U.S. Fish & Wildlife Service

PATHOGEN SCREENING AND GILL NA+/K+- ATPASE ASSESSMENT OF SOUTH DELTA CHINOOK AND STEELHEAD 2015 RELEASE GROUPS

Ken Nichols

October 2015

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

The California-Nevada Fish Health Center performed an assessment of the health and smolt development of steelhead trout and Chinook salmon utilized in south Delta salmonid survival studies in March-May, 2015. A suite of pathogen assays and gill Na^+/K^+-ATPase activity measurements were performed on cohorts of acoustic tagged release groups to help explain any performance and survival differences during the studies. In both steelhead and Chinook health assessment groups, mortality over the holding period was low (0-4%) and no significant pathogen infections were detected. Differences in gill Na^+/K^+-ATPase activity were observed in both steelhead and Chinook sample groups; however the differences were small and likely would not effect migration or survival.

Recommended citation for this report is:
Nichols, K. 2015. Pathogen screening and gill Na+/K+- ATPase assessment of south Delta Chinook and steelhead 2015 release groups. U.S. Fish & Wildlife Service, California-Nevada Fish Health Center, Anderson, CA. Accessible at: http://www.fws.gov/canvfhc.

Notice:
The mention of trade names or commercial products in this report does not constitute endorsement or recommendation for use by the Federal government. The findings and conclusions in this report are those of the author and do not necessarily represent the views of the US Fish and Wildlife Service.

BACKGROUND

As a component of the south Delta salmonid survival studies examining reach-specific survival and distribution of migrating juvenile Chinook salmon and steelhead trout in the San Joaquin River and Delta, the California-Nevada Fish Health Center conducted a general pathogen screening and smolt physiological assessment. Steelhead trout were sampled in support of the 6-year Study required by the 2009 Biological Opinion on the Central Valley Project and State Water Project operations (RPA IV.2.2). The health and smolt development of the study fish can help explain their survival and migration performance during the studies. Similar pathogen screening and physiological assessments have been conducted on Chinook used in various studies in the south Delta since 1996. In the majority of these past studies, juvenile Merced River Hatchery Chinook were utilized, and occasionally significant infections with the myxozoan parasite *Tetracapsuloides bryosalmonae*, the causative agent of Proliferative Kidney Disease (PKD), were observed (Foott, Stone and Nichols 2007; Foott 2012). In 2014, the source for the juvenile Chinook changed to Mokelumne River Hatchery due to health concerns. No significant health issues were observed in the Mokelumne River fish in 2014 (Nichols 2014). Steelhead trout from Mokelumne River Hatchery have been assessed for these studies since 2010 and no significant health issues have been observed in these fish to date.

METHODS

STUDY FISH

Both Chinook salmon and steelhead trout were obtained from the California Department of Fish and Wildlife Mokelumne River Hatchery. Health assessment groups were cohorts of acoustic tagged release groups and shadowed their tagged cohorts through handling, tagging (dummy tagged), transport, and in-river holding.

Steelhead – Groups of 24 yearling steelhead were sampled on 7 March, 28 March and 25 April, 2015. These groups were held in the San Joaquin River at the Durham Ferry release site for 48 hours prior to sampling.

Chinook – Groups of 30 juvenile Chinook salmon were sampled on 19 April and 4 May, 2015. The 19 April group was held in the San Joaquin River at the Durham Ferry release site for 48 hours before sampling. The 4 May group was held at the Mokelumne River Hatchery instead of the river due to elevated water temperatures at the release site. The 4 May Chinook group was held for 72 hours instead of 48 hours prior to sampling due to a schedule conflict.

SAMPLE COLLECTION

Fish were euthanized, fork length was recorded, any abnormalities were noted and tissues were sampled for lab assays. A sample of kidney tissue was aseptically collected and inoculated onto brain-heart infusion agar for bacterial culture (USFWS and AFS-FHS 2014). A kidney tissue imprint was collected to screen for *Renibacterium salmoninarum* (the bacteria that causes bacterial kidney disease) by fluorescent antibody test (USFWS and AFS-FHS 2014). Kidney and spleen tissue were collected in 3 fish pooled samples for viral tissue culture (USFWS and AFS-FHS 2014). Gill tissue was collected to assess smolt development by gill Na+/K+-Adenosine Triphosphatase (gill

ATPase) assay (McCormick 1993). For Chinook, samples of gill, liver, kidney and intestine tissues were collected from 12 fish from each group for histopathological examination (Humason 1979). In steelhead, samples of gill tissue were collected from all live fish for histopathological examination.

RESULTS AND DISCUSSION

FISH CONDITION

Steelhead – A total of 72 steelhead were examined (Table 1) and only one fish died over the 48 hour holding period. The mortality occurred in the 28 March group in a fish with moderate hemorrhaging at the suture site (Figure 1A), and it was likely this fish died due to complications following tagging. Pale gills were observed on one fish from the 28 March group, and significant scale loss (>50% of body) was observed in two fish from the 25 April sample group. Overall condition of the steelhead groups appeared good with no evidence to suspect survival differences.

Table 1. Fork length (FL), mortality, external abnormalities (Ext Abn), or internal abnormalities (Int Abn) in Steelhead health assessment groups. Observed external abnormalities included pale gills and scale loss.

Sample Date	FL ±SE (mm)	Mortality	Ext Abn	Int Abn
7 March	255 ±5	0/24	0/24	0/24
28 March	239 ±3	1/24 (4%)	1/23 (4%)	0/23
25 April	252 ±3	0/24	2/24 (8%)	0/24

Chinook – A total of 60 Chinook salmon were examined (Table 2) and there was no mortality in the health assessment groups over the holding period. Minor hemorrhaging (Figure 1B) or slightly pale gills (Figure 1C) were observed in 3 fish from the 19 April sample group. In the 4 May group, one fish had a small amount the liver extruding between the sutures with no hemorrhaging, and two other fish had minor hemorrhaging at the suture site. No evidence to suspect survival differences between Chinook groups due to fish condition was observed.

Table 2. Fork Length (FL), mortality, external abnormalities (Ext Abn), and internal abnormalities (Int Abn) in Chinook salmon health assessment groups. Observed external abnormalities included: pale gills, minor hemorrhaging and partly open sutures.

Sample Date	FL ±SE (mm)	Mortality	Ext Abn	Int Abn
19 April	102 ±0.9	0/30	3/30 (10%)	0/30
4 May	104 ±1.0	0/30	3/30 (10%)	0/30

PATHOGEN SCREENING

Steelhead – No obligate bacterial or viral pathogens were detected in the 71 trout sampled. Other bacteria isolates (presumptive environmental contaminates due to field sampling conditions) were observed in 11% (8/71) of fish sampled. Minor parasitic infections were observed in 29% (20/70) of gill tissues examined by histopathology, with no associated lesion or other signs of impairment. Gill infections included: *Capriniana piscium* (presumptive ID, formerly known as Trichophrya) 23% (16/70); cyst-like xenoma due to an unidentified microsporidian 4% (3/70); and an infection of

Ichthyophthirius multifiliis 1% (1/70). None of these infections were likely to cause differences in survival between steelhead release groups.

Chinook – No obligate bacterial or viral pathogens were detected in 60 salmon sampled. Other bacterial isolates (presumptive environmental contaminates) were observed in 20% (12/60) of samples. No infections or signs of impairment were observed by histopathology of gill, liver, kidney or intestine tissues from 25 Chinook. No differences in survival due to infections would be expected in the Chinook groups,

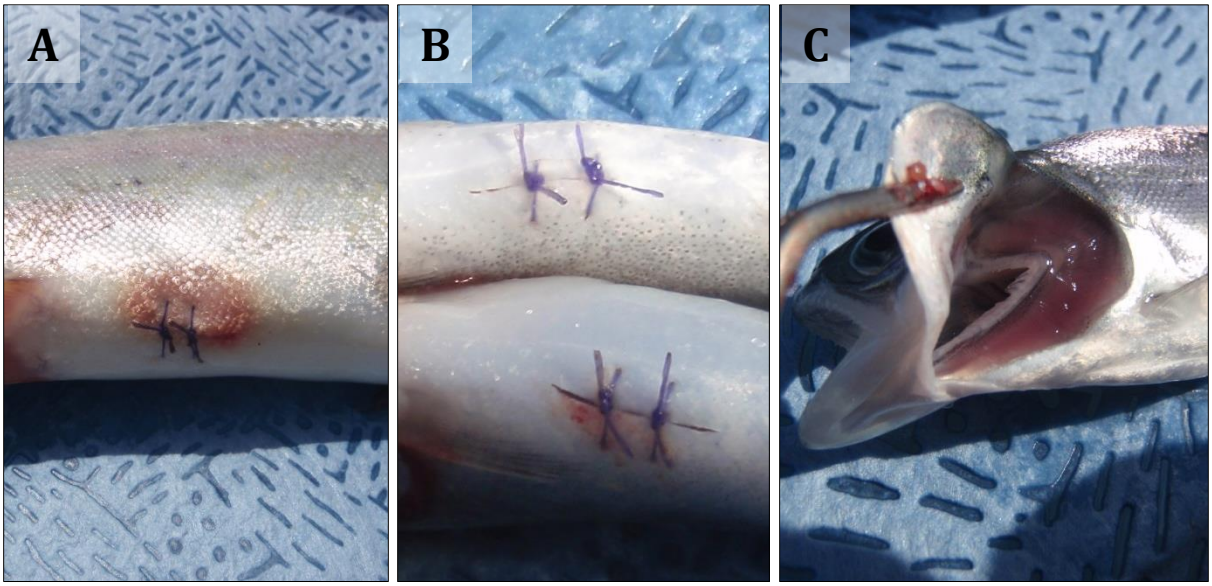

Figure 1. Examples of external abnormalities including: (A) Moderate hemorrhaging at suture site of a steelhead; (B) minor hemorrhaging (lower) and normal (upper) sutures in Chinook; and (C) slightly pale gills in Chinook.

Gill Na^+/K^+-ATPase Activity

Steelhead – Gill ATPase activity levels (µmol ADP*mg protein^{-1}*hr^{-1}) ranged from 0.3 to 6.9 in all groups (Figure 2). Median activity levels in the 7 March group were significantly lower than the later groups (Kruskal-Wallis test, $P<0.001$). Higher gill ATPase activity levels are associated with migrating smolts relative to their residual cohorts (Hanson et. al. 2011). In our experience, steelhead ATPase activity levels may increase over a short time period. While high levels may indicate salt-water readiness, lower values may have little biological significance due to this ability to rapidly change. Overall, the gill ATPase activity levels were low in all groups and the would not point to differences in migration or survival of the steelhead release groups.

Chinook – Gill ATPase activity levels ranged from 4.5 to 16.8 in all groups (Figure 3). Median activity levels in the 19 April group were higher than in the 4 May group. The modifications in holding conditions of the 4 May Chinook health assessment groups (mentioned above) and variability due to lab assay conditions likely contributed to the observed difference, and a direct comparison may not be useful. The majority of fish from both Chinook health assessment groups

had activity levels consistent with smolts (>6.7, CA-NV Fish Health Center unpublished data). Active migration in both release groups would be consistent with the observed activity levels.

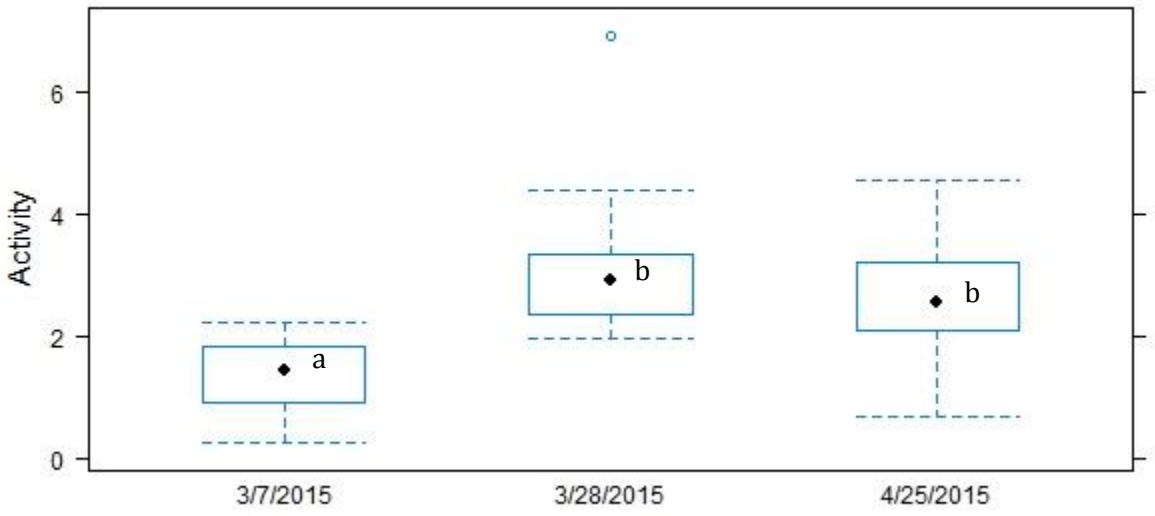

Figure 2. Boxplot of median gill ATPase activity in 2015 steelhead health assessment groups. Medians with the same letter are not significantly different (Kruskal-Wallis, P<0.001). Number of fish sampled for each group was 16.

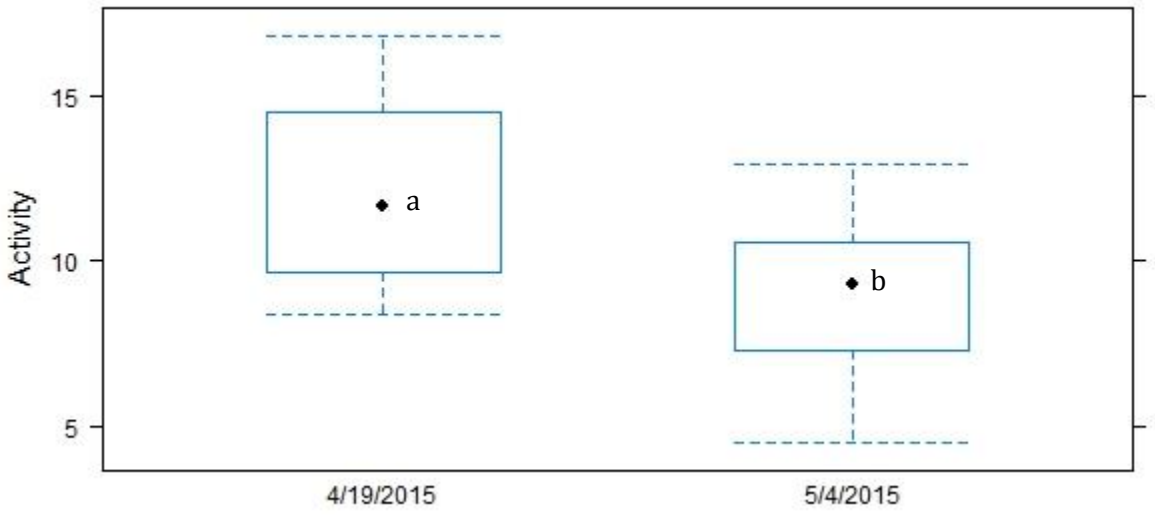

Figure 3. Boxplot of median gill ATPase activity in 2015 Chinook health assessment groups. Medians with the same letter are not significantly different (Kruskal-Wallis, P=0.005). Number of fish sampled from each group was 16.

REFERENCES

Foott JS. 2012. FY2012 Technical Report: Pathogen screening and gill Na-K-ATPase assessment of juvenile Chinook salmon used in south delta acoustic tag studies. U.S. Fish & Wildlife Service California-Nevada Fish Health Center, Anderson, CA. Accessible at: http://www.fws.gov/canvfhc.

Foott JS, R Stone and K Nichols. 2007. Proliferative Kidney Disease (Tetracapsuloides bryosalmonae) in Merced River Hatchery juvenile Chinook salmon: mortality and performance impairment in 2005 smolts. California Fish and Game 93: 57-76.

Hanson KC, WL Gale, WG Simpson, BM Kennedy and KG Ostrand. 2011. Physiological characterization of hatchery-origin juvenile steelhead *Oncorhynchus mykiss* adopting divergent life-history strategies. Journal of Fish and Wildlife Management 2(1): 61-71.

Humason G L. 1979. Animal Tissue Techniques, 4th edition. W H Freeman and Co., San Francisco.

McCormick S D. 1993. Methods for Nonlethal Gill Biopsy and Measurement of Na+, K+- ATPase Activity. Canadian Journal of Fisheries and Aquatic Sciences. 50: 656-658.

Nichols, K 2014. Pathogen Screening and Gill Na^+/K^+- ATPase Assessment of South Delta Chinook and Steelhead 2014 Release Groups. U.S. Fish & Wildlife Service, California-Nevada Fish Health Center, Anderson, CA. Accessible at: http://www.fws.gov/canvfhc.

USFWS and AFS-FHS (U.S. Fish and Wildlife Service and American Fisheries Society-Fish Health Section). 2014. Standard procedures for aquatic animal health inspections. In AFS-FHS. FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2014 edition. Accessible at: http://afsfhs.org/bluebook/bluebook-index.php.