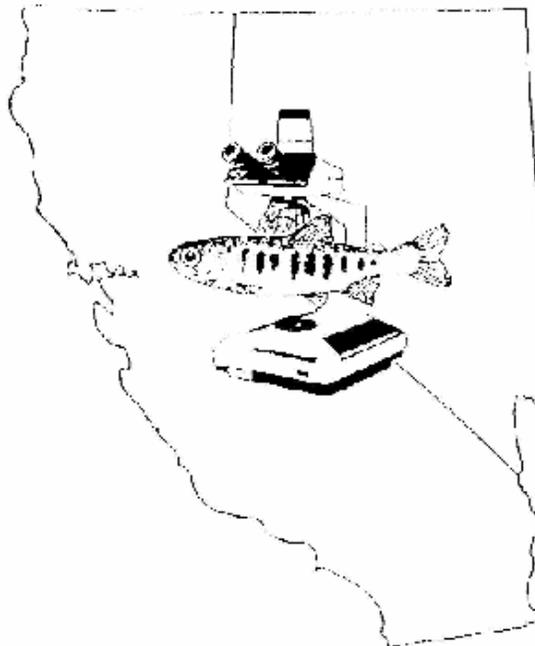


FY2005 Investigational Report:

The effect of Proliferative Kidney Disease on blood constituents, swimming performance and saltwater adaptation in Merced River Hatchery juvenile Chinook salmon used in the 2005 VAMP study.



**J. Scott Foott*, R. Stone and K. Nichols
U.S. Fish & Wildlife Service
California – Nevada Fish Health Center
24411 Coleman Hatchery Road
Anderson, CA 96007**

**Phone 530 - 365 – 4271
Scott_Foott@fws.gov**

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* **Direct Correspondence**

Summary: Merced River Hatchery (MRH) juvenile Chinook salmon, from tagged lots used in the 2005 Vernalis Adaptive Management Program (VAMP) study, were brought to the California-Nevada Fish Health Center wet lab 6 days prior to the first VAMP release and reared for 50 days at water temperatures similar to the San Joaquin River. At the time of transport, a fish health inspection showed that the population was generally healthy but had a low prevalence of an early stage infection by the myxosporean parasite, *Tetracapsuloides bryosalmonae*. This parasite has been detected in Merced River salmon for several decades and causes Proliferative Kidney Disease (PKD). The level of clinical PKD, as demonstrated by a combined kidney lesion and anemia score, markedly increased starting at 29 days post-transfer from MRH (**dpt**). Severe disease occurred in the study population after the last VAMP coded wire tag fish was recovered in the Chipp's Island trawl on 27 May. A total of 76 study salmon (27% cumulative mortality) died due to PKD beginning at 36 dpt through the final sample at 50 dpt. Both time post-transfer and disease state correlated with a decline in hematocrit and plasma magnesium as well as an elevation in circulating white blood cell number and plasma protein concentration. There was no observed PKD effect on time to exhaustion during a 120 min swim challenge until 50 dpt. Smolt development measurements indicated that the study fish were in an advanced stage of smoltification. Similar to swim performance, saltwater adaptation was not impaired until 50 dpt. *Tetracapsuloides bryosalmonae* was observed in 40% (17 of 43) of the kidney imprints collected from VAMP tag salmon recovered in the Chipp's Island trawl. These results indicate that while *T. bryosalmonae* infection is prevalent in Merced R. out-migrant salmon, it may not have a significant effect on VAMP recoveries. However, PKD could be a significant mortality factor for Merced River salmon smolts during their early seaward entry phase.

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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). Relatively short exposures (10 min), to ruptured bryozoans infected with *T. bryosalmonae*, results in the invasion of skin mucus cells and produced clinical infections within 8 weeks (Longshaw 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. 1987b). 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.

The objective of this study was to follow the health status and performance capabilities 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:

Chipp's Island trawl kidney imprints – A sub-sample of 97 adipose fin marked Chinook juveniles collected in the Chipp's Island trawl between 5 May and 27 May 2005 were sampled for kidney tissue by Stockton Fish & Wildlife Office biologists. Imprints of the kidney were made on numbered slides, fixed with isopropyl alcohol, and both the head tag and kidney sample number recorded. Imprints of 45 salmon with VAMP tag codes were later screened by an indirect fluorescent lectin assay utilizing biotin-labeled *Griffonia simplicifolia* agglutinin I lectin (GS-I) and fluorescein-labeled avidin stain (Vector Laboratories). The GS-1 stain reacts with carbohydrate moieties of *T. bryosalmonae* (Hedrick et al. 1992). The imprints were examined at 40x magnification with an Olympus BHS fluorescent microscope.

Fish handling – On 25 April 2005, two hundred and eighty two juvenile Fall-run Chinook salmon (*Oncorhynchus tshawytscha*), reared at the California Department of Fish and Game's Merced River hatchery (MRH) were transported to the Fish Health Center Wet Laboratory. Equal numbers of fish were removed from coded wire tagged lots used in the 2 release groups of the Vernalis Adaptive Management Plan (VAMP) study. The salmon were held in a 470 L circular tank supplied with aeration. A flow of 2-3 cm / s was selected to induce minimal swimming behavior in the population. Water temperature was maintained in the recirculation system by immersion heaters within a 300 L effluent sump. Makeup inflow for the system was set at 58 L / min. Water temperature was monitored every 2 hrs with Onset™ Stowaway temperature loggers. Daily mean water temperatures along the San Joaquin River migration route were obtained from a California Department of Water Resources real-time website (<http://cdec.water.ca.gov>). A commercial salmon diet was fed at 1.5% body weight per

day. 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.

Necropsy – Fish were captured by net and immediately euthanized in an overdose of MS222, measured for fork length and weight, and examined for pale gill (clinical sign of anemia). Fulton condition factor was calculated from the fork length ($KFL = \text{weight} / (\text{fork length})^3 \times 10^5$). The caudal peduncle was cut and blood collected into heparinized microhematocrit tubes. Sub-samples of blood were used to prepare a blood smear and 2.5 μL aliquot that was frozen for later hemoglobin measurement. The mean corpuscular hemoglobin concentration (MCHC), a measure of the average erythrocyte hemoglobin concentration, was calculated: $MCHC (\text{g} / \text{dL}) = [\text{Hb} (\text{g/dL}) / \text{HCT} (\%)] \times 100$

The microhematocrit tubes were centrifuged at 10,000 x g for 5 min. Packed red cell, buffy coat, and total fluid length was measured with a 30x dissection scope equipped with an ocular micrometer for calculation of both hematocrit and leukocrit (McLeay and Gordon 1977). Plasma was frozen on dry ice and stored at -70°C until assayed with colorimetric kits (Pointe Scientific Inc, Lincoln Park MI) for total protein, glucose, and magnesium. Upon dissection, the degree of spleen and kidney swelling was recorded for each fish. Anterior kidney was collected for a nitroblue tetrazolium assay and the posterior kidney was placed in Davidson's fixative, processed for 5 μm paraffin sections and stained with hematoxylin and eosin. On 25 April, kidney – spleen tissue from sixty fish at MRH were collected for viral assays (12, 5 –fish pool samples inoculated onto EPC and CHSE214 cell lines and incubated at 15°C for 18 d), *Renibacterium salmoninarum* DFAT assays performed on kidney imprints and bacteria isolation on BHI agar. Posterior kidneys from 16 MRH fish were collected for histological examination.

Nitroblue tetrazolium (NBT) assay – The respiratory burst activity of anterior kidney cell phagocytes was measured in a NBT assay by the method of Secombes 1991. One killing mechanism of activated phagocytes is the production of reactive oxygen species (O_2^-) and is referred to as respiratory burst (Slauson and Cooper 1982). Briefly, the anterior kidney was dissected from a sub-sample of 6 – 8 salmon, weighed to the nearest 0.01 g, placed into cold Hanks Buffered Salt Solution (HBSS), titerated to form a single cell suspension with a 21G needle mounted onto a 1 cc syringe, after a 10 μL sub-sample was fixed in Rees-Eckert solution for hemocytometer cell count the suspension was centrifuged (400x g , 4°C , 5 min) and the cell pellet re-suspended in 500 μL of HBSS without calcium or magnesium. One hundred microliters of the cell suspension was added to triplicate wells in a 96 well plate followed by 100 μL of NBT solution (1 mg / mL in HBSS with 1 μg / mL P horbol Myristate Acetate). The reaction was stopped after 20 min incubation at 25°C by centrifuging the plate (400x g, 4°C , 5 min). The supernatant was carefully poured off and the cell pellet fixed by repeat washing with 70% methanol and centrifugation. The cell pellet was air dried and NBT solubilized by the addition of 120 μL 2M KOH and 140 μL DMSO. The optical density of the solution was measured at 630 nm in a Biotek microplate reader. The data was expressed as $\text{mOD}_{630} / 10^5$ anterior kidney cell. The processing of the anterior kidney cells was begun within 2 h of collection.

Saltwater challenge - Groups of six fish were held for 24h in 19 L buckets containing 25 – 26 ppt saltwater (Instant Ocean aquarium salt mix) that were supplied with aeration. The buckets were held in a water bath set at 13 - 14°C . After 24 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, gill lamellae placed into SEI buffer and frozen at -70°C . Gill Sodium - Potassium - Adenosine

Triphosphatase activity (ATPase = μ moles ADP / mg protein / hr) was assayed by the method of McCormick and Bern (1989). After centrifugation, hematocrit and leukocrit were recorded for each blood sample. Plasma was frozen for later sodium (flame photometer), magnesium and total protein measurements (colorimetric assays).

PKD score – Each kidney section was scored a 0,1,2,or 3 for the relative number of parasites (0=none, 1 = < 10, 2 = 11- 30, 3 = > 30) and degree of inflammation (0 = none, 1 = < 10% of kidney, 2= 11 – 50%, and 3 => 50%). If the fish’s hematocrit was less than 20%, a score of 6 was assigned to the fish. An individual’s PKD score was the summation of the degree of anemia (hematocrit score of either 0 or 6), parasite load (2 multiplied by the parasite number value), and degree of inflammation (3 x inflammation value). PKD scores ranged from 0 (normal) to 24 (late stage clinical disease). PKD score was used to sort fish for statistically analysis.

Swim exhaustion performance - Swimming performance was determined by the amount of time a given fish could swim at a relatively high velocity before becoming exhausted (Jones and Moffitt 2004). Groups of 5 -10 salmon were placed into a 30 cm dia. x 56 cm long cylinder and allowed to acclimate for 20 min to a minimal 18.3 cm / sec flow generated by an electric trolling motor. This rate equated to approximately 2 body lengths / s (BL/s) and was close to the reported optimal 1 BL /s cruising speed for salmonids (Webb 1995). The ends of the cylinder were enclosed with 1 cm honeycomb screens to provide uniform flow pattern. The upstream half of the chamber was covered to encourage fish to swim in this region and an open hatch at lower end allowed for observation and capture of impinged fish. The entire cylinder was held in a 1400 L oval tank with a partial middle wall for circular flow. Water was kept at the same temperature as the rearing tank. Flow was measured in front of the rear screen by a Flowmate Model 2000 flowmeter (Marsh-McBirney Inc., Maryland). After acclimation, the flow was increased to 48.8 cm / sec for an additional 100 min. Once a given fish became impinged on the rear screen of the chamber, it was prodded to induce it to return to the upper portion of the chamber. Any fish that remained on the posterior screen was considered to be exhausted, the time recorded, and the fish was sampled in a manner similar to the weekly physiological group (weight, length, blood, and kidney histology).

Statistical analysis: For data sets that were not normally distributed, Kruskal-Wallis 1-ANOVA on ranks along with Dunn’s Multiple comparison method was used to compare significance among raw sample group data and is reported as an “H” value.

Results:

Relationship to VAMP tagged fish recovery and out-migration - Salmon were brought to the wet lab 6 days prior to the first release at Durham Ferry on 2 May and reared for 50 days (Table 1). Daily mean water temperature of the study fish tank ranged from 14.5 - 19.6° C and was similar to temperatures at Mossdale but averaged 2 °C lower than the San Joaquin River at Antioch (Figure 1). The Mossdale gauge was chosen to be reflective of the Durham Ferry and Dos Reis release sites while the Antioch gauge data should approximate the final capture location at Chipp's Island.

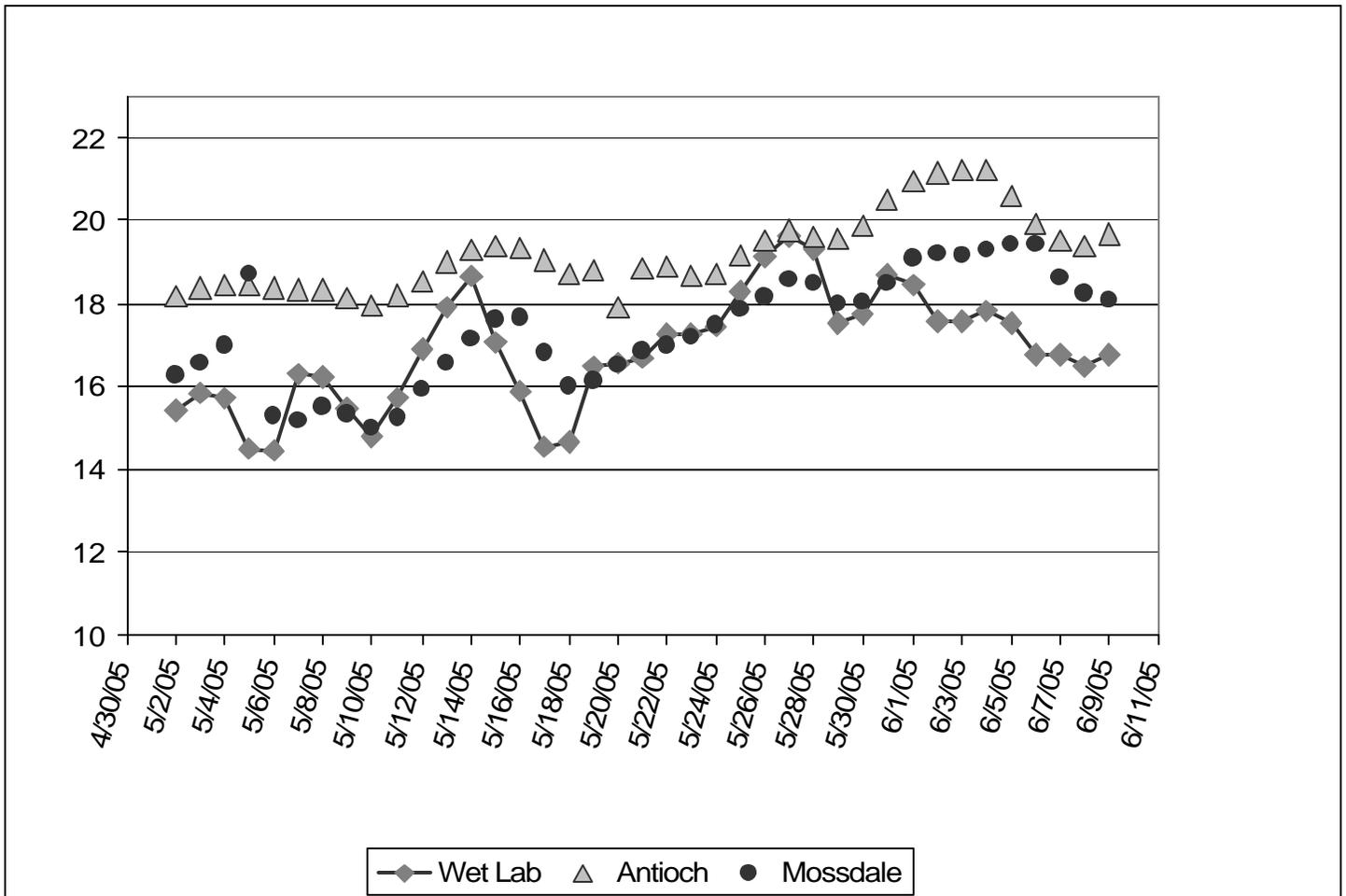
Tetracapsuloides bryosalmonae was observed in 40% (17 of 43) of the VAMP tag salmon kidney imprints collected from the Chipp's Island trawl (Table 1). It is unclear why the second Jersey Point group had such a low incidence (1 of 13 {8%}) in comparison to the other groups (67– 100%). The number of parasites per imprint was highly variable and ranged from 1 to over 100 with a mean of 13 (std. dev. 21). Neither virus nor *Renibacterium salmoninarum* was detected in the MRH population in the 25 April health inspection. *Aeromonas hydrophilia* was isolated in 2 of 42 bacterial cultures and 6 of the 16 histological samples of kidney contained very low numbers of *T. bryosalmonae* without associated inflammation.

Table 1.

VAMP study release sites, dates of release (REL) and last coded wire tag (L-CWT) recovery at Chipp's Island trawl, incidence of *Tetracapsuloides bryosalmonae* infection in kidney imprints (no. positive / total examined, Tb) and maximum days at large (DAL) value for each release group.

Release site	Group 1				Group 2			
	REL	L- CWT	Tb	Max. DAL	REL	L-CWT	Tb	Max.DAL
Durham Ferry (114rkm)	2 May	19 May	4 / 4	17	9 May	27 May	2 / 3	18
Dos Reis (82rkm)	3 May	12 May	2 / 3	9	10 May	18 May	1 / 1	8
Jersey Point (8 rkm)	6 May	15 May	15 / 18	9	13 May	20 May	1 / 13	7

Figure 1. Mean daily water temperature profile (°C) of study group at wet lab, DWR gauge data at Mossdale and Antioch.



Proliferative kidney disease (PKD) – The level of clinical disease, as portrayed by the PKD score, markedly increased starting with the 29 days post-transfer from MRH (dpt) sample (Fig 2). A severe disease state occurred in the study population after the last VAMP coded wire tag salmon was captured at Chipp’s Island on 27 May. A total of 76 study salmon (27% cumulative mortality) died due to PKD beginning at 36 dpt (Fig. 3). Clinical signs such as swollen kidney and spleen as well as pale gill were observed in the study fish beginning at 36 dpt and became more prevalent with time (Table 2). Late stage PKD was histologically characterized by hyperplasia and granuloma formation in the kidney interstitium and large numbers of *T. bryosalmonae* trophozoites.

Figure 2. Mean PKD score.

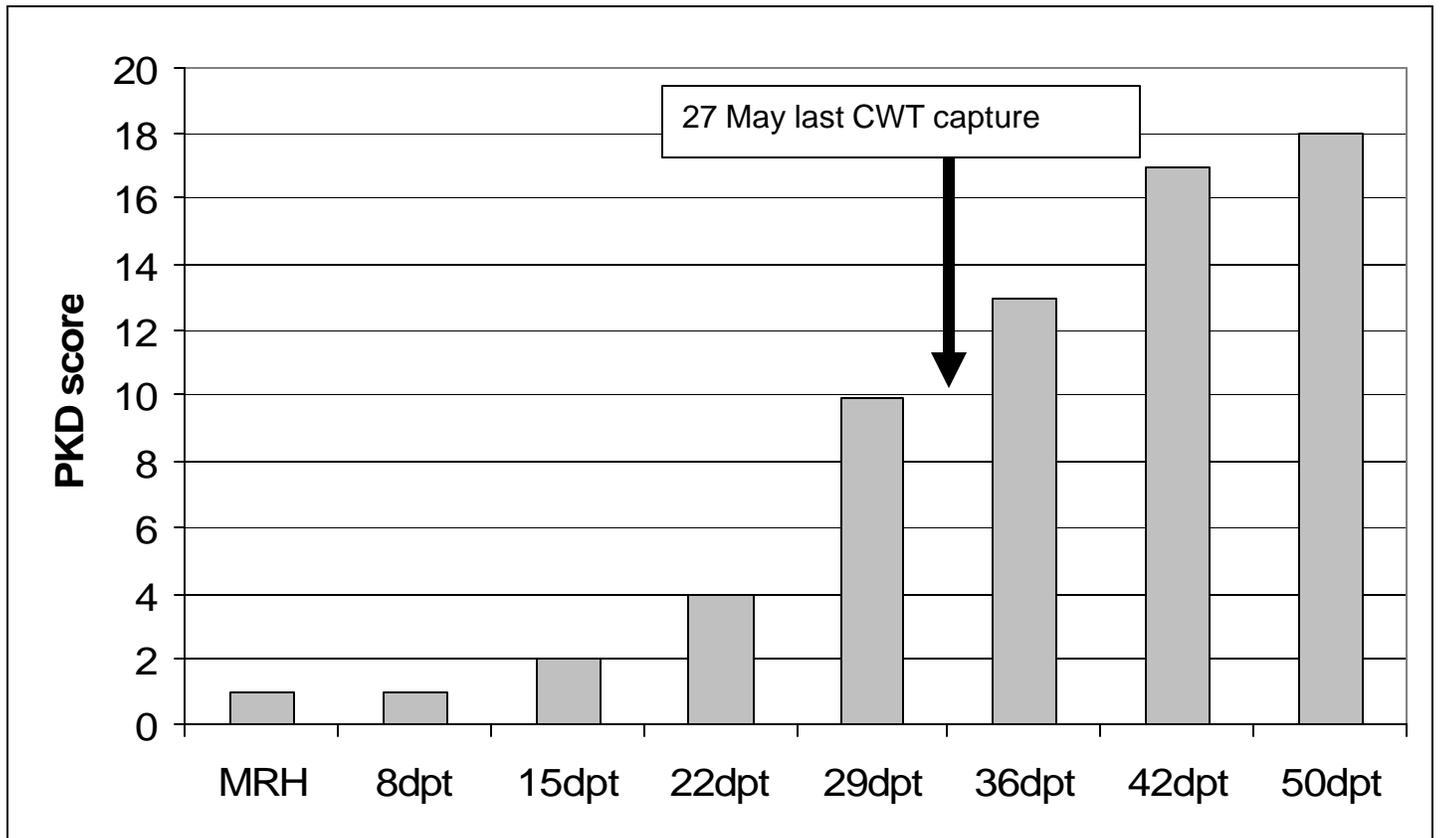
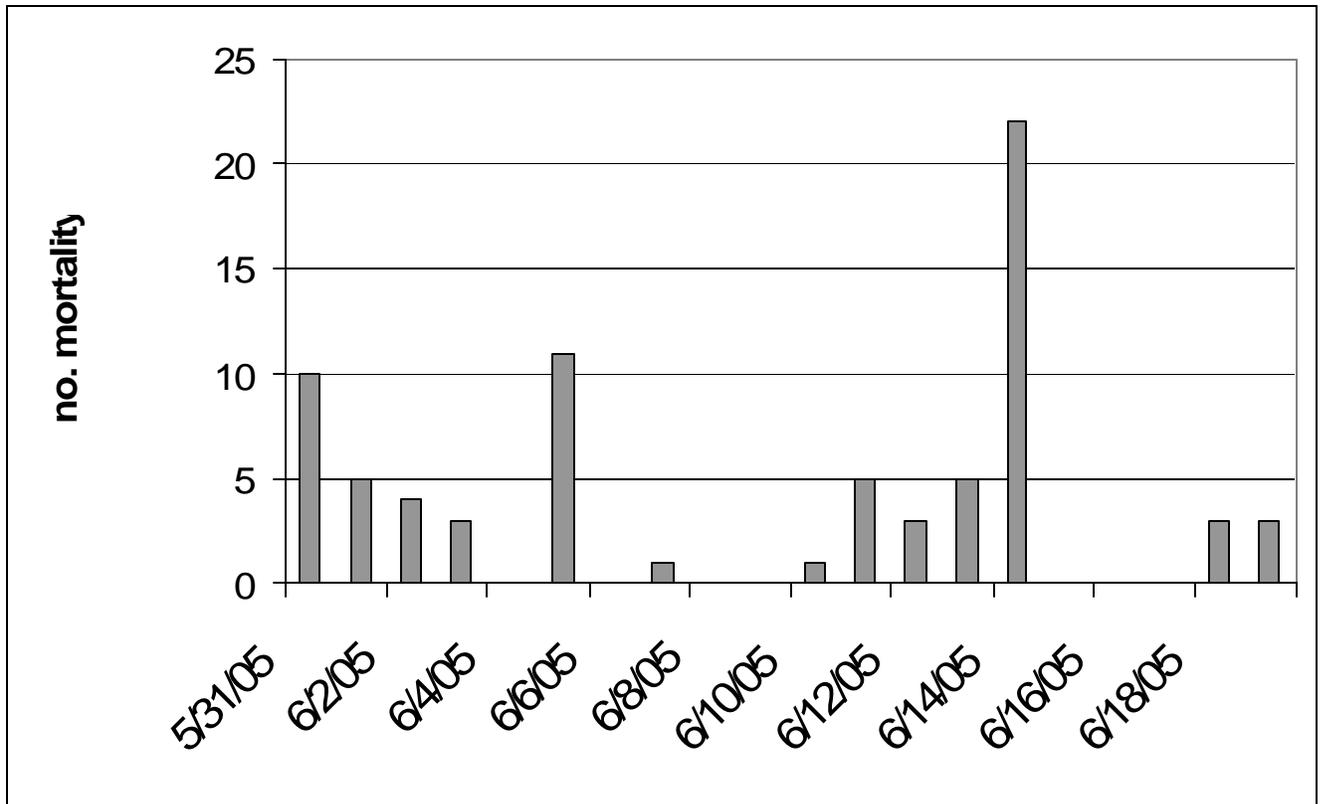


Figure 3.

Mortality data for the study. A total of 76 study fish died between 31 May and the termination of the study on 19 June.



Morphological and physiological characteristics -Minimal growth occurred in the study group over the 7 week period. Mean weight ranged from 5.5 to 6.8 g while mean fork length ranged from 81 to 86 mm (Table 2). Condition factor remained similar throughout the study with mean values ranging between 0.990 and 1.157 (Table 2). As estimated by hematocrit and leukocrit, erythrocyte numbers declined while white blood cell numbers increased starting at 42 dpt (Table 3, Fig. 2). The hematocrit of the MRH and 8 dpt sample groups were significantly higher than 29 – 50dpt groups ($H= 64.3, 7df, p<0.001$). Anemia was apparent by the high percentage of fish with pale gills at 42 and 50 dpt (Table 2). Both time and disease state correlated with reduced hematocrit and increased leukocrit. Linear regression analysis of hematocrit with sample time (dpt) and disease state (PKD score) demonstrated a R^2 of 0.66 and 0.58, respectively. Similarly, increased leukocrit values were moderately correlated with PKD score ($R^2 = 0.3$) and dpt ($R^2 = 0.42$). The leukocrit of the MRH and 8 dpt sample groups were significantly lower than 36 – 50 dpt groups ($H= 44.8, 7df, p<0.001$). Hemoglobin concentration and MCHC index declined after the 36 dpt sample group and were at their lowest points in the 42 dpt sample (Table 3). The MCHC of the 42 dpt group was significantly lower than MRH, 8, 15, and 29 dpt groups ($H= 38.1, 7df, p<0.001$). Days post-transfer was better correlated with MCHC decline ($R^2 = 0.21$) than PKD score ($R^2 = 0.15$). The white blood cell profile did not change to any great degree over the 7 weeks study with lymphocytes being the dominant cell type (Table 2). Both the highest

number of cells per gram of anterior kidney and the greatest NBT activity was observed at 36 dpt (Fig. 4, Table3). There was no correlation between PKD score and NBT reaction ($R^2 = 0.001$).

Table 2.

Mean (Standard deviation) fork length (mm), weight (0.1 g), condition factor ($(Wt / FL^3) \times 10^5$), and percent of examined group with swollen kidney or spleen (%Sw KD/Sp) and pale gill (%PG). Date of examination and days post transfer (dpt) from Merced River is stated for each examination group (n=16 fish each).

Group	Fork Length	Weight	KFL	%Sw KD/Sp	%PG
Merced R. SFH 25 April, 0 dpt	82 (5)	6.2 (1.2)	1.132 (0.068)	0	0
3 May 8 dpt	81 (5)	5.5 (1.1)	1.045 (0.062)	0	0
10 May, 15 dpt	85 (5)	6.2 (1.1)	0.990 (0.083)	0	0
17 May, 22 dpt	85 (7)	6.7 (1.0)	1.076 (0.125)	0	0
24 May, 29 dpt	86 (5)	6.8 (1.1)	1.062 (0.140)	0	0
31 May, 36 dpt	87 (5)	7.2 (1.2)	1.069 (0.053)	33%	0
7 June, 42 dpt	87 (2)	7.0 (0.7)	1.062 (0.062)	100 %	88%
13 June, 50 dpt	85 (5)	7.2 (1.3)	1.157 (0.048)	100%	100%

Table 3.

Blood cell characteristics and anterior kidney phagocytic activity. Mean (std. dev.) values of hematocrit (% HCT), hemoglobin concentration (g / dL, Hb), mean corpuscular hemoglobin concentration (g / dL, MCHC), leukocrit (% LCT), differential leukocyte count percentage of lymphocytes, thrombocytes, and neutrophils, the prevalence of bloodsmears containing > 1% immature erythrocytes (>1% IE), reduction of nitroblue tetrazolium dye (mOD/ 10⁵ cell) by the respiratory burst of activated anterior kidney cells (NBT), and the concentration of cells per gram of anterior kidney (AK cell / g x 10⁸) in sample groups (Merced R. Hatchery and 8-50 dpt).

	MRH	8	15	22	29	36	42	50
HCT	44 (3)	45 (6)	39 (4)	38 (5)	35 (5)	35 (5)	22 (3)	21(6)
Hb	7.5 (0.6)	7.4 (1.1)	6.5 (1.0)	5.8 (1.3)	6.6 (0.7)	4.7 (0.7)	1.7 (0.4)	2.5 (1.1)
MCHC	17 (1)	17 (3)	17 (2)	16 (5)	20 (3)	14 (3)	8 (2)	12 (4)
LCT	0.498 (0.167)	0.607 (0.177)	0.773 (0.133)	0.977 (0.231)	0.800 (0.192)	1.429 (0.808)	2.693 (1.00)	2.968 (2.336)
%lymphocyte	71 (16)	78 (12)	82 (10)	86 (9)	87 (7)	90 (4)	86 (12)	88 (5)
%thrombocyte	20 (16)	21 (12)	16 (10)	12 (8)	11 (3)	10 (4)	13 (11)	12 (5)
%neutrophil	3 (4)	0	2 (3)	2 (2)	0	0	0	1 (1)
> 1% IE	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	3 / 5 (60%)	4 / 5 (80%)
NBT	ND	24 (8)	12 (5)	23 (8)	14 (4)	34 (12)	18 (3)	16 (5)
AK cell /g (10 ⁸)	ND	9.4 (2.7)	8.6 (4.2)	8.3 (1.2)	11.8 (2.5)	15.4 (7.9)	ND**	10.7 (4.5)

Figure 3. Mean hematocrit (HCT%) and leukocrit (10x) values of study fish.

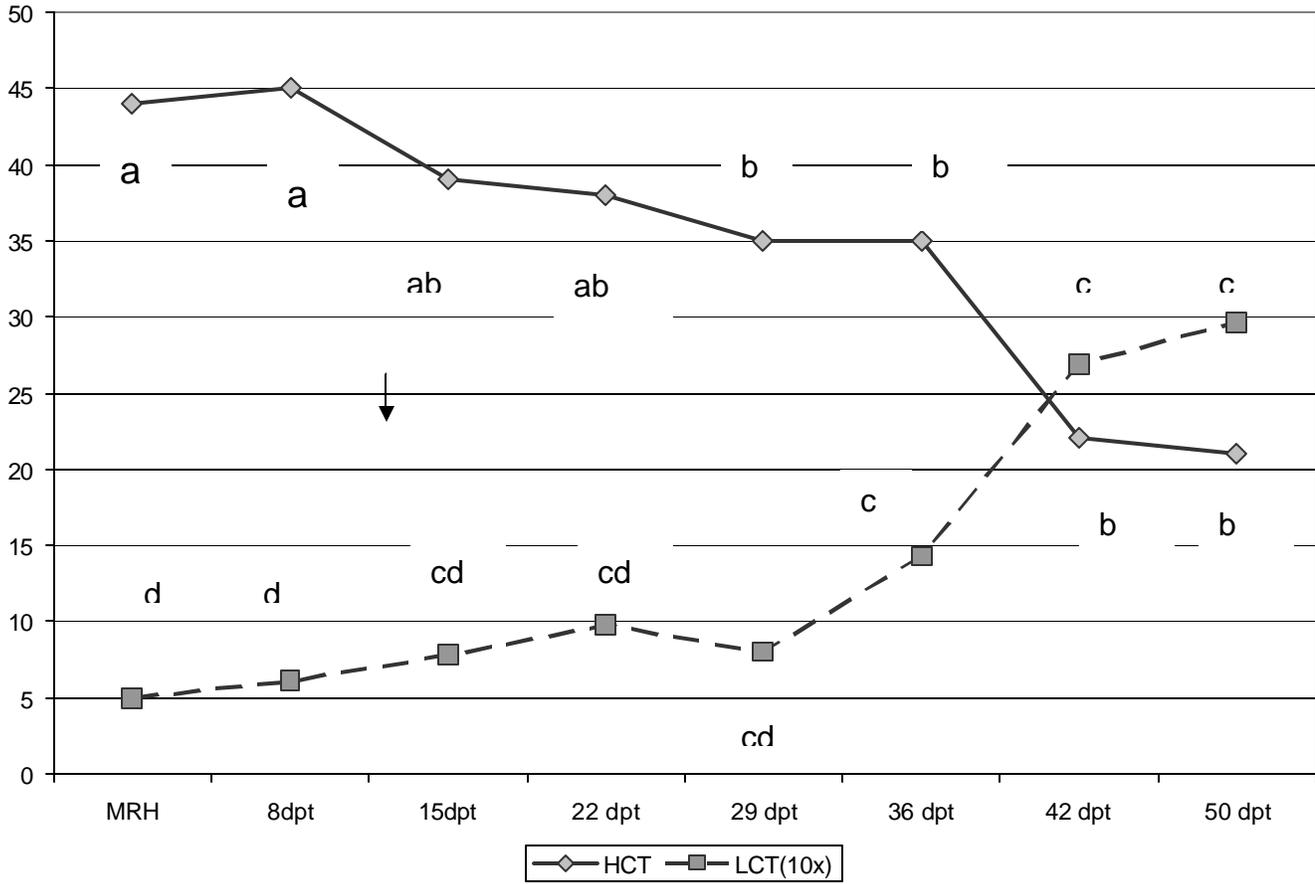
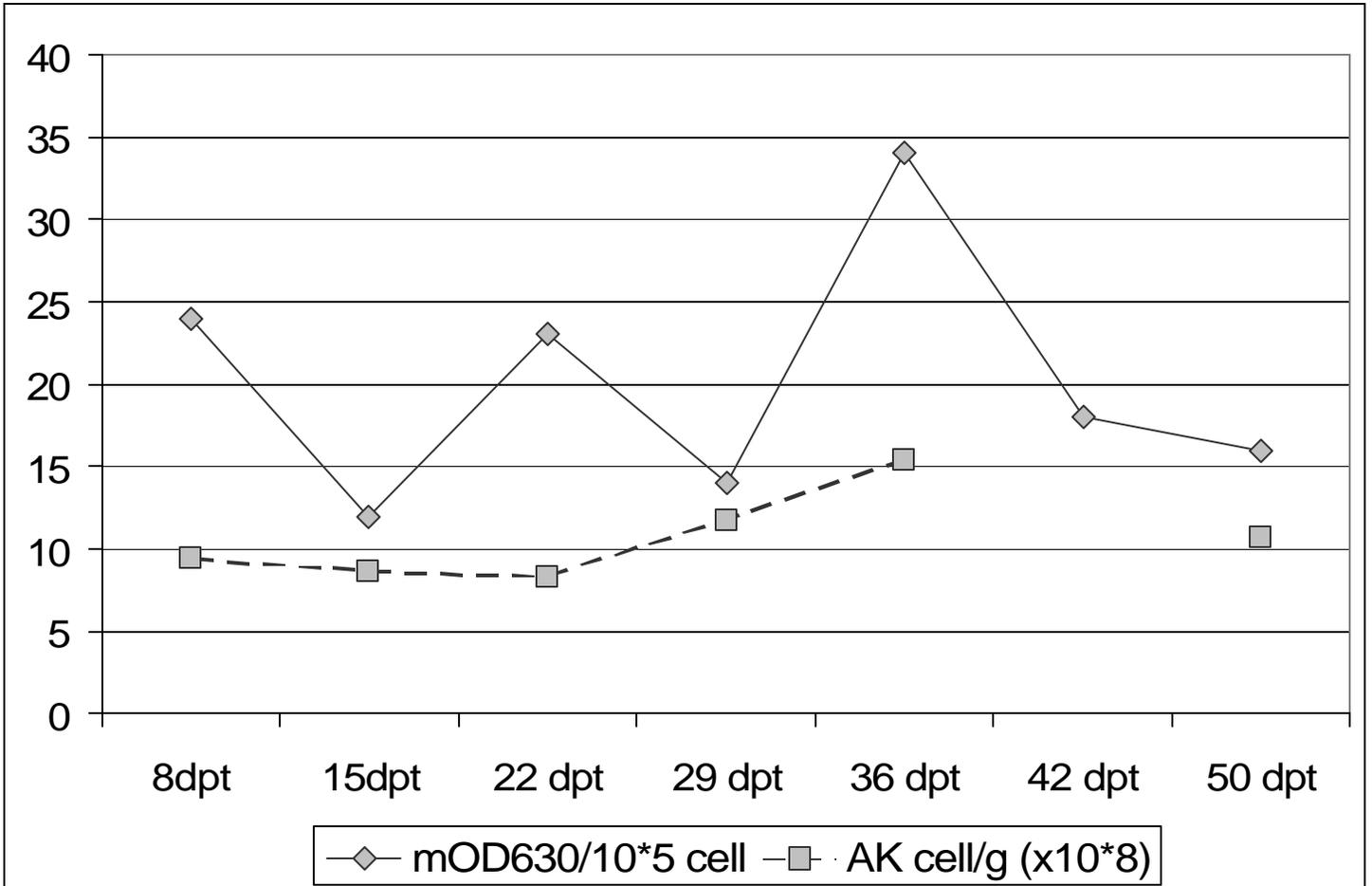


Figure 4. Cell numbers per gram of anterior kidney (AKcell / g $\times 10^8$) and nitroblue tetrazolium reaction (mOD630 / 10^5 cell) of activated kidney cells. Loss of 42 dpt group kidney weight data prevented the calculation of its anterior kidney cell numbers / g .



Plasma protein concentrations of salmon, sampled within 45 seconds (**45s**) of capture, increased beginning with the 29 dpt sample group. A maximum mean value of 3.8 g / dL occurred in the 42 dpt fish (Fig 5). Similarly, plasma protein levels in 42 and 50 dpt swim endurance fish were also elevated in comparison to the previous weeks (Table 4). The correlation between PKD score and increased plasma protein concentration was relatively low ($R^2 = 0.17$). Plasma glucose values remained relatively low in the 45s sample fish (< 90 g / dL) indicating that sampling occurred prior to a secondary stress response. In contrast, the swim challenge fish tended to have higher plasma glucose than their 45s cohorts (Table 4). Mean glucose values of 25 and 41 g / dL for the 50 and 29 dpt sample groups were significantly lower than the MRH, 8, and 42 dpt groups ($H= 47.7, 7df, p<0.001$). Similarly, glucose values for the 42 and 50 dpt swim challenge fish were significantly lower than 8 and 22 dpt groups ($H= 28.4, 6df, p<0.001$). Plasma magnesium values decline over time (Table 4, fig. 6). This decline was somewhat correlated to PKD score ($R^2 = 0.33$). The 29, 42, and 50 fish sampled within 45s of capture had significantly lower plasma magnesium values than MRH and 8 dpt groups ($H= 48.9, 7df, P<0.001$). Swim challenge fish showed a similar declining plasma magnesium trend.

Table 4

Mean (std.dev.) values for plasma protein(TP g/dL) , glucose (GLU g/dL), and magnesium (Mg mEq/L) of study fish held in freshwater and sampled within 45 sec of capture (45s) and after the swim challenge (swim). Letters denote statistical relationships between sample groups.

	MRH	8 dpt	15 dpt	22 dpt	29 dpt	36 dpt	42 dpt	50 dpt
TP 45s	3.1 (0.2) n=15 ab	2.5 (0.2) n=14 a	2.4 (0.3) n=15 a	4.2 (0.2) n= 8 a	2.6 (0.7) n=10 a	2.9 (0.9) n = 12 ab	3.8 (0.6) n = 8 b	2.9 (0.7) n=5 ab
TP swim	ND	2.6 (0.3) n=10 ab	2.5 (0.3) n=5 ab	2.5 (0.1) n = 5 ab	2.2 (0.2) n= 5 b	3.0 (0.4) n=9 ab	3.1 (0.3) n = 9 a	3.6 (1.1) n=7 a
GLU 45s	69 (10) n=15 a	65 (8) n=13 ac	57 (16) n=15 abc	47 (7) n=8 abc	41 (9) n=10 b	61 (53) n=12 bc	70 (24) n=8 ac	25 (4) n=4 b
GLU swim	ND	116 (26) n=10 a	69 (18) n=5 ab	131 (15) n=4 a	48 (9) n=5 ab	75 (33) n=9 ab	39 (25) n=9 b	50 (48) n=7 b
Mg 45s	2.6 (0.2) n=15 a	2.5 (0.4) n=14 ac	2.1 (0.4) n=15 abc	2.0 (0.6) n=8 abc	1.6 (0.3) n=10 b	2.0 (0.4) n=10 bc	1.7 (0.3) n=8 b	1.5 (0.2) n=4 b
Mg swim	ND	2.3 (0.3) n=10 a	2.0 (0.3) n=5 ab	1.5 (0.6) n=4 ab	1.7 (0.1) n=5 ab	2.0 (0.4) n=9 ab	1.5 (0.3) n=9 b	1.5 (0.4) n= 7 ab

ND = not done

Figure 5 Mean (Std Dev.) plasma protein concentrations (g / dL) of study fish held in freshwater and sampled within 45 sec of capture. Asterisk denotes statistical difference from all other groups.

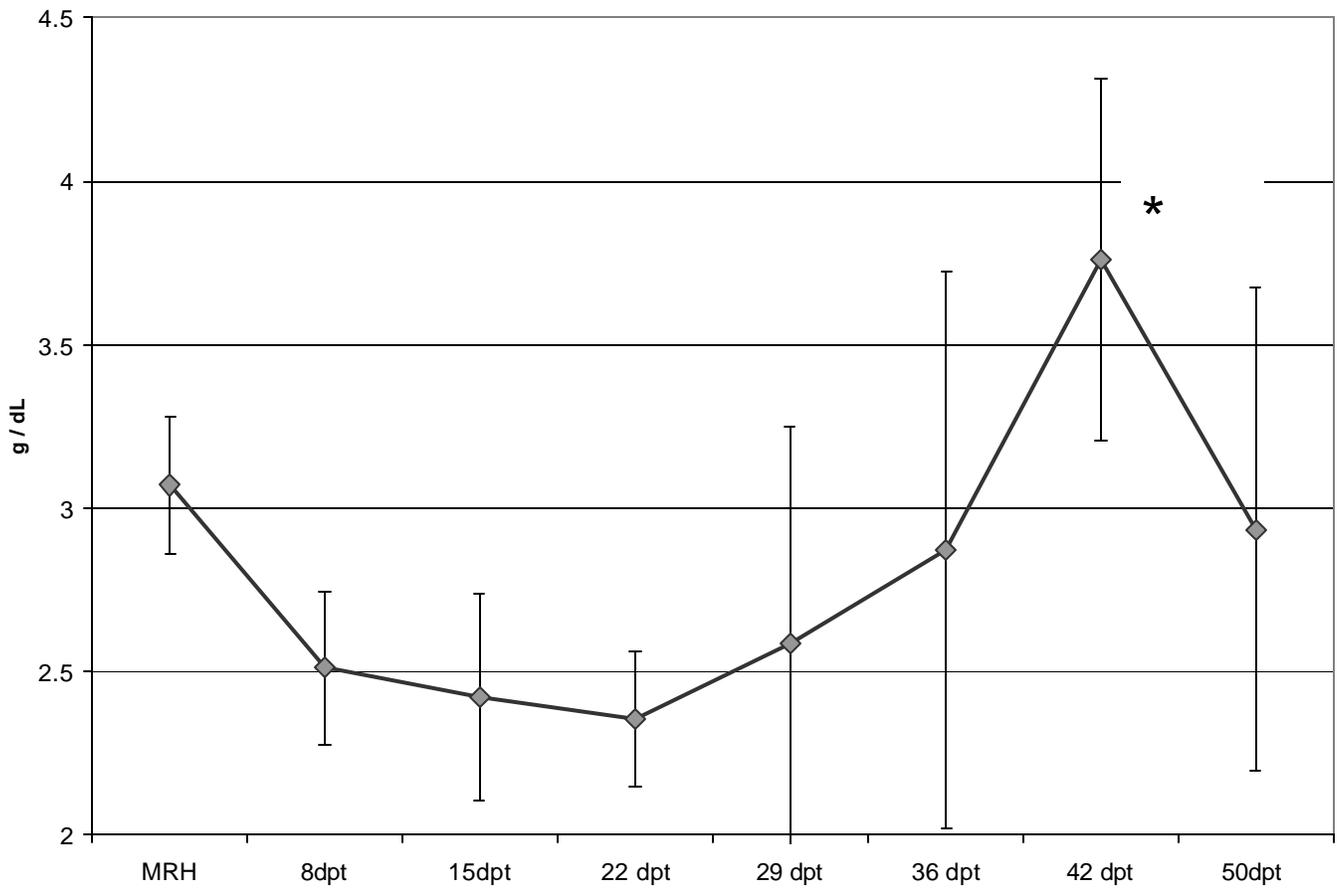
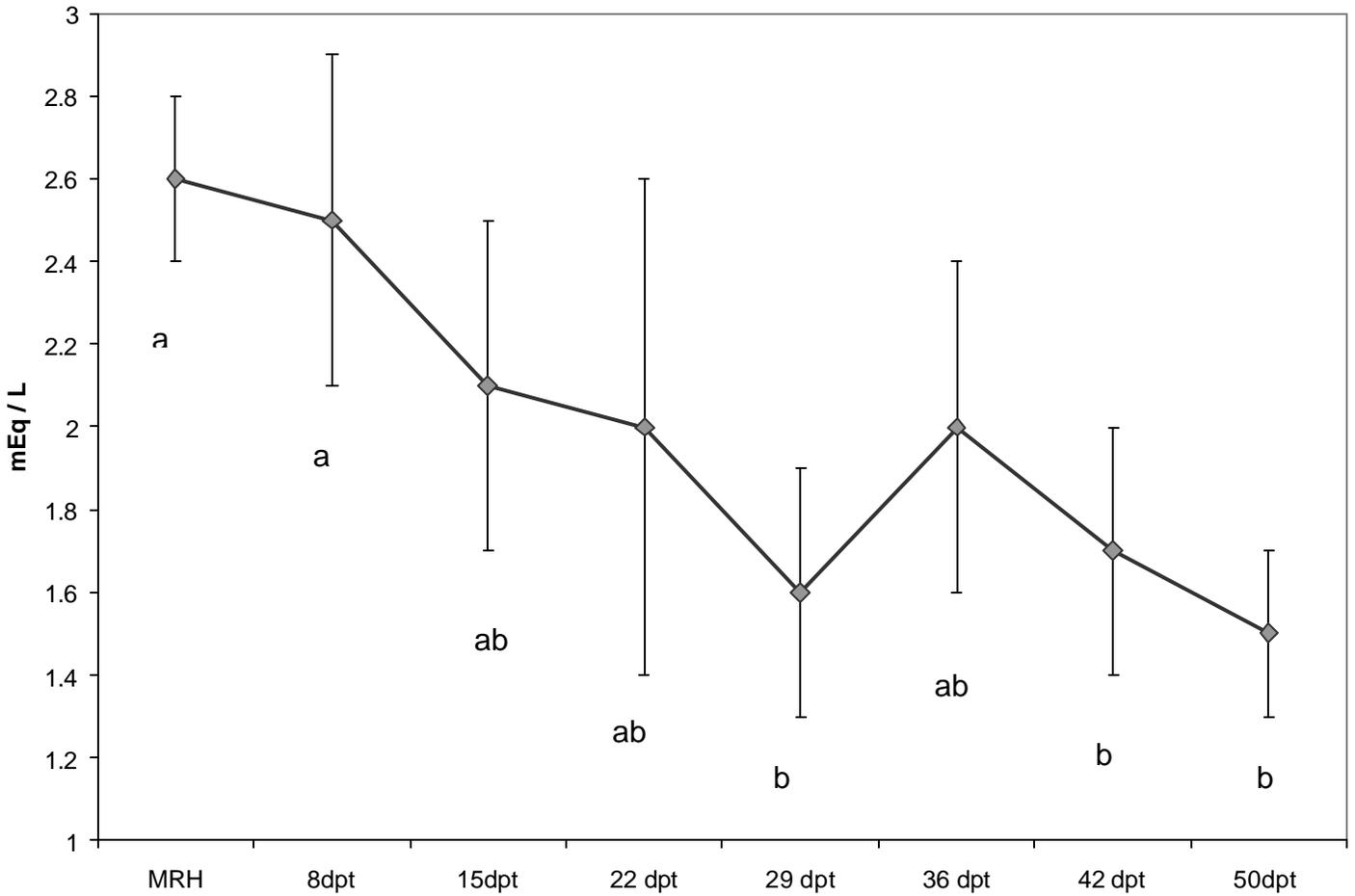


Figure 6. Mean plasma magnesium concentration (mEq / L) of study fish held in freshwater and sampled within 45 s of capture. Letters denote statistically difference ($P < 0.001$) and bars are standard deviation.



Swim Endurance – There was no observed PKD effect on the exhaustion swim challenge performance until 50 dpt (Table 5). Five of seven fish in this challenge did not complete the 120 min test. Lower hematocrit values in the 50dpt fish were weakly correlated with endurance time ($R^2 = 0.18$). The markedly lower hematocrit values and high PKD scores observed in the 42 dpt fish did not correspond to a drop in their swimming performance.

Table 5. Swim exhaustion data. Mean (std. dev.) Body length / sec at maximum flow (max BL / s), PKD score, and hematocrit (HCT). Also reported is the percent of test group that became exhausted (% exhaust) before 120 min. and the range of exhaustion times in minutes(Times).

	8 dpt	15 dpt	22 dpt	29 dpt	36 dpt	42 dpt	50 dpt
Max BL/s	3.3 (0.1)	6.0 (0.3)	5.9 (0.2)	5.9 (0.2)	6.0 (0.4)	5.7 (0.4)	7.9 (0.4)
% exhaust	0	0	0	0	0	0	71
Times (min-max)	na	na	na	na	na	na	41 - 82
PKD score	1	2	3	11	12	17	19
HCT	47 (5)	63 (6)	39 (6)	36 (3)	32 (5)	25 (4)	27 (8)

na not applicable

Saltwater adaptation – No fish died during the 24 h SW challenges performed between 22 and 50 dpt. Smolt development measurements indicated that the study fish were in an advanced stage of smoltification. Saltwater adaptation performance did not decline until at 50 dpt (Table 6). Four of twelve 50 dpt salmon had plasma sodium levels in excess of normal values (> 170 mmol /L) and the mean percent of normal FW condition factor fell to 87% for the group. Lower condition factors of SW fish indicate dehydration due to poor ion regulation. The degree of anemia, as measured by declining hematocrit, was poorly correlated with elevated plasma sodium concentrations ($R^2 = 0.11$). Similarly to the FW sample groups, plasma protein levels became elevated starting in the 42 dpt sample group but were only weakly correlated with PKD score ($R^2 = 0.12$). Plasma protein levels of fish with PKD scores > 12 were significantly higher than cohorts with lower PKD scores (Mann-Whitney Rank Sum test, $P < 0.001$). No difference in plasma magnesium concentration was observed between the groups ($H = 5.48$, 4df, $P = 0.241$). Magnesium concentration was not correlated with PKD score ($R^2 = 0.009$). The decline in gill Na-K-ATPase activities with time was not considered biologically significant ($H = 12.61$, 4 df, $P = 0.016$) and was poorly correlated with PKD score ($R^2 = 0.09$). It should be noted that the 29 dpt challenge occurred near the date of the last Chipp’s Island CWT recovery and that the 29 dpt fish perform quite well.

Table 6.

Smolt development measurements for fish tested between 0 and 50 days post transfer (DPT) in 6 sample groups (Merced R. Hatchery and 22 – 50 dpt). Percent of mean FW cohort condition factor (%FW KFL), plasma sodium (mmol / L), plasma protein (g/dL), plasma magnesium (mEq/L), and gill sodium-potassium adenosine triphosphatase activity (μ moles ADP / mg protein / hr) data reported as mean (std. dev.). PKD score for challenge group reported as median value. Number of gill samples reported (N) if different from total challenge sample.

	DPT	median PKD	% FW KFL	plasma sodium	plasma protein	plasma Mg	gill Na-K- ATPase
MRH	0	0	n/a	n/a	n/a	n/a	10.5 (2.3) N = 12
22 dpt n = 10	22	4	91(4)	139 (12)	2.3 (0.2)	4.0 (2.5)	11.2 (3.9) N = 9
29 dpt n = 12	29	8	96 (6)	154 (7)	2.4 (0.6)	3.3 (1.9)	10.5 (2.9)
36 dpt n=12	36	15	100 (8)	156 (13)	2.4 (0.2)	3.9 (2.0)	9.5 (2.1)
42 dpt n=32	42	16	100 (8)	153 (10)	3.1 (0.4)	3.0 (0.7)	7.2 (3.3)
50 dpt n = 12	50	18	87 (7)	170 (9)	3.5 (0.6)	2.9 (0.8)	7.0 (3.3) N = 7

n/a = not applicable as no SW challenge performed

Discussion

Merced R. Chinook smolts had a high incidence of *T. bryosalmonae* infection in 2005. Kidney imprints were collected from 73% (43 of 59) of the total VAMP-tag salmon collected at Chipp's Island trawl and *T. bryosalmonae* was detected in 40% of these samples. The prevalence of *T. bryosalmonae* infection ranged from 38% in the 25APR MRH inspection to 100% by 22 dpt. Since 2000, the incidence of *T. bryosalmonae* infection in MRH salmon juveniles has ranged from 4 – 100% (Harmon et al. 2004). These fish have been sampled in early May and the majority of these infections have been rated as moderate with minimal kidney swelling.

At the time of the last VAMP-tag recovery from the Chipp's Island trawl on 27May, the degree of PKD was judged to be relatively mild in the study fish. During the spring smolt migration, water temperatures in the Delta can be in excess of 18°C and are thus conducive to the rapid expression of PKD (Baker et al. 1995, Ferguson 1981, Foott et al. 1986). Approximately 3 weeks after the last VAMP fish recovery, MRH salmon were in a severe disease state with the study group having a 27 % cumulative mortality due to PKD. The severity of PKD was also exemplified by a high degree of kidney inflammation and associated physiological impairment. It is unlikely that PKD significantly influenced tagged fish recovery in 2005 however PKD could be a significant mortality factor for Merced River salmon smolts during their early estuary and ocean entry phase.

In the American River, the seasonal infectivity of *T. bryosalmonae* ranges from April through September (Foott et al. 1986). Given the early stage of the infection in the 25April MRH sample group, we assume a similar seasonality in the Merced River. Clifton-Hadley et al. (1987a & b) reported that PKD will follow a 20+ week course with fish eventually recovering from the infection. If we assume that the 50 dpt group was near the peak of the clinical disease stage, then the initial infections probably occurred 10 weeks earlier in late March or early April. As mentioned above, the timing of clinical disease coincides with the period when MRH smolts would be entering San Francisco Bay and the ocean.

The low incidence of light *T. bryosalmonae* infections observed at MRH on 25April did not indicate that the population would undergo severe PKD in less than 7 weeks. This observation provides a cautionary note to any health predictions made on late April –early May inspections. Clinical signs and morbidity increased with time and were not closely correlated to the current histological scoring system. For instance, anemia was better correlated to dpt ($R^2 = 0.66$) than PKD score ($R^2 = 0.58$). In the future, image analysis of kidney sections to determine the degree of interstitial hyperplasia may provide a better tool for the identification of different PKD stages. Conversely, a simpler system using gross renal and splenic swelling categories could be employed as done by Clifton-Hadley et al. (1987a).

The progressive patho-physiological changes observed in this study were consistent with other reports on PKD (Hedrick et al. 1986, Clifton-Hadley et al. 1987b, Foott and Hedrick 1990). The study fish showed signs of anemia and compensatory erythropoiesis by 42 dpt. The co-occurrence of a hematocrit below 20% and pale gill was matched with indicators of compensatory erythropoiesis such as increased numbers of immature erythrocytes in the blood and a reduction in the mean corpuscular hemoglobin content (MCHC). The range of hemoglobin concentration, hematocrit, and MCHC observed in the MRH and 8-22 dpt sample groups were similar to the range described for immature rainbow trout and Coho salmon (Wedemeyer and Chatteron 1971, McCarthy et al. 1975, Miller et al

1983). Foott et al. (1990) reported that rainbow trout, with asymptomatic PKD but having histological kidney lesions, had MCHC values of 17 in comparison to the 19 of the uninfected controls. The mean MCHC of the study fish did not drop below 16 until 36 dpt and were at 8 and 12 in the 42 and 50 dpt sample groups, respectively. Gallauger and Farrel (1998) state that rainbow trout with hematocrits < 20% were considered anemic. We used this value to delineate anemia as it was less than half of the hematocrit values of healthy salmon in the MRH and 8 dpt sample groups. Hematocrit < 20% was also associated with pale gill.

A number of reports have linked reduced swimming performance in salmonid fishes with a disease state (Tierney and Farrell 2004, Butler and Milleman 1971). Swimming performance was not closely correlated with low hematocrit values or PKD score. Despite severe PKD, most of the 42 and 50 dpt salmon could complete the 120 min swim challenge at rates of > 6 body lengths / sec. Brauner et al. (1993) experimentally reduced the concentration of functional hemoglobin in juvenile Chinook salmon and did not detect a decline in the critical swimming speed (Ucrit) until hemoglobin concentration was < 30 %. These authors hypothesize that other mechanisms could aid fish in maintaining swimming speed (i.e. anaerobic metabolism, increase cardiac output, shift of blood from internal organs to muscle, etc). Further investigation of this performance measure should examine infected smolts later in the course of the disease. The use of hematocrit as a measure of oxygen transport capacity in the blood has been criticized due to the variability imposed by sample technique and that transient deficits in oxygen carrying capacity can be met by changing cardiac output, ventilatory flow, or lamellar perfusion (Houston 1997). The lack of correlation between low hematocrit and swimming performance of the study fish could be explained by the compensatory mechanism mentioned above. We chose hematocrit as our measure of circulating erythrocyte numbers based on its ease of measurement in comparison to manual erythrocyte counts. Despite its limitations, we feel that hematocrit provided a valid estimate of erythrocyte concentration and could detect anemia in the population.

Kidney inflammation may have impaired divalent ion re-absorption as evident by the marked decline in plasma magnesium values. This trend was observed in salmon sampled immediately after capture (45 s) and after 120 min exhaustive swimming challenges. Magnesium levels in the healthy MRH and 8 dpt sample salmon were similar as those reported for normal Lake Trout (Edsall 1999). Complete kidney tubule dysfunction was not apparent in the study fish given their elevated plasma protein profile. Plasma glucose also tended to be within a normal range until 50 dpt when both the 45 s and stressed swim challenge salmon had relatively low values. It is possible that insufficient liver glycogen reserves could have contributed to this low glucose level. Feeding was poor during the 42 to 50 dpt period.

The salmon in the study appeared to be responding to *T. bryosalmonae* infection with a strong immune system response. Circulating white blood cell numbers increased steadily during the course of the study. Leukocrit values reached over 2% by 42 dpt, however we are uncertain on whether the cell density of immature erythrocytes would result in these cells being part of the buffy coat measured for leukocrit. This concern is prompted by the poor correlation between elevated leukocrit and the low numbers of white blood cells seen in corresponding blood smears taken at 42 and 50 dpt. Leucocytosis has been reported in trout affected by PKD (Clifton-Hadley et al. 1987, Angelidis et al. 1987, Foott et al. 1990, Chilmonczyk et al. 2002). The composition of the white blood cell population remained similar throughout the study with the lymphocytes being the dominant cell type. Chilmonczyk et al. (2002) reported that lymphocyte proliferation was the key cellular change during clinical PKD and is responsible for the renal hyperplasia. These authors also stated that phagocytosis of latex beads by

anterior kidney cells declined in trout showing clinical signs of disease. We did not observe a reduction in the reactive oxygen production by stimulated anterior kidney cells in the NBT assay. This observation was true for clinically diseased salmon sampled at 42 and 50 dpt. A similar observation was made by Foott et al. (1990) for NBT response in trout with subclinical PKD. The increased plasma protein levels seen in salmon sampled at 42 dpt could reflect an increase in acute phase proteins as reported in PKD affected trout (Scott 1984, Klontz et al 1986, Foott et al. 1990).

The MRH salmon were undergoing smoltification during the month of May. High gill ATPase levels and normal ion regulation in the saltwater challenges were seen in the study fish until 50 dpt. It is likely that advanced PKD was responsible for the impaired ion regulation seen in the 50 dpt group. Despite their elevated plasma sodium and signs of dehydration, no mortality occurred in this group during its 24 hr SW challenge. Given the severity of PKD observed in the study fish, osmoregulatory success of any MRH salmon entering saltwater after 50 dpt is questionable. The decline in gill Na-K-ATPase activity seen during the study may be a result of elevated water temperature (McCormick et al. 1999). We have observed a similar response to extended rearing in elevated water temperatures in juvenile Chinook from Trinity R. Hatchery. In saltwater, there is a marked decrease in urine flow (now isosmotic to the blood) and an active excretion of the divalent ion magnesium by the kidney tubules (Clarke and Hirano 1995). We did not observe elevated plasma magnesium levels in the SW challenge fish. Plasma magnesium (decrease) and protein (increase) of the SW fish followed the same trend as their FW cohorts. In summary, no significant kidney impairment for SW adaptation was observed in the study group until 50 dpt. Osmoregulatory performance began to decline at this point in the disease. In future studies, challenging uninfected control fish will provide an insight into any captivity-influenced changes independent of PKD. Proliferative kidney disease has been shown to follow the same disease course in saltwater as in freshwater (Hedrick and Arostien 1987). The authors noted that mortality was much lower in Chinook salmon juveniles held in the saltwater laboratory system compared to their freshwater cohorts due to the arrest of FW ectoparasites and external bacteria. Merced R. smolts moving into the bay may also benefit from cooler water temperature and effects of salinity on FW ectoparasites.

T. bryosalmonae spores have been shown to be thin walled and fragile with the absence of harden valves as a key characteristic of the newly established order Malacosporea (Canning et al. 2000). Spore infectivity did not exceed 24 h in study by de Kinkelin et al. (2002) and suggested that reducing byrozoan habitat directly upstream of fish culture facilities could be a viable disease management strategy. Such modifications should be considered for MRH to reduce the infectious spore load moving through the facility.

In summary, the population was near the peak of clinical PKD at the time the study ended in early June. Anemia is the most significant factor negatively influencing survival of the infected smolts. Future work should examine the performance of an infected MRH population for a longer period and compare it with uninfected cohorts.

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