

U.S. Fish & Wildlife Service

California Nevada Fish Health Center FY2008 Investigational Report:

Evaluation of sonic tagged Chinook juveniles used in the 2008 VAMP study for delayed mortality and saltwater survival – Effects of Proliferative Kidney Disease.

J.Scott Foott* and R. Stone



U.S. Fish and Wildlife Service
California-Nevada Fish Health Center
24411 Coleman Hatchery Road
Anderson, CA 96007
PH: (530) 365-4271 FAX: (530) 365-7150
September 2008



*direct correspondence

Summary: A subset of sonic tagged Merced River Hatchery (MRH) juvenile Chinook salmon, used in the 2008 Vernalis Adaptive Management Program (VAMP) study, were brought to the California-Nevada Fish Health Center wet lab and reared for 42 days at water temperatures similar to the San Joaquin River. These fish experienced a 20% cumulative mortality due to Proliferative Kidney Disease (PKD) with the majority of sampled fish showing signs of severe clinical disease by June. Despite PKD, the salmon had a high survival rate in 96 h saltwater challenges. Histopathology rating of the kidney (PKD score) was not informative for predicting saltwater adaptation. It is unlikely that PKD affected the short-term performance of tagged salmon released in early May. Whether PKD significantly reduces MRH smolt survival during early ocean rearing is unclear and will require longer term SW rearing studies.

The correct citation for this report is:

Foot JS and R Stone. 2008. FY2008 Investigational Report: Evaluation of sonic tagged Chinook juveniles used in the 2008 VAMP study for delayed mortality and saltwater survival – Effects of Proliferative Kidney Disease. U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA.

Notice

The mention of trade names or commercial products in this report does not constitute endorsement or recommendation for use by the Federal government.

Introduction:

Proliferative Kidney Disease has been diagnosed in Merced River Hatchery (MRH) juvenile Chinook salmon for several decades (Hedrick et al 1986). This trout and salmon disease is caused by the myxosporean parasite of freshwater bryozoans, *Tetracapsuloides bryosalmonae* (Canning et al. 2002). The progressive kidney inflammation and associated hypoplastic anemia is likely to reduce the fitness and performance of affected fish (Clifton-Hadley et al. 1987). Nichols and Foott (2002) report *T. bryosalmonae* infections in natural juvenile Chinook salmon collected in the Merced and Tuolumne Rivers. The bryozoan *Fredericella* is reported as a host for *T. bryosalmonae* and was observed at the water intakes of MRH (Okamura and Wood 2002). These authors speculate that salmonid fish may be an accidental host for this bryozoan parasite given the strong inflammatory response characterized by PKD and the observation that infections can occur from water supplies without fish. The incidence of *T. bryosalmonae* infection in MRH salmon inspected prior to and shortly after release has ranged from 4 – 100% (Harmon et al. 2004). The vast majority of these infections have been deemed early and the fish were asymptomatic. In 2005, the performance of MRH Chinook was tracked in swim and saltwater challenges through mid-June (Foott et al. 2007).

The objective of this study was to follow the health status and saltwater adaptation performance of *T. bryosalmonae* infected MRH juvenile Chinook salmon used for the Vernalis Adaptive Management Plan (VAMP) out-migrant salmon study. These fish were reared at temperatures similar to the San Joaquin River at the California-Nevada Fish Health Center wet laboratory for a period of time that encompassed the out-migration of the VAMP study population.

Methods:

On May 2, 2008, forty “dummy tagged” (implanted with sonic tag but not activated) Chinook juveniles were transported from the Merced River Hatchery (MRH) to the California Nevada Fish Health Center wet lab. The fish had been tagged in 2 separate lots and were held in separate sections of a 750 L rectangular tank supplied with 19 L min⁻¹ of single-pass, ozone- treated water at temperatures similar to the San Joaquin River. Water temperature was monitored hourly with an Onset™ Stowaway temperature logger. Daily mean water temperatures at Mossdale (<http://cdec.water.ca.gov>) were examined to approximate the San Joaquin River temperatures experienced by the MRH released salmon. A commercial salmon diet (Silvercup Salmon #2) was fed at 1.2% body weight per day. Kidney tissue was collected from mortalities for imprints, histology, and bacterial culture. The laboratory effluent was treated with 1-2 mg / L chlorine for a minimum of 50 min and discharged into a 1.3 ha abatement pond.

Saltwater (SW) challenge – Four to seven salmon were held in a 0.03 m³ cage within a 628 L tank supplied with 10 – 27 mg /L saltwater (Instant Ocean aquarium salt mix). The water was re-circulated through a chiller (12- 13°C) and aerated. The salinity was raised from 10 to 20 mg / L at 32h and from 20 to 27 mg /L at 64 h of the challenge. At 96 h, all fish were rapidly netted and euthanized with an overdose of MS222 in saltwater, gently dried, weighed to the nearest 0.1 g and the fork length measured (mm), bled into a heparinized microhematocrit tube from the severed caudal peduncle, and gill lamellae placed into SEI buffer and frozen at -70°C. An imprint was made with kidney tissue for *Renibacterium salmoninarum* direct fluorescent antibody testing and the remaining kidney was fixed in Davidson's fixative for 24h, transferred to 50% ethanol, and later processed for 6µm paraffin sections stained with hematoxylin and eosin. After centrifugation, hematocrit was recorded for each blood sample. Plasma was frozen for later sodium (flame photometer) as well as magnesium and total protein measurements (colorimetric assays). Gill Sodium- Potassium - Adenosine Triphosphatase activity (ATPase = µmoles ADP / mg protein / hr) was assayed by the method of McCormick and Bern (1989). Condition factor was calculated as: $KFL = (Wt / FL^3) * 10,000$. Plasma chemistry data was analyzed by ANOVA (1-way on means or Kruskal-Wallis on ranks).

PKD score – Each kidney section was scored 0, 1, 2, or 3 for *T. bryosalmonae* (Tb) location in the kidney and occurrence of kidney inflammation. These scores were multiplied by 3 to obtain weight factors.

0 = no Tb observed, no inflammation
0x3 = 0 Tb score

1 = Tb only observed in blood sinuses with no inflammation {early stage infection}
1x3 = 3 Tb score

2 = Tb observed in the kidney interstitium with minor to moderate level of inflammation
2x3 = 6 Tb score

3 = similar to #2 but severe inflammation and /or granulomas observed (disease state). 3x3 = 9 Tb score

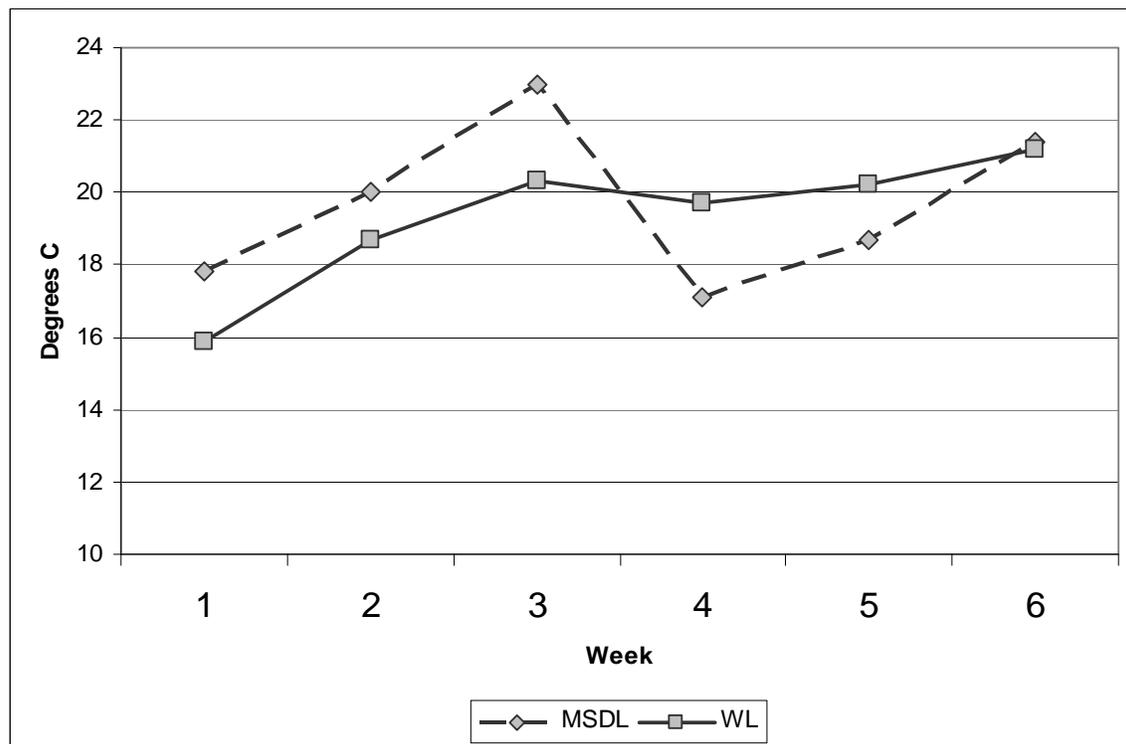
A fish was considered anemic if its hematocrit was $\leq 25\%$ and it was given an anemia score of 6. The PKD score was a summation of the Tb (0,3,6,9) and anemia (0,6) score. PKD scores ranged from 0 (normal) to 15 (clinical disease).

Results and Discussion:

Mean weekly water temperature was increased from 16° to 21°C over the 6 week study and was relatively similar to the temperature profile at Mossdale (Fig 1). The salmon showed a poor feed response throughout the study. Eight mortalities (8 / 40 = 20%) occurred to salmon held in freshwater between 14May (9 days post-transferred {dpt}) and 12June (40 dpt). All exhibited clinical signs of

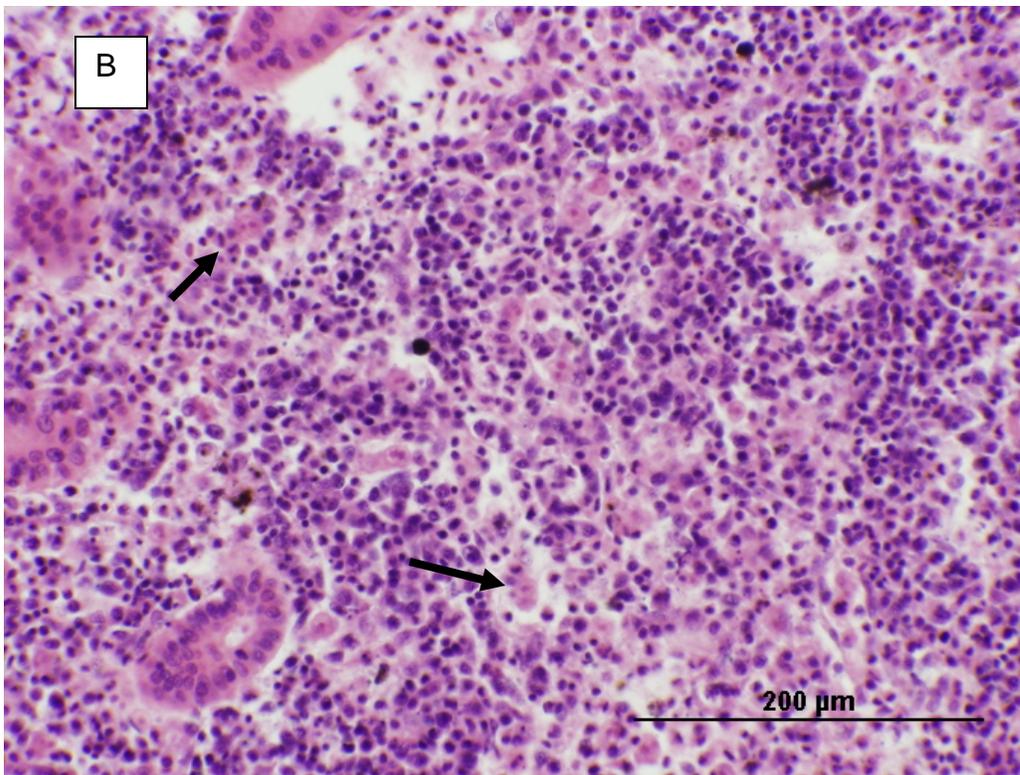
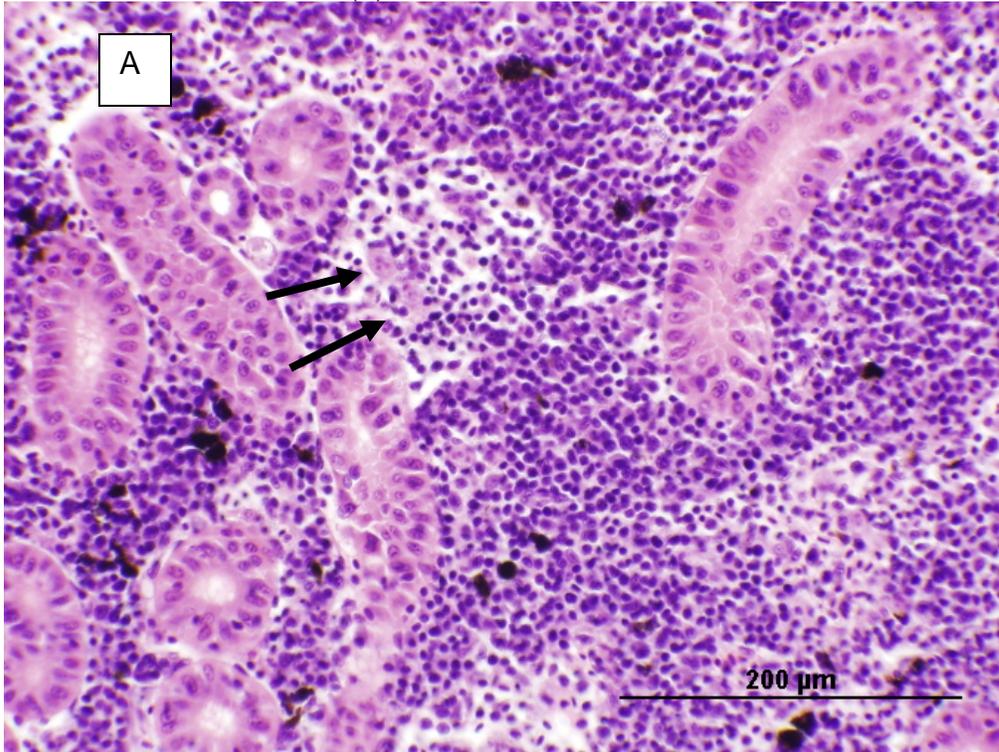
Proliferative Kidney Disease such as pale gills (anemic) as well as swollen spleen and kidney. Aeromonid bacteria (motile gram-negative, cytochrome oxidase positive) were isolated from 2 of 3 mortalities assayed. It is assumed that these opportunistic bacteria were not the primary cause of death but were secondary infections. Histological examination of mortalities did not demonstrate significantly different kidney pathology than live cohorts sampled at similar times. There was no difference in mortality between the 2 tag lots and the population was combined on 23May (21 dpt). One mortality had shed its tag and another showed hemorrhage associated with the tag suture. One to three cells resembling *Renibacterium salmoninarum* were observed in 2 of 39 kidney DFAT imprints. This low-level infection has been seen in previous MRH release group and does not appear to be a health threat for the smolts (Nichols and Foott 2002). It appears that Proliferative Kidney Disease was predominate cause of death.

Figure1. Mean weekly water temperature in wetlab (WL) tank and in the San Joaquin River at Mossdale (MSDL).



Histological results - It appears that the population was experiencing clinical PKD at the time of the first SW challenge on 23May (21 dpt). Parasites were observed in the kidney interstitium and were often associated with varying degrees of inflammation (Fig. 2). There was a 62% incidence of clinical PKD (score ≥ 9) observed in all 39 salmon sampled for kidney histology. The prevalence of clinical PKD ranged from 50% in the 23 May sample to 69% in the 6June sample. It can be argued that the 6June challenge population was affected by PKD to the greatest degree as 6 of the 13 fish in this SW challenge were judged to be anemic. This data is reflective in the higher mean PKD score (table 1).

Figure 2. Micrographs of kidney rated as (a) Tb2 = within interstitium associated with minor to moderate level of inflammation and (b) Tb3 = severe inflammation and/or granulomas. Parasites are denoted by arrows. Note the low proportion of tubules to interstitium in (b) due to inflammation. H&E stain.



Saltwater challenge- MRH salmon had high survival and maintained normal plasma constituent levels after 96 h of increasing salinity. Hedrick and Aronstien (1987) reported similar findings with *T. byrosalmonae* – infected juvenile Chinook held in saltwater. The only mortality occurred in the 13June challenge. No statistically significant difference ($P < 0.05$) was observed in condition factor (KFL), plasma protein or plasma magnesium values (Table 1). The 13June (42 dpt) challenge group had significantly higher plasma sodium levels than the 23May (21 dpt) group however all sampled fish had concentrations below 170 mmol / L. Blackburn and Clarke (1987) report that 170 mmol / L is a threshold value for successful ion regulation in juvenile Chinook in 24 h SW challenges.

While not statistically significant, fish in the 13June challenge had 4 indicators (reduced KFL, elevated magnesium and sodium, lower gill ATPase activity) of osmoregulatory impairment. It is unclear how PKD is related to these changes as the kidney histopathology was not judged to be different from the 06June sample group. It is possible that chronic stress due to disease and high water temperature rearing were affecting osmoregulation. Reduced condition factor can occur when the fish is dehydrated and altered divalent ion (Mg^{2+}) regulation would indicate kidney dysfunction (Clarke and Hirano 1995).

Sodium regulation occurs primarily in the gill and should not be directly affected by kidney inflammation. A freezer failure resulted in the movement of gill ATPase samples from $-80^{\circ}C$ to $-20^{\circ}C$ for several days. The effect on activity is unknown but could have caused a general reduction in the entire sample set. The range of ATPase activity values (1 to 6 mmol ADP/ mg protein/ h) were much lower than gill samples from previous VAMP studies (Table 1). The 2008 data is viewed as comparative between challenge groups but is suspect for accurate activity levels. The 23May group had significantly higher activities than the 06June group ($F = 7.217$, $P = 0.003$).

Table 1. Saltwater challenge data for MRH Chinook groups. Mean (std) for weight (g), fork length (mm), condition factor (KFL), plasma sodium (mmol/L), plasma protein and magnesium (g/dL), gill ATPase activity (mmol ADP/ mg protein/ h), and mean PKD score. Plasma data from one fish in the 6June challenge was excluded due to extreme values indicating probable contamination. Subscripts (a,ab,b) indicate statistically significant relationships among groups (P< 0.05, ANOVA).

| | 23May | 06 June | 13June |
|-------------|---------------|----------------------|--------------------|
| WT | 16.68 (2.8) | 18.88 (3.8) | 17.34 (4.1) |
| FL | 115 (6) | 118 (6) | 118 (7) |
| KFL | 1.10 (0.11) | 1.14 (0.12) | 1.03 (0.10) |
| Plasma | | | |
| No. sampled | 12 | 12 | 7 |
| Sodium | 147.5 (4.7) a | 151.6 (std = 8.7) ab | 162 (std = 11.9) b |
| Protein | 1.54 (0.35) | 1.70 (std = 0.19) | 1.68 (std = 0.21) |
| Magnesium | 2.35 (0.56) | 2.25 (std = 0.05) | 3.06 (std = 1.29) |
| Hematocrit | 32% (1) | 27% (6) | 27% (8) |
| ATPase** | 3.79 (1.12) a | 2.27 (0.78) b | 3.02 (1.04) ab |
| PKD score | 8 | 10 | 9 |

** ATPase activities likely affected by storage temperature variation and should be viewed as comparative data for this study only.

Significance to VAMP study – It is unlikely that PKD affected the short-term performance of the 2 VAMP release groups (29April-1May, 6May-8May) as the first SW challenge occurred 2 weeks after the first tagged cohort had been released into San Joaquin river. The 23May group appeared to be just entering a clinical phase of disease (44% with a moderate PKD-6 score and only 17% anemic). Only one freshwater mortality occurred prior to 26May.

The 2008 MRH salmon responded in a similar manner as in 2005 (Foott et al 2007). Anorexia and anemia were prevalent in the PKD affected salmon. Cumulative mortality due to PKD was 27% in 2005 compared to 20% in 2008. Survival in seawater was high in both years. It is unclear how to separate the effects of PKD from extended rearing in high water temperatures on SW adaptation. As in 2005, histopathology rating of the kidney (PKD score) was not informative for predicting SW adaptation. In order to examine the effect of PKD on early estuary and ocean survival, it is advisable to employ longer term SW rearing (example Bodega Marine Laboratory).

Acknowledgements:

Partial funding for this work came from the Stockton FWO and IEP Vernalis Adaptive Management Program (FWS account 81230 –1933-PY01). We thank California Department of Fish and Game Merced River Hatchery, USGS BRD, and Resource Scientist for access to the tagged Chinook salmon used in the study.

Reference:

Blackburn J and WC Clarke. 1987. Revised procedure for the 24 hour seawater challenge test to measure seawater adaptability of juvenile salmonids. Canadian Technical Report of Fisheries and Aquatic Sciences no. 1515, Department of Fisheries and Oceans Pacific Biological Station, Nanaimo British Columbia V9R 5K6.

Canning EU, S Tops, A Curry, TS Wood, and B Okamura. 2002. Ecology, Development and pathogenicity of *Buddenbrockia plumatellae* Schroder, 1910 (Myxozoa, Malacosporea) (syn. *Tetracapsula bryozoides*) and establishment of *Tetracapsuloides* n. gen. for *Tetracapsula bryosalmonae*. Journal of Eukaryotic Microbiology. 49(4):280-295.

Clarke, W.C. and T. Hirano. 1995. Chapter 5 Osmoregulation. pp 319 - 377. *In*: Physiological ecology of Pacific salmon. eds. C. Groot, L. Margolis, and W.C. Clarke. UBC Press, Vancouver, Canada.

Clifton-Hadley RS, RH Richards and D Bucke. 1987. Further consideration of the haematology of proliferative kidney disease (PKD) in rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Diseases 10:435-444.

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(2): 57 – 76.

Harmon R, K Nichols, and JS Foott. 2004. FY 2004 Investigational Report: Health and Physiological Assessment of VAMP Release Groups – 2004. US Fish and Wildlife Service, California-Nevada Fish Health Center, Anderson, C A (<http://www.fws.gov/canvfhc/reports.asp>).

Hedrick RP, ML Kent, and CE Smith. 1986. Proliferative kidney disease in salmonid fishes. Fish Disease Leaflet 74, Fish and Wildlife Service, Washington D.C. 20240.

Hedrick RP and D Aronstien. 1987. Effects of saltwater on the progress of proliferative kidney disease in chinook salmon (*Oncorhynchus tshawytscha*). Bulletin of the European Association of Fish Pathologists 7(4): 93-96.

McCormick, SD and HA Bern. 1989. In vitro stimulation of Na⁺/ K⁺ ATPase activity and ouabain binding by cortisol in coho salmon gill. AM. J. Physic. 256: R707-715.

Nichols K and JS Foott. 2002. Health monitoring of hatchery and natural fall-run Chinook salmon juveniles in the San Joaquin River and tributaries, April – June 2001. US Fish and Wildlife Service, California-Nevada Fish Health Center, Anderson, C A (<http://www.fws.gov/canvfhc/reports.asp>).

Okamura B. and TS Wood. 2002. Byrzoans as hosts for *Tertracapsula bryosalmonae*, the PKX organism. Journal of Fish Diseases 25:469 – 475.