U.S. Fish & Wildlife Service

FY2009 Technical Report:
Health and Physiological Assessment of VAMP Release Groups

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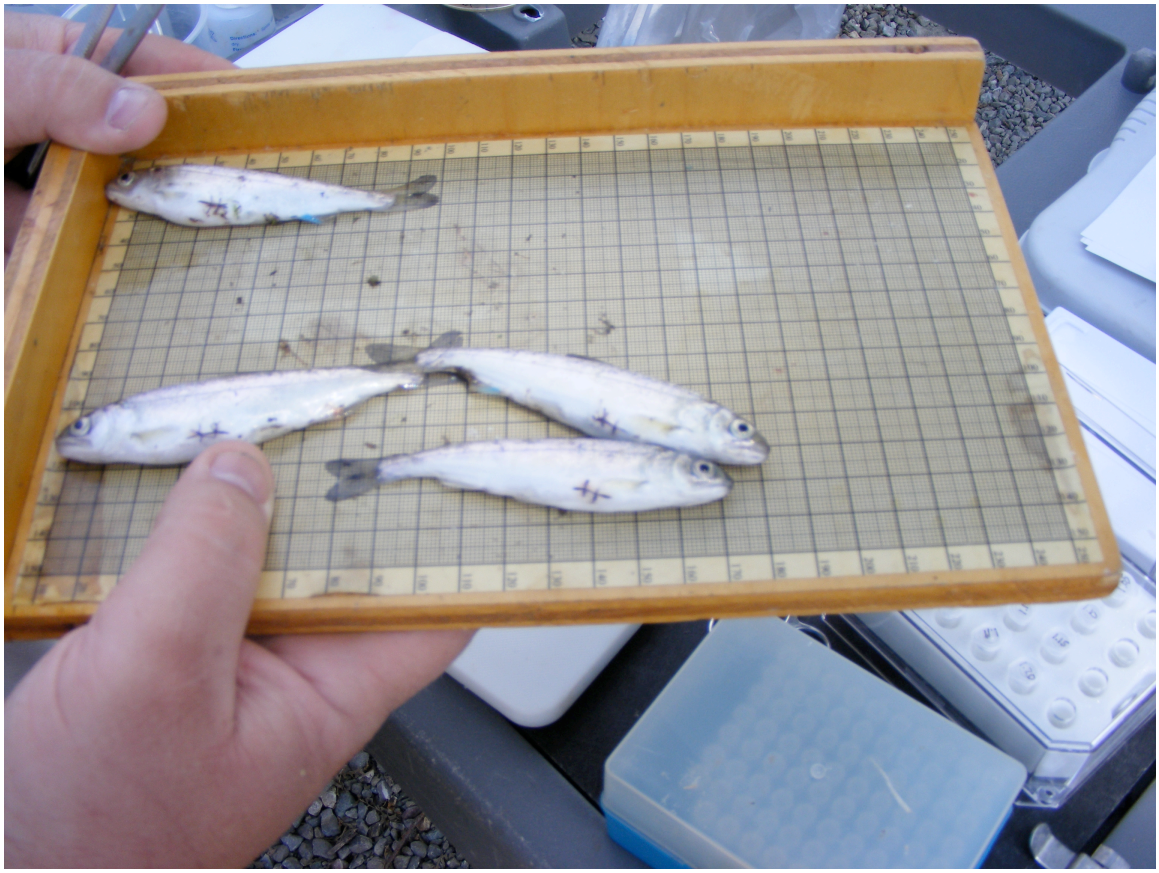

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Summary: In support of the 2009 VAMP survival and distribution studies, the California-Nevada Fish Health Center performed pathogen screening and bioassays. Infections with the parasite *Tetracapsuloides bryosalmonae* and bacteria in the *Aeromonas*/*Pseudomonas* complex were detected, but no signs of clinical disease were observed. Most of the fish had undergone or were in the process of smoltification. No differences were detected in survival, blood chemistry, or white blood cell counts. No significant tissue abnormalities was observed in histological sections of gill, kidney, or liver from all bioassay groups. The data indicates that the juvenile Chinook population used for the VAMP study was healthy, undergoing smoltification, and did not incurred overt impairment in 40 hour bioassays at either Durham Ferry or adjacent to the Stockton Wastewater treatment plant outfall.

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INTRODUCTION

As a component of the 2009 Vernalis Adaptive Management Plan (VAMP) study 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 a bioassay to aid in evaluating fish performance. Pathogen screening during past VAMP studies has detected infection with the myxozoan parasite *Tetracapsuloides bryosalmonae* (causative agent of Proliferative Kidney Disease). This parasite has been shown to cause mortality in Merced River Hatchery salmon held until June (Foott, Stone and Nichols 2005). In the 2007 VAMP study, a significant number of acoustic tags from juvenile Chinook salmon were "motionless" in the San Joaquin River just upstream of the Stockton wastewater treatment facility (SJRGA 2008). The cause of the mortality was unknown, and it was recommended that the site be monitored to identify similar a mortality event during the 2009 study. The objectives of this project was: 1) survey the juvenile Chinook population used for the VAMP study for specific fish pathogens and smolt development (gill ATPase), and 2) determine if 40 hour bioassays in the San Joaquin River, at either the Durham Ferry release site or adjacent to the Stockton Wastewater treatment plant outfall, resulted in mortality, reduced white blood cell counts, or abnormalities in gill, kidney, or liver tissues.

METHODS

Fish

Juvenile Chinook salmon used in this study were cohorts of acoustic tagged Chinook used in the 2009 VAMP survival and distribution studies. Fish for the pathogen screening and bioassay were held in separate live cages for approximately 40 hours in the San Joaquin River at the Durham Ferry site. An additional bioassay group went to the river near the Stockton water treatment facility (WTF). Bioassays began on Tuesday of each week with fish sampled 40 hours later on Thursday. Sampling was performed each week of the VAMP study releases: April 23 (Week 1), April 30 (Week 2), May 7 (Week 3) and May 14 (Week 4).

Pathogen and Physiology

Eighteen fish held at Durham Ferry were sampled weekly for bacteriology, virology and gill ATPase assays as described below:

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 2007). 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 and incubated for 24 days (including a 14 day blind pass) at 15ºC. (USFWS and AFS-FHS 2007.)

Gill ATPase - Gill Na^+, K^+-Adenosine Triphosphatase activity (ATPase) was assayed by the method of McCormick and Bern (1989). Briefly, gill lamellae were dissected and frozen in sucrose-EDTA-Imidazole (SEI) buffer on dry ice. The sample was later homogenized, centrifuged and the pellet sonicated prior to the assay. ATPase activity was determined by the decrease over time in optical density (340 nm) as NADH is converted to NAD+. This activity was reported as μmol ADP/mg protein/hour as 1 mol of NAD is produced for each mol of ADP generated in the reaction. Gill ATPase activity is correlated with osmoregulatory ability in saltwater and is located in the chloride cells of the lamellae. This enzyme system transports salts from the blood against the concentration gradient in saltwater. Data analysis was performed by ANOVA and Tukey multiple comparison test.

Bioassay

Each week, fish for the bioassay were surgically implanted with a non-functioning acoustic (dummy) tag which mimicked, as closely as possible, the treatment of the VAMP study groups. Groups of 10 dummy tagged fish per site were placed in live cages and transported to both the WTF and Durham Ferry sites. Following the 40 hour exposure period, mortality was recorded and both blood and histology samples collected. Blood was collected from the severed caudal vessel using heparinized Natelson tube.

Histopathology – The gills, liver 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 at low (40X) and high magnification (400X).

Apoptosis assay – Anterior kidney and thymus tissues were processed for histopathology as above. Apoptosis (programmed cell death) in cells within these organs was used as a potential biomarker for environmental stress (Sweet et al. 1999). Molecular changes to the DNA signifying apoptosis were visualized on the section by TUNEL assay using an *in situ* detection kit (Trevigen Inc, Gaithersburg, MD; Catalog# 4828-30-BK). Each tissue was examined and rated on a scale relative to control tissues. Five tissue sections from each site in weeks 1 and 4 were examined by this assay (5 samples x 2 sites x 2 weeks = 20 samples total).

Plasma Total Protein and Chloride - Plasma was separated in the field by centrifuge and stored at -80ºC until analyzed. Total protein was measured using colorimetric analysis reagents from Point Scientific (Canton, Michigan, kit T7528) and bovine serum albumin as a standard. Plasma chloride was measured using colorimetric analysis reagents from Point Scientific (kit C7501). Data analysis was performed using the Mann-Whitney test on medians. Plasma was not collected in week 2 due to an equipment problem.

WBC Counts - Blood was diluted 200x in Rees-Ecker fluid and stored cool. White blood cell (WBC) counts were performed by hemocytometry (Manner 1992). Counts were reported as WBC/mm^3 whole blood. Data analysis was performed using the Mann-Whitney test on medians.

RESULTS

Pathogen Screening

Summary results of weekly pathogen testing are presented in Table 1. No obligate viral or bacterial pathogens were detected however *Aeromonas-Pseudomonas* bacteria were isolated in 26% of the bacterial samples. This gram-negative bacterial group 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. No clinical signs of bacterial septicemia were observed in these fish. Due to high winds during sampling, bacterial culture plates became contaminated with airborne bacteria and fungi. Approximately 25% of the samples were so overgrown with contaminates that they were discarded due to human health concerns. *Tetracapsuloides bryosalmonae* (the causative agent of proliferative kidney disease) was detected in 13% (10 / 76) of the kidney samples examined by histology. All of the *Tb* infections were in the early stages and no significant inflammation or kidney damage was associated with the infections.

Table 1. Summary of pathogen screening of 2009 VAMP study fish. Assays included: virology by tissue culture of pooled kidney and spleen samples; bacteriology by culture of individual kidney samples on BHIA media; fluorescent antibody test for *Renibacterium salmoninarum* (*Rs*-FAT) by individual kidney imprints using polyclonal antiserum.

Assay	Samples	Total Fish	# Pos (%)	Pathogen
Virology	14	58	0	No virus detected
Bacteriology	80	80	0	No obligate bacterial pathogens detected
			21 (26%)	*Aeromonas/Pseudomonas*
Rs-FAT	58	58	0	None detected

Gill ATPase - Weekly mean ATPase activity ranged from 7.3 to 10.4 μmol ADP/mg protein/hr (Figure 1). ATPase activities in fish sampled in week 4 were higher than those observed in weeks 1 (P=0.048) and 3 (P=0.020). The majority of fish sampled (74%) had ATPase activities consistent with Chinook salmon smolts (data not presented). Other than a likely increase in ATPase activity with time, no other trends were observed.

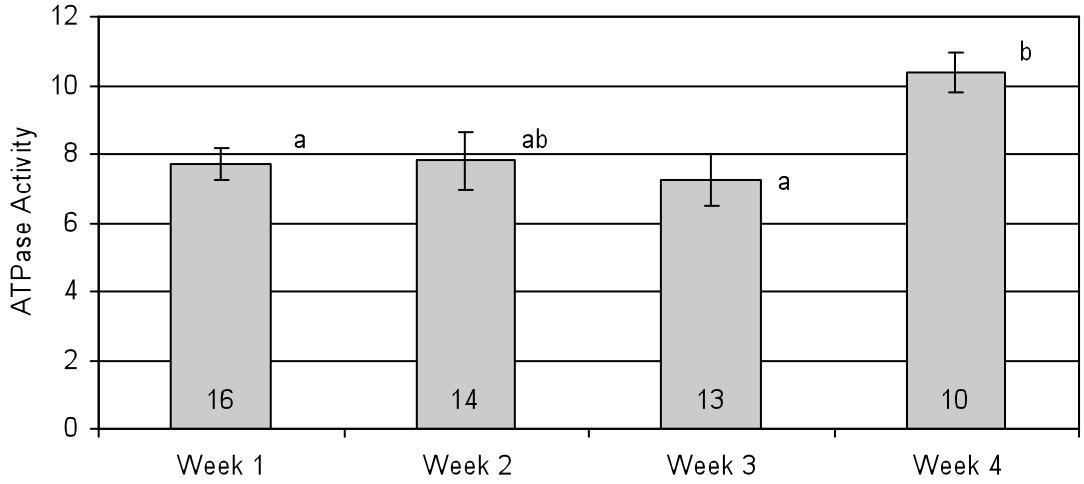

Figure 1. Gill Na^+, K^+-ATPase activities (µmol ADP/mg protein/hr) for cohorts of acoustic tagged Chinook salmon utilized in the 2009 VAMP study. Data presented as mean ± SE and sample number at base of each bar. Groups with letters in common are not statically different (P=0.021, ANOVA).

Bioassay

There was no difference in 40 hour fish survival between WTF and Durham Ferry sites. During the 4 weekly bioassays 95% (38/40) of the fish survived at both sites (Table 2). No cause of death was determined for the 4 mortalities.

Table 2. Survival of bioassay Chinook held for 48 hours in live cages adjacent to the Stockton Wastewater Treatment Facility (WTF) or Durham Ferry release site.

Site	Week 1	Week 2	Week 3	Week 4
WTF	10/10	9/10	9/10	10/10
Durham Ferry	8/10	10/10	10/10	10/10

Histopathology

No biologically significant differences were detected between fish held at the WTP and Durham Ferry in any of the weeks. Fish from both locations in Week 1 had deposits of microscopic colored spheres associated with macrophages. It is believed the beads are associated with colored fin marks observed on this group of fish (Figure 2). No microscopic spheres were observed in fish from Weeks 2-4. Minor kidney lesions and gill edema were observed in fish from other weeks, but these changes were not likely to significantly affect fish performance or lead to acute mortality. No evidence of apoptosis was evident in any of the anterior kidney or thymus sections from the WTF or Durham Ferry fish sampled in Weeks 1 and 4.

Plasma Total Protein

Median plasma total protein concentration in bioassay groups ranged from 21 to 26 mg/ml (Figure 3). No significant differences were detected between fish held at the WTF

and control sites in week 1 (P=0.690), week 3 (P=1.000), week 4 (P=0.421) or when all weeks were combined (P=0.367). No testing was done in week 2 due to an equipment problem.

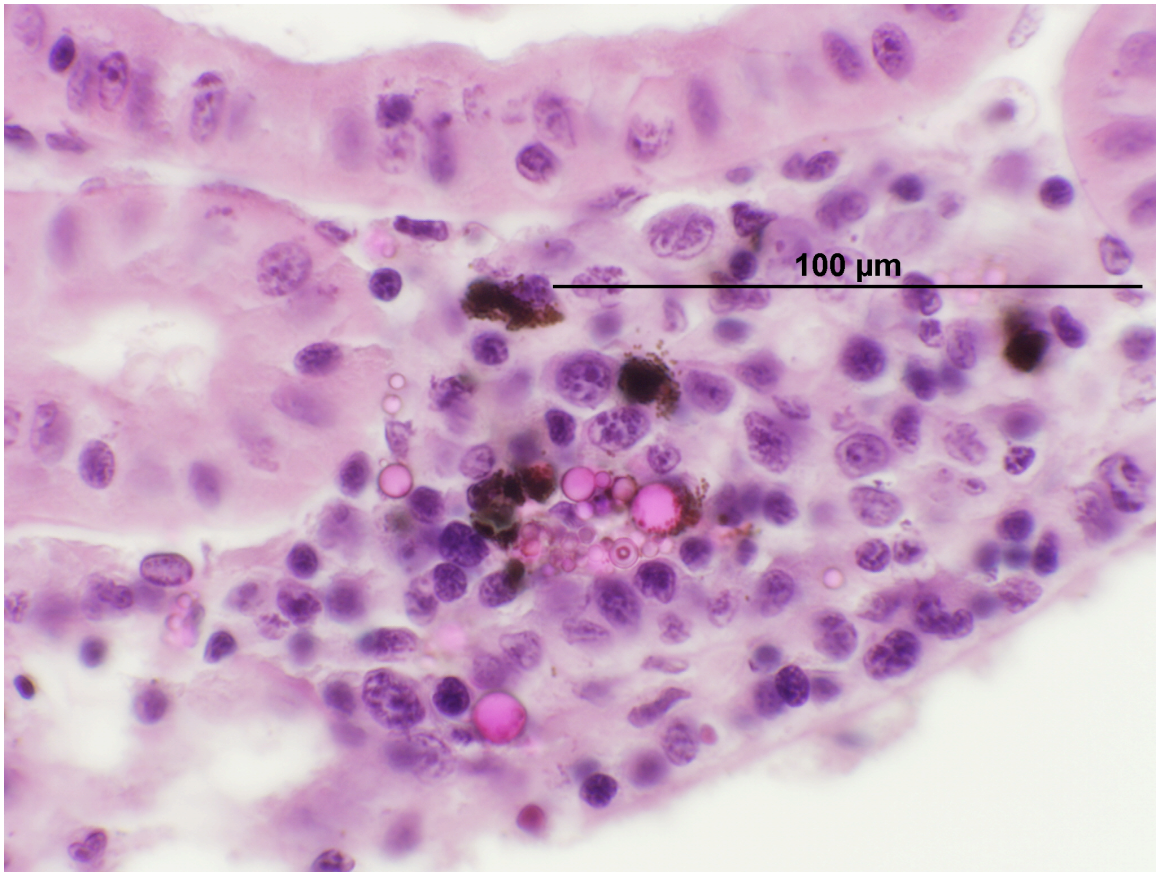

Figure 2. Microscopic colored spheres observed in all of the kidneys from juvenile Chinooks salmon from Week 1 of the VAMP study. The spheres above are pink, however yellow and blue were observed in other fish.

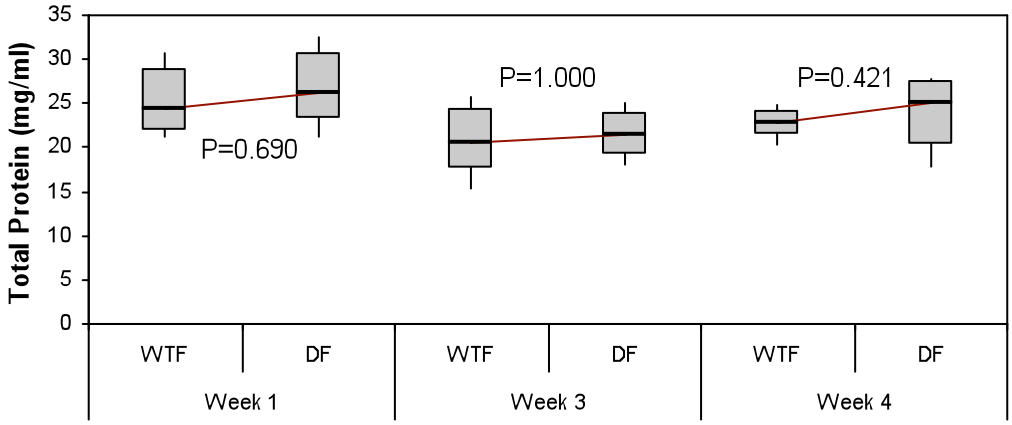

Figure 3. Box plots of median plasma total protein concentrations for bioassay fish groups held in the San Joaquin River near the Stockton Wastewater Treatment Facility (WTF) and Durham Ferry release site (DF). Samples consisted of 5 fish at each site each week.

Plasma Chloride

Median plasma chloride concentration in bioassay groups ranged from 102 to 107 mEq/l (Figure 4). No significant difference was detected between fish held at the WTF or Control sites in week 1 (P=1.000), week 2 (P=1.000), week 3 (P=0.690) or when all weeks were combined (P=0.838).

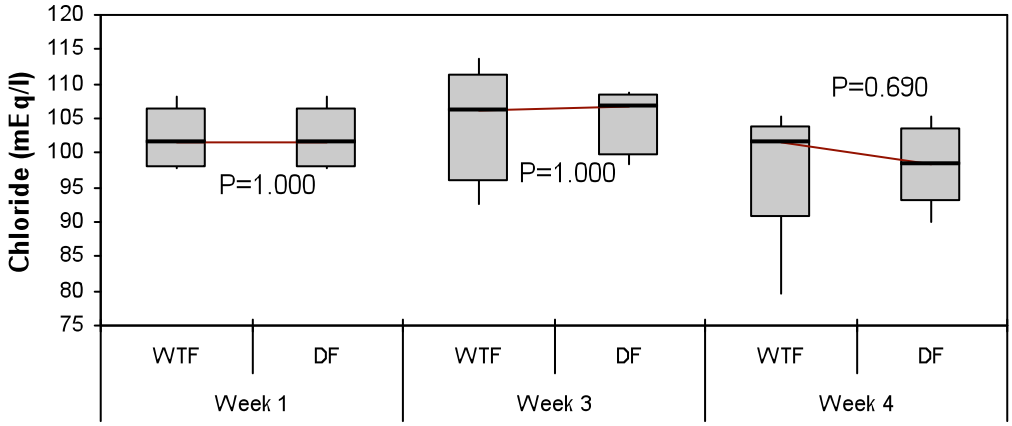

Figure 4. Box plots of median plasma chloride concentrations for bioassay fish groups held in the San Joaquin River near the Stockton Wastewater Treatment Facility (WTF) and Durham Ferry release site (DF). Samples consisted of 5 fish at each site each week.

WBC Counts

Median counts for the bioassay groups ranged from 4750 to 16250 cells/mm^3 (Figure 5). In week 3, the fish held at the Durham Ferry site had elevated WBC counts compared to fish held at the WTF (P=0.032). When all weeks were combined, there was no significant difference (P=0.103) in the median WCB count at the WTF (7750 cells/mm^3) compared to the Durham Ferry site (9750 cells/mm^3). Elevated WBC count is nonspecific, but can indicate infection (Barton, Morgan and Vijayan 2002).

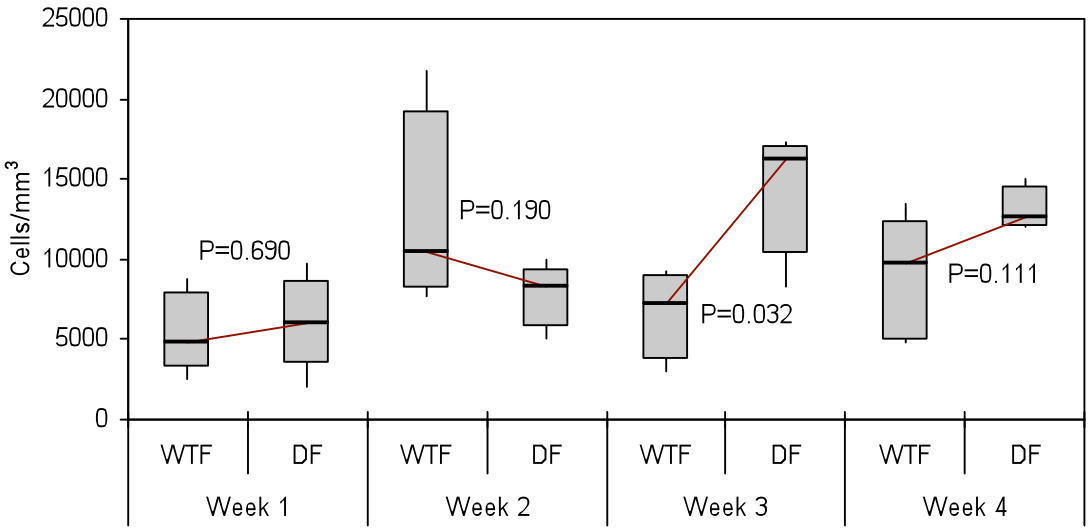

Figure 5. Box plots of Median WBC counts for bioassay fish groups held in the San Joaquin River adjacent to the Stockton Water Treatment Facility (WTF) or Durham Ferry release site (DF). Sample number was 5 fish for all samples except Week 2 - WTF and Week 4 - Control samples consisted of 4 fish.

CONCLUSIONS

No significant health or physiological problems were detected in the 2009 VAMP release groups. Light infections of *Tetracapsuloides bryosalmonae* were detected, but all infections were at very early stages and would not likely impact survival during the VAMP study period. It is possible that a portion of the population had asymptomatic infections from opportunistic bacteria. Most fish had undergone or were in the process of smoltification.

No differences were observed in bioassay groups held adjacent to the WTP or Durham Ferry sites. While low mortality in the bioassay groups was observed, it occurred at both sites and was likely a result of handing and transport. No indications of significant tissue changes were observed at either site by histology. Minor gill edema was observed in fish from both locations in several weeks. It was not know if these changes were due to water

quality at the site or handling of the sample groups. Blood clinical chemistry and WBC count data did not demonstrate any consistent difference between bioassay groups. The elevated WBC count observed at the Control group in Week 3 was the only exceptional observation and may have been caused by infections of *Tb* or an adverse reaction to tagging.

Acknowledgments
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