U.S. Fish & Wildlife Service

PATHOGEN SCREENING AND GILL NA^+/K^+- ATPASE ASSESSMENT OF SOUTH DELTA CHINOOK 2016 RELEASE GROUPS

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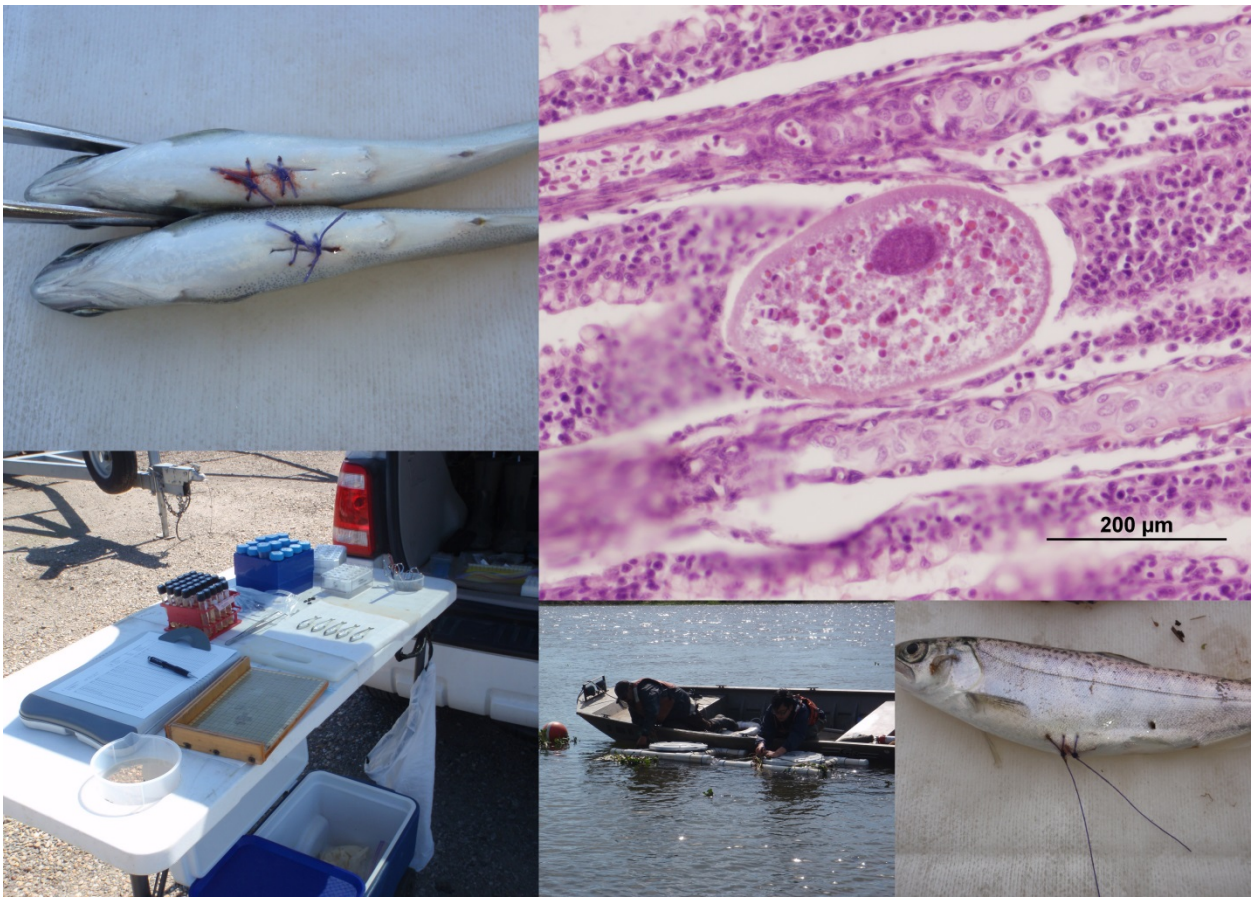

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SUMMARY

The California-Nevada Fish Health Center performed a general pathogen screening and smolt physiology assessment of Chinook release groups. Health assessments were performed on dummy-tagged cohorts of the 15 April, 17 April, 22 April and 24 April release groups. A total of 120 fish (30 fish per health assessment group) were examined after a 48 hour holding period at the release sites. Some minor hemorrhaging around the suture site and slight inflammation of the kidneys were noted. No bacteria or viral pathogens were detected in the 120 fish examined. Light *Ichthyophthirius multifiliis* (Ich) infections were observed on 12% (7/60) of gills, and early stage infections of the kidney parasite *Tetracapsuloides bryosalmonae* (cause of proliferative kidney disease) was observed in 9% (11/117) of fish. Median gill Na^+/K^+-ATPase activity levels were highest in the 22 April release group; however, the majority of fish from all 4 health assessment groups had levels consistent with Chinook smolts. None of these findings were significant enough to expect differences in survival or outmigration performance between release groups.

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BACKGROUND

As a component of the south Delta salmonid survival studies examining reach-specific survival and distribution of migrating juvenile Chinook salmon in the San Joaquin River and Delta, the California-Nevada Fish Health Center conducted a general pathogen screening and smolt physiological assessment. The objective of the health assessments were to evaluate the juvenile Chinook used in the studies for specific fish pathogens and assess smolt development to help explain differences in survival and migration performance between release groups. 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).

METHODS

STUDY FISH

Juvenile Chinook salmon were from the California Department of Fish and Wildlife Merced 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. Groups of 30 juvenile Chinook salmon were sampled on 15 April, 17 April, 22 April and 24 April, 2016 (Table 1). Fish were held for 48 hours in the San Joaquin River at either the Durham Ferry or River Point release sites before fish health assessments were performed. The objective of the health assessments were to evaluate the juvenile Chinook used in the studies for specific fish pathogens and assess smolt development to determine potential differences between release groups.

Table 1. Sample date, location and average fork length (FL, ±SE) of juvenile Chinook salmon fish health assessment groups.

Date	Location	FL ± SE (mm)
15 April	Durham Ferry	84.3 ±0.4
17 April	River Point	84.4 ±0.4
22 April	Durham Ferry	87.9 ±0.7
24 April	River Point	89.6 ±0.7

SAMPLE COLLECTION

Fish were euthanized, fork length was recorded, any abnormalities were noted and tissues were sampled for lab assays (USFWS and AFS-FHS 2014). Gill tissue was collected to assess smolt development by gill Na^+/K^+-Adenosine Triphosphatase (gill ATPase) assay (McCormick 1993). Samples of gill, liver, kidney and intestine tissues were collected from 12 fish from each group for histopathological examination (Humason 1979).

RESULTS AND DISCUSSION

FISH CONDITION

A total of 120 Chinook salmon were examined from the health assessment groups. There was no mortality over the holding period. Minor hemorrhaging at the suture site (Figure 1) and slight kidney inflammation were noted (Table 2); however, there were no significant differences between the health assessment groups. No evidence to suspect survival differences between Chinook groups due to fish condition was observed.

PATHOGEN SCREENING

No obligate bacterial or viral pathogens were detected in 120 salmon sampled. Other bacterial isolates (presumptive environmental contaminates) were observed in 19% (23/120) of samples. Infections with an external protozoan parasite, *Ichthyophthirius multifiliis* (Ich), were observed in 12% (7/60) of gills examined by histopathology (Table 2); however no significant lamellar hyperplasia was associated with the Ich infections. Early infections in kidneys with the myxosporidean parasite *Tetracapsuloides bryosalmonae* (proliferative kidney disease) was observed in 9% (11/117) of fish (Table 2,); the parasites were observed in the sinuses of the kidney with no associated interstitial hyperplasia. While these infections could progress, no significant lesions affecting fish survival were observed in the health assessments.

Table 2. Prevalence of minor suture hemorrhaging (Suture Hem), minor kidney inflammation (Kd inf), *Ichthyophthirius multifiliis* (Ich) gill infection, and *Tetracapsuloides* bryosalmonae (*T. bryosalmonae*) kidney infection in Chinook salmon health assessment groups. Significant differences between sample dates (*) were detected in Ich prevalence (P=0.002, Fisher's Exact Test).

Sample Date	Suture Hem	Kd Inf	Ich *	*T. bryosalmonae*
15 April	3/30 (10%)	0/30	0/15	1/27
17 April	5/30 (17%)	0/30	0/15	2/30
22 April	0/30	1/30 (3%)	1/15	4/30
24 April	2/30 (7%)	0/30	6/16	4/30

GILL NA^+/K^+-ATPASE ACTIVITY

Median gill Na^+/K^+ ATPase activity levels were highest in the 22 April group (Figure 2, $p<0.001$, Kruskal-Wallis test). While statically significant the differences between release groups were likely not biologically significant. The majority of fish from the 4 Chinook health assessment groups had activity levels consistent with Chinook smolts (>6.7, CA-NV Fish Health Center unpublished data).

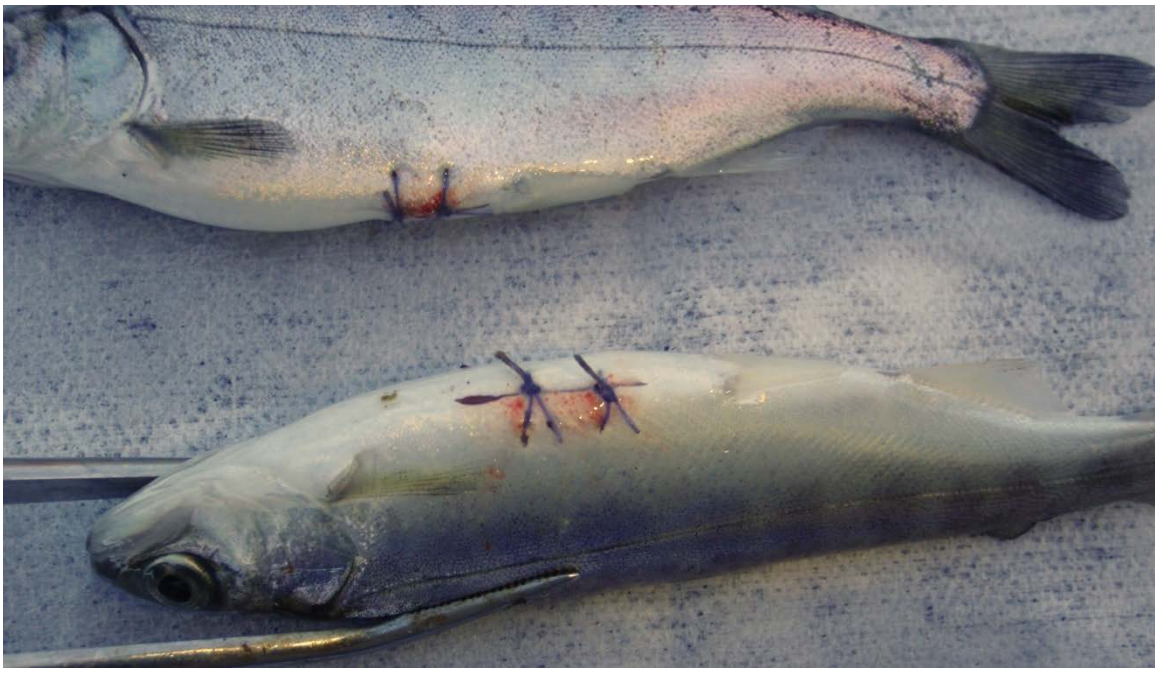

Figure 1. Minor hemorrhaging around sutures on dummy-tagged Chinook after 48 hours holding at the release site.

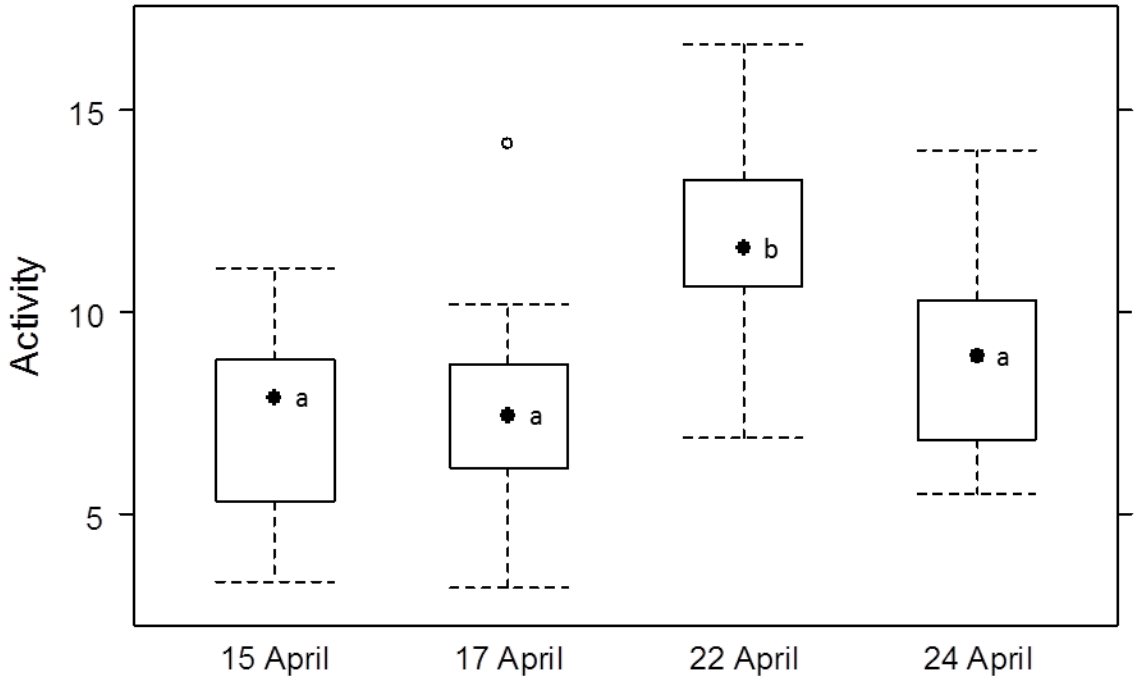

Figure 2. Boxplot around median gill Na^+/K^+-ATPase activity (µmol ADP * mg protein^{-1} * hr^{-1}) for Chinook health assessment groups. Medians with the same letter are not significantly different (P<0.001, Kruskal-Wallis test). There were 16 fish sampled from each health assessment group.

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SUPPLEMENT TO 2016 SOUTH DELTA PATHOGEN SCREENING:

COMPARISON OF TAGGED CHINOOK TO UNTAGGED CONTROLS AT MERCED RIVER HATCHERY

In conjunction with the 2016 South Delta survival studies, the potential negative consequences of surgically implanting acoustic tags on disease progression were investigated. The Merced River Hatchery (MRH) Chinook stock used in the 2016 study had a history of *Tetracapsuloides bryosalmonae* (cause of proliferative kidney disease, PKD) infection in past years, so this comparison included measures of proliferative kidney disease progression. Typical clinical signs of PKD include anemia (pale gills and low hematocrit), swollen kidney and enlarged spleen.

On 8 April, 30 tagged and 30 untagged MRH Chinook were transferred to live cages and held for 13 days at the hatchery before sampling. Fish were examined on 21 April; any abnormalities were noted; and tissue samples were collected for lab assays. Kidneys from a subsample of fish with swollen spleen or kidney inflammation (possible signs of PKD) were examined by histopathology to confirm parasite infection. Gill tissues were assayed for Na^+/K^+-ATPase activity levels (gill ATPase) as a measure of smolt development.

Survival was high in both tagged and untagged groups. One tagged fish died during holding and no obvious cause was noted. No *T. bryosalmonae* infections were detected by histopathology in the 8 kidney samples from fish with possible signs of PKD (data not presented). There was no difference in gill ATPase activity between tagged and untagged groups (**Table 3**, $p=0.070$, Kruskal-Wallis test). Tagged fish were observed to have a lower average hematocrit ($p<0.001$, t-test), enlarged spleens, and external fungal infections (**Table 3**). The fungal infections were common at the suture site and were accompanied by minor hemorrhaging in the surrounding skin. Fungus was also noted on a few (3/30) of the untagged fish. The fungal infections were likely an artifact of the holding conditions. The observations of low hematocrit and enlarged spleen may also have been associated with the fungus infections or possibly a lingering effect of tagging surgery.

Table 3. Comparison between tagged (tag) and untagged (NT) juvenile Chinook salmon held at Merced River Hatchery for 13 days. Observations included fork length (FL), number of fish that survived holding (survival), pale gills, hematocrit (HCT), enlarged spleen, fungus growth on sutures (fungus), and mean gill Na^+/K^+-ATPase activity (ATPase, µmol ADP * mg protein^{-1} * hr^{-1}, n=9 per group).

Group	FL (mm)	Survival	Pale gills	HCT	Enlarged spleen	Fungus	ATPase
Tag	82.0	29/30 (97%)	0/29	36%	6/29 (21%)	22/29 (76%)	6.3
NT	82.2	30/30 (100%)	0/30	43%	0/30	3/30 (10%)	7.9