

Health and physiology monitoring of chinook and steelhead
smolts in the Trinity and Klamath Rivers.

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Abstract. - - Infectious disease is one of many life history factors which can influence smolt survival. It is important to distinguish between infection of a pathogen and the disease state. A fish may be infected yet not be in a disease state or have its performance significantly compromised. A cooperative project, among offices of the USFWS and the CDFG, to monitor the health and physiology of chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*O. mykiss*) smolts in the Klamath River basin has been conducted since 1991. Hatchery fish have been sampled from Iron Gate and Trinity River State Fish Hatcheries prior to their release, at lower Klamath and Trinity River traps, and in the Klamath River estuary. Natural fish have also been collected at the latter two sites. The project objectives have evolved from a general pathogen survey to now include monitoring of physiological parameters associated with smoltification and energy reserves. The bacterium, *Renibacterium salmoninarum*, causative agent of Bacterial Kidney Disease, and the trematode parasite, *Nanophyetus salmincola*, have been identified as the most significant pathogens affecting smolt health in the basin. *Nanophyetus salmincola* infection is particularly severe in the Trinity River. Hatchery and natural chinook have similar levels of infection of *Renibacterium salmoninarum* and *Nanophyetus salmincola*. Hatchery chinook immunodefense and energy measurements declined after their release while gill Na⁺-K⁺-Adenosine Triphosphatase activity increased.

Background

A survey of pathogens and the general health condition of steelhead and chinook smolts in the Trinity and Klamath R. was conducted in 1991 and 1992. Fish were sampled at Iron Gate Hatchery (IGH), Trinity R. Hatchery (TRH), a rotary screw trap at Klamath River km 81 near Big Bar (BB), a rotary screw trap at Trinity River km 34 near Willow creek (WC), and in both the upper and lower Klamath R. estuary. The bacterium *Renibacterium salmoninarum* and the trematode *Nanophyetus salmincola*, were identified as the most significant pathogens infecting both natural and hatchery smolts (Table 1). Several trends observed in hatchery smolts were scale loss, rapid declines in condition factor with migration, and gas bubble trauma (Fall release- Trinity R. Hatchery).

In 1993, we concentrated our efforts on detection of *R. salmoninarum*, *N. salmincola*, and the intestinal parasite *Ceratomyxa shasta* in natural and hatchery chinook smolts. While reported by Hendrickson et al (1989) to be enzootic to the Klamath R., we have rarely found either spores or other life stages of *Ceratomyxa shasta* in fish collected from the Klamath. In addition, physiological indicators of smoltification, energy, and immune defense status were examined in sub-samples from the collected chinook (Table 2). Each fish collected was assigned a unique identifier and a database kept on every test run on that particular fish. These physiological tests examined how stress, smoltification development, migration, and infection affected the performance of the fish. Distinction between infection of a pathogen(s) and disease is important. Salmon typically are infected with several pathogens during their lifecycle, however, a high intensity of infection (how many organisms per host) and stressful conditions (crowding in raceways, release from a hatchery into a

river environment, high temperatures, etc.) must usually occur before the host-parasite balance favors the parasite (pathogen) and a disease state occurs in the fish.

Table 1. Significant pathogens and disease conditions detected in salmonid smolts from the Trinity and Klamath Rivers, 1991-1993.

Bacteria:		Viral Disease:	
<i>Renibacterium salmoninarum</i>		Infectious hematopoietic necrosis (IHNV)	
<i>Yersinia ruckeri</i>		Erythrocytic Inclusion Body Syndrome (EIBS)	
<i>Aeromonas hydrophila</i>			
Parasites:		Environmental:	
<i>Nanophyetus salmincola</i>		Gas Bubble Disease (GBD)	
<i>Diplostomum</i> sp.		Nephrocalcinosis	
<i>Sanguinicola</i> sp.			
<i>Gyrodactylus</i> sp.			
<i>Ceratomyxa shasta</i>			
Glochidia (immature mollusk)			
Myxosporidia (kidney tubules)			

Table 2. Tests performed on chinook smolts in 1993.

Measurement Category	Test (Reference)
General health condition	Organosomatic assay (Foott 1990)
Pathogens:	
<i>R. salmoninarum</i> antigen in Kidney	ELISA
<i>N salmincola</i> / g kidney	Direct count of squashed kidney
Parasites in gill, gut, heart, kidney	Histology
Smoltification	
Osmoregulation	24 hr Saltwater challenge (Blackburn & Clarke, 1987)
Plasma sodium of SW fish	Kodak DTE analyzer
Gill Na ⁺ -K ⁺ -Adenosine Triphosphatase	ATPase (McCormick & Bern, 1989)
Energy	
Plasma triglyceride	Kodak DT60 blood analyzer
Visceral fat score	Organosomatic analysis
Change in body conformation	Condition factor (Wt/TL ³)
Estimate of liver glycogen stores	Hepatosomatic index
Energy stores for stress response	Plasma glucose (Mazeaud et al., 1977)
Immune Defense	
Estimate of blood Leukocyte numbers	Leukocrit (McLeay & Gordon, 1977)
Estimate globular serum proteins	Albumin / Globulin ratio (Ravel, 1973)

Pathogens

Renibacterium salmoninarum

Renibacterium salmoninarum and *N. salmincola* are common salmonid pathogens endemic to the N.E. Pacific drainages and have evolved with their salmonid hosts. *R. salmoninarum* is the bacterium responsible for Bacterial Kidney Disease (BKD) of salmonid fishes throughout the world. Typically, the infection is chronic (months in duration) and can progress after the infected smolt enters saltwater. *R. salmoninarum* can be transmitted both horizontally (fish to fish) and vertically (female to her progeny). One important virulence factor is the bacterium's ability to survive and even multiply in host phagocytic cells.

Beginning in 1992, we have used an enzyme linked immunosorbent assay (ELISA) to test kidney tissue for the concentration of *R. salmoninarum* antigen as described by R. Pascho and D. Mulcahy (1987). ELISA is a method for indirectly detecting infection, but does not necessarily indicate the presence of *R. salmoninarum* (unlike Fluorescent Antibody Technique (F.A.T.) where the bacteria themselves are visualized). In the ELISA technique, a surface-bound antibody selectively extracts *R. salmoninarum* antigen from homogenized kidney extract. The captured antigen is then detected by addition of antigen-specific antibody conjugated with a horseradish peroxidase color indicator. A color reaction is produced which is proportional to the amount of antigen present in the sample. We have chosen to report our ELISA results into 3 categories:

- 1) Below Negative Cutoff (BNC) = Sample Optical Density value (O.D.) below a non-infected kidney O.D. (mean + 2 std. dev.);
- 2) Suspect (SUS) = sample O.D. > BNC < 0.2; and
- 3) Positive (POS) = sample O.D. > 0.2. (indicates an active infection with or without clinical signs of disease)

The O.D. value of 0.2 was chosen based on our experience that it was possible to confirm an active infection in kidney samples with this O.D. by direct observation of the cells (F.A.T.).

Natural steelhead smolts in the Trinity river were found to have incidence of *Renibacterium salmoninarum* infection much higher than the Trinity R. hatchery stock (Table 3). In 1991, over twice the percentage of natural steelhead (21 %) as hatchery fish (10 %) were found to be infected with *R. salmoninarum*. ELISA data from the 1992 survey showed that 53 % of the natural steelhead were in the "positive" category. No signs of clinical BKD was observed in the either hatchery or natural steelhead. It is possible that steelhead can tolerate *R. salmoninarum* infections better than chinook or coho salmon.

Both natural and hatchery chinook smolts in the Klamath and Trinity rivers had similar *R. salmoninarum* infection profiles by ELISA (Table 4). The majority (56 - 100 %) of the fish sampled in 1992 and 1993 had *R. salmoninarum* antigen concentrations in the low-level "suspect" range. Iron Gate chinook released in the spring of 1993 and Trinity R. Hatchery chinook yearlings released in the fall of 1992 were 2 groups which did show moderate incidence of ELISA positives (20 - 38 % POS).

It is unclear what happens to these "suspect" category salmon. *Renibacterium salmoninarum* infection can develop into clinical BKD in the marine environment and if the fish is placed under stress (Banner et al., 1986). In 1993, 16 of the 33 TRH chinook smolts (CWT) sampled for *R. salmoninarum* in the Klamath estuary had high antigen levels. This is a much higher incidence of high-level infection than at either the river traps or the hatcheries. No trend in salinity preference (freshwater upper compared with brackish lower estuary) has been seen in the *R. salmoninarum* infected fish collected in the Klamath estuary (Table 5). ELISA data from adult chinook collected at Trinity R. hatchery in 1992 and 1993 showed that only 2 - 17 % of the fish were in the positive category (high) while 40 - 58 %

were in the suspect category (low). It is quite possible that a sizable percentage of the smolts with low-level *R. salmoninarum* infections die of clinical BKD prior to spawning.

Table 3. *Renibacterium salmoninarum* ELISA antigen levels or direct observation of bacteria by Fluorescent Antibody Technique (FAT+) in kidney tissue from Trinity R. hatchery (TRH) and natural Trinity R. (NAT) steelhead smolts collected at Willow Creek (WC) or prior to hatchery release (PR) in 1992 and 1993. Data reported as percent of sample group in each category. Antigen categories are Below Negative Cutoff (BNC), Suspect (SUS), or Positive (POS).

Group	Sample Date	% BNC	% SUS	% POS	% FAT(+)
TRH-PR	11MAR91	ND	ND	ND	0
TRH-WC	APR-MAY91	ND	ND	ND	10
NAT-WC	APR-MAY91	ND	ND	ND	21
TRH-PR	16MAR92	80	20	0	ND
TRH-WC	APR-MAY92	6	88	6	ND
NAT-WC	APR-MAY92	12	34	53	ND

ND Not done.

Table 4. *Renibacterium salmoninarum* ELISA antigen levels in kidney tissue from Trinity R. Hatchery (TRH), Iron Gate Hatchery (IGH), and Natural chinook juveniles collected at Willow Creek (WC), Big Bar (BB), or prior to hatchery release (PR) in 1992 and 1993. Data reported as percent of sample group in each category. Antigen categories are Below Negative Cutoff (BNC), Suspect (SUS), or Positive (POS).

Group	Sample Date	% BNC	% SUS	% POS
<u>1992</u>				
TRH-PR	22JUN	32	59	9
TRH-WC	02JUL	7	93	7
TRH-PR	29SEP	0	75	25
TRH-WC	07OCT	0	93	7
TRH-WC	14OCT	3	77	20
<u>1993</u>				
TRH-PR	15JUN	0	90	10
NAT-WC	10JUN	6	88	6
TRH-WC	22JUN	0	100	0
IGH-PR	07JUN	6	56	38
NAT-BB	04-18JUN	15	77	8
IGH-BB	27JUN-14JUL	0	78	22
TRH-PR	28SEP	17	78	5
TRH-WC	05-12OCT	6	86	8
IGH-PR	26OCT	5	90	5

Table 5. *Renibacterium salmoninarum* ELISA antigen levels in kidney tissue from chinook juveniles of mixed origin collected in 1992 and 1993 from the upper (KEU) and lower Klamath estuary (KEL). Data reported as percent of sample group in each category. Antigen categories are Below Negative Cutoff (BNC), Suspect (SUS), or Positive (POS).

Group	Sample Date	% BNC	% SUS	% POS
KEU	SPRING92	25	50	25
KEL	SPRING92	10	84	6
KEU	SPRING93	0	74	26
KEL	SPRING93	0	41	59

Nanophyetus salmincola

Salmonids are one intermediate host of the digean trematode, *Nanophyetus salmincola*, the final host being a fish-eating mammal (Millemann and Knapp, 1970). Eggs, shed with the final host's feces, hatch into free-swimming miracidia and infect aquatic snails of the genus *Oxytrema*. Free-swimming cercaria later leave the snail and penetrate the fish's skin. The parasite, now referred to as metacercaria, enters the circulatory system, encysts in various tissues, and stays with the fish for its entire life. *Nanophyetus salmincola* is itself parasitized by the rickettsial organism *Neorickettsia helminthoeca* which causes "salmon poisoning" in canines (Farrell et al., 1964). While metacercaria elicit little inflammation in fish, the parasite cysts cause obstruction and pressure injury to infected tissues (Wood and Yasutake, 1956).

Nanophyetus salmincola was not detected in fish at either TRH or IGH, however, TRH chinook captured in the river 2 days post-release were found to be infected with the parasite. In 1993, we quantified the intensity of infection by counting the number of metacercaria in squash preparations of individual kidney and dividing by the weight of the sample to obtain a Metacercaria / gram tissue value. Natural Trinity R. chinook had both high incidences of infection and carried large numbers of metacercaria (Table 6). While TRH chinook had somewhat lower incidence and intensity of infection than the natural population, these hatchery fish acquired this parasite load in a matter of days. This acute challenge of metacercaria may act to weaken the hatchery fish and reduce their performance. *Nanophyetus salmincola* infection appears to be particularly severe in the fall as seen in TRH yearling release groups in 1992 and 1993. Whether the infective cercaria stage is more abundant in the fall is unclear. Generally, both natural and hatchery chinook captured in the Klamath R. had lower intensity metacercarial infections than Trinity R. fish. It is tempting to use high metacercaria loads (>100 Metacercaria / g) as a "biological tag" for Trinity R. chinook.

As evidenced by the presence of chinook smolts with high intensity infection (2500/ g) in the brackish lower Klamath Estuary (Table 6) and common finding of 2000 - 4000 metacercaria/g in returning adults at TRH (data not shown), it appears that chinook can tolerate relatively high parasite loads. *Nanophyetus salmincola* has been documented to be pathogenic to salmonids if fish are subjected to acute challenges of large numbers of cercaria (Baldwind et al., 1967). Necomb et al. (1991) observed an inverse relationship between the number of metacercaria in the kidney of infected coho smolts and their survival in saltwater. There may be "threshold" level of infection and challenge duration which causes a reduction in performance and smolt survival.

Table 6. Incidence (Incid.) and intensity of *Nanophyetus salmincola* infection in Trinity R. Hatchery (TRH), Iron Gate Hatchery (IGH), Natural, and mixed-stock (MIX) chinook juveniles collected at Willow Creek (WC), Big Bar (BB), upper Klamath estuary (KU), lower Klamath estuary (KL), or prior to hatchery release (PR). Intensity data reported as mean (std. dev.) Metacercaria / g kidney tissue and maximum Metacercaria / g (MAX MET/g).

Group	Collection Period	Incid. (%)	MET/g	MAX MET/g
TRH-WC	SPRING93	46	74 (134)	464
NAT-WC	SPRING93	80	607 (933)	5912
TRH-WC	SPRING92	41	ND	ND
NAT-WC	SPRING92	91	ND	ND
TRH-WC	FALL93	60	306 (651)	4094
TRH-WC	FALL92	90	ND	ND
IGH-BB	SPRING93	53	27 (55)	327
NAT-BB	SPRING93	46	14 (0.4)	491
MIX-KL	SPRING93	37	188 (367)	2500
MIX-KU	SPRING93	72	66 (153)	948

ND Not done

Organosomatic Data

A necropsy based fish condition assessment system developed by Ron Goede of the Utah Division of Wildlife Resources (Goede and Barton, 1987) was modified for the smolt organosomatic assays (Foott, 1990). The organosomatic assay is a method for ordered observation and reporting of the gross morphology of selected organs, hematological parameters, and size criteria of each individual in a 20 fish sample. A numeric "severity" score (0,1,2,3) is assigned to each tissue where zero is normal and 3 represents severe abnormality. All of the fish's tissue scores are tallied to obtain an abnormality score (AS). A low AS represents an apparently healthy fish. The mean sample AS is a relative health indicator of a population and can be used for comparison between populations.

Most groups sampled in both the Trinity and Klamath rivers had low AS values (0.00 - 2.40). Factors which influenced the higher AS values (0.70 - 2.40) seen in TRH chinook released in the fall of 1992 and 1993 included eye damage due to Gas Bubble Trauma (gas emboli from supersaturated water) and copepod infection of the gill. In 1992, TRH yearlings with eye damage were also collected post-release at Willow creek. Although collection methods were identical, scale loss was much more prevalent in hatchery fish compared to natural chinook.

Physiological Measurements

Smolt Development

Gill ATPase -- The gill Sodium-Potassium adenosine triphosphatase (ATPase) activities of individual chinook ranged from 1.172 to 12.750 μ moles ADP/mg protein/hr. These values agree with those reported for coho smolts by McCormick and Bern (1989). The NA-K-ATPase system transports sodium ions out of the blood of saltwater fish and helps to maintain a normal ion balance (Folmar and Dickhoff, 1980). During smolt development there is a significant increase in ATPase activity in the chloride cells of

the gill. The ATPase activities of IGH chinook in the spring and TRH fish in the fall increased significantly ($P < 0.05$) after hatchery release (Table 7).

Saltwater challenge -- Natural chinook tended to have better ion control in saltwater, as measured by plasma sodium concentration and the percent of surviving fish which showed osmotic stress (>170 mmol Na⁺/L), in 24 hr. saltwater challenges than hatchery fish released in the spring (Table 8). Unlike Gill ATPase, no trend for increased hypoosmoregulation was seen in hatchery fish after release. The apparent difference between the Fall pre-release and 05OCT93 Willow creek challenge done on TRH chinook was probably due to an error in salt concentrations of the pre-release challenge.

Although the number of chinook challenged in saltwater with either "positive" ELISA values or Metacercaria / g values >100 was low, no effect of either infection on hypoosmoregulation or gill ATPase activities was observed. We will need to test fish with a range of infection levels to determine if there is a "threshold" level of infection which compromise's the osmoregulatory ability of the smolts.

Table 7. Gill Sodium-Potassium Adenosine Triphosphatase activity (ATPase) in Trinity R. Hatchery (TRH), Iron Gate Hatchery (IGH), and Natural chinook juveniles collected at Willow Creek (WC), Big Bar (BB), or prior to hatchery release (PR). Data reported as mean μ moles ADP/mg protein/hr (Standard Deviation) and mean total (TL) and fork length (FL) of sampled fish.

Group	Sample Date	ATPase	Sample No.	TL (mm)
TRH-PR	15JUN93	4.18 (1.57)	10	103
NAT-WC	10JUN93	6.77 (1.55)	6	97
IGH-PR	07JUN93	3.29 (1.34)	10	76
IGH-BB	23JUN93	9.59 (2.33)	7	101
IGH-BB	14JUL93	4.49 (1.12)	7	107
				<u>FL (mm)</u>
TRH-PR	28SEP93	3.57 (1.01)	8	146
TRH-WC	05OCT93	4.00 (1.85)	11	137
TRH-WC	12OCT93	4.02 (1.23)	11	135
IGH-PR	26OCT93	3.16 (1.10)	3	169

Energy

Triglyceride -- While having little visceral fat, natural Trinity R. chinook smolts had similar plasma triglyceride (TG) concentrations and condition factors as TRH chinook collected prior to release (Table 9). Hatchery fish rapidly depleted their fat reserves after release. This occurrence is evident by both the low mean TG values, the percent of hatchery fish with no visible visceral fat, and TG < 15 mg/dL (sensitivity limit of TG assay). The fall release TRH chinook (05OCT93) showed significant reductions in plasma TG within 5 days of release ($P < 0.01$). It appears that visceral fat reserves are an important energy source for hatchery smolts during their transition to the wild and for the high energy demands of emigration.

Glucose -- Stress invokes a series of neurohormonal events which change many aspects of an animal's physiology. One secondary metabolic change associated with stress is the mobilization of the body's glycogen stores to produce an elevation in blood glucose. While the "resting" blood glucose concentration in salmonids varies with species, age, and nutrition, we have observed values of between 60 - 90 mg/dL in juvenile chinook sampled quickly from raceways.

Table 8. Survival and plasma sodium measurements of 12 chinook per group challenged for 24 hrs in 27-30 ppt saltwater. Hatchery fish from Trinity R. Hatchery (TRH) and Iron Gate Hatchery (IGH) were challenged prior to release (PR) or at trap sites at Willow Creek (WC) and Big Bar (BB). Natural chinook (NAT) were challenged at the trap sites. Data reported as percent of challenged fish which survived (%SURV.), mean plasma sodium (Standard Deviation), percent of surviving fish showing osmoregulatory stress (plasma Na⁺ >170 mmol/L), and mean Total length.

Group	Challenge Date	%SURV.	Plasma Na	Percent > 170	TL(mm)
TRH-PR	15JUN93	100	161.7 (14.0)	17	92.7
TRH-WC	23JUN93	100	162.4 (16.0)	8	97.4
NAT-WC	10JUN93	100	146.8 (21.2)	10	94.1
IGH-PR	08JUN93	100	163.4 (9.2)	33	73.8
NAT-BB	17JUN93	100	153.7 (24.4)	9	116.8
TRH-PR	29SEP93	75*	164.6 (9.1)	22	138.8
TRH-WC	06OCT93	100	157.7 (3.3)	8	141.3

* Poor challenge as saltwater was 34 and 32 ppt in the replicate units and dissolved oxygen was 11.2 ppm.

Plasma glucose declined significantly (P<0.01) for both IGH and TRH fish after their release (Table 10). This decline in glucose response was particularly striking in yearling chinook released from TRH in the fall. Together with the decline in TG, it appears that the hatchery smolts had expended a great deal of energy and had few reserves left to respond to stress.

The hepatosomatic index (HSI) of hatchery chinook juveniles did not significantly (P<0.05) change after release. Hepatocytes store large quantities of glycogen and the HSI will to some extent reflect the glycogen content of the liver. Brighenti et al. (1991) reported that trout rapidly deplete their liver glycogen during starvation periods. Emigration stress could also cause a reduction of liver glycogen due to the effects of cortisol on the liver (Soengas et al., 1992). It is unclear why the decline in plasma glucose response did not correspond with a decline in HSI. Perhaps hepatic glycogen was replaced by cytoplasmic fluid thereby maintaining the same liver weight.

Table 9. Plasma triglyceride mg/dL (TG), percent of sample group with TG below 15 mg/dL limit of assay (%TG<15), percent of sample group with visible mesentery fat (%FAT>0), and Condition factor (KTL= Weight (g)/Total Length(mm)³) in Trinity R. Hatchery (TRH), Iron Gate Hatchery (IGH), and Natural chinook juveniles collected at Willow Creek (WC), Big Bar (BB), or prior to hatchery release (PR). Data reported as mean (Standard Deviation).

Group	Sample Date	TG	%TG <15	%FAT >0	KTL
TRH-PR	15JUN	86.4 (36.8)	0	100	0.841 (.046)
NAT-WC	10JUN	110.3 (53.2)	0	31	0.836 (.168)
TRH-WC	21JUN	37.4 (10.2)	36	47	0.737 (.043)
IGH-PR	07JUN93	62.0 (34.9)	0	100	0.788 (.046)
IGH-BB	28JUN-14JUL	45.2 (22.0)	0	22	ND
NAT-BB	18JUN93	43.8 (12.7)	0	25	0.807 (.054)
TRH-PR	28SEP93	77.3 (23.8)	0	100	0.876 (.040)
TRH-WC	05OCT93	42.2 (20.7)	53	60	0.859 (.066)
TRH-WC	12OCT93	37.6 (26.1)	25	67	0.819 (.127)
IGH-PR	26OCT93	88.8 (21.6)	0	100	1.104 (.148)

ND Not done

Table 10. Plasma glucose (mg/dL), as a stress-response measurement, and hepatosomatic index (HSI= liver wt(g)/body wt(g)x100), as a glycogen storage indicator, in Trinity R. Hatchery (TRH), Iron Gate Hatchery (IGH), and Natural chinook juveniles collected at Willow Creek (WC), Big Bar (BB), or prior to hatchery release (PR). Data reported as mean value (Standard Deviation).

Group	Sample Date	Glucose	HSI
TRH-PR	15JUN93	175.4 (40.1)	0.994 (.265)
TRH-WC	21JUN93	111.4 (38.5)	0.978 (.245)
NAT-WC	10JUN93	139.7 (49.8)	1.008 (.395)
IGH-PR	07JUN93	141.3 (49.8)	1.120 (.293)
IGH-BB	14JUL93	109.2 (47.3)	ND
NAT-BB	18JUN93	148.8 (55.6)	1.143 (.327)
TRH-PR	28SEP93	115.6 (24.7)	1.151 (.165)
TRH-WC	05OCT93	81.5 (15.8)	0.902 (.195)
TRH-WC	12OCT93	69.1 (20.5)	0.947 (.565)
IGH-PR	26OCT93	142.6 (50.5)	1.050 (.148)

ND Not done

Immune Defense Indicators

Both leukocrit (LCT = measure of white blood cell quantity) and the albumin : globulin ratio (A/G) tended to be different in hatchery fish captured at the traps compared to their pre-release samples (Table 11). With the exception of the TRH chinook sampled on 12OCT93 and nine IGH chinook captured at BB on 14JUL93, leukocrits declined after release. The A/G

ratios of hatchery chinook collected in-river was significantly higher than cohorts sampled prior to release ($P < 0.01$). This A/G increase was due to a decline in globular protein concentration as plasma albumin level remained similar (data not shown). Immunoglobulin and many non-specific immunodefense proteins are found in the globular protein fraction of plasma. While the leukocrit values of the natural Trinity R. chinook did not significantly ($P < 0.05$) differ from the TRH chinook captured at WC, the A/G ratio was lower. Specker and Schreck (1982) report that coho juveniles show marked rises in blood cortisol levels during smoltification which reduces their immunodefense. Stress can also cause reductions in leukocrit (McLeay & Gordon, 1977). It appears that the immunodefenses of the hatchery smolts were reduced during outmigration.

Table 11. Leukocrit (LCT) and albumin : globular protein ratio (A/G) in Trinity R. Hatchery (TRH), Iron Gate Hatchery (IGH), and Natural chinook juveniles collected at Willow Creek (WC), Big Bar (BB), or prior to hatchery release (PR). Data reported as mean (Standard Deviation).

Group	Sample Date	LCT	A/G
TRH-PR	15JUN93	1.116 (.699)	0.469 (.160)
TRH-WC	21JUN93	0.505 (.272)	0.959 (.223)
NAT-WC	10JUN93	0.657 (.430)	0.660 (.430)*
IGH-PR	07JUN93	0.229 (.271)	ND
IGH-BB	14JUL93	1.023 (.781)	0.704 (.130)
NAT-BB	18JUN93	1.269 (.865)	0.743 (.222)
TRH-PR	28SEP93	0.588 (.300)	0.379 (.065)
TRH-WC	05OCT93	0.264 (.362)	0.495 (.171)
TRH-WC	12OCT93	1.151 (.369)	0.532 (.168)
IGH-PR	26OCT93	0.970 (.393)	0.371 (.041)

* Some samples were pooled with another fish due to small volumes.

Summary

We observed similar levels of pathogen infection in both hatchery and natural chinook smolt populations with *Renibacterium salmoninarum* and *Nanophyetus salmincola* being the most prevalent pathogens. *Nanophyetus salmincola* infection is particularly intense in Trinity R. fish. Most chinook smolts sampled from 1991 - 1993 have had low level infections of *R. salmoninarum*, however, it is unclear if the infection progresses and compromises survival. Natural steelhead smolts in the Trinity R. had relatively high levels of *R. salmoninarum* infection without obvious signs of disease. The incidence of *Ceratomyxa shasta* infection was quite low in Klamath stocks and not detected in Trinity R. fish. Infectious diseases may have some effect on smolt survival in the Trinity R. but is probably not a major survival factor in the Klamath R. chinook smolts.

Smolt migration is energy intensive. Hatchery fish rapidly depleted their lipid reserves. The immunodefences of hatchery smolts showed signs of decline during out-migration. Gill NA-K-ATPase activity increased in hatchery fish during outmigration. Natural chinook showed better hypoosmoregulation control in saltwater challenges than hatchery cohorts. Although our data is limited, no trend for compromised smolt development with infection was observed in the groups. One long-term goal of our research is to determine whether there is an upper "threshold" of *R. salmoninarum* or *N. salmincola* infection which compromises smolt performance and survival.

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