

U.S. Fish & Wildlife Service

Survival and Physiological Evaluation of Chinook Salmon held in the San Joaquin River near the Stockton Wastewater Treatment Plant, May 2008.

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SUMMARY

As a component of the 2008 VAMP study of the survival and distribution of migrating Chinook salmon in the San Joaquin River and delta, the CA-NV Fish Health Center conducted a bioassay to determine acute water quality effects. In 2007, acoustic tags from juvenile Chinook salmon were detected “not moving” near the Stockton Waste Water Treatment Plant (WWTP). The purpose of this component of the VAMP study was to determine if there was localized acute mortality occurring in the vicinity of the WWTP. Exposure sites included: the WWTP outfall, Burn’s Cut about 0.5 miles downstream of the water treatment plant, and a control site at the Bryant Bridge water quality monitoring station approximately 8 miles upstream of the WWTP. All fish survived the exposures and no significant site specific, sublethal effects were identified. The incidence of *T. bryosalmonae* was high in the all the exposure groups, but the infections were in an early stage and unlikely to influence the performance of the fish.

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Notice

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Introduction

As a component of the 2008 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 bioassay to assess acute water quality effects on salmon. In 2007, acoustic tags from juvenile Chinook salmon were detected “not moving” near the Stockton Waste Water Treatment Plant (WWTP). Due to the mortality observed in 2007, aquatic bioassays were conducted in the critical reach near the WWTP during the initial 24 hours of the VAMP study releases.

Methods

Fish

Juvenile Chinook salmon used in this study were reared at the California Department of Fish and Game Merced River Hatchery (MRH) and were cohorts of the acoustic tagged Chinook used in the VAMP survival and distribution studies. Exposures began on the same days as the acoustic tagged fish were released at Durham Ferry. The first exposure began on May 1 and the second exposure on May 8. Prior to transport, weight and fork length were measured from 20 fish of the tank population used for the study. This data was not collected at the exposure sites to speed necropsy. Another 60 salmon were transported in an aerated 80 gal tank to the bioassay sites in 6 live cages (10 fish / cage). Total transport time averaged 2 hours.

Sites

The three exposure sites were at the Stockton WWTP outfall (WWTP), Burn’s Cut about 0.5 miles downstream of the water treatment plant (Downstream), and a control site at Bryant Bridge approximately 8 miles upstream of the WWTP (Control) (Figures 1-4). Two live cages were placed at each site. Temperature and dissolved oxygen were measured at each site using a Hach HQ10 portable LDO meter at the end of each exposure.

Sampling

One live cage at each of the 3 sites was sampled after 4 and the other at 24 hours post exposure. Fish were euthanized in an overdose of MS222 and immediately bled into heparinized microhematocrit tubes from the severed caudal peduncle. Blood was used to prepare a blood smear, assay for methemoglobin, centrifuged to obtain the hematocrit value and collect plasma. Plasma was frozen on dry ice for transportation back to -80°C storage. Tissues were collected for histology, and samples of gill, liver and kidney were frozen in liquid nitrogen for Dr. Inge Warner (UC Davis).

Assays

Histopathology – The gills, viscera (intestinal tract, pyloric caeca, heart, liver and spleen) 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).

Methemoglobin (metHb) Assay – Elevated nitrite levels can induce methemoglobinemia in fish (Wedemeyer 1996). A blood sample was tested for percent methemoglobin using a method modified from Fairbanks and Klee (1994). In short, 20 μ l of blood was diluted in 1980 μ l phosphate buffer (0.067M, pH 6.7). The samples were mixed and split into 2 cuvettes (A and B). A solution of 20% $K_3Fe(CN)_6$ was added to cuvette B and allowed to react for 2 min. The absorbance (630 nm) of both cuvettes was then read in a spectrophotometer (A_1 and B_1) using 50% phosphate buffer in water as a blank. A drop of neutralized cyanide (6% acetic acid and 5% sodium cyanide in water) was added to all cuvettes. The sample was mixed and absorbance read again (A_2 and B_2). The percent metHb was then calculated as $100 \times (A_1 - A_2) / (B_1 - B_2)$.

White blood cell count – Blood smears were stained with a Diff-Quick stain kit (Dade-Behring, Newark DE) and read at 1000X magnification. A total of 100 white blood cells were counted and identified to lymphocyte, thrombocyte, neutrophil, or monocyte. The ratio of leukocytes to granulocytes (neutrophil) was calculated.

Plasma total protein and chloride – Plasma was stored at -80°C 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).

Results

Fish

Average (SE) fork length and weight was 96.8mm (0.3mm) and 9.8g (0.1g) for the May 1 exposure, and 106.0mm (0.6mm) and 12.3g (0.2g) for the May 8 exposure. All fish survived the exposures. Due to a failure of the anchor system, one of the two live cages was lost at the Burn's Cut site in the May 1 exposure. The 10 fish from the remaining live cage were split for the 4 and 24 hr samples.

Sites

The Stockton Waste Water Treatment Plant was discharging effluent during the May 1 (first) exposure period, but the plant was not discharging during the May 8 (second) exposure. The plant was down for maintenance and did not discharge for the entire 24 hour exposure period. Dissolved oxygen measurements at the exposure sites ranged from 7.8-9.8 mg/L. Water temperature measurements ranged from 17.1 to 19.6 $^\circ\text{C}$ on the May 1 exposure and 19.4 to 21.5 $^\circ\text{C}$ on the May 8 exposure.

Assays

Histopathology – Most of the fish in the experiment were infected with *Tetracapsuloides bryosalmonae* (Tb) with associated kidney inflammation apparent in only a few samples (Tables 2 and 3). There was no difference in Tb infection between exposure groups or sites. The incidence of microvesicular hepatocyte vacuoles (Fig. 5) in the liver was higher in fish exposed May 1 compared to fish exposed on May 8. Similarly, edema of the gill epithelial layer (Figure 6) was noted in a few fish from all groups. These changes were observed in fish from all exposure sites with no evidence of a difference between sites.

MetHb assay – This assay failed to perform under field conditions. Results were highly variable ranging from negative values to well over 100% MetHb.

Hematocrit – Values were all within normal range (25-55 %, Table 1). The only significant difference detected was between the May 8 Downstream and WWTP 24 hour exposure groups ($P < 0.001$, ANOVA). None of the fish had HCT values suggesting anemia.

White blood cell count – No obvious differences between groups were observed in the WBC counts, but a high number of lysed cells made counts difficult and possibly biased. This assay was not used in any analysis.

Plasma total protein – No differences were detected between fish at any of the sites in the May 1 exposure groups or May 8 exposure groups ($P > 0.05$, ANOVA) (Table 1). A potential hyperproteinemia (≥ 40 mg/dl) was observed in several (7 of 107) fish. These fish were noted at all sites and no pattern in exposure time or location was evident (data not shown)

Plasma chloride – A difference was observed in the May 1 (24 h) exposure groups between the WWTP and the control site ($P = 0.028$, ANOVA). No differences were detected in any of the other exposure groups. Three of the 10 fish in the May 1 WWTP 24 h exposure group were hyperchloremic (> 140 mEq /L) which caused this group to stand out. In total, 7 of 108 fish were potentially hyperchloremic and were detected in samples from all 3 sites. One fish from the May 8 Downstream 24 h exposure group appeared hypochloremic.

Table 1. Blood chemistry data for 2008 VAMP fish used in live cage bioassays at the Stockton Waste Water Treatment Plant outfall (WWTP) 0.5 miles downstream of the WWTP (Downstream) and 8 miles upstream of the WWTP (Control). Exposures corresponded with the 2008 VAMP release groups on May 1 and May 8 and fish were held in live cages at the sites for 4 and 24 hours before sampling. Data presented as mean \pm SE (n).

Exposure	Time	Site	HCT (%)	TP (mg/dl)	Cl ⁻ (mEq/l)
May 1	4 hrs	Control	39.0 \pm 1.9 (10)	29.3 \pm 2.1 (10)	124 \pm 3 (10)
		WWTP	40.5 \pm 1.3 (10)	37.2 \pm 3.4 (10)	134 \pm 6 (10)
		Downstream	45.6 \pm 4.5 (5)	29.1 \pm 1.7 (5)	138 \pm 10 (5)
	24 hrs	Control	33.5 \pm 1.5 (10)	29.0 \pm 2.1 (9)	148 \pm 11 (9)
		WWTP	36.1 \pm 1.9 (10)	25.6 \pm 1.1 (10)	128 \pm 3 (10)
		Downstream	35.8 \pm 0.5 (4)	26.5 \pm 1.2 (4)	117 \pm 1 (5)
May 8	4 hrs	Control	42.0 \pm 0.9 (10)	32.5 \pm 1.1 (10)	120 \pm 7 (10)
		WWTP	42.9 \pm 1.7 (9)	35.6 \pm 1.2 (10)	117 \pm 5 (10)
		Downstream	40.3 \pm 1.2 (10)	33.6 \pm 1.1 (10)	117 \pm 6 (10)
	24 hrs	Control	38.3 \pm 1.4 (9)	25.0 \pm 0.8 (9)	119 \pm 1 (9)
		WWTP	42.0 \pm 0.8 (10)	28.4 \pm 1.6 (10)	110 \pm 6 (10)
		Downstream	34.3 \pm 1.2 (10)	31.0 \pm 4.0 (10)	123 \pm 3 (10)

Table 2. Histological evaluation of gill, liver, and kidney from MRH salmon used as sentinels for VAMP release 1 (29April-1May). Data recorded as number of fish showing abnormality over total fish sampled at 4 or 24h at the control, WWTP outfall, or downstream of outfall. Also listed is the incidence of *T. bryosalmonae* (Tb) infection and number of infected fish showing severe interstitial hyperplasia (inflammation).

	Control		WWTP		Downstream	
	4h	24h	4h	24h	4h	24h
Epithelial edema in >10% of gill	3/10	1/10	1/10	1/9	0/5	1/5
Vacuolated hepatocytes	3/9	3/8	2/9	2/9	2/4	1/5
Incidence of Tb infection	80%	100%	50%	66%	80%	80%
Interstitial inflammation	1/8	3/10	0/10	0/9	0/5	0/5

Table 3. Histological evaluation of gill, liver, and kidney from MRH salmon used as sentinels for VAMP release 2 (6May-8May). Data recorded as number of fish showing abnormality over total fish sampled at 4 or 24h at the control, WWTP outfall, or downstream of outfall. Also listed is the incidence of *T. bryosalmonae* (Tb) infection and number of infected fish showing severe interstitial hyperplasia (inflammation).

	Control		WWTP		Downstream	
	4 hr	24 hr	4 hr	24 hr	4 hr	24 hr
Epithelial edema in >10% of gill	0/10	0/10	1/10	0/10	2/10	0/10
Vacuolated hepatocytes	1/10	1/10	3/10	0/10	5/10	3/10
Incidence of Tb infection	80%	90%	60%	90%	70%	100%
Interstitial inflammation	1/10	0/10	0/10	0/10	0/10	2/10

Discussion

The purpose of this VAMP study component was to determine if there was localized acute mortality or morbidity associated the WWTP effluent as observed in 2007. None of the fish in this study died and no significant site specific sub-lethal effects were identified. In past monitoring of VAMP study fish, the most significant health finding for study fish was infection with *T. bryosalmonae* (Foott et al. 2007). While the incidence of *T. bryosalmonae* (causative agent of Proliferative Kidney Disease or PKD) was high in the all the exposure groups, it appeared that the infections were in early stages and would not influence the fish's performance. The Merced River Hatchery Fall Chinook become infected with *T. bryosalmonae* at the hatchery, and mortality due to PKD does not occur until June after the VAMP studies are completed (Foott et al. 2007). Elevated plasma protein and chloride values (hyperproteinemia and hyperchloremia) were observed in fish from all sites and both exposure periods. These elevated plasma chemistry values were likely not a result of the exposure site, but rather changes due to our handling of the fish or samples. Possible explanations of these elevated plasma chemistry values include: plasma reduction due to shock, contamination of the plasma in the field, or desiccation of the plasma.

A difference in HCT values was detected between the WWTP and downstream sites during the May 8 exposure, but the difference was not large enough to have any biological significance as none of the fish were anemic. Elevated HCT can result from the stress response of splenic contraction and erythrocyte swelling (Wells and Weber 1991). The histopathological changes observed in the gills did not appear to be related to exposure site, and were more likely artifacts of delayed fixation. Hepatocyte vacuoles appeared to be a mix of fat and glycogen. This condition is not atypical for hatchery salmon fed high energy diets.

Two of the assays attempted in this study did not perform well enough to be included in the analysis. The metHb assay relied on the ability to quickly and consistently process the blood sample in the field. Several factors which may have interfered with the metHb assay including: multiple fish were processed at a time delaying some steps; warm weather likely caused blood to clot and reagents react and degrade faster than expected; bright sunlight and dust may have interfered with the spectrophotometer. This assay may perform much better in the lab where a single fish could be processed at a time and environmental conditions could be better controlled. The white blood cell count assay was impaired by high numbers of smudge and ghost cells which may have been affected by field conditions including temperature and delayed processing.

Acknowledgments

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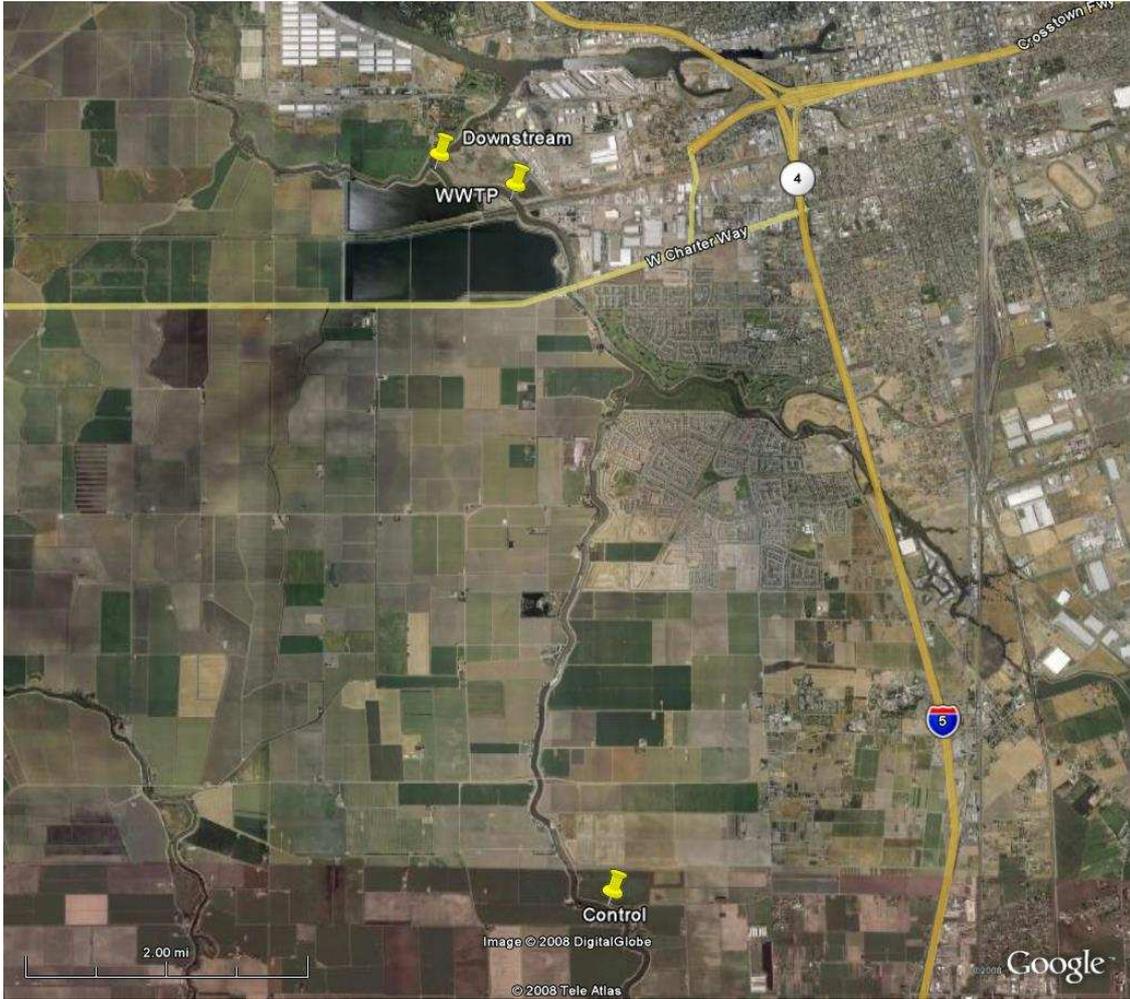


Figure 1. Exposure sites for the live cage bioassay component of the 2008 VAMP studies. Sites include the Stockton Waste Water Treatment Plant (WWTP), 0.5 miles downstream of the WWTP (Downstream), and a control site about 8 miles above the WWTP (Control).



Figure 2. Downstream exposure site about 0.5 miles downstream WWTP near Burn's Cut.

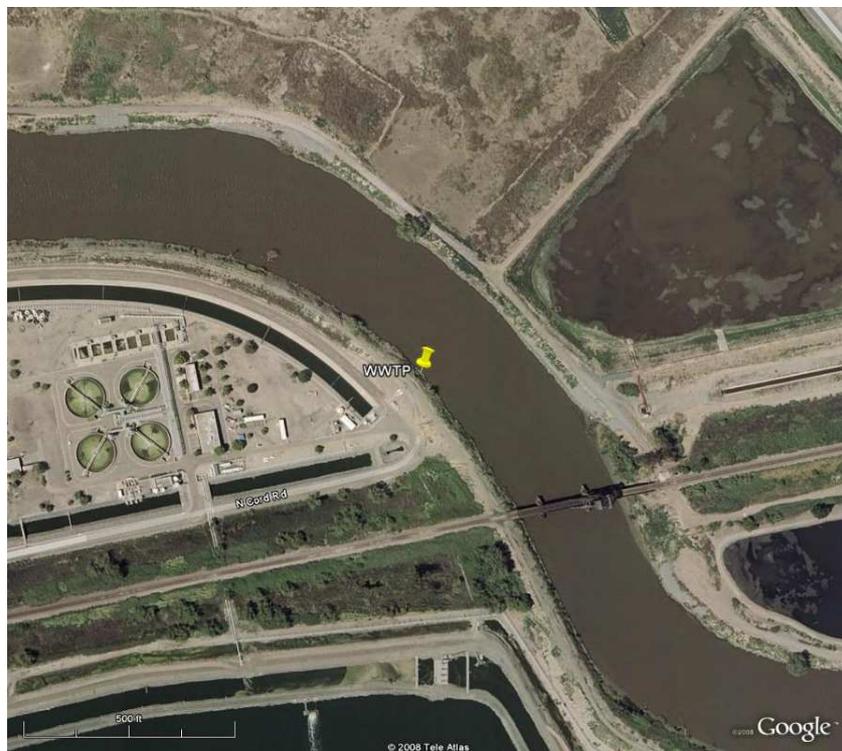


Figure 3. WWTP site located near the Stockton Waste Water Treatment Plant outfall.



Figure 4. Control site about 8 miles upstream of the WWTP near the Brandt Bridge water quality station.

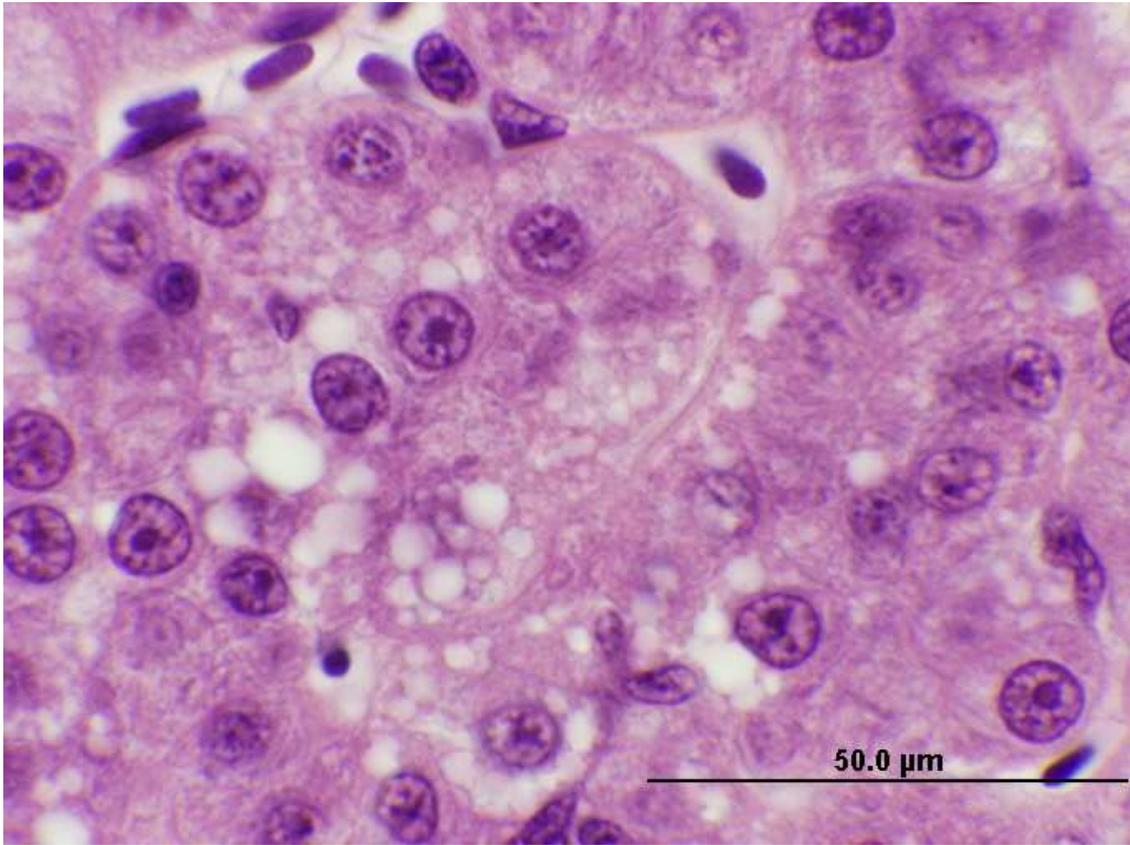


Figure 5. Photomicrograph of Microvesicular hepatocyte vacuoles in the liver of juvenile fall Chinook salmon. H&E stain.



Figure 6. Photomicrograph of epithelial edema in the gill of juvenile fall Chinook salmon. H&E stain.