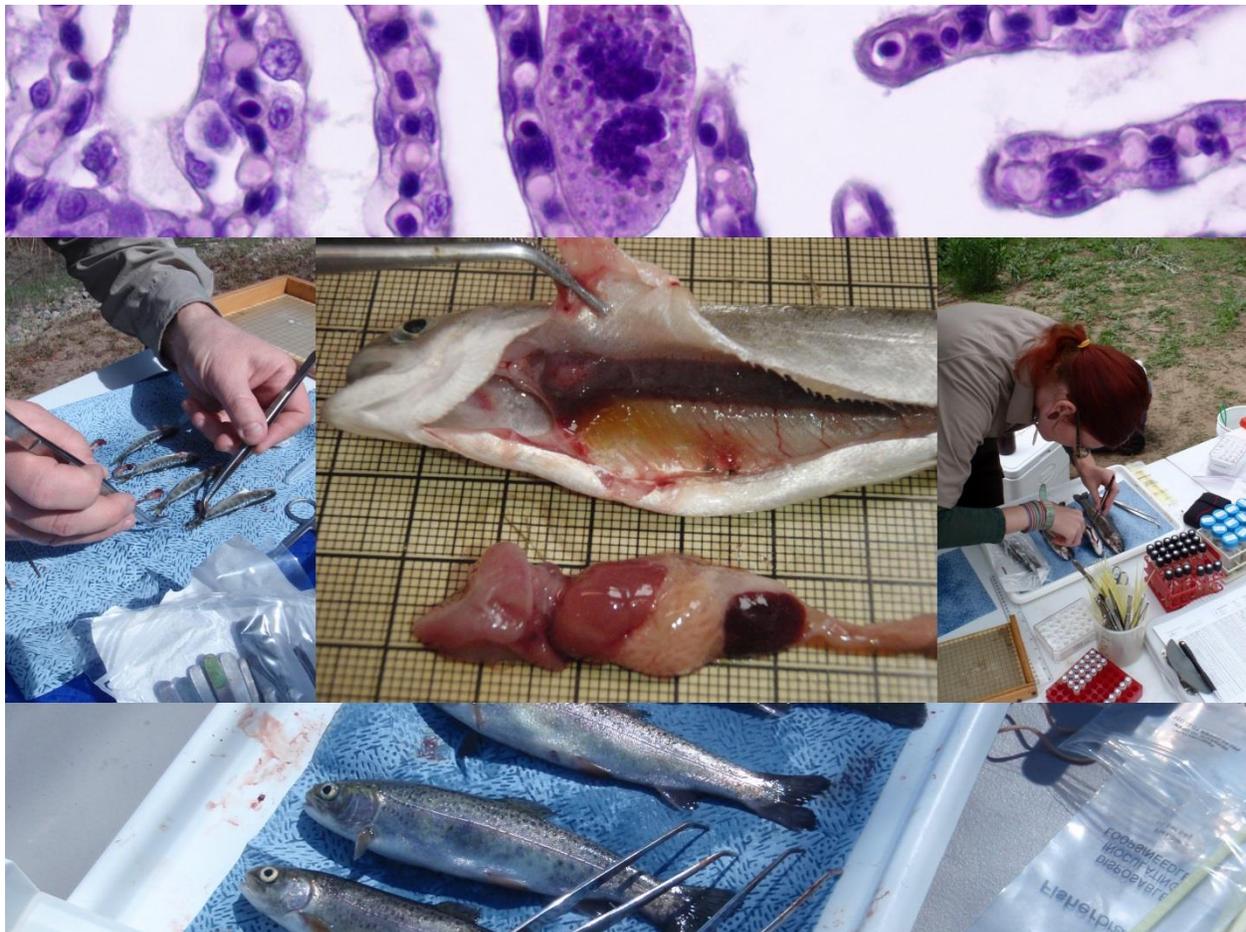


U.S. Fish & Wildlife Service

FY2013 Technical Report: Pathogen Screening and Gill Na^+/K^+ -ATPase Assessment of South Delta Chinook and Steelhead 2013 Release Groups

Ken Nichols



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US Fish and Wildlife Service
California-Nevada Fish Health Center
24411 Coleman Fish Hatchery Rd
Anderson, CA 96007
(530) 365-4271 Fax: (530) 365-7150
<http://www.fws.gov/canvfhc/>

Summary

As a component of studies examining the reach-specific survival and distribution of migrating juvenile Chinook salmon and steelhead in the San Joaquin River and Delta, the CA-NV Fish Health Center conducted a general pathogen screening and smolt physiological assessment. Juvenile Chinook salmon and steelhead trout were surveyed for specific fish pathogens and smolt development using gill Na⁺/K⁺-ATPase (gill ATPase) activity levels. The health and physiological condition of the study fish can help explain their performance and survival during the studies. In both steelhead and Chinook release groups, survival over the 24 holding period was high. The myxozoan parasite *Tetracapsuloides bryosalmonae* was detected at moderate to high levels in a majority of the Chinook sampled. Anemia associated with late stage PKD was not observed. The infection was progressive and impacts on survival could occur within the study period (30 days). No other significant pathogen infections were detected in either the Chinook or steelhead. Gill ATPase activity levels were lower in later release groups of both Chinook and Steelhead suggesting these later groups were beyond the peak of smoltification.

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BACKGROUND

As a component of studies examining the reach-specific survival and distribution of migrating juvenile Chinook salmon and steelhead in the San Joaquin River and Delta, the CA-NV Fish Health Center conducted a general pathogen screening and smolt physiological assessment. Steelhead trout were examined in support of the 6-year Study required by the 2009 Biological Opinion on Central Valley Project and State Water Project operations (RPA IV.2.2). The health and physiological condition of the study fish can help explain their performance and survival during the studies. Similar pathogen screening and physiological assessments have been conducted on Chinook used in various south delta studies since 1996. These past examinations have identified the myxozoan parasite *Tetracapsuloides bryosalmonae*, the causative agent of Proliferative Kidney Disease (PKD), in juvenile Merced River Hatchery Chinook. This parasite has been shown to cause mortality in Chinook salmon with increased mortality and faster disease progression in fish at higher water temperatures (Ferguson 1981; Foott et al. 2007). In 2013, juvenile Chinook salmon and steelhead trout were surveyed for specific fish pathogens and smolt development using gill Na⁺/K⁺-ATPase activity levels.

METHODS

FISH SAMPLING

All study fish were cohorts of acoustic tagged release groups and shadowed each release group through handling, tagging (dummy tagged), transport, and in-river holding. Study fish were held for 48 hours at the Durham Ferry release site on the San Joaquin River before sampling. Groups of 30 juvenile Merced River Hatchery Chinook salmon were sampled on 5 May and 19 May, 2013. Groups of 24 Mokelumne River Hatchery yearling steelhead trout were sampled on 9 March, 6 April and 11 May, 2013. Fish were euthanized; fork length (FL), weight (Wt) and any abnormalities were noted; and tissue samples for lab assays were collected. In addition to the release groups, an additional 30 Chinook were sampled at Merced River Hatchery on 3 May, 2013 (MRH group). Only kidney tissue for the histopathology assay was collected from the MRH group.

LAB ASSAYS

Bacteriology – A sample of kidney tissue was collected aseptically and inoculated onto brain-heart infusion agar. Bacterial isolates were screened by standard microscopic and biochemical tests (USFWS and AFS-FHS 2010). These screening methods would not detect *Flavobacterium columnare*. *Renibacterium salmoninarum* (the bacteria that causes bacterial kidney disease) was screened by fluorescent antibody test of kidney imprints.

Virology – Three fish pooled samples of kidney and spleen were inoculated onto EPC and CHSE-214 at 15°C as described in the AFS Bluebook (USFWS and AFS-FHS 2010) with the exception that no blind pass was performed.

Histopathology – The gill and/or posterior kidney were removed from the fish and immediately fixed in Davidson's fixative. In the lab, the tissues were processed for 5 µm paraffin sections and stained with hematoxylin and eosin (Humason 1979). All tissues for a

given fish were placed on one slide and identified by a unique code number. Each slide was examined under a light microscope and observations of abnormalities were noted. Gill was sampled from both Chinook and steelhead release groups and examined for signs of external parasite infection. Kidney was sampled from Chinook release groups and screened for the *T. bryosalmonae* parasite. Infections of the myxozoan parasite *T. bryosalmonae* were rated for intensity of parasite infection and associated tissue inflammation. Intensity of infection was rated as none (zero), low (<10), moderate (11-30) or high (>30) based on number of *T. bryosalmonae* trophozoites observed in the kidney section. Severity of kidney inflammation was rated as normal, focal, multifocal or diffuse.

Gill ATPase – Gill Na⁺/K⁺-Adenosine Triphosphatase (gill ATPase) activity was assayed by the method of McCormick (1993). Gill ATPase activity is correlated with osmoregulatory ability in saltwater, and high concentrations are found in the chloride cells of the lamellae.

RESULTS

FISH CONDITION

Chinook – The size and condition of the release groups are summarized in Table 1. No mortality occurred with either sample group. Externally, there were no observations of pale gills, significant scale loss or external hemorrhaging. Sutures were all in good condition with minor inflammation noted in 3% (1/30) of fish on 5 May and 7% (2/30) of fish on 19 May. Internally, clinical signs of PKD (swollen kidney and/or spleen) were observed in 23% (7/30) of fish on 5 May and 23% (7/30) fish on 19 May.

Table 1. Mean (\pm standard deviation) fork length (FL), weight (Wt), Fulton condition factor (KFL) and sample size (N) for Chinook salmon release groups.

Group	FL (mm)	Wt (g)	KFL	N
5 May	113.9 \pm 5.0	17.0 \pm 2.4	1.15 \pm 0.06	30
19 May	117.2 \pm 5.9	18.6 \pm 2.9	1.15 \pm 0.04	30

Steelhead – The size and condition of the release groups are summarized in Table 2. No mortalities prior to sampling occurred in the March group, one moribund (dying) fish was observed in the April group, and there was one mortality and one moribund fish in the May group. All fish were euthanized at once on the March sample, so some fish were dead up to 2 hours before sampling. In the April and May samples, fish were euthanized in three fish groups immediately before sampling. No pale gills, excessive scale loss or external hemorrhaging were observed; however one fish with a missing eye and another with a healed wound on the belly were noted in the March fish group. No problems with sutures were noted in the fish sampled in March (0/23); minor inflammation at the suture site was noted in 17% (4/24) of the April fish; and 8% (2/24) of the May fish had poorly healed partly open sutures. Internally, an unidentified kidney cyst was observed in one (1/23) fish from the March group, and no other gross internal abnormalities were observed in the steelhead examined in March, April or May.

Table 2. Mean (\pm standard deviation) fork length (FL), weight (Wt), Fulton condition factor (KFL) and sample size (N) for steelhead sample groups.

Group	FL (mm)	Wt (g)	KFL	N
March	201 \pm 21	79 \pm 27	0.94 \pm 0.08	23
April	209 \pm 19	84 \pm 23	0.89 \pm 0.06	24
May	221 \pm 14	102 \pm 18	0.93 \pm 0.10	24

BACTERIOLOGY AND VIROLOGY

In both Chinook and steelhead sample groups, no virus or other cytopathic effects were observed by cell culture over the 21 day incubation period. No obligate fish pathogens were detected, and other isolates were isolated in 5-23% of sample groups (Table 3). These other isolates were common fauna in the environment and fishes GI tract (Aoki 1999) and were likely contaminants due to field sampling conditions.

Table 3. Summary of bacteria isolated from the kidneys of dummy tagged fish. These isolates were likely contaminants from which are commonly found in surface water, soil or the fish's GI tract.

Species	<i>Aeromonas /Pseudomonas</i>	various Gram positive bacteria
Chinook	5% (3/60)	23% (14/60)
Steelhead	6% (4/71)	10% (7/71)

GILL HISTOLOGY

Chinook – No parasite infections or significant inflammation was seen in gill sections from the 5 May or 19 May Chinook sample groups.

Steelhead – The majority of the fish sampled in March demonstrated epithelial edema which was most likely a post mortem change due to premature euthanization of this group. Minor gill edema was observed in 33% (8/24) of steelhead in the April sample and 4% (1/24) in May, but no significant inflammation or gill lesions were observed in any of the sample groups. An unidentified protozoan parasite (Figure 1A) was observed in 39% (9/23) of fish sampled in March, 63% (15/24) of fish in April and 8% (2/24) of fish sampled in May. The prevalence of this protozoan parasite was highest in April ($P < 0.001$, Kruskal-Wallis test). Cyst-like zenomas of an unidentified Microsporidia (Figure 1B) were noted in 8% (2/24) of fish from the April and May samples groups, but were not observed in fish from the March group. As noted above, there was no significant gill inflammation or other signs of gill damage associated with these infections.

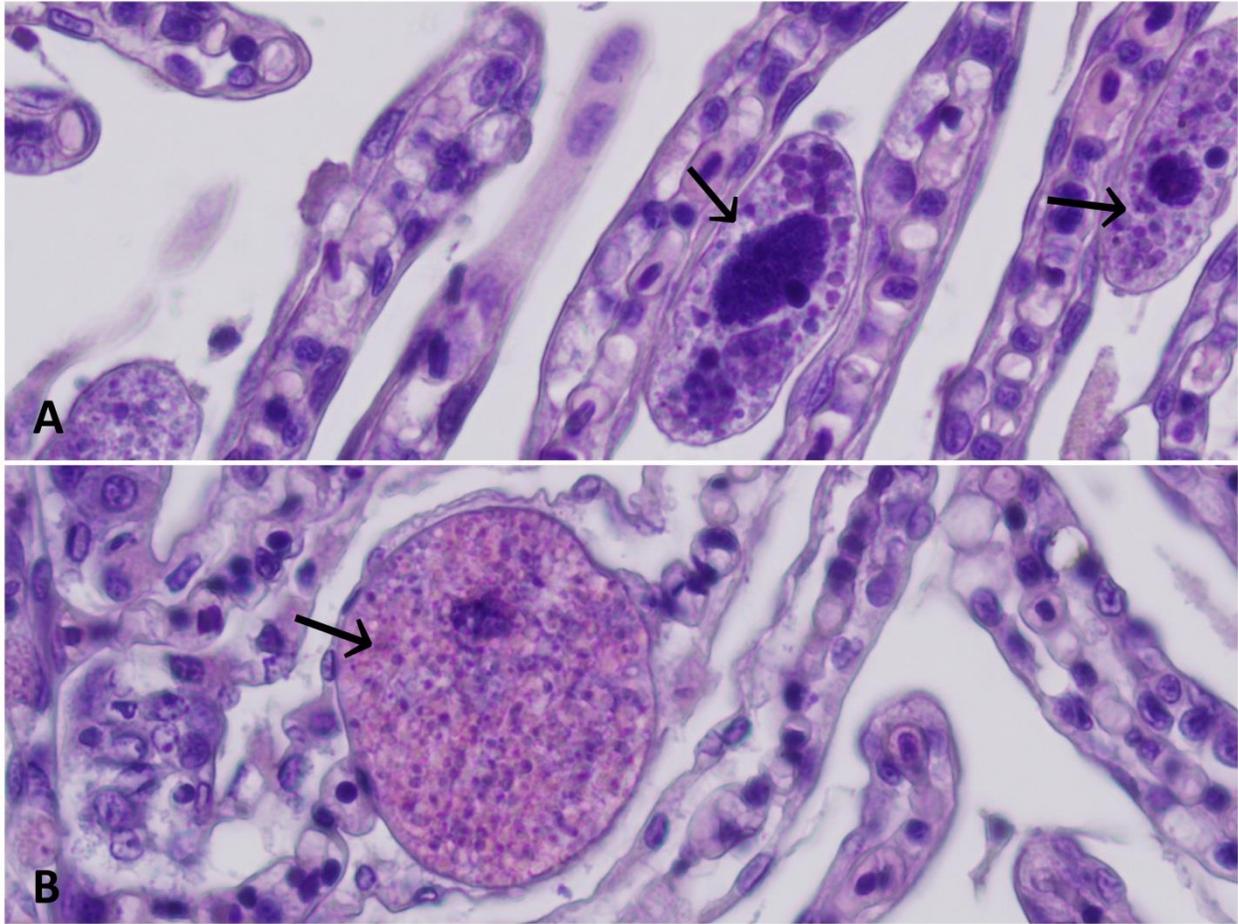


Figure 1. Parasite infections observed in histopathological examination of steelhead gills. No inflammation or other tissue damage was associated with these infections. (A) Unidentified external protozoan observed on steelhead gills from March, April and May release groups. (B) Zenoma of an unidentified Microsporidea observed in April and May release groups.

KIDNEY HISTOLOGY

Chinook – The *T. bryosalmonae* parasite was detected in fish from all three Chinook release groups, with 80% to 100% of the fish infected. The intensity of the infections (based on number of parasites) was rated as high in over half of the fish from each release group (Table 4). There was no significant difference detected in the severity of the infections between release groups (Table 5, $p=0.089$, Fisher's exact test for count data).

Table 4. Prevalence and intensity of *T. bryosalmonae* infection in kidney tissue of juvenile Chinook. Data presented as number of fish with zero (None), few than 10 (Low), 11-30 (Moderate) or greater than 30 (High) parasites observed in kidney tissue by histopathology. No significant difference was detected between release groups (p=0.101, Fisher's Exact Test for Count Data).

Group	None	Low	Moderate	High
MRH (3 May)	1	10	2	16
5 May	5	5	1	14
19 May	0	9	5	16

Table 5. Severity of kidney inflammation associated with *T. bryosalmonae* infection in juvenile Chinook. Data presented as the number of fish with kidney inflammation rated as normal, focal, multifocal or diffuse by histopathology. No significant difference was detected between release groups (p=0.089, Fisher's Exact Test for Count Data).

Group	Normal	Focal	Multifocal	Diffuse
MRH (3 May)	4	11	11	3
5 May	5	9	7	4
19 May	0	12	8	10

GILL ATPASE ACTIVITY

Chinook – Gill ATPase activity levels ($\mu\text{mol ADP} \cdot \text{mg protein}^{-1} \cdot \text{hr}^{-1}$) ranged from 2.8 to 19.3. The activity levels in the 5 May release group was significantly higher than 19 May (Figure 2, $P < 0.001$, Wilcoxon rank sum test)).

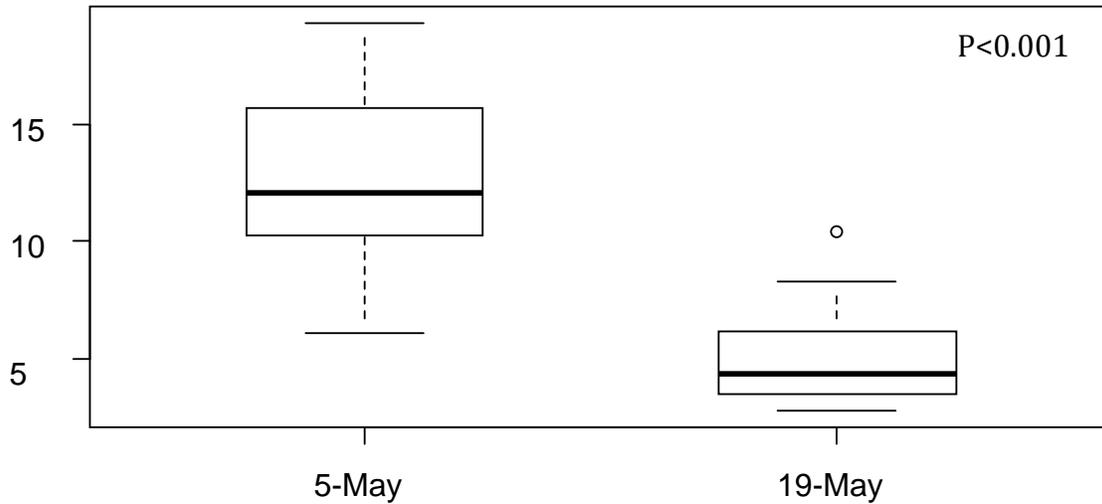


Figure 2. Boxplot of median gill ATPase activity ($\mu\text{mol ADP}\cdot\text{mg protein}^{-1}\cdot\text{hr}^{-1}$) in juvenile Chinook salmon sampled from the 5 May and 19 May release groups. A significant difference was detected between the release groups ($P < 0.001$, Wilcoxon rank sum test).

Steelhead – Gill ATPase activity levels ($\mu\text{mol ADP}\cdot\text{mg protein}^{-1}\cdot\text{hr}^{-1}$) ranged from 0.78 to 10.34. Activity levels were greatest in the March release group and decreased in the April and May groups (Figure 3, $P < 0.001$, ANOVA)

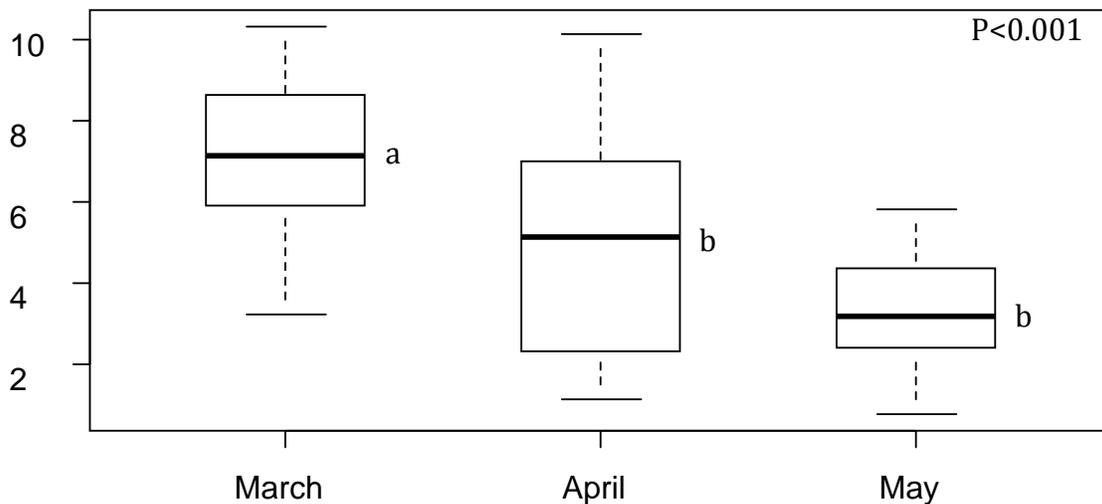


Figure 3. Boxplot of median gill ATPase activity ($\mu\text{mol ADP}\cdot\text{mg protein}^{-1}\cdot\text{hr}^{-1}$) in juvenile steelhead from the March, April or May release groups. Groups with letter subscripts in common were not significantly different ($P < 0.001$, ANOVA).

DISCUSSION

The most significant health problem observed was the *T. bryosalmonae* infection in the Chinook release groups. Anemia associated with late stage PKD was not observed. The infection is progressive and may have impacted survival of the Chinook release groups within the typical (30 day) battery life of the acoustic tags (Ferguson 1981; Foott, Stone and Nichols 2007). In past VAMP studies where fish were held in the laboratory for monitoring, total mortality due to the disease was low at 20%-27% (Foott, Stone and Nichols 2007; Foott and Stone 2008). Direct and indirect mortality rates due to PKD in study fish which must actively traverse the Delta are not known.

In the steelhead release groups, the minor abnormalities observed (gill edema, suture inflammation, parasite infections) were not judged to be a cause of poor performance or survival at the time of sampling. The gill edema common in the March release group was believed to be an artifact of the sampling procedure and not an indication of poor fish condition. Whether the parasite infections progressed after release to the point fish performance was affected was not known.

Gill ATPase activity levels in both the Steelhead and Chinook release groups were lower in the later release(s) which suggests activities were beyond peak levels and declining in those groups. Gill ATPase activity in salmonids typically increases and peaks near the time of most active migratory behavior (Duston, Saunders and Knox 1991; Ewing, Ewing and Satterthwaite 2001; Wedemeyer 1996). Decreases in gill ATPase activity can also occur due to increases in water temperature (Duston et al. 1991). More active migratory behavior in the 5 May Chinook and March steelhead release groups would be consistent with the gill ATPase levels.

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