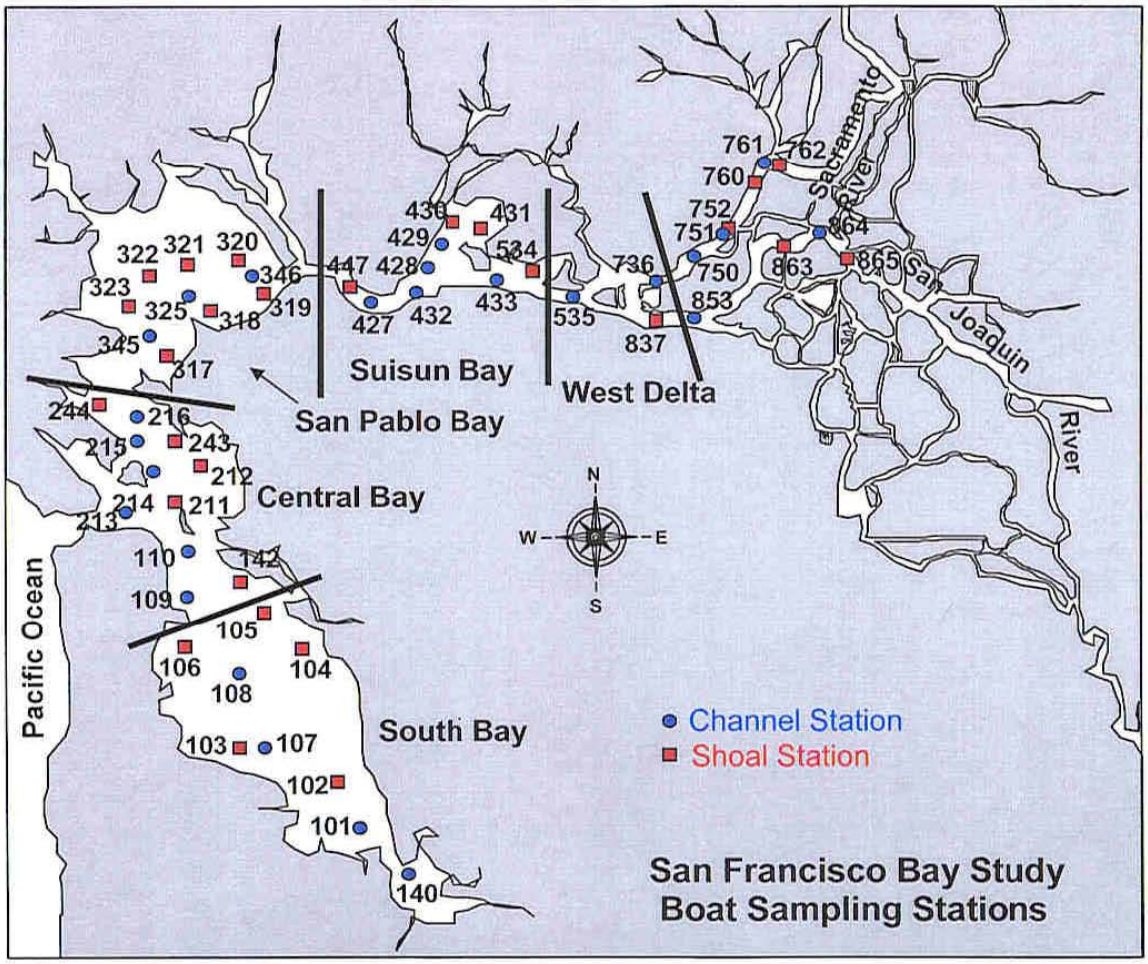


Figure 3. San Francisco Bay Study stations.



Results and Discussion:

Longfin smelt - A total of 169 larvae were collected, for viral or histological examination, in the West Delta and Suisun Bay between 24 April and 18 June 2007 (Table 1). Twenty-three juveniles were collected for histology in the fall (11Sept – 15Nov) from Suisun, San Pablo, Central San Francisco, and South San Francisco bays.

Despite the inherent difficulties of obtaining sagittal sections containing all organs, four targeted organs (liver, kidney, intestine, and gill listed in bold) were observed in >71% of sections (Table 3).

Table 3.
Percent of all Longfin smelt (LFS) and Threadfin shad (TFS) sections examined with various tissues. Whole fish (< 70mm FL) were sectioned in a sagittal plane and the percent of each tissue reported for this subset (sagittal).

	Olfactory organ	Brain	Gill	Eye	Heart	Liver	Pancreatic tissue	Kidney	Intestine	Skin / Muscle
LFS	27	64	**78**	56	71	**81**	58	**74**	**82**	76
LFS sagittal	29	75	**78**	72	66	**71**	65	**78**	**74**	85
TFS	4	92	**88**	18	25	**95**	81	**96**	**75**	23
TFS sagittal	12	92	**58**	73	58	**81**	77	**65**	**35**	92

Beginning in the 23May sample and continuing through November, a low number of intestines were observed to have one or more trematodes (7%, 5 of 74 sections) or cestodes (1%, 1 of 74 sections). No inflammation or lesion was associated with these parasites. No parasites were observed in other tissues nor was any virus isolated from the 106 larval samples.

Hepatocyte vacuoles were observed in 76% (16 / 21) liver sections from LFS juveniles collected between September and November. In 2006, similar hepatocyte vacuolation was observed in 25% of the 77 LFS sampled between July and October (Foott et al. 2006). An individual hepatocyte would either contain numerous small vacuoles (microvesicular) or 1 to 2 large vacuoles with distinct membranes (Fig 4). The interior of the vacuoles tended to have a faint eosinophilic staining reaction indicative of protein. They did not stain positive for glycogen by PAS stain (Fig. 5). Lipids are lost from paraffin sections due to the solvents used in histological processing. Based on this, we presume that the vacuoles observed in LFS juveniles had contained a lipoprotein (such as the phospholipoprotein vitellogenin or “egg yolk protein”). We cannot confirm the connection between hepatocyte vacuolation and ova maturation as the sex of the dissected juveniles was not determined and gonads were not removed for examination. This correlation with ovarian development would help discern whether the presumed lipoprotein is vitellogenin.

Figure 4 LFS liver with both microvesicular (black arrow) and macrovesicular vacuoles (white arrow). Note pale eosinophilic material (stains pink) within vacuoles. H&E stain.

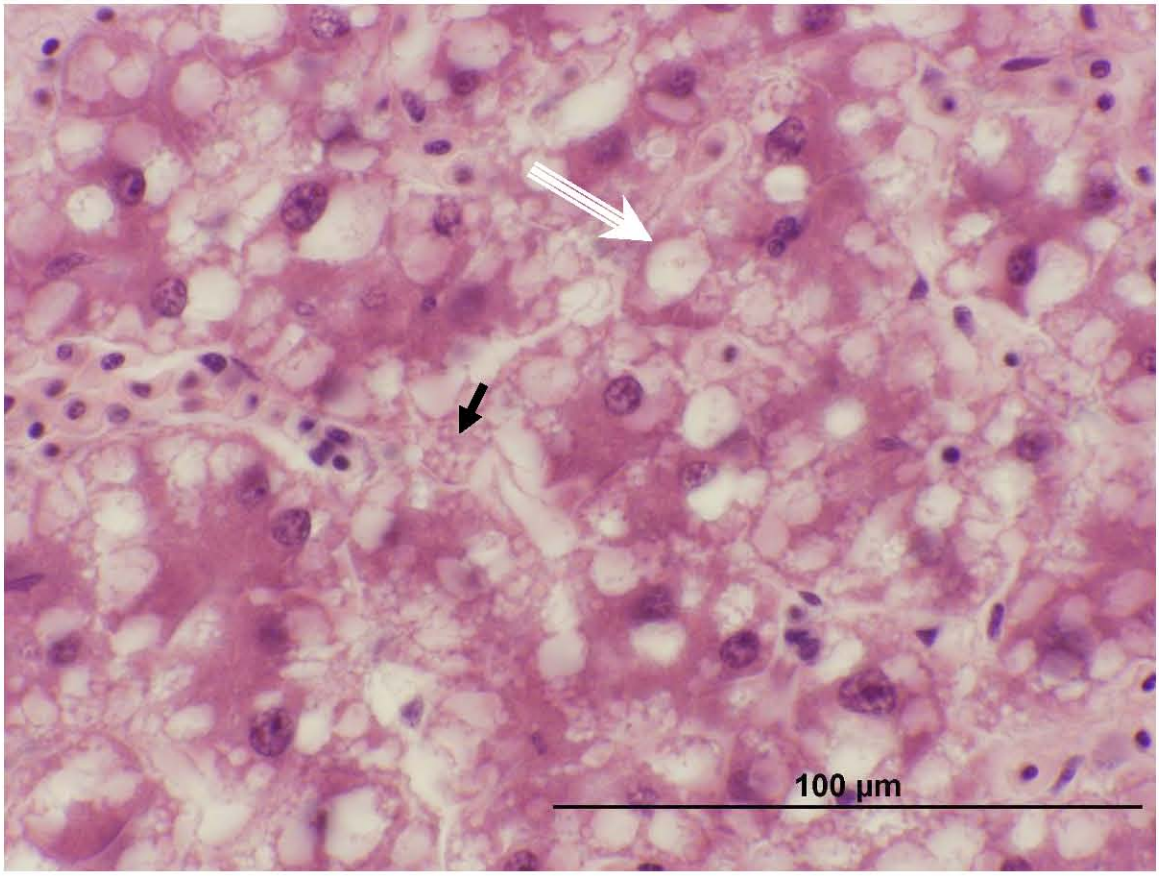
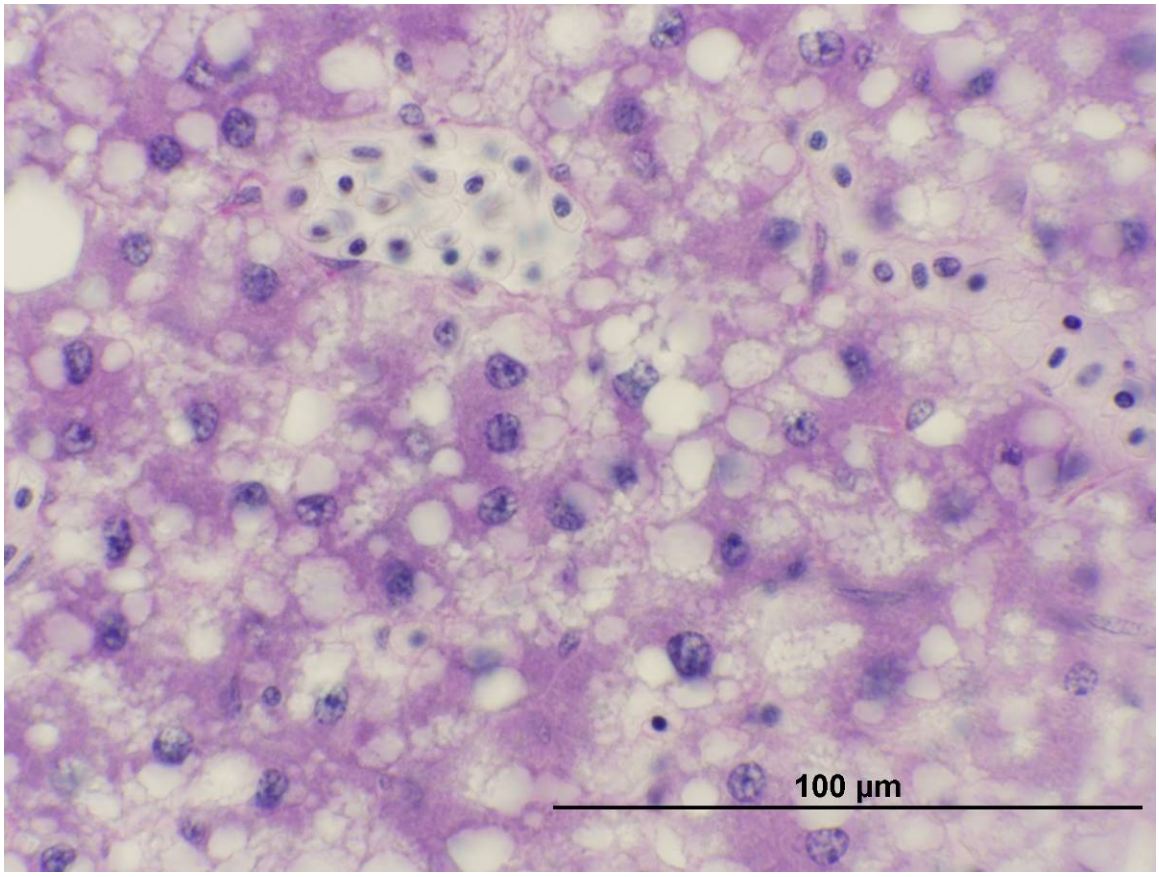


Figure 5. Vacuolated LFS liver (same fish as figure 4) stained for glycogen reaction (note that vacuoles do not stain pink for glycogen). PAS stain .



Hinton and Lauren (1990) discuss the various factors associated with fatty change in fish hepatocytes that include exposure to toxicants, nutritional state, and vitellogenesis in maturing females. Before hepatocyte vacuolation can be used as a biomarker for contaminant exposure it will be necessary to distinguish it from normal developmental processes by examining the seasonal changes in healthy LFS. Another hepatocyte abnormality, hydropic vacuolation, has been reported as a teleost biomarker for contaminate exposure (Stehr et al 1998) however it was not observed in the LFS livers. The nucleus of a cell with hydropic vacuolation is eccentrically displaced by the large vacuole. Electron microscopy of some marine flatfish collected from contaminated bays has shown that extreme dilation of the perinuclear space is the characteristic cell change associated with hydropic vacuolation (Stehr et al 1998). The LFS hepatocyte nuclei were normal in shape and intracellular location. Additionally, we did not observe cell changes suggestive of neoplasmic changes (megalocytic hepatocytes, enlarged nucleus, bile duct alterations).

We presumed that hypoxic stress associated with capture was responsible for the 63% incidence of cell swelling in kidney tubule epithelium. Other histological observations of interest include the large olfactory organ located in the 2 nasal

pits (Fig. 6) in both larvae (first seen in April samples) and juvenile LFS and the "triangle" swimbladder of larvae (Fig. 7). The well developed olfactory organs seen in the larvae suggest an early capability for chemosensory evaluation.

Figure 6. Olfactory organ (arrow) of LFS larvae captured in April 2007 (~18mm FL). Higher magnification (A) of the organ showing the bipolar neurosensory cells (arrow) whose dendrite joins the axon of the olfactory bulb nerve. H&E stain.

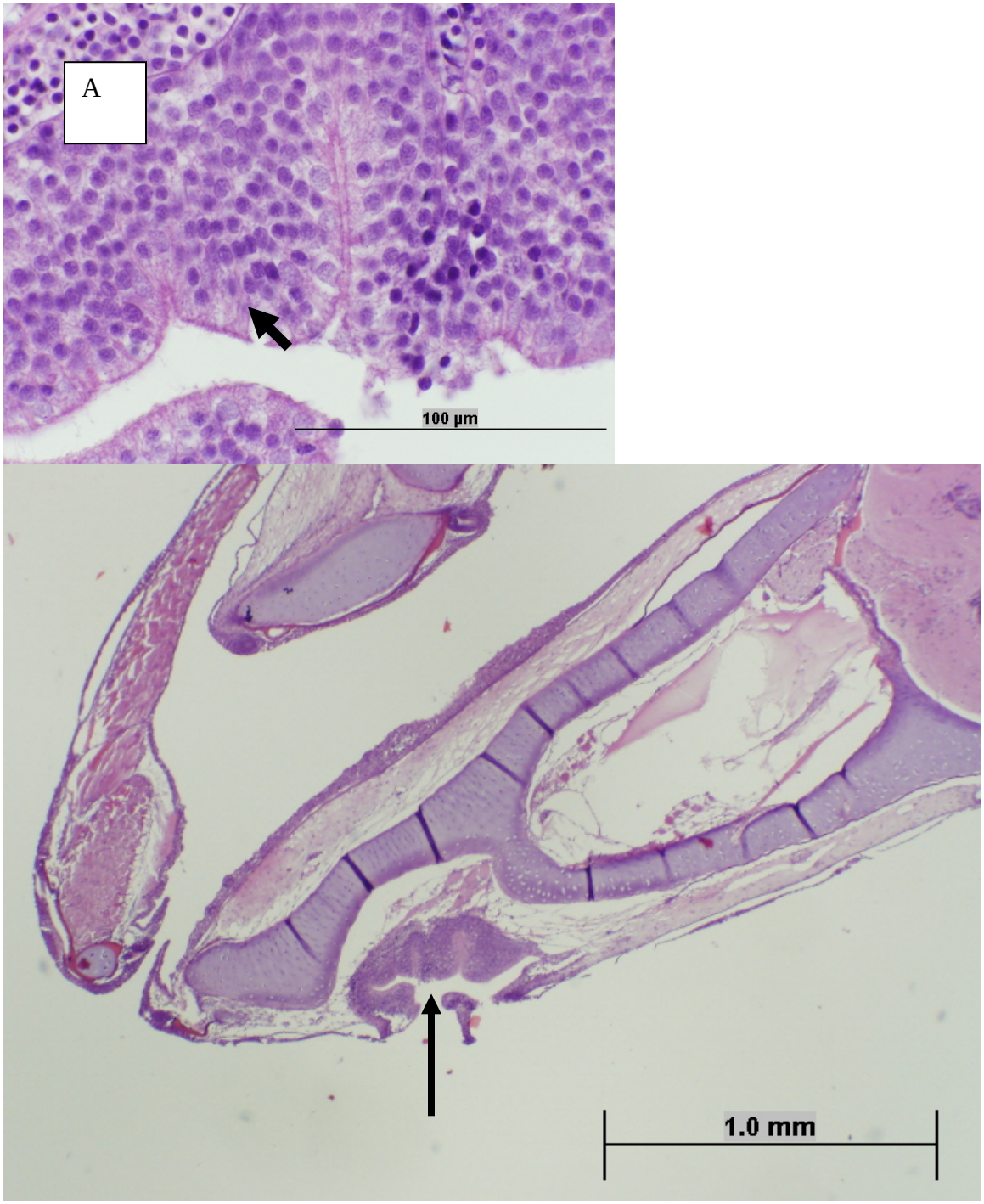
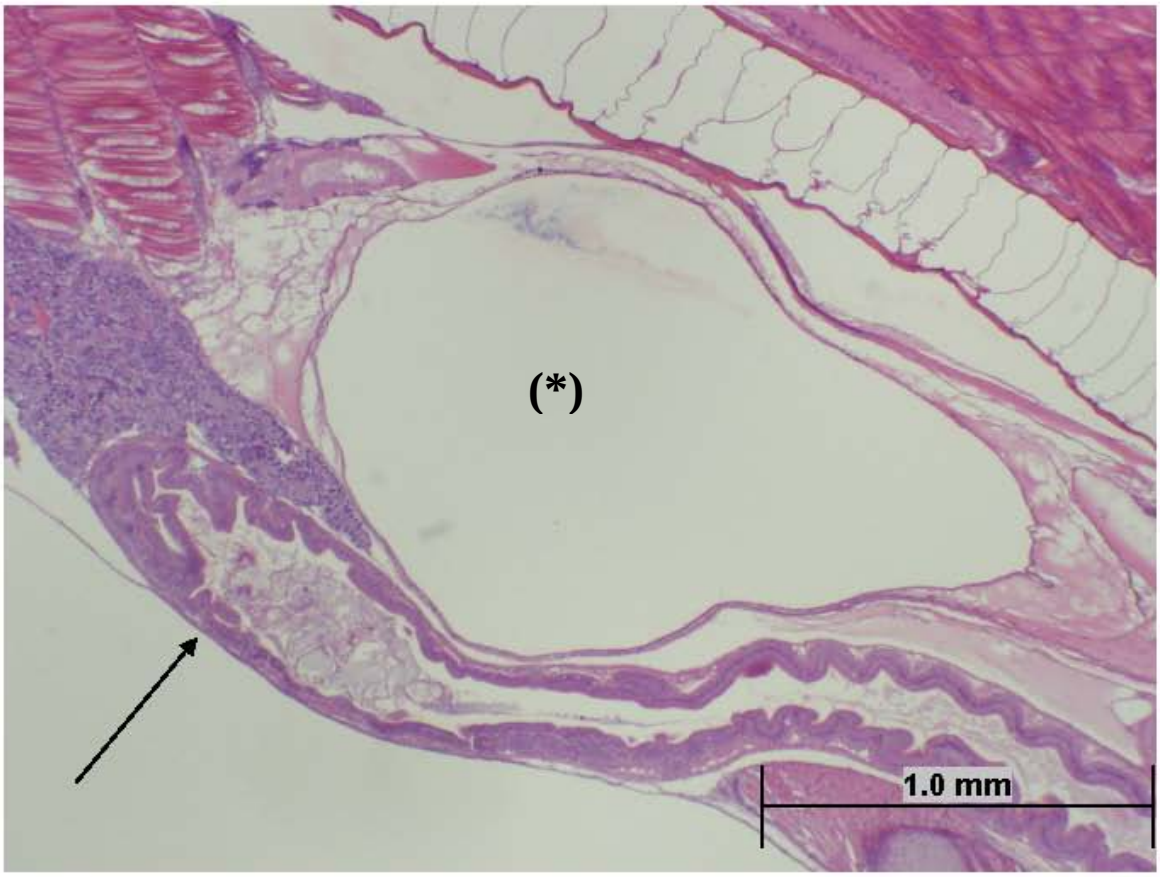


Figure 7. Triangular-shaped swim bladder in LFS larvae (*). Arrow points towards ventral surface of fish adjacent to the intestinal tract. H&E stain)



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Threadfin shad – Larval and juvenile (0+) shad were collected in the lower Sacramento and San Joaquin Rivers between 17July and 23 October 2007. A total of 90 TFS, designated as larvae (<40mm FL), were collected in July and August with an additional 390 juveniles collected in September and October (Table 2). The four targeted organs (liver, kidney, intestine, and gill listed in bold) were observed in >58% of sections (Table 3). Intestine within sagittal sections (35%) was an exception.

Epitheliocystis infection was observed in 20% (3 of 15) of larval and 37% (33 of 89) of juvenile gill sections for an overall 35% incidence of infection (Fig. 8). The cysts were not associated with necrosis or inflammation and appeared to be benign infections. Collaborative diagnosis was based on the TFS cysts staining positively for DNA (methyl green-pyronin stain), gram negative, and having a positive reaction with Giemsa stain. Wolke et al. (1970) reported similar staining characteristics for epitheliocystis infection in striped bass and white perch. Novak and La Patra (2006) describe fish epitheliocystis as obligate, intracellular gram-negative bacteria in the order Chlamydiales. The bacteria induce hypertrophy of the infected epithelial cell however most infections tend to be benign. Similar observations were made in 2006 (Foott et al. 2006).

A presumptive branchiuran crustacean (*Argulus* sp.) was seen on the gill surface in 16% (14 / 89) of TFS gill sections (Fig. 9). *Argulus* is both a marine and freshwater fish parasite with an oval dorsal shield that covers the appendages, 2 sucker-like first maxillae, and a pair of dorsal compound eyes (Kabata 1988). No inflammation was associated with this organism. A presumptive amoeba in the intestinal lumen was seen in 18% (16 / 89) of small intestine sections from the juveniles (Fig. 10). This organism had the characteristic large nucleus with central karosome and peripheral chromatin of amoeba. It did not penetrate the intestinal epithelium nor was it associated with any lesion.

Vacuoles were not observed in the 112 liver sections examined and glycogen content was judged to be moderate in both hematoxylin and eosin and Periodic Acid – Schiff stain sections. No virus was isolated from the 362 fish sampled between July and October.

Figure 8. Epitheliocystis in TFS gill. Hypertrophied gill epithelial cell containing chlamydial bacteria. H&E stain.

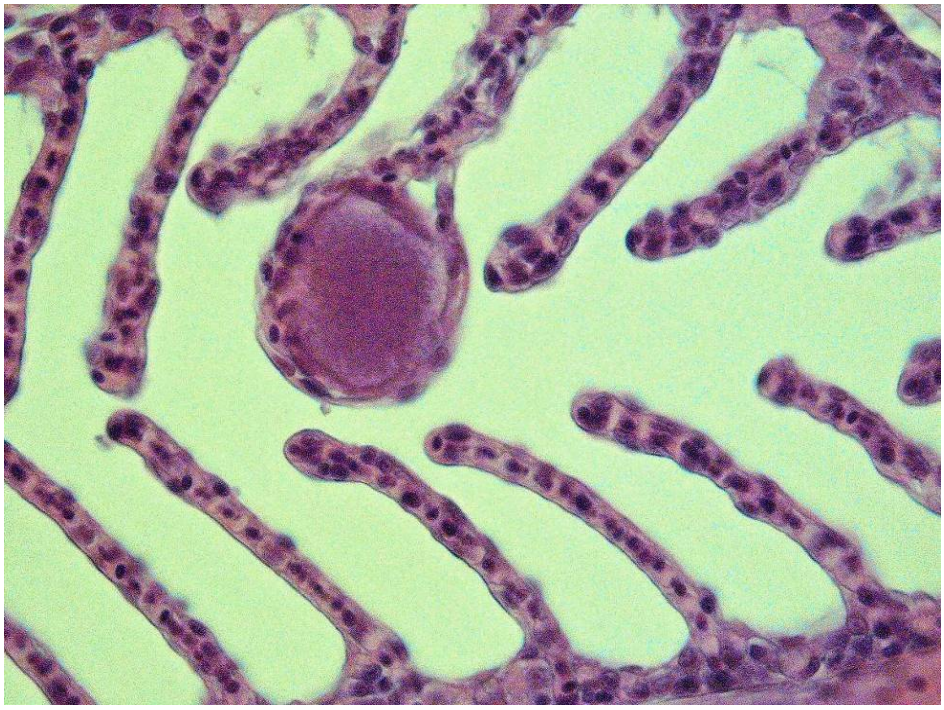


Figure 9. Presumptive Branchiuran parasite on TFS gill. Note dorsal compound eyes (arrow) and possible appendages under dorsal shield (arrowhead). H&E stain.

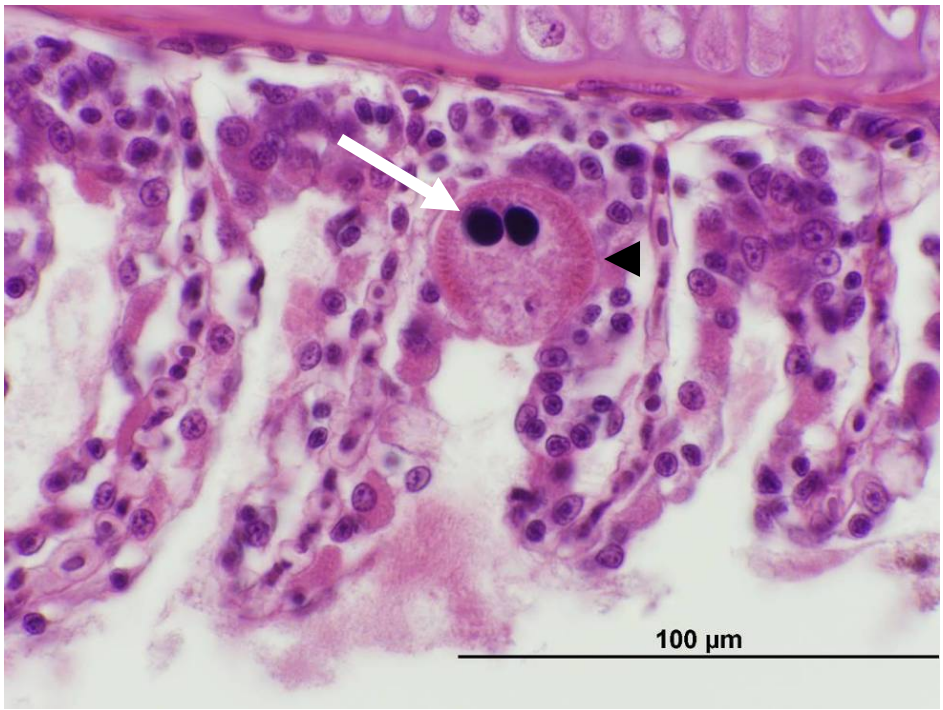
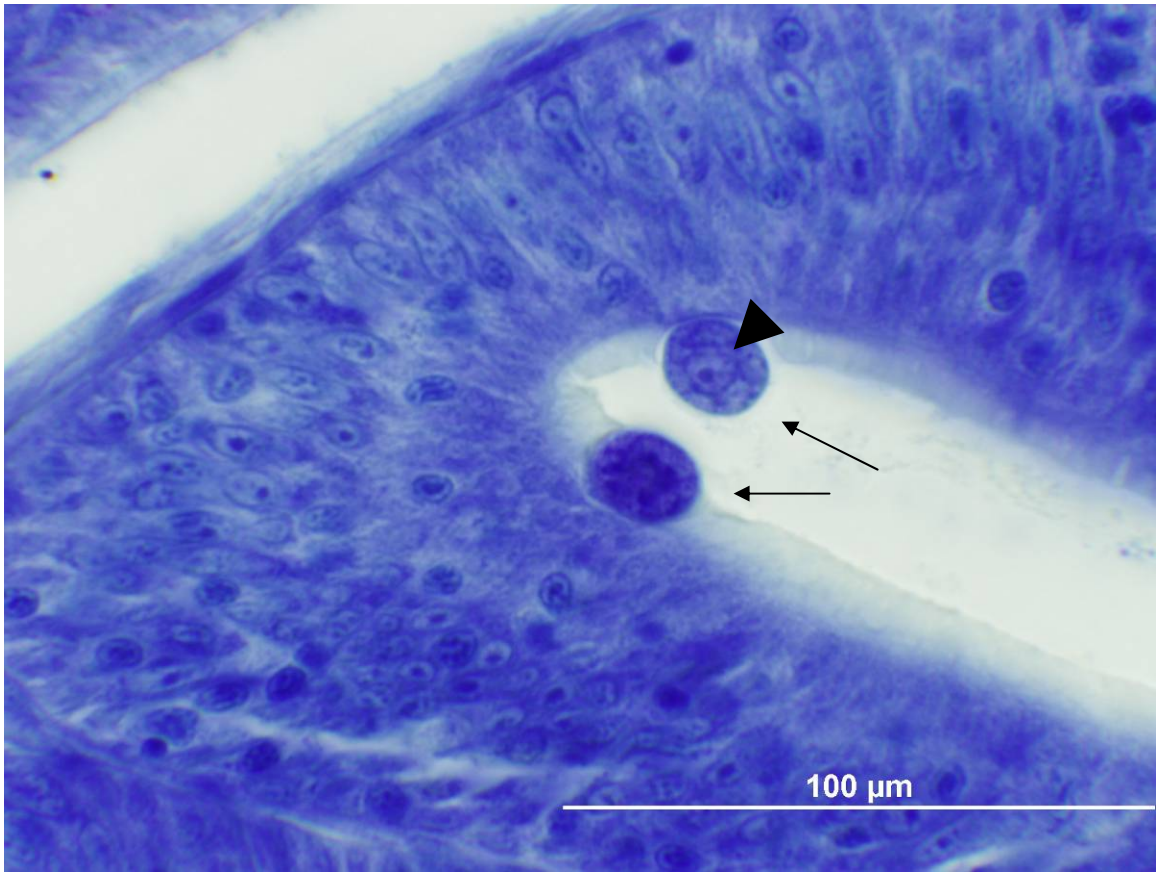


Figure 10. Presumptive amoeba (arrows) along brush border of intestinal epithelium of juvenile TFS. Note peripheral chromatin (arrowhead) and large karyosome of nucleus. Giemsa stain.



Summary- No significant health problem was detected in either TFS or LFS juveniles in 2006 or 2007. No virus was isolated in over 800 samples and the low incidence of parasitic infection was not associated with tissue damage or inflammation. In both 2006 and 2007, hepatocyte vacuolation was seen in many juvenile LFS livers from fish collected primarily in the fall. It is unknown whether fatty liver is normal for LFS or associated with toxic insults. If future studies are conducted to elucidate the cause of LFS vacuolated hepatocytes, 3 steps should be considered:

1. Prior to field sampling, empirically determine the effect of capture technique (time in net) and sorting on liver morphology of either LFS or another age 0+ smelt surrogate.

2. If capture artifacts can be minimized, continue histological analysis of juvenile and adult LFS (including immunohistochemistry assays for metallothionein, apoptosis, cytochrome p-450). A subset of fish should be fixed for Transmission Electron Microscopy analysis. Microarray analysis of up-regulated genes associated with detoxification could also be informative.

3. Analyze a subset of each LFS catch for body burden of specific contaminants (polycyclic aromatic hydrocarbons, metals). Analyze diet items (copepod and opossum shrimp) or stomach contents of larger juveniles or adult LFS for similar contaminants.

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