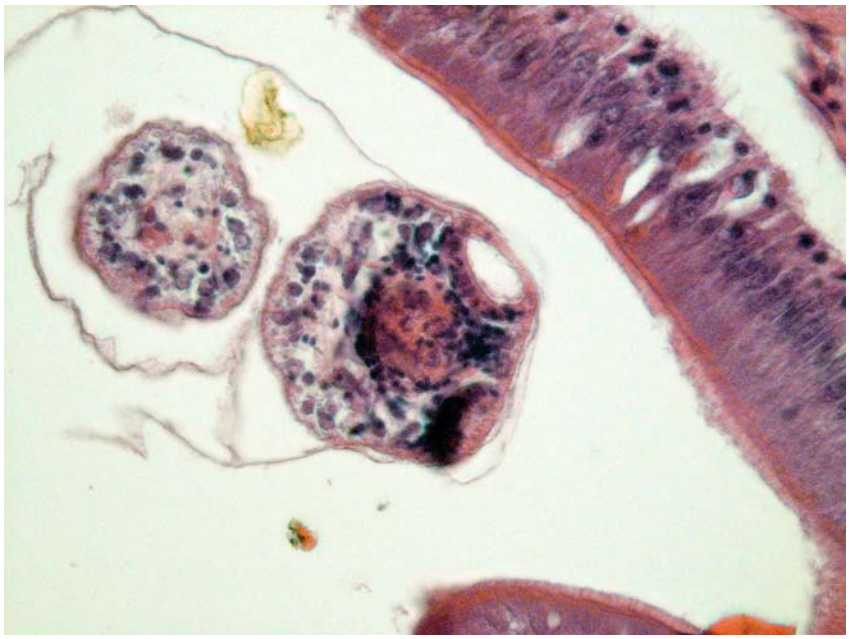
U.S. Fish & Wildlife Service

California Nevada Fish Health Center
FY2006 Investigational Report:

Histological Evaluation and Viral Survey of Juvenile Longfin Smelt (*Spirinchus thaleichthys*) and Threadfin Shad (*Dorosoma petenense*) collected from the Sacramento – San Joaquin R. Delta, April – October 2006.

J.Scott Foott*, K. True, and R. Stone



U.S. Fish and Wildlife Service
California-Nevada Fish Health Center
24411 Coleman Hatchery Road
Anderson, CA 96007
PH: (530) 365-4271 FAX: (530) 365-7150
December 2006

*direct correspondence

Introduction:

Significant declines in pelagic fish abundance in the Sacramento – San Joaquin River Delta over the last decade has prompted the Interagency Ecological Program (IEP) to augment its monitoring program with a disease screening effort in 2006 for juvenile Longfin smelt, Delta smelt, and Striped bass. The disease survey focused on virus isolation and histological examination. Delta smelt and Striped bass were examined by two UC Davis research teams headed by Drs Swee Teh and David Ostrach, respectively. The California-Nevada Fish Health Center (FHC) was tasked to examined Longfin smelt (**LFS**, *Spirinchus thaleichthys)* and Threadfin shad (**TFS**, *Dorosoma petenense*) juveniles collected between April and October 2006.

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. Locations sampled are referenced to the survey sampling gear in use at the time; Summer Townet stations represent a subset of those for the 20mm Survey, so Townet stations are not referenced in text or documented on a separate map (Table 1, Figure 1). As soon as LFS or TFS from a given tow were sorted and measured for fork length, a subset was placed in either 10% buffered formalin (Z-fix, Anatech) or Davidson's fixative, transferred to 70% ethanol after 24 – 48 h, processed for 5 μm paraffin sections (sagittal whole body or dissected organs in fish >75mm) and stained with hematoxylin and eosin (Humason 1979). Specimens from the September and October collection were de-calcified for 24h prior to processing. Initial collections were placed into 10% buffered formalin, however, poor tissue morphology resulting from shrinkage artifacts necessitated changing to Davidson's fixative starting with the 10May collection. The general sectioning protocol was to place a "shallow" (~ 50 - 100μm from the epidermis) and "deeper" (midline of fish) section onto each slide. All tissues for a given fish were placed on one slide and identified by a unique code number. Each slide was examined at both low (40X) and high magnification (400X) without knowledge of collection group.

Fish selected for virological assays were placed into cold antibiotic-mycotic solution in pools of 5 or less fish and a 40 and 100x dilution of whole body homogenate was inoculated onto both Epithelioma Papulosum Cyprini (EPC) and Chinook Salmon Embryo (CHSE214) cell lines. Cultures were held at 15°C for 18 – 21 d and examined for cytopathic effects. Suspect cultures were subsequently filtered (0.2μm) and re-inoculated on new cell cultures.

The RNA:DNA ratio growth index was determined from 20 LFS caudal sections (including tail) frozen on dry ice after collection and held at -70°C until assayed by a modification of the method of Kaplan et al. (2001). Briefly, the sample was digested with proteinase K (45°C, 50min in 10μg PK /mL TE buffer) and the supernatant assayed for total RNA and DNA with Quant- iT ™ RNA and dsDNA fluorometric kits by Molecular Probes (Eugene OR).

Results and Discussion:

Longfin smelt histology - A total of 142 of the 147 LFS processed for histology were examined for parasites and abnormalities (Table 1). Despite the inherent difficulties of obtaining sagittal sections containing all organs, four targeted organs (liver, kidney, intestine, and gill) were observed in the majority of sections (Figure 2).

Table 1. Longfin smelt (*Spirinchus thaleichthys*) collection dates, monitoring survey station numbers and their referenced survey and estuary location, fork length (FL), and histological sample numbers examined in 2006.

Date	Station No.	FL (mm)	Sample No.
29March	336,340 20mm Survey, eastern San Pablo Bay and lower Napa River	13-32 Avg=21	10
12April	320,336,340 20mm Survey, eastern San Pablo Bay and lower Napa River	12-34 Avg=22	20
26April	340 20mm Survey, lower Napa River	16 – 27 Avg=23	1
10May	328,329,334,335, 336 20mm Survey, eastern San Pablo Bay	18 – 39 Avg=25	25
24May	334,335,336 20mm Survey, eastern San Pablo Bay	16 – 30 Avg=25	10
18July	318 – 323,325,346 Bay Study Survey, throughout San Pablo Bay	27 – 80 Avg=42	27
29August	418,504,602 Fall Midwater Trawl Survey, Suisun and Grizzly bays	29 – 95 Avg=50	19
26-27October	517,519,504,601,609,610 20mm Survey and Fall Midwater Trawl Survey, Honker Bay and Montezuma Slough	45 – 98 Avg=60	35
	total		147

Figure 1. Map of 20mm Survey sample station numbers

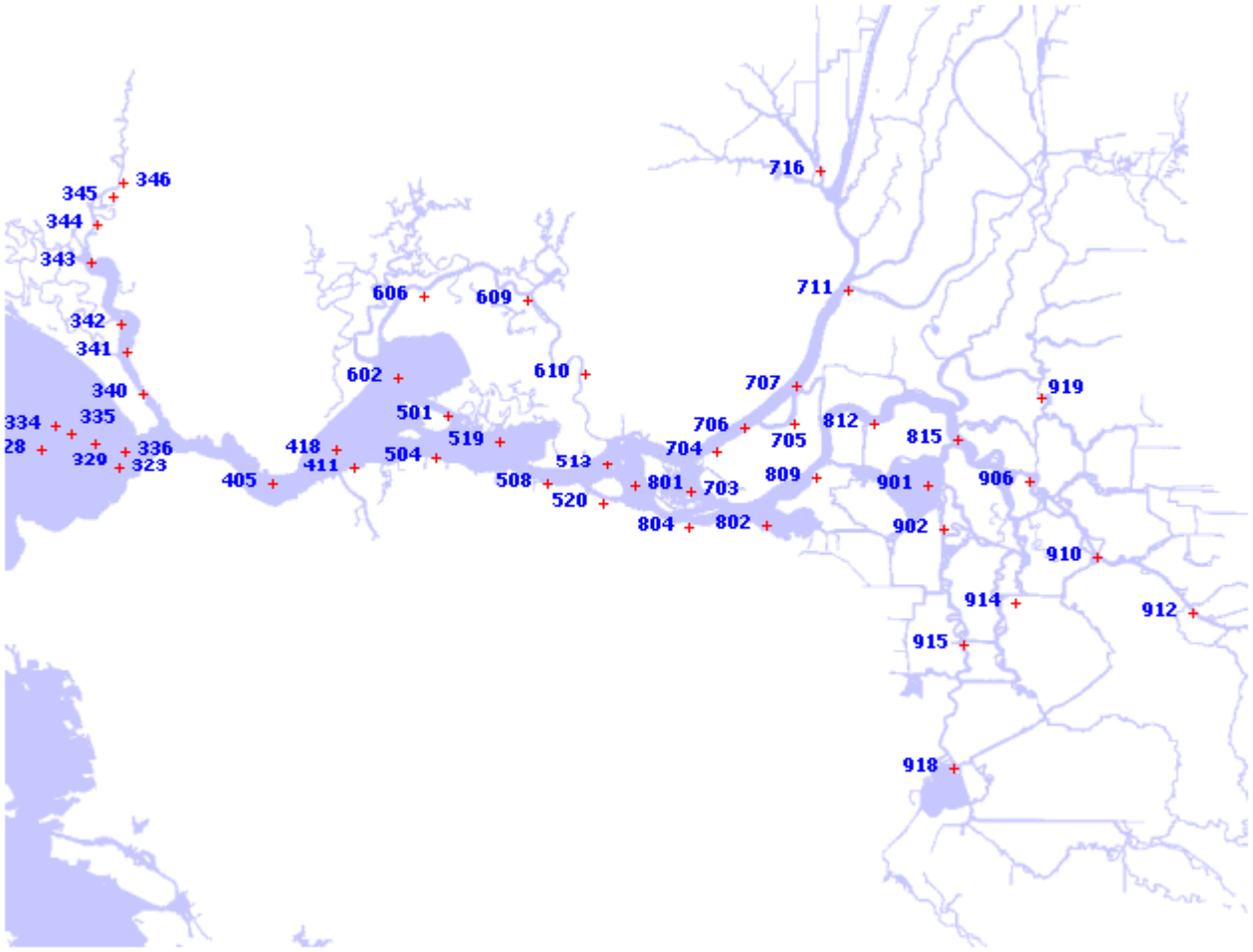
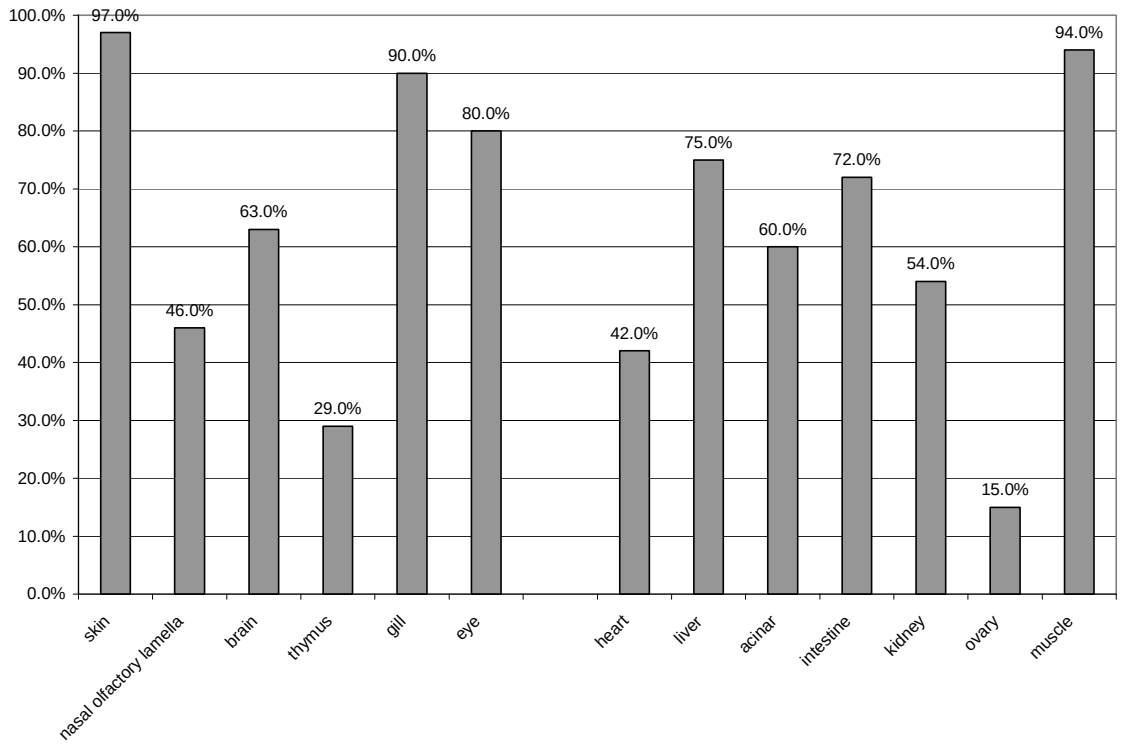
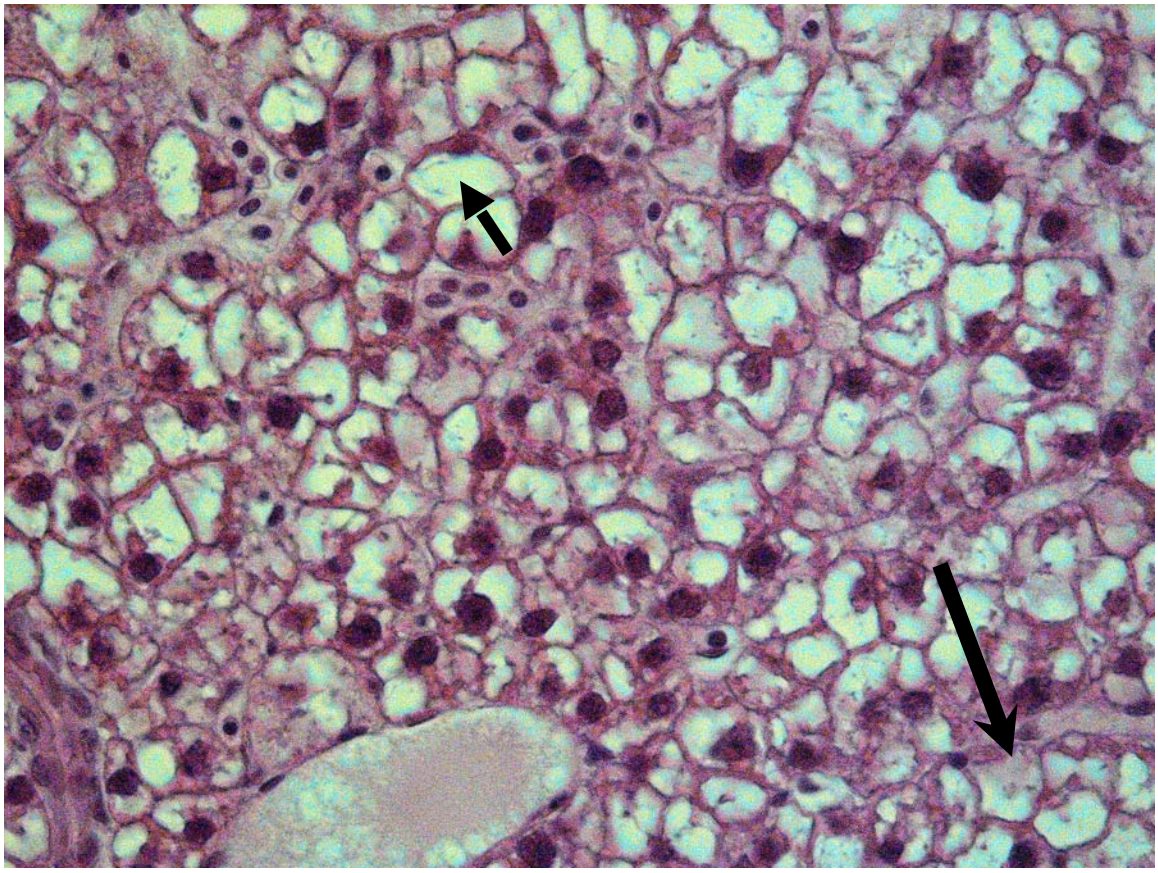


Figure 2 Percent of Longfin smelt histological sections containing specific organs.



No abnormalities were observed in the LFS tissues. Hepatocyte vacuoles (vacuolated hepatocytes in $\geq$ 30% of the liver section) were seen in 14 of 107 liver sections (13%) beginning with the 18JULY collection. It is the lead author's opinion that this condition indicates that the smelt were storing excess energy reserves during the summer months and is not an abnormality. This opinion is based on previous work done on fatty liver diagnosis in juvenile hatchery salmon (Free and Foott, 2000). The 2 types of vacuoles observed in the LFS livers contained either fat (defined vacuole wall with cleared interior that is PAS negative and cryosections from the same liver stain positive with Oil Red-O) or glycogen (microvesicular or with poorly defined walls containing eosinophilic granular material that is PAS positive). The LFS livers contained varying percentages of both types of vacuoles and no nuclear abnormalities or necrotic changes were associated with these cytoplasmic vacuoles (Figure 3).

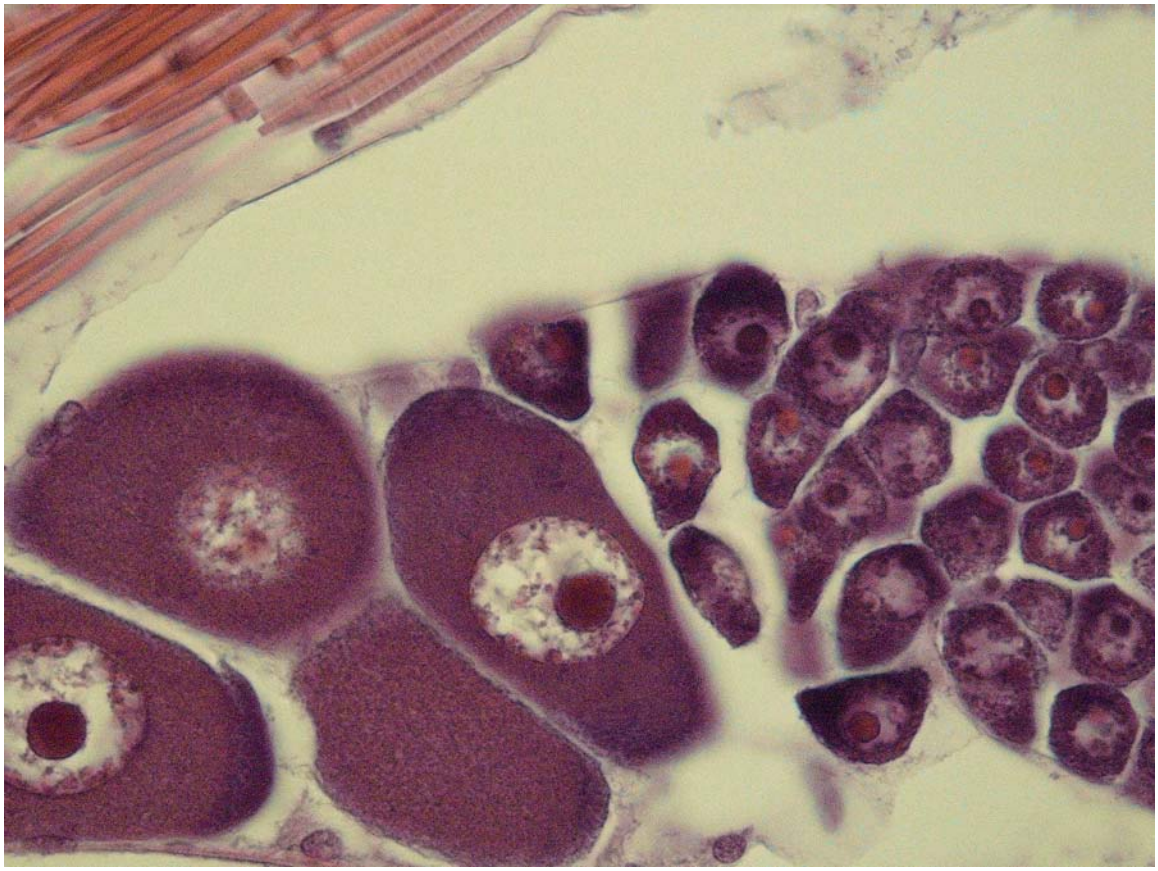
Figure3. LFS liver section containing vacuolated hepatocytes. Presumptive fat-containing (short arrow) and glycogen (long arrowhead) containing vacuoles (400x total magnification, Davidson's fixative H&E stain).



Small immature cestodes, resembling the pleurocercoid stage of *Phyllobothrium salmonis,* were observed in the intestinal tracts of 16 smelt (16% incidence of infection). The first detection occurred in a 10May sample and infected fish occurred through the final 26OCT sample. This presumptive identification is based on the observation of at least 3 suckers on the scolex without an apical sucker (see photomicrograph on title page) and the common occurrence of this cestode in adult Sacramento River salmon. No inflammation or necrosis was associated with these cestodes. Copepods, an element in the LFS's diet, are an intermediate host for the cestode's procercoid stage that is infectious to fish. No external parasites were observed on the skin or gills with the exception of 2 fish collected on 27OCT. Several "ciliate / ameba-like" parasites were seen on gill lamellae without any associated inflammation. Histological processing can result in the loss of unattached external parasites however, inflammatory signs of external parasite infection was not observed in the sample set.

Ovary was observed in 15 LFS (11%) while testis was identified in only one fish. The first observation of ovary occurred in a 10MAY fish. The most mature ova stage seen was the primary oocyte with little to no yolk vesicles (Fig.4).

Figure 4. Ovary section from LFS female collected 26OCT06 (400x total magnification, Davidson's fixative H&E stain).



Longfin smelt viral samples – A total of 404 whole fish homogenate samples were assayed for virus (Table 2). No viral isolates were obtained from the sample set. Cytopathic (CPE) changes, such as syncytium formation, in EPC cell cultures were observed in several samples from the 29March, 29August, and 26October collection but did not replicate further when a 0.22µm filtrate of the original culture was passed onto fresh EPC cultures. It is unclear whether the initial CPE was due to toxicity or a virus.

Table 2. Longfin smelt (*Spirinchus thaleichthys*) collection dates, monitoring survey station numbers and their referenced survey and estuary location, and viral sample numbers in 2006. Sample collections that showed suspected Cytopathic Effects (CPE) in the initial EPC culture are identified.

Date	Station No.	Sample No.	CPE
29March	336,340 20mm Survey, eastern San Pablo Bay and lower Napa River	60	+
12April	320,336,340 20mm Survey, eastern San Pablo Bay and lower Napa River	76	
26-27April	328,336,340 20mm Survey, eastern San Pablo Bay and lower Napa River	19	
10May	328,329,334,335,336 20mm Survey, eastern San Pablo Bay	80	
24May	334,335,336 20mm Survey, eastern San Pablo Bay	60	
21June	335, 341 20mm Survey, eastern San Pablo Bay and lower Napa River	7	
18July	318 – 323,325,346 Bay Study Survey, throughout San Pablo Bay	60	
29August	418,501,602 Fall Midwater Trawl Survey, Suisun and Grizzly bays	20	+
26-October	504,517,519,601,606,609,610 20mm Survey and Fall Midwater Trawl Survey, Honker Bay and Montezuma Slough	22	+
	total	404	

Pilot effort in RNA:DNA ratio analysis - The total quantity of DNA per cell is relatively constant while RNA content tends to reflect protein synthesis activity. The basis of the R:D ratio as a growth measurement is that growing tissue will contain cells with high RNA to DNA ratios. The mean R:D ratios obtained from the 16 caudal sections was within the values reported for juvenile mummichog white muscle samples (Kaplan et al. 2001) although the authors used a different RNA assay (Table 3). The coefficient of variation was extremely high for the sample set. We observed poor proteinase K digestion of the fin tissue and speculate that fin would contribute a different R:D ratio than white muscle. If this

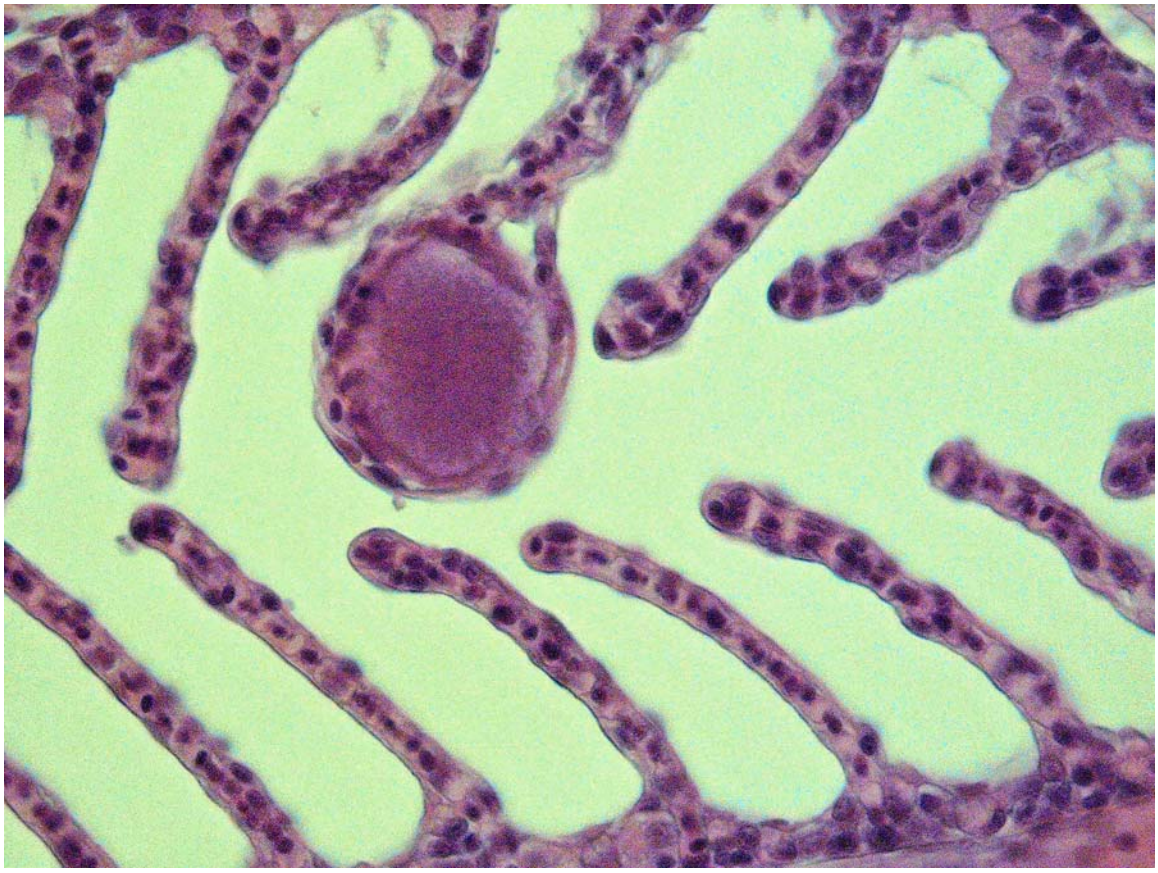
growth index is desired for future surveys, only caudal muscle should be included in the sample.

Table 3. Mean RNA:DNA ratio (Std. Dev).

Collection	R:D ratio
26April, n = 1	0.83
21June, n = 7	1.67 (1.58) high 95% CV
18July, n= 8	1.68 (0.74) 44%CV

Threadfin shad - One viral sample group (n=11) was collected on 26OCT and no viral CPE was observed in the cultures. A total of 15 juvenile TFS were processed for histological examination from the 46 collected. These fish were collected by CDFG on 29AUG (n = 3, site 602) and 19SEP (n =43, sites 511,512,609, and 703). Only 12 of the 43 TFS in the 19SEP collection were processed due to poor fixation issues (too many fish in a given tube of fixative). Epitheliocystis (chlamydia) infection was observed in the gill lamellae of 11 TFS (73%). The few cysts observed per section appear to be relatively benign and did not elicit an inflammatory response (Fig. 5). Five TFS sagittal sections contained immature ovaries and 2 fish had livers with vacuolated hepatocytes similar to the LFS. No other abnormalities were observed in sections.

Figure 5. Epitheliocystis cyst in TFS gill lamellar cell (400x total magnification, Davidson's fixative H&E stain).



Summary- LFS and TFS juveniles collected in 2006 appeared to be relatively healthy and did not demonstrate significant parasitic infections or tissue abnormalities. We did not isolate virus in the tissue cultures (EPC and CHSE214 cell lines) employed for the survey but this area could benefit from addition of other fish cell lines such as Fathead Minnow. The growth index, RNA:DNA ratio, can be measured from larval LFS under the current collection protocols however fin tissue should be excluded from the sample. Given the labor required for the histological analysis (>60 h for processing and another 30+ h for microscopic examination) and lack of observed abnormalities, we do not recommend that future health LFS surveys employ this same level of histological sampling.

Acknowledgements: Partial funding for this project was obtained from the Interagency Ecological Program. We thank the following cooperators for their assistance with field collection and logistics: CDFG Bay-Delta Program biologists (in particular Julio Samii and Steve Slater), Gouhua Zhang (UC Davis, T Sweh Laboratory), and Dr. David Ostrach. , Both L. Rosten and J. Grey helped with viral assays.

Reference:

Free D and JS Foott. 2000. FY97 investigational report: Effects of diet composition, feeding rate, and water temperature on liver lipoid disease, growth, smolt development, and adult return in Coleman NFH Late-Fall Chinoook Salmon

(broodyear 1996). USFWS Ca-NV Fish Health Center, Anderson CA (http://www.fws.gov/canvfhc , Activities / reports).

Humason GL. 1979. Animal tissue techniques. 4th ed., WH Freeman and Co., San Francisco.

Kaplan LA, J Leamon, and JF Crivello. 2001. The development of a rapid and sensitive, high-through-put protocol for RNA:DNA ratio analysis. Journal of Aquatic Animal Health 13:276 – 279.