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FY2011 Technical Report:
Health and Physiological Assessment of VAMP and SDTB 2011 Release Groups

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SUMMARY
Health assessments were conducted on cohorts of acoustic tagged Merced River Hatchery juvenile Chinook salmon used in the Vernalis Adaptive Management Plan (VAMP) and South Delta Temporary Barriers (SDTB) project studies corresponding to 19 May, 26 May and 16 June 2011 study fish releases. Health assessment control (HAC) groups were transferred to the CA-NV Fish Health Center wet lab, and sampled at 1 and 30 days post transfer. No obligate viral or bacterial pathogens were detected in any of the 3 HAC groups sampled 1 day post transfer. External infections with *Flavobacterium columnare* (the bacteria which causes columnaris disease) and *Ichthyophthirius multifiliis* (the protozoan which causes ich or white spot disease) were observed on fish from all 3 HAC groups sampled 30 days post transfer. *Tetracapsuloides bryosalmonae* parasites, the causative agent of proliferative kidney disease (PKD), were detected in 0-7% of fish in HAC groups at 1 day post transfer and 27-46% of fish from HAC groups sampled at 30 days post transfer. Survival for the 30-day holding periods was high and ranged from 96-100%. Gill ATPase activity levels were consistent with fish undergoing smoltification in all except the 26 May HAC group. Overall, HAC groups demonstrated low mortality and only mild PKD prevalence; indicating, fish health was not a concern in survival of 2011 VAMP and SDTB study fish.

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INTRODUCTION

As a component of the 2011 Vernalis Adaptive Management Plan (VAMP) and South Delta Temporary Barrier (SDTB) project studies on reach-specific survival and distribution of migrating Chinook salmon in the San Joaquin River and delta, the CA-NV Fish Health Center conducted a general pathogen screening and smolt physiological assessment. The health and physiological condition of the study fish can help explain their performance and survival during the studies. Pathogen screenings during past VAMP studies using Merced River Hatchery (MRH) Chinook have regularly found infection with the myxozoan parasite *Tetracapsuloides bryosalmonae*, the causative agent of Proliferative Kidney Disease (PKD). 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, Stone and Nichols 2007). The objectives of this project were to: Survey the juvenile Chinook salmon used for the studies for specific fish pathogens including *Tetracapsuloides bryosalmonae*, assess smolt development (gill Na^+/ K^+-ATPase), and diagnose any mortality in health assessment control fish held for 30 days in the laboratory.

METHODS

Sample groups – Three health assessments were conducted on cohorts of acoustic tagged MRH juvenile Chinook salmon used in the VAMP and SDTB studies corresponding to 19 May, 26 May and 16 June 2011 study fish releases. Target sample number for each health assessment control (HAC) groups consisted of 24 dummy-tagged (DT) Chinook and 60 Chinook that were not tagged (NT). The second (HAC2) group included an extra 12 DT fish, and limitations on available fish reduced the sample size for the third (HAC3) group (Table 1). The DT fish received surgical implant of a tag identical to the VAMP and SDTB acoustic tagged fish. The primary purpose of the DT groups was to monitor and diagnose mortality over a 30 day holding period. The NT group was included as a control for surgery effects and to increase sample numbers without sacrificing DT fish. Each HAC group was sampled at two time points: after an overnight acclimation (1d) and after approximately 30 days (30d).

Fish handling and holding – Fish handling attempted to shadow treatment of acoustic tagged fish used in the tracking studies. All HAC groups were split from the larger study population at tagging of the acoustic tagged cohort before transfer to the CA-NV Fish Health Center wet lab in Anderson, CA (wet lab). Each DT groups was held for 48 (±12) hours in the San Joaquin River during the release period for their acoustic tagged cohort before transfer. The NT groups were transferred directly from the tagging facility. In the wet lab, temperature of the single-pass water supply was allowed to fluctuate with ambient conditions. Once temperatures began to exceed 18°C (29 July), the water supply was switched to a constant 17°C source due to concerns of *Flavobacterium columnare* infection. Fish were fed a pelleted salmon diet daily. Tanks were checked daily for dead or moribund fish. Diagnostic sampling was performed on sick or dead fish to identify

any associated pathology. In addition to HAC groups, a reference sample of 30 unmarked Chinook was sample at Merced River Hatchery on May 18 (MRH).

Table 1. Number of fish in health assessment control (HAC) groups transferred to the wet lab. Groups include: 1-day untagged (1d-NT), 30-day untagged (30d-NT) and 30-day dummy tagged (30d-DT).

Group	*1d-NT*	*30d-NT*	*30d-DT*
HAC1	30	30	24
HAC2	30	30	36
HAC3	20	14	22

Sample collection – For the HAC 1d and MRH samples, 30 NT fish were euthanized; FL and any abnormalities noted; and tissue samples for bacteriology, virology, histopathology, and gill ATPase assays collected. For the HAC 30d samples, all surviving fish (both DT and NT) were euthanized; fork length, weight and any abnormalities were noted. Tissue samples for bacteriology and histopathology assays were collected from the HAC 30d-DT groups only.

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 – Four 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, liver, intestine and posterior kidney were rapidly removed from the fish and immediately fixed in Davidson's fixative, 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. 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. Data analysis was performed using R version 2.11.1 using Fisher's Exact Test for Count Data.

Gill ATPase - Gill Na^+/K^+-Adenosine Triphosphatase (ATPase) activity was assayed by the method of McCormick (1993). Gill ATPase activity is correlated with osmoregulatory ability in saltwater and is located in the chloride cells of the lamellae. Data analysis was performed using R version 2.11.1 by Kruskal-Wallis rank sum test.

RESULTS

Holding Conditions – While the target holding period in the wet lab was 30 days, fish were sampled at 29-32 days to facilitate laboratory workflow (Table 2). The HAC3 group was sampled at 29 days due to concerns the fish would not survive to 32 days because of a significant external parasite infection (*I. multifiliis*). Fish were fed 1.1% to 1.8% body weight/day and average fish length increased during the 30-day monitoring period (data not shown).

Table 2. Holding period and water temperature (T) for health assessment control (HAC) groups held in the wet lab.

Group	*Start Date*	*End Date*	*Total (days)*	*T Mean (°C)*	*T Range (°C)*
HAC1	19 May	20 June	32	14.5	11.9-16.6
HAC2	26 May	27 June	32	15.4	12.1-18.7
HAC3	16 June	15 July	29	17.3*	15.7-18.8

* The water supply for the HAC3 group was switched from an ambient temperature supply to a constant 17°C supply on 29 June.

Survival – Of the 156 fish held in the wet lab, two mortalities were observed. Survival in individual PC groups ranged from 96-100%. The first mortality occurred 31 May (day 12) in the HAC1 DT group. The second mortality occurred 27 June (day 32) in the HAC2 DT group. Both mortalities occurred overnight, so had been dead too long for a full pathology examination. No survival comparison between DT and NT groups was performed due to the low number of mortalities.

Pathogen assays – Summary results of pathogen testing are presented in Table 3. No obligate viral or bacterial pathogens were detected; however, Aeromonas and Pseudomonas bacteria were isolated in 5% of the kidney samples cultured for bacteria. This group of gram-negative bacteria is ubiquitous in soil and water as well as the intestinal tract of fish (Aoki 1999). It is often classified as an opportunistic fish pathogen. External hemorrhaging, which may be a sign of bacterial septicemia, was observed in one fish that died during holding. This fish died overnight and was in poor condition; therefore, no bacterial isolation was attempted on this fish. External infections with *Flavobacterium columnare* were observed by gross examination (Figure 1) in 3% (2/66) of the HAC2 30d group and 2% (1/36) of the HAC3 30d group. External infections with *Ichthyophthirius multifiliis* were observed by gross examination (Figure 2) in 100% of the HAC3 30d group.

Table 3. Summary of pathogen screening of 2011 VAMP study fish. Assays included: virology by tissue culture; bacteriology by culture; fluorescent antibody test for *Renibacterium salmoninarum* (*Rs*-FAT).

Assay	Samples	Total Fish	# Pos (%)	Pathogen
Virology	37	110	0	No virus detected
Bacteriology	178	178	0	No obligate bacterial pathogens
			9 (5%)	*Aeromonas/Pseudomonas*

Rs-FAT	110	110	0	*Rs* not detected

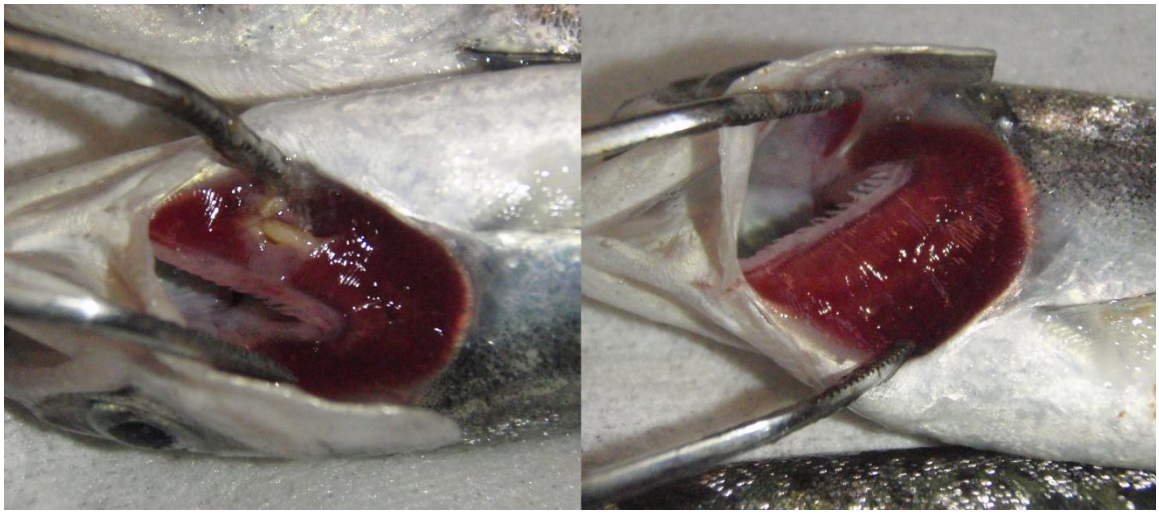

Figure 1. Juvenile Chinook salmon with a bacterial *F. columnare* gill lesion (left) and normal gill (right).

Figure 2. Example of *Ichthyophthirius multifiliis* infection (ich or white spot disease) observed in health assessment control group 3 (HAC3).

Histopathology –External infections of *I. multifiliis* (Figure 3) and kidney infections with *T. bryosalmonae* were observed by histopathology in all HAC 30d groups.

Ichthyophthirius multifiliis infections were not detected in HAC 1d groups, but moderate to heavy infections were observed on the gills of 91% to 100% of all three HAC 30d groups. Infections with *T. bryosalmonae* were observed in 15% (27/177) of all kidney histopathology samples. In HAC 1d groups, no difference was detected in *T. bryosalmonae* infection intensity (P=0.32, Table 4) or severity of the associated lesion (P=1, Table 5). In HAC 30d groups, no difference was detected in *T. bryosalmonae* infection intensity (P=0.40, Table 4) or severity (P=0.08, Table 5). Increased intensity and severity of *T. bryosalmonae* infection were observed between 1d and 30d samples in all HAC groups (P<0.05)

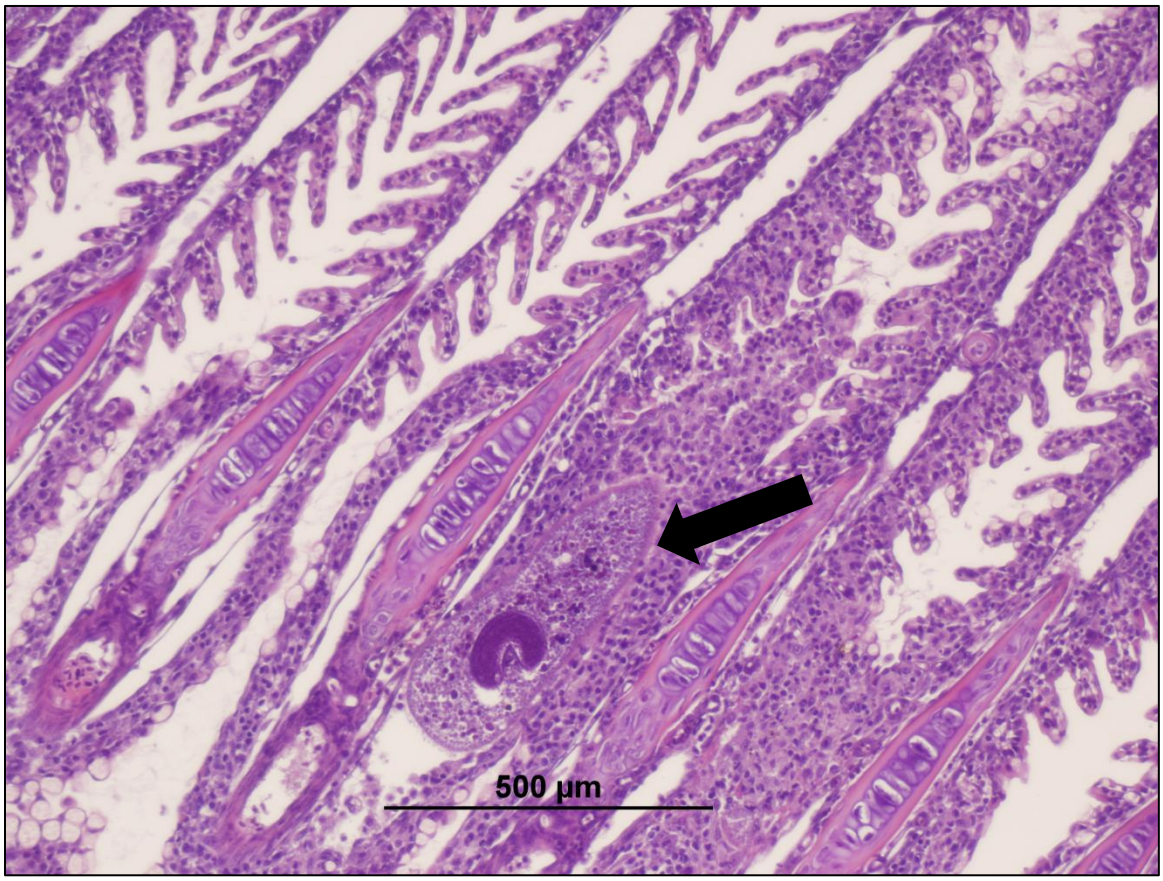

Figure 3. Example of *Ichthyophthirius multifiliis* parasite observed in gill histopathology samples of all health assessment control (HAC) groups at the end of the 30 day holding period. Note the classic horseshoe shaped nucleus.

Table 4. Intensity of *T. bryosalmonae* infection in Chinook salmon. Data presented as the number of fish with zero (None), <10 (Low), 11-30 (Moderate) or >30 (High) *T. bryosalmonae* parasites observed in kidney tissue by histopathology.

Group	Time Point	None	Low	Moderate	High
MRH	--	27	0	0	0
HAC1	1d	29	0	1	0
	30d	16	6	1	0
HAC2	1d	28	2	0	0
	30d	13	10	1	0
HAC3	1d	18	0	0	0
	30d	16	5	0	1

Table 5. Severity of clinical proliferative kidney disease lesion in Chinook salmon. Data presented as the number of fish with kidney inflammation rated as normal, focal, multifocal or diffuse by histopathology.

Group	Time Point	Normal	Focal	Multifocal	Diffuse
MRH	--	27	0	0	0
HAC1	1d	29	1	0	0
	30d	17	5	1	0
HAC2	1d	29	1	0	0
	30d	15	3	6	0
HAC3	1d	18	0	0	0
	30d	16	5	0	1

Gill ATPase – Activity ranged from -0.1 to 15.7 µmol ADP·mg protein^{-1}·hr^{-1}. Significant differences between groups were observed, with the PC2 group having significantly lower ATPase activity levels compared to the other two PC groups ($P<0.001$, Figure 4).

DISCUSSION

No health problems were detected that would have a significant effect on mortality of the VAMP or SDTB study fish in 2011. *Tetracapsuloides bryosalmonae* infection intensity was very low in all groups and significant PKD lesions were observed in only a few fish. External bacterial (*F. columnare*) and parasite (*I. multifiliis*) infections necessitated water temperature control and a shorter holding period during HAC3 group. Of these pathogens, the *T. bryosalmonae* infection status provides the best insight into the health of acoustic tagged cohorts in the river.

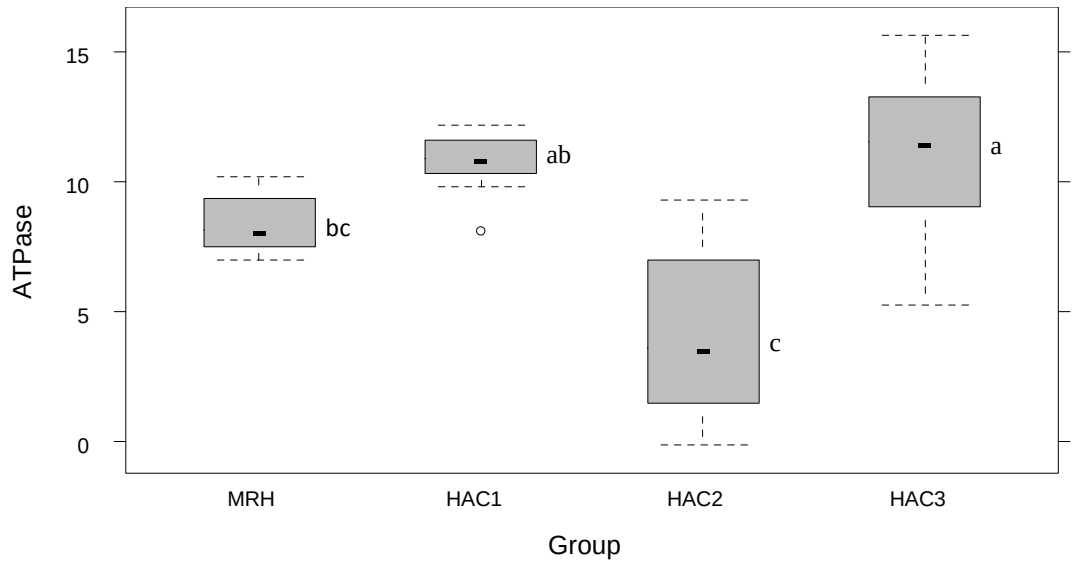

Figure 4. Boxplot of median gill ATPase activity (µmol ADP·mg protein^{-1}·hr^{-1}) in juvenile Chinook salmon sampled at Merced River Hatchery (MRH) or in health assessment control groups sampled in the wet lab (HAC1-HAC3). Groups with letter subscripts in common were not significantly different (Kruskal-Wallis rank sum test, P<0.001).

Given the historic presence of PKD at MRH, the *T. bryosalmonae* infections most likely occurred in all MRH fish in 2011. Infections were light in 2011, and the histopathology assay likely missed low level infections in the MRH and HAC 1d sample groups. Proliferative kidney disease is progressive and dependent on water temperature (Ferguson 1981; Foott, Stone and Nichols 2007). It was expected that infection intensity and PKD severity would increase during the study period. Since this parasite is not transmitted horizontally fish-to-fish, infection rates and mortality observed in the wet lab would be correlated with study fish released into the river. In past VAMP studies where fish were held for monitoring, total mortality due to the disease was low at 20%-27% (Foott, Stone and Nichols 2007; Foott and Stone 2008). In 2011, incidence of *T. bryosalmonae* was lower than these previous studies and mortality due to all causes was very low (0-4%). There was no indication that PKD was a health concern in the 2011 VAMP or SDTB study fish.

The *I. multifiliis* and *F. columnare* infections were of concern in fish held in the wet lab. Infections were likely intensified in the HAC 30d groups by holding conditions (confinement at high density) in the wet lab (Dickerson and Dawe 1995). It is unclear from the limited sampling performed whether *I. multifiliis* infections impaired the health and performance of the smolts prior to the 30d examination. Given the high value of each acoustic tagged fish, measures should be considered to reduce the risk of exposure to these pathogens including: cooler water temperatures during rearing and tagging; water disinfection such as high wattage UV; and formalin or antibiotic treatment.

Gill ATPase activity levels were consistent with smolting Chinook except for the HAC2 group. ATPase activity appeared to be suppressed in the VAMP study group released

25-26 May. 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 ATPase activity can also occur due to increases in water temperature (Duston et al. 1991). Experience has shown that this indicator can change rapidly once fish enter salt water. Low ATPase levels will not reliably predict poor migratory performance, but may corroborate other observed differences between groups. Poor migration behavior in acoustic tagged cohorts of the HAC2 group would be consistent with the low gill ATPase observation.

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