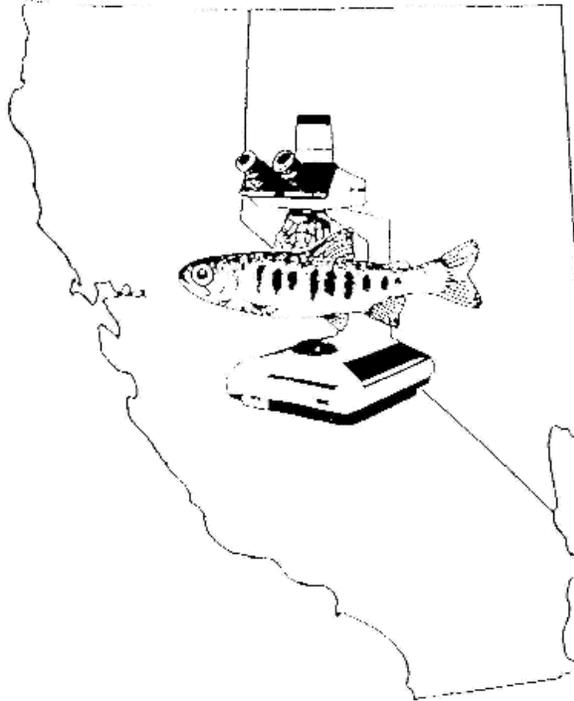


FY95 Investigational Report :
Health, physiology, and migration characteristics of
Iron Gate Hatchery Chinook, 1995 Releases.



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Summary: Infectious disease significantly affected the survival of juvenile chinook (broodyear 1994) released from Iron Gate Hatchery into the Klamath River during 1995. Ceratomyxosis was quite prevalent in the June release out-migrants with a high of 92 % incidence of infection occurring in the 3rd week post-release. This parasitic infection was associated with intestinal hemorrhage, anemia and high mortality. Elevated river temperatures appear to exacerbate the disease as the Iron Gate Hatchery stock tends to be resistant to *Ceratomyxa shasta* infection at temperatures ≤ 16 °C. Immunodefenses were stimulated in the early stages of Ceratomyxosis, however, the disease progressed until death. Inflammation of the acinar cells (pancreatitis) and associated adipose cells (steatitis) occurred in the majority of June out-migrants. Energy reserves were rapidly depleted in the June release group but to much lesser degree in the November release fish. The health and condition of the June out-migrants captured at Big Bar dramatically improved at 5 weeks post-release. We speculate that these fish may have used thermal refugial habitat prior to their capture. An estimated 25 % of the November yearling release group suffered from advanced Bacterial Kidney Disease and were likely to die of the disease.

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abbreviations used in report:

ANOVA	Analysis of Variance (1-way)
ATPase	Gill Sodium – Potassium- Adenosine Triphosphatase {Na-K-ATPase}
ADP	Adenosine diphosphatase
BKD	Bacterial Kidney Disease
BBT / BBS	Big Bar Trap / Seine
°C	degree Celsius
CDFG	California Department of Fish and Game
cfs	cubic foot per second
cm	centimeter
CWT	Coded wire tag
Clipped	Adipose fin clipped fish (Iron Gate Hatchery mark)
dL	deciliter = 0.1 liter
dia.	Diameter
D.O.	dissolved oxygen
EDTA	ethylenediaminetetraacetic acid
ELISA	Enzyme Linked ImmunoSorbent Assay
FBS	Fetal Bovine Serum
FCS	Fall-run chinook salmon
FL	Fork length (millimeters)
g	gram
hrs	hours
HSI	hepatosomatic index
IOI	Incidence of Infection
INC	Indian Creek
L	Liter
μmole	micromole
μm	micrometer
μL	microliter = 0.001 mL
M	mole
mg	milligram
mmol /L	micromole / liter
mm	millimeter
mL	milliliter
MS222	3-aminobenzoic acid ethyl ester (anesthetic)
NAD (NADH)	Nicotinamide Adenosine Dinucleotide (reduced form)
nm	nanometer (10 ⁻⁹ meters)
OD	Optical Density (μm)
ORL	Orleans
PBS	Phosphate buffered saline (pH 7.2)
POI	Prevalence of Infection
ppm	parts per million
RS	<i>Renibacterium salmoninarum</i>
RCC	Red Cap Creek
rpm	revolutions per minute
U/mL	international units per milliliter
w / v	weight per volume

Introduction

Declining chinook populations in the Klamath basin has prompted an intense restoration effort of this valuable resource and a key element of the State's aquatic biodiversity. Both infectious and non-infectious disease are important factors in smolt survival. Ceratomyxosis is one of several significant infectious disease identified in the Klamath River (Hendrickson et al. 1989). Elevated water temperatures, often in excess of 18° C during the late spring and summer have been identified as a negative factor for anadromous fish in the Klamath River (Klamath R. Basin Fish. Task Force 1991).

This study examined Iron Gate Hatchery chinook juveniles captured in the mid-Klamath river during both their spring and autumn releases in 1995. The general health and a number of physiological indicators of immune defense, energy reserves, and smolt development were evaluated in the study groups.

Methods and Materials

Study groups – Sample sites in this study are identified in figure 1 and include Iron Gate hatchery, the Klamath River below the confluence of Indian Creek, Red Cap Creek, below the Highway 96 bridge at Orleans, and below Big Bar. Broodyear 1994 Fall-run Chinook Salmon juveniles were examined at Iron Gate Hatchery (**IGH**) just prior to their June 14 - 16, 1995 release and again prior to their November 13-15, 1995 release. At dusk on each release day, approximately 1/3 of the total release group was planted into the Klamath R. just below the hatchery. This hatchery is operated by the California Department of Fish & Game (CDFG) as a mitigation facility for Iron Gate Dam and is located at rkm 306. The June release group totaled 4,913,457 and included 194,644 adipose fin clipped / Coded Wire Tagged {CWT} fish (CDFG planting receipt KS- 94-P1). Out-migrant juveniles were collected with a 50 ft. beach seine and at the rotary screw trap below Big Bar (Table 1). Adipose fin clipped fish were selected when available, however, only 4 % of the June release group was marked. Six of the 10 down-river collections were composed of both adipose-fin clipped and unmarked fish. As the unmarked fish could either be hatchery (similar size and appearance as marked fish) or natural, these sample groups are considered as "mixed" origin (Table 1). The first in-river sample was scheduled for 1 week post-release, however, no marked fish were captured at the Big Bar Trap on or prior to this June 21 sample. The June 21 salmon are considered to be of natural origin. Approximately 2 weeks post-release (June 26), the first marked chinook were captured at the sample sites .

The November release group had been reared at both IGH and the Fall Creek satellite facility and totaled 1,076,445 including 86,570 adipose fin / CWT fish (CDFG Planting receipt KS-94-P3). The first in-river sample occurred approximately 2 weeks post-release on November 20 (Table 1). All yearling chinook captured at Indian Creek and Orleans in November and on December 5 were considered to be IGH fish as few chinook of similar size would likely be found in this part of the river during late Fall (pers. comm. Jim Craig, USFWS, Arcata). Also, the unmarked fish were similar in size and appearance to the 5 marked fish captured on December 5. The coded wire tags from these 5 marked fish identified them as part of the IGH November release (06-63-21, 06-63-29, and 06-57-01).

Figure 1. Sample sites.

Physical measures taken at each sample site included: water temperature and dissolved oxygen concentration (0.3 m depth with YSI model 55 meter), total dissolved solids (Cole Palmer TDSTestr1 model 1980-00 meter), and percent total gas saturation (Weiss satrometer and barometer). Spring river flows at the USGS Orleans gauge provide an estimate of river flow on the day of the sample (Table 3 results).

Field Collections- At IGH, equal numbers of fish were netted from the middle section of 3 raceways representing the size (age) span of the hatchery population (raceway c,e,h). Captured fish were held in aerated containers of ambient temperature water. Groups of 5 -10 fish were euthanized with an overdose of a benzocaine solution, rapidly examined for external organosomatic parameters, weighed, measured for total and fork length, and bled from the caudal peduncle into 75 mm heparinized microhematocrit tubes. In the spring, elevated water temperatures made it imperative that all blood work was done quickly to avoid hemolysis. The blood sample was centrifuged (10,000 RPM, 7 min.) for measurement of hematocrit, leukocrit, and collection of plasma. Heparinized microtainer tubes (1.0 mL volume) were also used to collect plasma from the larger November release chinook. Plasma, liver (see liver glycogen assay) and a gill sample (see ATPase assay) were frozen on dry ice and held at -80° C. After an internal organosomatic examination, various tissues were removed for histological examination and the anterior 2/3 of the kidney for both *R. salmoninarum* antigen assay (ELISA) and metacercarial counts. For the November release, the percent moisture of the liver was determined from 3 - 8 fish per group. Upon necropsy, the livers were weighed and frozen intact within pre-weighed aluminum foil pouches. These samples were later dried at 75 ° C for 12 - 18 hrs and weighed to the nearest 0.0001 g with a Mettler balance. Percent moisture was calculated ((wet wt. - dry wt)/wet wt. x100). For measurement of plasma glucose response to capture stress, the time removed from the trap or raceway and the time of euthanization was recorded for each fish.

Table 1 Sample sites, dates of collection, sample group designation, and percent of hatchery marked fish in collection group.

June Release (spring= s)

Sample Site	(rkm)	Sample Date	Week Post-release	Group Designation	Marked fish Total collection (%)	notes
Iron Gate Hatchery	(360)	13JUN	0	IGH-s0	NOT APPLICABLE	
Big Bar Trap	(80)	21JUN	1	BBT-s1	0 / 12 (0%)	NATURALS
Big Bar	(80)	28JUN	2	BBS-s2	11 / 11 (100%)	SEINE
2mi above Indian Creek	(174)	28JUN	2	2IC-s2	9 / 9 (100%)	
Big BarTrap	(80)	29JUN	2	BBT-s2	9 / 32 (28%)	
Red Cap Creek	(85)	06JUL	3	RCC-s3	0 / 12 (0%)	
Big Bar Trap	(80)	06JUL	3	BBT-s3	9 / 9 (100%)	
Indian Creek	(172)	07JUL	3	INC-s3	19 / 19 (100%)	
Red Cap Creek	(85)	11JUL	4	RCC-s4	0 / 14 (0%)	
Big Bar Trap	(80)	11JUL	4	BBT-s4	4 / 20 (20%)	
Indian Creek	(172)	12JUL	4	INC-s4	5 / 15 (33%)	
Big Bar Trap	(80)	18JUL	5	BBT-s5	12 / 27 (71%)	

November Release (fall = f)

Iron Gate Hatchery	(360)	11NOV	0	IGH-f0	NOT APPLICABLE
Indian Creek	(172)	20NOV	1	INC-f1	0 / 11 (0%)
Orleans	(96)	28NOV	2	ORL-f2	0 / 27 (0%)
Orleans	(96)	05DEC	3	ORL-f3	5 / 42 (12%)

Organosomatic analysis- Fish were evaluated by a modified organosomatic assay (Goede and Barton 1990, 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. Features evaluated included skin condition (scale loss), gill, visceral fat, presence of food in the gastrointestinal tract, and any gross abnormalities of internal organs. Hematocrit (% packed erythrocyte volume) and leukocrit (% white blood cell volume) were determined from centrifuged microhematocrit tubes containing heparin. Fulton condition factor was calculated from both the fork and total length ($K = \{ \text{weight (g)} / \text{length (mm)}^3 \times 10^5 \}$), however, only total length condition factors are reported. The criteria for the severity scores are listed below in Table 2.

Table 2 Organosomatic analysis criteria scores

Skin	0 = normal scale number, no lesions 1 = some scale loss, 5 - 20 % of body surface 2 = focal hemorrhages, scale loss 21 - 40 % of body 3 = open wound, scale loss > 40 % of body surface
Gill	0 = normal condition, color 1 = pale 2 = clubbed, frayed, nodules, mild parasite load 3 = necrotic zones, fungi or bacterial lesions, hemorrhagic
Vfat	0 = no visceral fat on pyloric ceca or peritoneal cavity 1 = < 50 % coverage of ceca and/or cavity fat dia. < ceca vol. 2 = >50 % but not covering ceca and/or cavity fat dia. = ceca vol. 3 = ceca and cavity completely filled with fat, organs obscured by fat
Food	(+) = food in any part of GI tract (-) = no food seen in GI tract

Saltwater challenge: A 30 ppt saltwater (SW) static aquarium (sealed bucket) was produced by adding 900 g Instant ocean™ salt to 30 L of freshwater from the fish's environment. Salinity was checked by salinity refractometer. The buckets were held in ambient water to maintain temperature, supplied with bubbled air, and a lid was securely fasten. At BBT, the buckets were suspended in the river from small cataraft and aeration supplied by a compressed air cylinder (dive tank and modified oxygen regulator). Test fish were quickly captured and placed in the SW buckets (6 fish / bucket). After 24 hrs, the surviving fish were quickly netted, euthanized in a SW-MS222 solution, weighed, measured for total and fork length, and bled from the caudal peduncle into heparinized tubes. The tubes are centrifuged soon after collection and the plasma frozen for later sodium analysis. One measure of smolt development is the ability of a fish in SW to maintain its plasma sodium concentration below 170 mmol /L (Blackburn & Clarke1987). We increased this cutoff to 174 to take into account intra-assay variability.

Histology - Gill, intestinal tract, pyloric ceca, lower 1/3 of the kidney, and liver tissue was rapidly removed from the fish after death, fixed for 24 hrs. in Davidson's fixative (Humason 1979), processed for 5 μm paraffin sections, and stained with hematoxylin and eosin. Tissue abnormalities and parasite infections were evaluated by light microscopy.

Pathogens- The anterior 2/3 kidney was removed, stored in a cryovial and frozen for later enumeration of metacercaria and *R. salmoninarum* antigen. Antigen was tested by a polyclonal Enzyme Linked Immunosorbent Assay (ELISA). After thawing, the kidney sample was weighed, homogenized with an applicator stick in a 1x (w / v) volume of PBS, and a 40 μL subsample removed for metacercaria counts. The weight of this 40 μL sub-sample and the number of metacercaria observed with stereomicroscope (40- 70 x magnification) was recorded to determined a severity index referred to as "metacercaria / gram" of kidney tissue. The remainder of the kidney suspension was further diluted with a 3x (w / v) volume of PBS - Tween20, boiled for 15 min., centrifuged, and the supernatant tested for *R. salmoninarum*

antigen. Many of the June 13 IGH fish were of such small size that replicate ELISA samples were not possible. The optical density (O.D. = absorbance at 405 nm) of the labeled antibody-antigen reactions were averaged between sample replicates. A blank O.D. was subtracted from the mean sample OD values, log transformed, and the data analyzed by ANOVA. Three semi-quantitative categories are used to view the transformed data:

- 1) **BNC** = sample O.D. **Below** the **Negative** kidney **Control** value
- 2) **Suspect** = sample O.D. above BNC and less than -0.7 (= Log 0.2., O.D of 0.2 is the subjective value which there is the likelihood of confirming infection by DFAT), and
- 3) **Positive** = sample O.D. > -0.7

Physiological Assays - Plasma was assayed for total protein, triglyceride, sodium, and glucose using a Johnson & Johnson DT60 Clinical Chemistry Analyzer and reagents. Plasma protein electrophoresis was performed with a 7 μ L sample run on a CIBA agarose gel (1 M barbital buffer, 90V for 45 min.). The stained gels were scanned and the percent area of each fraction determined with Seprascan™ software. Analysis of Variance was performed on percent area values for each fraction (or combined fractions). Gill Adenosine Triphosphatase activity (ATPase = μ moles ADP / mg protein / hr) was assayed by the method of McCormick and Bern (1989). Briefly, gill lamellae were dissected and frozen in sucrose-EDTA-Imidazole (SEI) buffer on dry ice. The sample was later homogenized, centrifuged, supernatant sonicated, protein content assayed, and the ATPase activity determined by the decrease over time in optical density (340 nm) as NADH is converted to NAD⁺. This activity was reported as μ mole ADP / mg protein / hr as 1mole of NAD is produced for each mole of ADP generated in the reaction. Gill Na-K-ATPase activity is correlated with osmoregulatory ability in saltwater and is located in the “chloride cells” of the lamellae. This enzyme system transports salts from the fish against the concentration gradient to the saltwater.

Liver glycogen was determined by the method of Murat and Serfaty (1974). Briefly, the liver was removed from the fish, weighed, placed in Citrate buffer (0.09 M, pH 4.8, 2.5 mg sodium fluoride / mL) and frozen on dried ice. The sample was later defrosted, homogenized, a 200 μ L sub-sample tested for free glucose, the remainder digested with amyloglucosidase at 37 °C for 12 hrs, the glucose concentration determined (0-toluidine method), and the difference between digest glucose and free glucose equaling the amount of polysacchride present in the liver sample. This value was expressed as mg polysacchride / 100 mg liver.

Phagocytic characteristics of anterior kidney cells were tested with a modification of methods reported by Enane et al (1993) and Dalmo and Seljelid (1995). The cephalic horn region of the anterior kidney was aseptically removed and transported to the laboratory in cold L-15 media with 2 % FBS and 100 U/mL penicillin-streptomycin, a single cell suspension produced by forcing the tissue through a 21G needle several times, the suspension seeded onto replicate 1 cm dia. slide wells, incubated at 15 °C for 2 hrs, the slide washed with cold PBS, the glass-adherent cells (GAC) were then exposed to a latex bead suspension (opsinized with chinook serum) for 30 minutes, washed, fixed in methonal, air dried, cleared in xylene to dissolve extracellular beads and stained with safranin red. One hundred GACs were examined at 1000x magnification per well and the number of cells with engulfed beads counted (Phagocytic index = GAC with beads / 200) as well as the number of GAC's with > 5 beads (Phagocytic capacity = GAC w/ > 5 beads / 200). In the spring, 1 μ m beads were used for the assay, however, it was quite difficult to consistently distinguish the beads and 3 μ m beads were later used in the November release samples.

Data analysis / Statistics- Group data was tested for normality and either analyzed by the parametric (T-test, 1-way ANOVA) or non-parametric tests (Mann-Whitney rank sum test, Kruskal-Wallis ANOVA on ranks). If significant differences among the groups were detected in the ANOVA tests, Student-Newman-Keuls multiple comparison (pairwise) tests were performed to identify which group was different. An alpha (type I "false difference" error) value of $P \leq 0.05$ was chosen for all tests. Both Lotus 1-2-3™ spreadsheets and SigmaStat™ software was used for data manipulation and analysis.

Results- June Release

Sample Site Characteristics- The IGH chinook were released directly into the Klamath River below the hatchery and experienced a rapid change in water temperature. The hatchery water supply is taken from a lower level of Iron Gate reservoir and ranged from 11 - 12 ° C in early June. In contrast, river water was in excess of 16° C in early June. At 2 weeks post-release (June 28), water temperatures at BBT and INC were 18 - 19 ° C and remained in this range until the 21.9 ° C temperature measurement of the July 18 BBT sample (Table 3). River flows at Orleans declined from 8510 cfs on June 21 to 3730 cfs by July 18.

The effect of cooler tributary water on adjacent mainstem habitat was better demonstrated at INC than at RCC. Both on July 7 and July 12, INC temperature was 5.1 - 5.6° C less than the river. Our sampling site was 5 -15 meters directly below the mouth and had temperatures 2.9 - 3.1 ° C cooler than the upstream river measurement (Table 4). We observed that although there was considerable human activity (raft launching) at INC, chinook remained in the area during the late morning collections. At RCC, water temperature measurements at 25 cm depth indicated less than 1.0 ° C difference between the sample site below the creek mouth and the river. The presence of juvenile chinook at the RCC seine site (a 15 - 20 m dia. pool formed by a gravel bar at the creek mouth and a rock face on the downstream portion) suggests some attractant feature such as microhabitat temperature (gravel upwelling from creek ?) or slack water refuge for the smolts.

With the exception of the IGH-s0 and the BBT-s3 sample, dissolved oxygen (D.O.) concentrations were in the 7 - 9 ppm range and above the 5 - 6 ppm threshold for oxygen stress in salmonids (Wedemeyer 1996). At IGH, the inflow D.O. measured 8.7 ppm while the outflow D.O. from 3 chinook raceways (D,E, F) ranged from 3.4 - 3.8 ppm. This 5 ppm decline in oxygen was also measured with another meter (hatchery's Oxyguard™ unit) and indicates the raceways contained more fish than was prudent to hold. It was also interesting that the calculated D.O. value at 100% saturation (11° C, 2200 ft elevation) of 10 ppm was much greater than the 8.7 ppm measured in the inflow (Rawson's nomograph pg 5, *In*: Piper et al. 1982). Other sample site water measurements such as total gas saturation (%SAT), total dissolved solids (TDS), and pH were not judged to be adverse for the fish.

Table 3. Water quality data from the June and July collection sites: temperature (25 cm. depth), dissolved oxygen (D.O., ppm), pH, flow (cfs) from USGS gauge at Orleans or IGH raceway, total gas % saturation (%SAT) and Total Dissolved Solids (TDS, ppm).

Sample date	site	° C	D.O. ppm	pH	flow	%SAT.	TDS
13JUN	IGH-s0	11.1	3.8	7.5	3	101	ND
21JUN	BBT-s1	14.8	8.4	ND	8510	104	60
28JUN	BBS-s2	18.8	9.4	8.0	8340	101	50
28JUN	2IC-s2	19.6	8.9	8.2	8340	102	60
29JUN	BBT-s2	19.1	9.8	8.1	7550	103	50
06JUL	RCC-s3	19.6	7.3	ND	5040	103	60
06JUL	BBT-s3	18.9	6.2	7.8	5040	103	60
07JUL	INC-s3	17.5	8.4	8.4	4860	103	70
11JUL	BBT-s4	18.2	8.8	8.4	4620	102	70
11JUL	RCC-s4	18.7	9.1	8.3	4620	ND	70
12JUL	INC-s4	18.4	8.3	8.3	4440	101	80
18JUL	BBT-s5	21.9	7.5	8.4	3730	100	80

ND Not done.

Table 4 Temperature (°C) of tributary sample sites and mainstem Klamath River.

		Creek	River (Upstream)	Below creek mouth River (seine site)
07JUL	INC	14.8	20.4	17.5
12JUL	INC	13.6	18.7	15.6
06JUL	RCC	18.6	19.6	19.5
11JUL	RCC	18.3	18.8	18.7

Morphometries and Organosomatic Data- The fork length of all post-release capture groups were similar to each other (mean F.L. 88 - 96 mm) and significantly larger than the mean of 75 mm observed in the pre-release IGH sample (ANOVA $P < 0.001$, Table 5). Condition factor (mean 0.800×10^5) was similar for all 3 size groups at IGH. Condition factors were variable among the different in-river collections (Table 5). Out-migrants collected at BBT showed a peak in condition factor at 3 weeks post-release with the BBT-s5 group having a mean condition factor (0.8043) similar to the IGH-s0 sample group (Fig. 2). Chinook collected around Indian Creek showed a condition factor increase similar to BBT, however, condition

factor continued to increase past 3 weeks post-release . RCC-s3 fish had the highest mean condition factor (0.8872) of any sample group. The RCC-s4 condition factors declined to levels similar to the BBT-s4. Condition factors for the natural chinook collected on June 21 could not be accurately estimated due to inaccurate weight measurements (fish dried prior to weighing).

Observations of gross abnormalities included:

- a) lamprey bite wounds (3 fish total),
- b) scale loss (0 - 67 % of any particular sample groups),
- c) swollen / pale gills (0 - 33 % of any particular sample group),
- d) swollen / hemorrhagic intestinal tracts (no count data recorded, however, up to 10 % incidence was noted in a July BBT group)

Scale loss tended to be more prevalent in chinook recovered from BBT (prevalence in date order) 19, 31, 45 and 47 %) in comparison to the July beach seine collections at INC (16 and 27 %) and RCC (0 and 23 %). This increased prevalence of scale loss at BBT is likely density dependent as it tended to correspond to large catches at the trap. Abrasion during netting is believed to be a major factor in this observation, however, the condition of fish prior to capture is unknown. All chinook sampled during the spring showed some degree of silvering. The gill anomalies were related to the anemia and osmoregulatory problems induced by *Ceratomyxa shasta* infections.

Table 5. Fork length, weight, and condition factor ($\{wt.(g) / \text{total length (mm)}^3 \times 10^5\}$) of June and July collection groups. Data reported as mean (\pm SEM).

	Fork length (mm)	Weight (g)	Condition Factor
IGH -s0 n = 20	75 (\pm 1) ++	4.6 (\pm 0.2)	0.800 (\pm 0.011) ab
BBT-s1 (NAT.) n = 12	99 (\pm 3)	ND **	ND**
BBS -s2 n = 11	96 (\pm 1)	9.3 (\pm 0.3)	0.800 (\pm 0.019) a
BBT-s2 n = 32	94 (\pm 1)	9.0 (\pm 0.3)	0.819 (\pm 0.008)
BBT-s3 n = 13	91 (\pm 2)	8.6 (\pm 0.4)	0.864 (\pm 0.022)
BBT-s4 n = 19	92 (\pm 2)	8.6 (\pm 0.5)	0.845 (\pm 0.024)
BBT-s5 n = 12	95 (\pm 2)	9.1 (\pm 0.5)	0.804 (\pm 0.013) a
2IC-s2 n = 9	91 (\pm 1)	9.1 (\pm 0.4)	0.917 (\pm 0.022)
INC-s3 n = 14	88 (\pm 2)	8.3 (\pm 0.5)	0.861 (\pm 0.013)
INC-s4 n = 15	89 (\pm 1)	8.0 (\pm 0.4)	0.882 (\pm 0.018)
RCC-s3 n = 12	91 (\pm 3)	8.8 (\pm 0.7)	0.887 (\pm 0.014)
RCC-s4 n = 14	93 (\pm 2)	9.1 (\pm 0.6)	0.848 (\pm 0.019)

ND not done

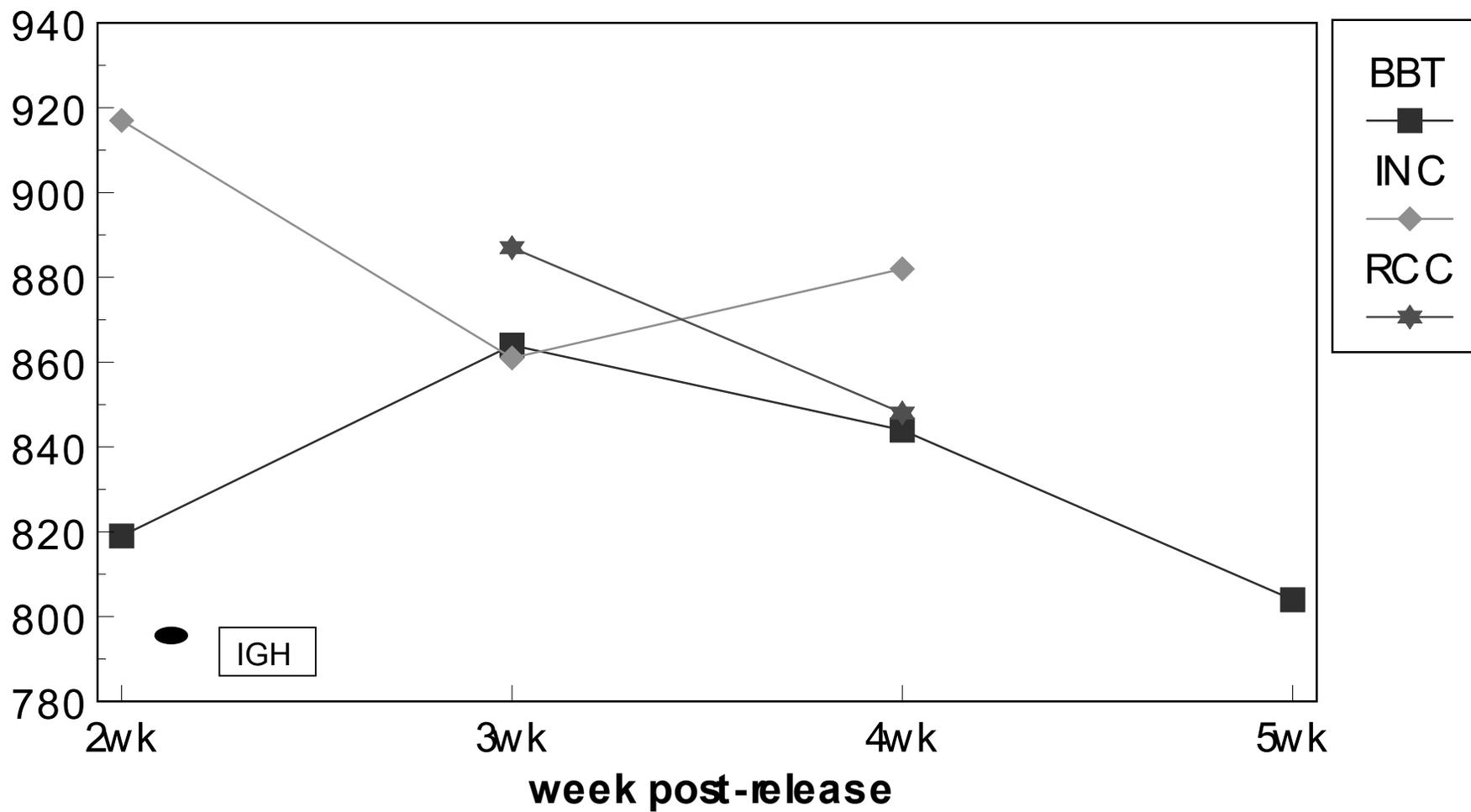
a Significantly less ($P < 0.05$) than 2IC-s2

b Significantly less ($P < 0.05$) than RCC-s3 and INC-s4.

++ Significantly less than all downriver collection groups (due to small fish in Raceway H).

** A 30 min. delay in weighing and hot conditions resulted in severe drying of the BBT-s1 sample group. The accuracy of the measured weights (mean = 10.6g) and condition factors (mean = 0.755) are suspect.

Figure 2. Condition Factors ($Wt / Total\ length^3 \times 10^8$) of June and July Samples.



Energy Reserves- Hepatosomatic index (HSI), which relates liver mass to body mass, was variable within sample groups and between the different site collections. It was not correlated to either condition factor ($r^2 = 0.074$ for RCC and -0.0075 for BBT) or liver glycogen content. There was a temporal trend to the HSI data as values peaked in the 3rd week and declined in week 4 at all 3 sites (Fig. 3). Hepatosomatic indices increased significantly after 3 weeks post-release (Kruskal-Wallis $P < 0.05$, Table 6) even though mean liver glycogen concentration dropped 65 - 71 % during this time period. The 2 RCC collections had similar HSI values (means of 1.873 and 1.516). While these values were lower than the BBT and INC groups for corresponding sample dates, the differences were not statistically significant (Kruskal-Wallis, $P > 0.05$). At BBT, liver glycogen was extremely low in the BBT-s3 group but returned to earlier levels in week 4 and 5. The BBT-s5 fish were noteworthy as their mean glycogen of 0.734 mg polysaccharide / 100 mg liver was similar to week 2 measurements and none of the samples had undetectable glycogen levels. Glycogen values at INC followed a similar pattern in which the week 3 fish had the lowest level followed by an increase in week 4. Both RCC chinook groups had similar glycogen values (mean 0.371 and 0.415 mg polysaccharide / 100 mg liver) as well as the number of samples with undetectable glycogen (42 and 43 %). Histological examination of out-migrant livers showed no obvious glycogen deposits within the hepatocyte except in several BBT-s5 fish. Natural fish collected on June 21 had a mean liver glycogen of 0.133 with 42 % of the sample group having undetectable levels. Up to 85% of some sample groups had "undetectable" liver glycogen levels. The glycogen assay is based on the difference between the "free" glucose in the raw sample and the glucose produced in the glycogen digest. In many cases, glucose values were higher in the "free" than digested sample which resulted in a negative or "undetectable" glycogen value.

Capture stress would have resulted in the conversion of liver glycogen to plasma glucose (Mazeaud et al. 1977). It is difficult to standardize the degree of capture stress experienced by a fish collected in the river. Particularly at BBT, we do not know how long a given fish was in the trap livebox prior to necropsy. Plasma glucose (GLU) values are thus reflective of the magnitude and duration of capture stress as well as the glycogen reserves of the fish. At IGH, the relatively low plasma glucose values are a result of the quick capture-to-sample protocol (≤ 10 minutes). As mentioned above, IGH-s0 liver glycogen was the highest of all the sample groups. Mean glucose values varied from a low of 52 mg/ dL (2IC) to 124 mg / dL (BBT-s1 Naturals, Table 7). The 2IC group was captured in 2 seine sets with over 50 minutes from initial capture until the first fish was euthanized for sampling. It is likely that this long period of stressful handling may have influenced the low circulating GLU concentration. Most other chinook sampled for plasma were held for < 30 minutes in an aerated bucket prior to bleeding. We have observed that most juvenile salmonids (hatchery stocks) will have $GLU > 90$ mg / dL when sampled 20 - 40 minutes following capture stress. No single pattern emerges when the percentage of high GLU (>90 mg/dL) responders is examined for each sample group, (Table 7). At BBT, the out-migrants in week 2 showed a normal response to capture stress with over 88% having > 90 mg/dL GLU. High responders dropped to 25 % in week 3 and 33 % by week 4. Corresponding to their higher liver glycogen values, 83 % of the BBT-s5 group were also high responders. This glucose response was similar to the BBT-s2 fish. At Indian Creek, both the mean GLU and the percentage of high responders dropped between week 3 and 4. The RCC fish also showed low GLU and few high responders (11 and 0 %) at week 3 and 4.

Figure 3. Mean Hepatosomatic index values for June – July sample groups.

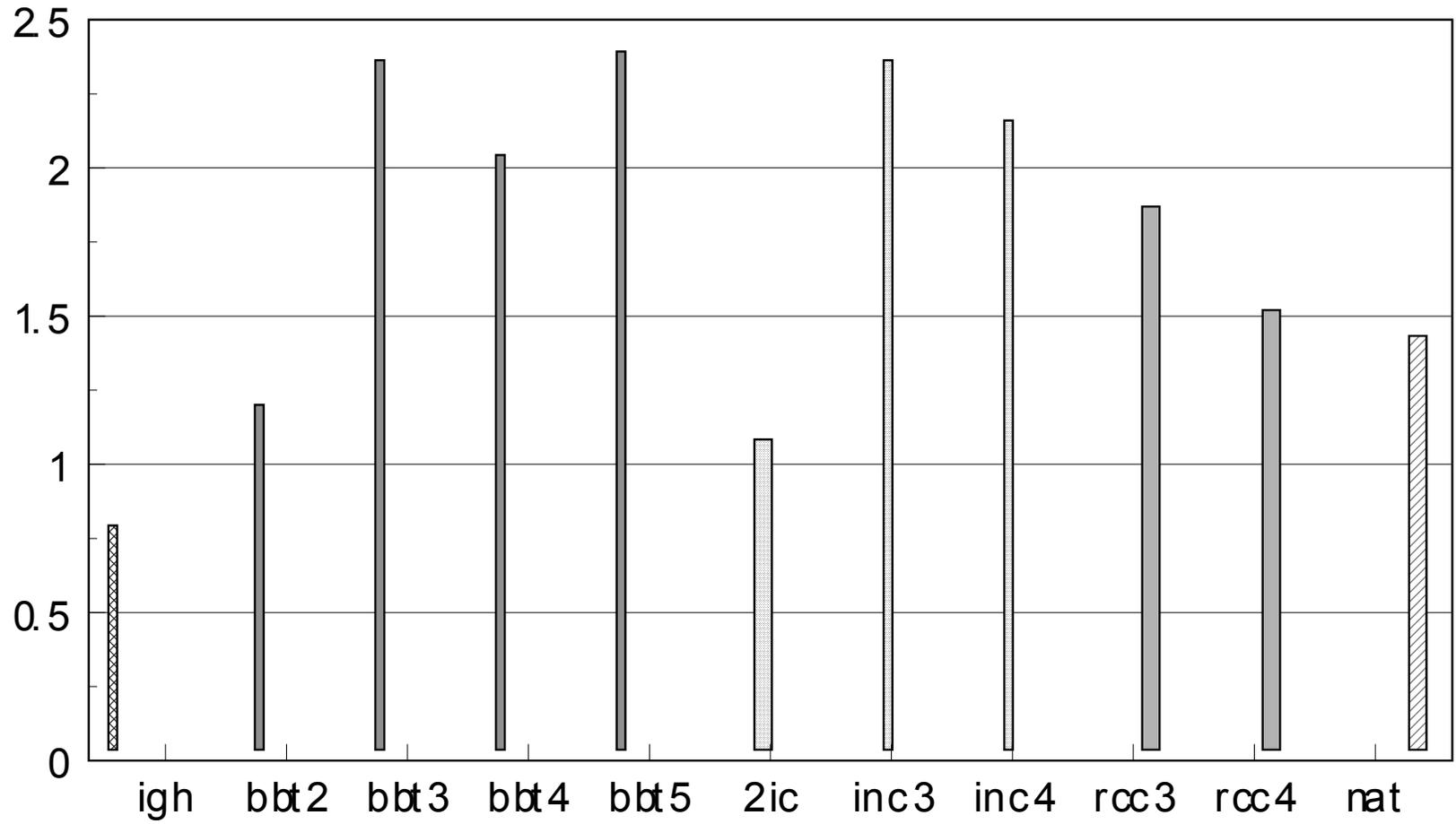


Table 6.

Hepatosomatic index (liver wt / body wt X100), Liver glycogen (mg polysaccharide {Ps} / 100 mg liver and percent of 10-12 liver samples below assay detection limits (% BDL)** of June - July groups. Hepatosomatic index and liver glycogen values are reported as Average \pm Standard error of the mean. Only positive liver glycogen values were used for the calculations.

	Hepatosomatic Index	Ps mg / 100 mg LIVER	%BDL
IGH-s0	0.7927 (\pm 0.0708) a-f	2.682 (\pm 0.342)	0
BBT-s1 (nat.)	1.4390 (\pm 0.1783)	0.133 (\pm 0.046)	42
BBS-s2	1.1469 (\pm 0.0853) a-c	0.781 (\pm 0.118)	0
BBT-s2	1.1920 (\pm 0.0513) a-c	0.774 (\pm 0.046)	0
BBT-s3	2.3760 (\pm 0.0620)	0.021 (\pm 0.017)	85
BBT-s4	2.0409 (\pm 0.1789)	0.438 (\pm 0.090)	15
BBT-s5	2.3995 (\pm 0.1849)	0.734 (\pm 0.175)	0
RCC-s3	1.8730 (\pm 0.2140)	0.371 (\pm 0.132)	42
RCC-s4	1.5155 (\pm 0.1776)	0.415 (\pm 0.120)	43
2IC-s2	1.0980 (\pm 0.1395) a-d	0.944 (\pm 0.161)	11
INC-s3	2.3581 (\pm 0.1142)	0.149 (\pm 0.062)	58
INC-s4	2.1743 (\pm 0.0869)	0.597 (\pm 0.048)	0

a-f Significant difference from: a) BBT-s3, b) INC-s3, c) BBT-s5, d) INC-s4, e) BBT-s4, and f) RCC-s3 at P<0.05.

nd not done

** Liver glycogen value derived from equation: Glucose of enzyme digested sample - Free glucose of sample. When free glucose is higher than stored glycogen, the liver glycogen value is negative and not used in statistical calculations.

Table 7. Plasma glucose (mg/dL) data of June - July groups. Values of samples above assay detection limits reported as mean (\pm SEM). Percent samples above 90 mg/dL (% > 90).

	Glucose	% > 90
IGH-s0	76.5 (\pm 3.5) n=2	0
BBT-s1 (natural)	123.8 (\pm 23.6) n=8	75
BBS-s2	82.0 ** n=1	na
BBT-s2	103.6 (\pm 6.6) n=8	88
BBT-s3	73.8 (\pm 9.0) n=4	25
BBT-s4	71.7 (\pm 15.9) n=9	33
BBT-s5	119.0 (\pm 19.9) n=6	83
RCC-s3	71.2 (\pm 8.6) n=9	11
RCC-s4	64.0(\pm 5.4) n=8	0
2IC-s2	52.0 (\pm 11.0) n=3	0
INC-s3	71.8 (\pm 7.8) n=8	38
INC-s4	61.1 (\pm 5.9) n=8	0

** Value is from a single sample which was within assay detection range.

na not applicable as all samples below assay detection limits.

Plasma triglyceride (TG) significantly declined after release. The mean TG for the IGH-s0 sample was 108 (\pm 10) mg / dL. At 2 weeks post-release, average TG dropped 2- 3 X to 25 - 41 mg / dL (Table 8). Average TG values for all out-migrant groups ranged from 18 - 67 mg / dL, however, a high percentage of samples were below the 15 mg / dL limit for the assay (Table 8). The “ below detection limit” data shows that by 3 weeks over 58 % of each sample group had extremely low levels of circulating TG (Fig. 4). In a similar fashion, natural chinook sampled on June 21 averaged only 45 mg/dL with half of the TG samples below 15 mg / dL. Visceral fat declined after release (Table 8). Out-migrants captured at BBT showed a low prevalence of visceral fat reserves ranging from 0 % to 23 % (Fig 5). The percentage of chinook with visceral fat captured at INC and RCC was much higher than BBT groups captured on corresponding dates. By week 4, both the BBT-s4 and INC-s4 had few fish (0 and 7 %) with visceral fat compared to 36% in the RCC capture group (Fig. 5). The percent of each sample group with food in the intestinal tract ranged from 20 - 93 %. Absence of food was usually related to *C. shasta* enteritis.

Table 8. Plasma triglycerides (mg / dL) and percent of sample observed to have visceral fat (% VFAT > 0) . Plasma triglycerides data reported as mean (\pm SEM) and prevalence of samples below analyzer range of 15 mg / dL (% <15 mg/dL).

	Triglyceride		% < 15 mg / dL	%VFAT > 0
IGH-s0	107.5 (\pm 10.4)	n=11	0	100
BBT-s1 (nat)	45.0 (\pm 8.2)	n=10	50	0
BBS -s2	24.5 (\pm 1.3)	n=4	0	nd
BBT-s2	26.5 (\pm 4.6)	n=12	50	9
BBT-s3	32.0 **	n=10	90	23
BBT-s4	27.8 (\pm 4.1)	n=11	58	10
BBT-s5	67.0 **	n=11	91	0
RCC-s3	37.7 (\pm 8.3)	n=12	42	42
RCC-s4	18.0 **		89	36
2IC-s2	41.7 (\pm 6.4)	n=7	14	67
INC-s3	33.0 (\pm 7.8)	n=12	83	58
INC-s4	Na		100	7

** Value is from a single sample which was within assay detection range.

na not applicable as all samples below assay detection limits.

nd not done.

Figure 4. Percentage of sample group with plasma triglycerides (TG) below 15 mg /dL detection limit.

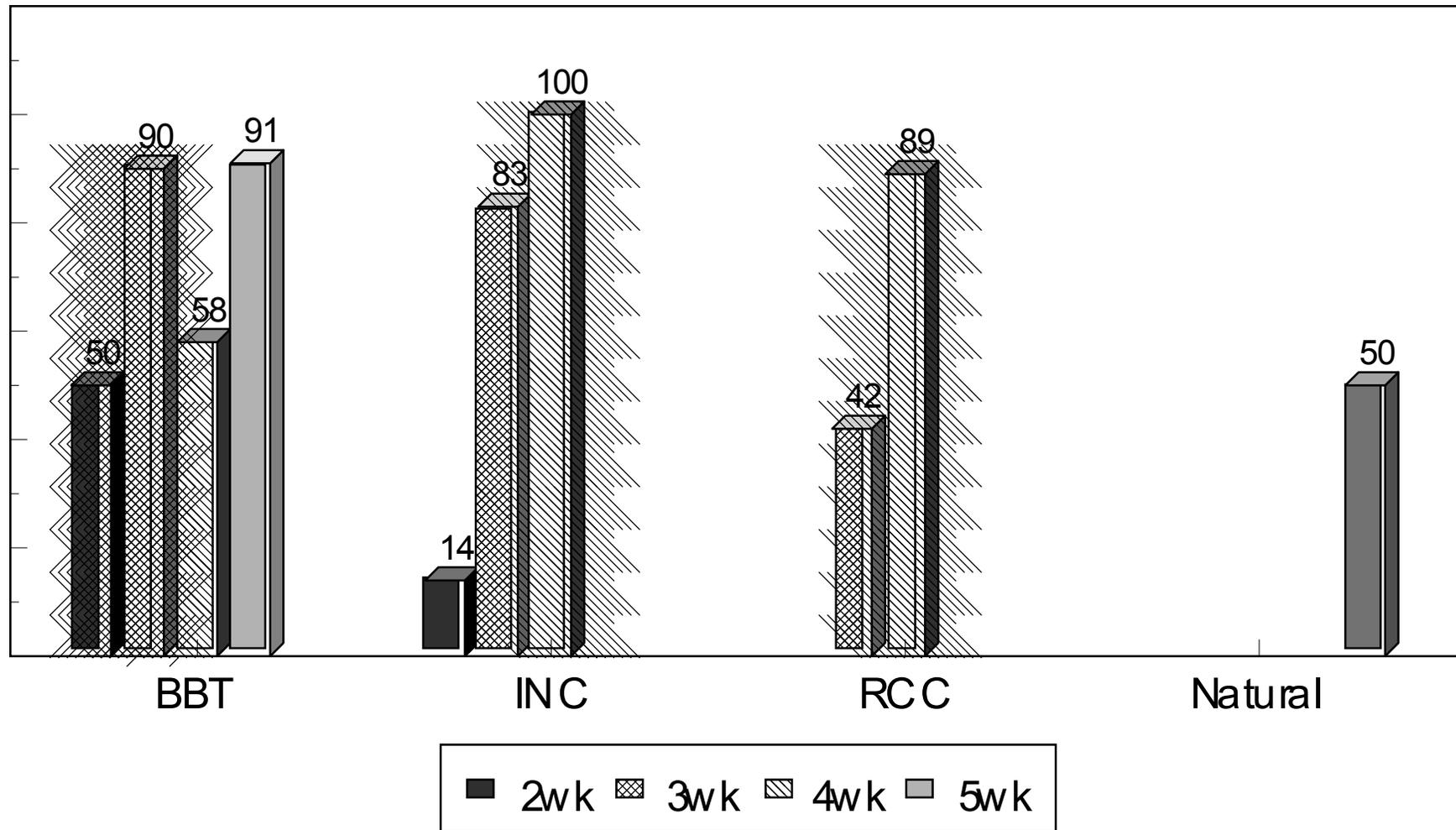
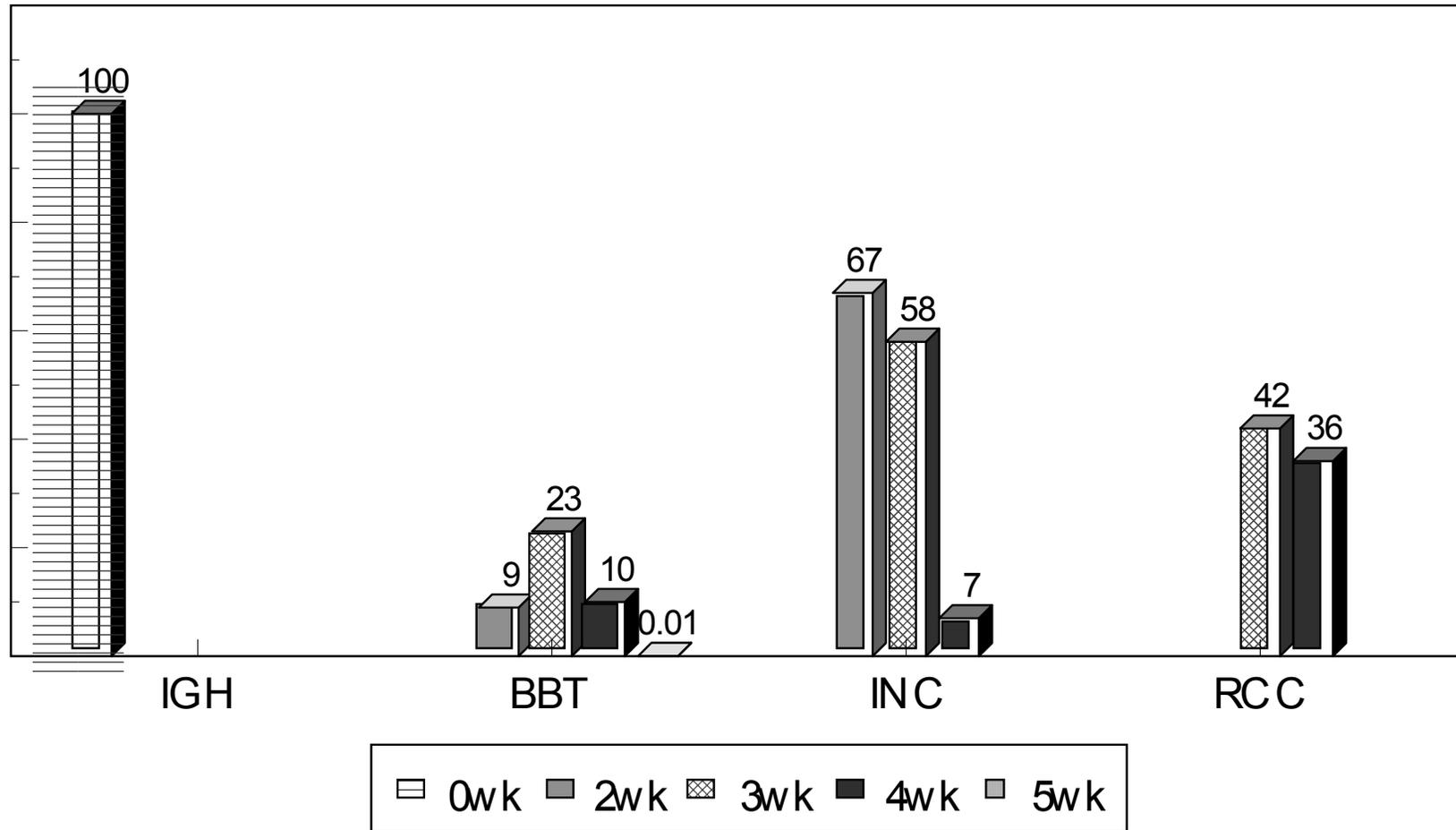


Figure 5. Percentage of sample group with observed visceral fat (%VFAT >0).



Smolt Development BBT-s2 chinook had a significantly higher mean gill Adenosine Triphosphatase Activities (ATPase) than all other out-migrant groups as well as the IGH-s0 ($P < 0.05$, Table 9). The next highest ATPase values were from the BBT-s1 naturals and the IGH-s0 fish (Fig. 6). These two groups of fish had similar ATPase values in spite of their difference in origin (hatchery – natural) and size (mean FL 75 & 99 mm, respectively). ATPase activities from sample groups collected after week 2 tended to be much lower than the preceding samples. In particular, the BBT-s4, INC-s3, and INC-s4 groups were significantly less than above mentioned groups ($P < 0.05$). Saltwater (22 - 27 ppt) adaptation was judged to be rather poor for IGH-s0 fish as well as the BBT-s3 and BBT-s4 out-migrants. The mean fork length of the BBT SW test groups (85 and 86 mm) was significantly greater than the IGH group (mean 76 mm FL), however, the BBT fish had low survival and poor sodium regulation (Table 9). While few fish in the IGH test died during the 24 hr challenge or demonstrated dehydration (reduced condition factor or hematocrit over freshwater fish), 67 % of the group could not maintain a normal plasma sodium level of < 174 mmol / L (Table 9). Fish size in the IGH challenge did not appear to be a factor in sodium regulation success. The IGH-s0 challenge occurred at the ambient water temperature of 11° C and all the fish used for the test appeared healthy. In contrast, the two BBT challenge groups were held in containers suspended in the $18 - 20^{\circ}$ C river water and were probably infected with *C. shasta* parasites (no histology done on SW fish). Dissolved oxygen concentrations at the end of each challenge was > 6.3 ppm and were not considered as a factor in the poor osmoregulatory performance.

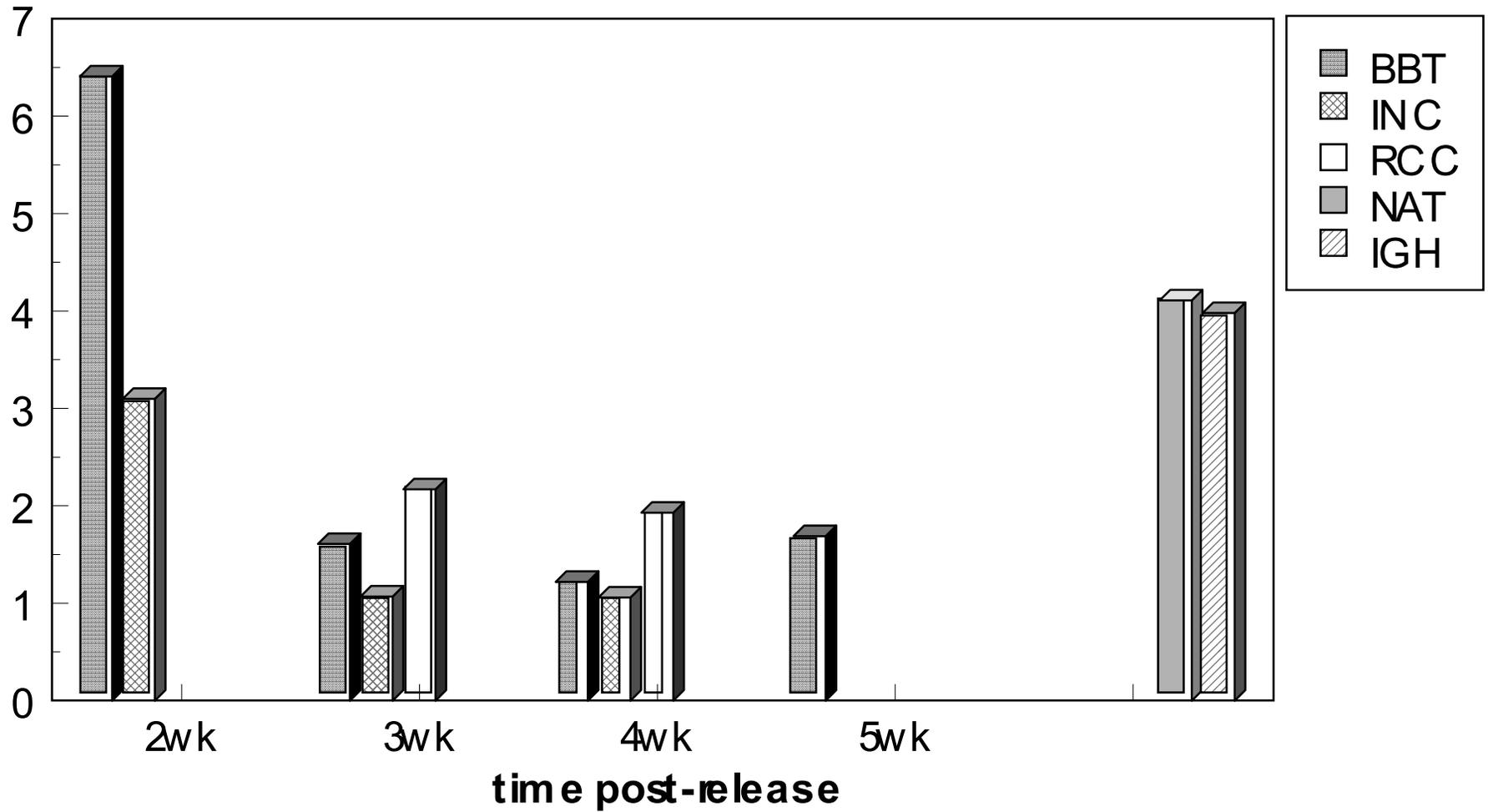
Table 9. Smolt development of June and July collection groups. Gill adenosine triphosphatase activity (ATPase = $\mu\text{mole ADP} / \text{mg protein} / \text{hr.}$), number of fish surviving a 24 hour 22 - 27 ppt saltwater challenge / total challenged (% SW Surv.), and percent of SW challenge survivors showing osmoregulation problems that had plasma sodium values > 170 mmol / L (% > 170). ATPase activity data from 9 - 12 fish per sample group is reported as mean (\pm SEM) and maximum measurement per sample group (MAX).

	ATPase	ATPase Max.	% SW Surv.	% > 170
IGH-s0	3.975 (\pm 0.474)	7.413	17 / 18 (94%)	8 / 12 (67%)
BBT-s1 (nat)	4.115 (\pm 0.499)	7.706	ND	ND
BBS-s2	2.769 (\pm 0.303)	3.888	ND	ND
BBT-s2	6.405 (\pm 0.485)	9.337	ND	ND
BBT-s3	1.608 (\pm 0.155)	2.496	5 / 18 (28%)	3 / 5 (60%)
BBT-s4	1.223 (\pm 0.215) abc	2.867	1 / 18 (6%)	1 / 1 (100%)
BBT-s5	1.689 (\pm 0.227) a	2.924	ND	ND
2IC-s2	3.096 (\pm 0.399)	4.904	ND	ND
INC-s3	1.075 (\pm 0.179) abcd	2.178	ND	ND
INC-s4	1.056 (\pm 0.139) abcd	1.834	ND	ND
RCC-s3	2.169 (\pm 0.468) a	5.089	ND	ND
RCC-s4	1.935 (\pm 0.238) a	3.692	ND	ND

a-d Significant difference (ANOVA, $P < 0.05$): a) BBT-s2, b) BBT-s1 (naturals), c) IGH-s0, and d) 2IC-s2.

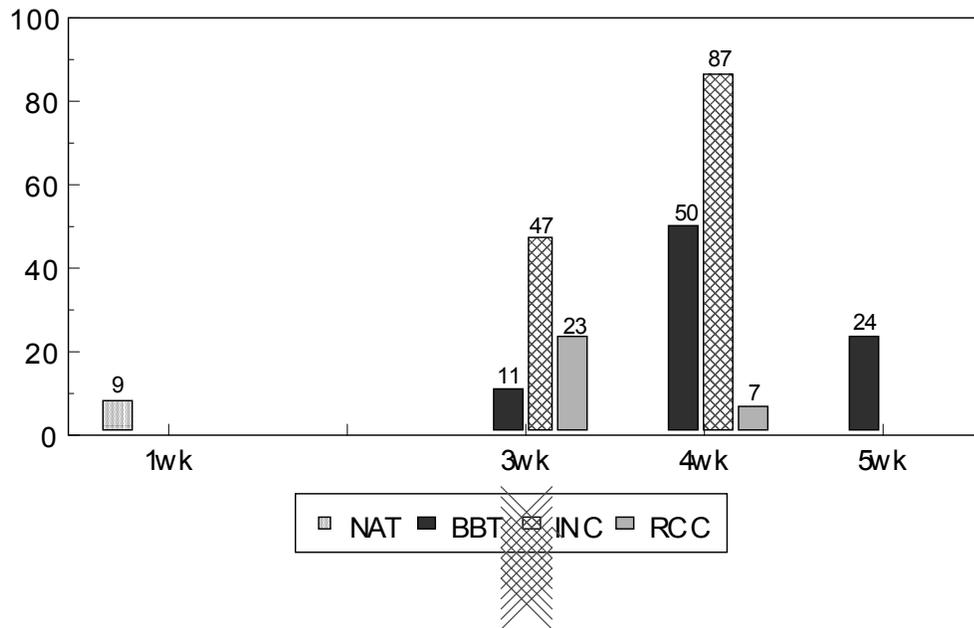
ND Not done.

Figure 6. Mean Gill ATPase Activities ($\mu\text{moles ADP} / \text{mg protein} / \text{hr}$) of June – July Sample Groups.



Blood cells and Immune defenses- Blood cell measurements were taken from 8 to 36 fish of each sample group. The mean hematocrit (HCT) declined from 47% at IGH to 37 - 40% in the 2nd week sample groups (Table 10). Hematocrits below 25 % are considered indicative of anemia in salmonids (Wedemeyer 1996). The severity of Ceratomyxosis in the out-migrant groups was demonstrated by the increased number of anemic fish sampled in week 3 and 4 at both BBT and INC (Fig 7). The BBT-s4 and INCs4 out-migrants were particularly affected by Ceratomyxosis with 50 % or more considered anemic and close to death (Fig 7). Intestinal bleeding associated with advanced Ceratomyxosis is the likely cause of the anemia. The BBT-s5 group showed an improvement in erythrocyte numbers with only 24 % of the group considered anemic. Few RCC fish had such low HCT values. Lower incidence of *C. shasta* infection in the RCC fish and the BBT-s5 group were also reflected in their higher mean HCT values of 33 and 37 % , respectively.

Figure 7. Percentage of anemic fish in each sample group (hematocrit \leq 25). No fish in week 2 were anemic.



Circulating white blood cell numbers, as indicated by leukocrit (LCT), tended to increase post-release. This condition, called leukocytosis, was probably in response to the *C. shasta* infection (Table 10). No significant difference between groups was detected in a Kruskal - Wallis 1-way ANOVA on ranks ($H= 19.6, P= 0.0509$). Intra-sample variance was quite large (Coeff. of Variation 27 - 48 %) and probably influence the ANOVA. Anemic fish with hematocrits less than 20% also had low LCT values. Phagocytic assays were difficult to quantify due to the small size of the 1 μ m latex beads used as phagocytic targets and only 5 of the 12 groups could be fully evaluated. Between 6 and 27 % of the glass adherent cells from the kidney preparations were judged to be macrophages due to their positive esterase staining and cytoplasmic melanin granules (Ellis 1981). Both the Phagocytic Index (PI) and Phagocytic Capacity (PC) tended to decrease after release and was negatively affected by ceratomyxosis. For example, the uninfected IGH-s0 mean (PI) of 89 and mean (PC) of 62 is in contrast to the

infected BBT-s3 group's (PI) of between 0.7 - 78 with all (PC) values below 7. Mean values for several other sample groups are listed below:

Group		PI	PC
IGH	n = 10	89	62
Nat 21jun	n = 10	83	54
RCC 06JUL	n = 2	64	31
RCC 11JUL	n = 6	55	6
BBT 11JUL	n = 8	48	6
BBT 18JUL	n = 3	54	27

Plasma protein concentration declined after release and was negatively influenced by Ceratomyxosis (Table 10). Only 7% of all out-migrant plasma samples were above the lowest quartile value (lower 25 % level = 2.8 g/dL) of the IGH group. After week 2, the percentage of fish with plasma protein concentrations below the analyzer's lower range of < 2.0 g / dL was between 56 - 100 % of the sample group. The large number of samples below analyzer range precluded statistical comparisons between groups and limits the significance of the mean values reported in Table 10. This data was only calculated from those samples > 2.0 g/dL.

Electrophoresis of plasma samples revealed different profiles for the IGH-s0 fish and the later out-migrants (Table 11). Analysis of these changes was limited to sample groups rather than individual fish in various health conditions due to an error in sample identification. The electrophoresis data was also biased towards those fish with total protein values > 2.0 g/dL. Even with these limitations, the pre-albumin and albumin concentrations of the IGH fish was significantly higher than all in-river capture groups (Kruskal-Wallis ANOVA on ranks, P<0.05). The 3rd globulin protein fraction (Glob-3) was significantly elevated in the BBT and INC out-migrants in comparison to the IGH-s0 fish. This trend was not seen in the RCC groups. There was an initial drop in the 4th and 5th globulin fractions (Glob-4/5) in the week 2 out - migrants, however, subsequent groups had concentrations similar to the IGH-s0 fish. The 6th and 7th globulin fractions (Glob-6/7 = region where immunoglobulin is located) was elevated in several 3rd - 4th week groups (RCC-s3, INC-s3 & s4) in comparison to the IGH-s0 group. It is likely that increases in globular proteins were in response to Ceratomyxosis and can be illustrated by the albumin (+pre-albumin) : globulin ratio data. The IGH fish had a mean ratio of 2.5 compared to the < 1.0 ratio seen in all out-migrant groups (Fig. 8).

Figure 8. Albumin : Globulin ratios of June – July sample groups.

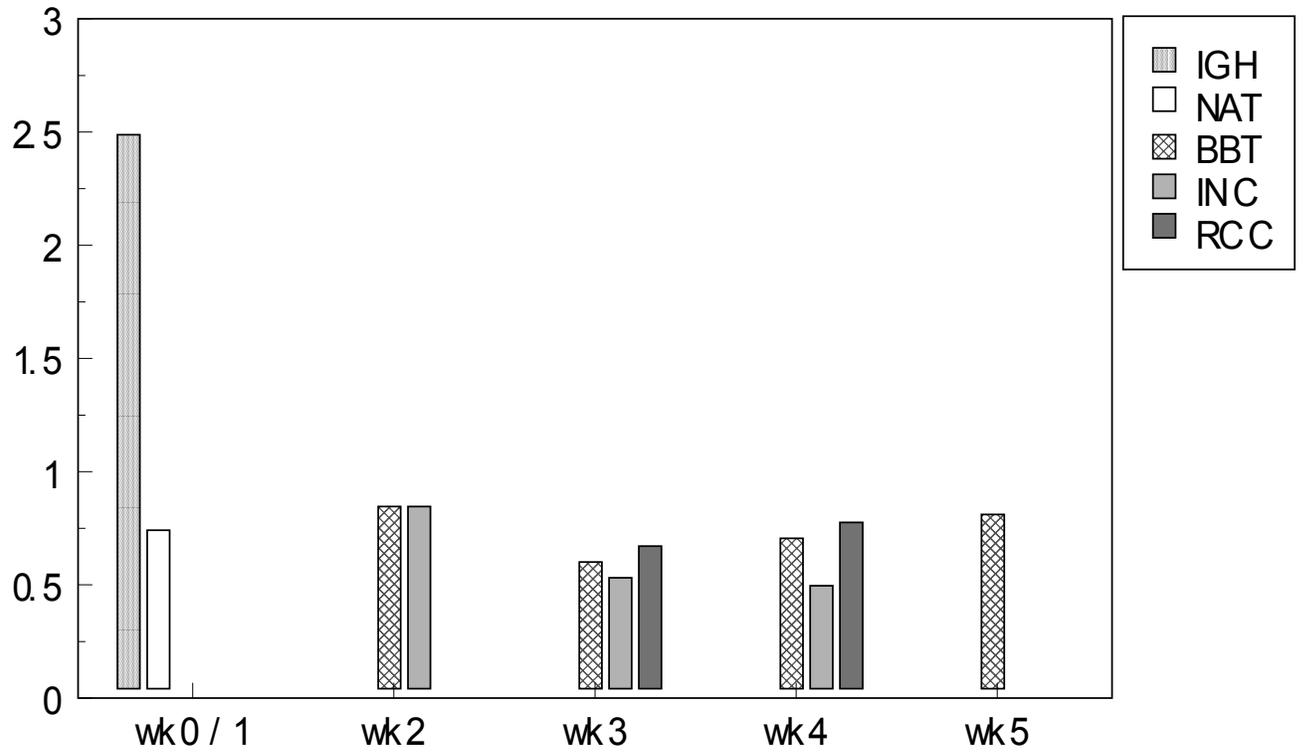


Table 10. Blood data from June and July collection groups. Hematocrit (% packed erythrocytes vol. = HCT), Leukocrit (% packed white blood cell vol. = LCT), and those plasma total protein samples within analyzer range (TPRO g / dL) are reported as group mean (\pm SEM). Plasma samples below the analyzer range of 2.0 mg / dL are reported as % TPRO < 2.0.

	HCT	LCT	TPRO	%TPRO < 2.0
IGH-s0	47 (\pm 1)	0.735 (\pm 0.086)	2.9 (\pm 0.1)	0
BBT-s1 (nat)	34 (\pm 3)	0.839 (\pm 0.240)	3.4 (\pm 0.4)	38
BBS - s2	40 (\pm 3)	2.237 (\pm 0.118)	3.1 (\pm 0.7)	0
BBT-s2	39 (\pm 1)	1.063 (\pm 0.105)	2.9 (\pm 0.3)	20
BBT-s3	30 (\pm 2) a	2.119 (\pm 0.652)	2.1 *	89
BBT-s4	25 (\pm 3) ab	0.607 (\pm 0.128)	2.4 (\pm 0.3)	75
BBT-s5	33 (\pm 2) a	1.118 (\pm 0.202)	2.5 (\pm 0.2)	56
RCC-s3	32 (\pm 3) a	1.293 (\pm 0.289)	2.3 (\pm 0.3)	78
RCC-s4	35 (\pm 2)	1.289 (\pm 0.209)	NA	100
2IC-s2	37 (\pm 1)	0.628 (\pm 0.256)	2.4 (\pm 0.3)	43
INC-s3	27 (\pm 3) ab	1.827 (\pm 0.436)	NA	100
INC-s4	21 (\pm 2) abc	1.110 (\pm 0.179)	NA	100

- only 1 of 9 samples above protein detection limit
- NA not applicable as all sample below detection limit
- a HCT significantly different from IGH (Kruskal Wallis ANOVA P<0.05)
- b HCT significantly different from 29JUN BBT (Kruskal Wallis ANOVA P<0.05)
- c HCT significantly different from 28JUN BBS (Kruskal Wallis ANOVA P<0.05).

Table 11. Plasma protein fractions (pre-albumin, albumin, globulin fractions 1-7) of spring collection fish. Data reported as mean (\pm SEM) of percent area of electrophoretic bands. Sample size variance ($n < 7$) reflect the inability to detect bands in low protein plasma from sick fish and bias the mean values towards healthier animals.

	Pre-Alb.	Albumin	Glob-1	Glob-2	Glob-3	Glob-4/5	Glob-6/7
IGH-s0 1 n= 7	24.7 (\pm 1.4)	46.4 (\pm 1.7)	5.7 (\pm 0.6)	3.7 (\pm 0.6)	4.4 (\pm 0.4)	24.7 (\pm 1.4)	1.5 (\pm 0.3)
Naturals BBT-s1 n= 8	7.5 (\pm 1.3)	34.2 (\pm 2.1)	11.2 (\pm 1.5)	10.7 (\pm 1.2)	7.3 (\pm 0.6)	24.0 (\pm 1.2)	5.2 (\pm 0.9)
BBS-s2 n= 7	4.7 (\pm 0.6)	40.9 (\pm 1.8)	12.0 (\pm 1.2)	6.2 (\pm 0.6)	15.5 (\pm 1.2)	14.9 (\pm 1.5)	5.9 (\pm 0.6)
BBT-s2 n = 8	8.5 (\pm 1.0)	35.6 (\pm 2.9)	11.3 (\pm 0.8)	11.6 (\pm 1.9)	11.1 (\pm 0.5)	19.4 (\pm 2.6)	2.5 (\pm 0.4)
BBT-s3 n= 6	8.1 (\pm 1.8)	28.7 (\pm 1.9)	12.0 (\pm 1.5)	7.5 (\pm 1.1)	12.0 (\pm 0.8)	27.1 (\pm 2.1)	4.6 (\pm 0.9)
BBT-s4 n= 3	9.5 (\pm 3.1)	30.8 (\pm 2.1)	14.3 (\pm 2.3)	8.5 (\pm 0.7)	7.2 (\pm 0.4)	24.9 (\pm 1.0)	4.8 (\pm 0.7)
BBT-s5 n= 6 **	11.1 (\pm 2.5)	31.1 (\pm 7.2)	14.8 (\pm 2.6)	7.0 (\pm 1.4)	10.8 (\pm 0.9)	22.8 (\pm 2.3)	4.2 (\pm 0.6)
2IC-s2 n= 8	10.2 (\pm 1.2)	35.4 (\pm 1.3)	10.2 (\pm 0.6)	8.2 (\pm 0.5)	14.5 (\pm 0.9)	17.1 (\pm 0.9)	4.4 (\pm 0.6)
INC-s3 n = 6	3.5 (\pm 1.0)	31.2 (\pm 2.0)	13.8 (\pm 1.7)	9.2 (\pm 0.8)	12.4 (\pm 1.5)	24.4 (\pm 1.1)	5.6 (\pm 0.8)
INC-s4 n= 5 **	4.2 (\pm 1.4)	28.0 (\pm 2.5)	13.6 (\pm 1.1)	11.8 (\pm 3.6)	14.7 (\pm 2.3)	26.5 (\pm 2.0)	6.5 (\pm 1.1)
RCC-s3 n= 7	5.7 (\pm 1.0)	33.6 (\pm 3.5)	12.4 (\pm 1.8)	9.1 (\pm 2.0)	9.5 (\pm 1.0)	24.9 (\pm 2.9)	6.4 (\pm 0.8)
RCC-s4 n= 8	9.9 (\pm 2.4)	32.3 (\pm 1.9)	12.0 (\pm 2.7)	9.4 (\pm 1.5)	10.0 (\pm 1.1)	22.0 (\pm 1.5)	5.4 (\pm 1.0)

** Majority of plasma samples with low protein content and only 2 distinct peaks.

Histopathology and pathogen assays - Infection with *R. salmoninarum* did not appear to be a significant health factor for the spring collection fish. Antigen concentrations, as measured by ELISA, were low in the majority of the 236 fish tested in the spring (Table 12). Only 3 % of the chinook tested had antigen levels suggestive of an active infection ("POSITIVE") with 54 % incidence of no detectable antigen (Below Negative Cutoff). The IGH-s0 group had significantly higher antigen levels than all other sample groups except BBS-s2, INC-s4, RCC-s4, and BBT-s1 (Natural) (Kruskal-Wallis, $P < 0.001$). No clinical signs of Bacterial Kidney Disease were observed in any sampled fish.

Trematode metacercariae were observed in gill lamellae, heart, and kidney tissue of chinook captured in-river. The parasite was not seen in fish collected at IGH. The prevalence of metacercarial infection (**POI**) in squashed kidney preparations ranged from 0 % - 50 % (Table 12). The severity of infection, as measured by the index of "metacercaria / gram kidney", was judged to be rather light. No obvious trend in severity or POI was seen for either site or date of capture. Mean severity values ranged from 0 - 260 metacercariae / g for the out-migrant groups. In contrast, severity values of 5900 metacercariae / g are found in Trinity R. out-migrant chinook in the spring and up to 33,000 in the fall (unpublished FHC data). Metacercariae were more commonly observed lodged in gill blood vessels than in the kidney. Histological examination data from out-migrant chinook, revealed that 84 % of the gill sections (21 of 25) had metacercariae compared with only 7 % (2 of 27) of the kidney sections (Tables 13 & 14). Many of these metacercarial infections of the gill resulted in proliferation of the branchial cartilage, however, gill function did not appear to be impaired. Metacercarial infection of Trinity River chinook tend to be concentrated in the kidney (Foott et al. 1997). We presume that the trematode, *Nanophyetus salmincola*, is the parasite observed in the Trinity river, however, the preference for gill may indicate another species is affecting Klamath R. salmonids. The morphology of the Klamath R. metacercariae in both wet mount and histological preparations appears to be the same as those seen from Trinity R. fish. These parasites did not appear to be a significant health factors in the IGH spring release.

A pre-sporogonic stage of a myxosporean parasite was observed in the glomeruli and within the renal tubule lumens of 85 % of the out-migrant chinook kidney sections. It was associated with interstitial hyperplasia and glomerulonephritis. As with the metacercariae, IGH fish became infected after their release. The severity of associated lesions increased with time post-release. No genus identification was possible due to the lack of identifying spores, however, several possible candidates include *Myxidium minteri*, *Chloromyxum majori*, *Parvicapsula sp.*, and *Sphaerospora sp.* (Yasutake and Wood 1957, Kent et al. 1994). It is likely that kidney function would be impaired due to the glomerulonephritis. No gross signs of infections such as swollen or mottled kidneys were observed, however, the full course of the disease may not have been reached with the last sample group on July 18.

The myxosporean parasite, *Ceratomyxa shasta*, were observed in the liver and intestinal tract of 3 natural chinook (BBT-s1) collected on June 21 (Table 13). Each of these fish had severe enteritis (CS#2 histological category) and were anemic (Fig. 9). Early stage *C. shasta* infections were first observed in 33 % of the BBT-s2 fish. In week 3, both the POI and severity markedly increased in fish captured at BBT and INC (Fig. 9). The POI for the BBT-s4 out-migrants was 92 % and 69 % for the INC-s4 fish. RCC-s3 chinook had a 29% POI of which half of the infections were considered early stage (Fig. 9). While the POI at BBT and INC declined in the 4th week, the incidence of severe clinical signs such as anemia and hemorrhagic intestinal tracts were higher than at week 3. Five weeks after the hatchery release, the trapping crew reported a dramatic improvement in the out-migrant health condition. Of the 17 intestine samples examined from the BBT-s5 group, *C. shasta* parasites were only detected in 1 fish (6%). Systemic infections of *C. shasta* was apparent in some fish as trophozoites (pre-spore stage) were observed in blood vessels of the gill, kidney, and liver (Tables 13 & 14). The severity of Ceratomyxosis in out-migrants captured at BBT is also demonstrated by the weekly cumulative percent mortality

which jumped from 1- 6 % in late June and early July to reach 27 % in week 4. Although river temperature remained high (21.9 ° C), trap mortality rate dropped to 5 % in the 5th week .

Mild inflammatory cell infiltration could be observed in pancreatic (pancreatitis) and associated mesenteric adipose tissue (steatitis) of several IGH chinook from the June 13 pre-release sample. The incidence and severity greatly increased in the out-migrant groups (Tables 13 & 14). Inflammation appeared to originate in the periportal region of the acinar cell masses and expand outwards. The acinar cells of the pancreas was eventually replaced with granulomatus tissue in some fish collected in July. While *C. shasta* trophozoites could be observed associated with pancreatitis in week 4, this lesion was also seen in fish without detectable *C. shasta* infection. The effect on survival of this lesion is uncertain.

Table 12 Prevalence (POI) and intensity of metacercaria (Metac) and *Renibacterium salmoninarum* infection data for the June and July samples. *R. salmoninarum* antigen categories: Below Negative Cutoff (BNC), low level or Suspect (SUS), and moderate - high level or Positive (POS). Not all fish screened for metacercaria were sampled for ELISA (n= elisa samples).

	Trematode		<i>R. salmoninarum</i> antigen		
	POI Metac.	Metac. / g	BNC	SUS	POS
IGH -s0 n = 58	ND	ND	6	91	3
BBT-s1 natural n =12	ND	ND	58	42	0
BBS-s2 n = 11	0 / 11 (0)	0	50	50	0
BBT-s2 n = 32	6 / 32 (19)	24 (\pm 10)	74	26	0
BBT-s3 n = 41	9 / 41 (22)	150 (\pm 15)	87	8	5
BBT-s4 n= 19	4 / 33 (12)	92 (\pm 1)	74	26	0
BBT-s5 n = 12	3 / 12 (25)	144 (\pm 12)	75	25	0
2IC-s2 n = 9	1 / 9 (11)	190	ND	ND	ND
INC-s3 n = 15	2 / 15 (13)	98 (\pm 2)	53	47	0
INC-s4 n = 12	1 / 12 (8)	93	83	17	0
RCC-s3 n= 12	3 / 12 (25)	260 (\pm 48)	75	25	0
RCC-s4 n= 14	7 / 14 (50)	197 (\pm 27)	50	50	0
Totals (%)	36 / 192 (19)		127 / 236 (54)	101 / 236 (43)	8 / 236 (3)

ND Not done

Table 13. Histological examination results of Pre-Liberation (IGH), Natural Smolts, and Big Bar trap (BBT) and seine site (BBS) samples collected in June and July 1995. Lesions observed included steatitis (ste), pancreatitis or inflammation of the acinar cell regions (pnc), enteritis (ent), interstitial hyperplasia (ihp), and glomerulonephritis (gmn). Parasite infections of metacercaria (meta), miracidia (mira), Cestode or nematodes (helminths = helm), *Ceratomyxa shasta* trophozoites (Csha), and pre-sporogonic myxosporean in the kidney (myxo). Prevalence data reported as number of specimens positive / total number of specimens of a given tissue.

Sample Site / Date	Gill	Kidney	Liver	Intestine
IGH -s0 Lesion	0 / 5	0 / 5	0 / 5	3 / 23 ste 1 / 13 pnc
Parasites	0 / 5	0 / 5	0 / 5	0 / 31
BBT-s1 Natural Lesion	0 / 4	3 / 4 gmn	0 / 4	7 / 7 pnc 3 / 14 ent
Parasites	4 / 4 meta 2 / 4 mira	2 / 4 myxo	1 / 4 Csha	3 / 14 Csha
BBS-s2 Lesion	ND	ND	ND	2 / 4 pnc
Parasites				3 / 10 Csha
BBT-s2 Lesion	0 / 2	0 / 2	0 / 3	19 / 22 ste 13 / 16 pnc 6 / 30 ent
Parasites	2 / 2 meta	0 / 2	0 / 3	10 / 30 Csha 2 / 30 Helm
BBT-s3 Lesion	0 / 5	5 / 5 gmn	0 / 4	10 / 11 ste** 6 / 9 pnc **
Parasites	3 / 5 meta 2 / 5 Csha	5 / 5 myxo 1 / 5 Csha	1 / 4 Csha	24 / 26 Csha
BBT-s4 Lesion	0 / 4	1 / 4 gmn 3 / 4 ihp	0 / 4	4 / 5 ste 7 / 9 pnc
Parasites	0 / 4	3 / 4 myxo 2 / 4 Csha	3 / 4 Csha	11 / 18 Csha
BBT-s5 Lesion	0 / 5	1 / 5 ihp 5 / 5 gmn	0 / 5	6 / 6 ste
Parasites	3 / 5 meta	1 / 5 meta 4 / 5 myxo	0 / 5	1 / 17 Csha

ND Not done

** Most of these abnormalities associated with *C. shasta* trophozoite infection in the same tissue section.

Table 14. Histological examination results of samples collected in June and July 1995 at the mouths of Indian creek (INC) and Red Cap creek (RCC). Lesions observed included steatitis (ste), pancreatitis or inflammation of the acinar cell regions (pnc), enteritis (ent), interstitial hyperplasia (ihp), and glomerulonephritis (gmn). Parasite infections of metacercaria (meta), miracidia (mira), Cestode or nematodes (helminths = helm), *Ceratomyxa shasta* trophozoites (Csha), and pre-sporogonic myxosporean in the kidney (myxo). Prevalence data reported as number of specimens positive / total number of specimens of a given tissue.

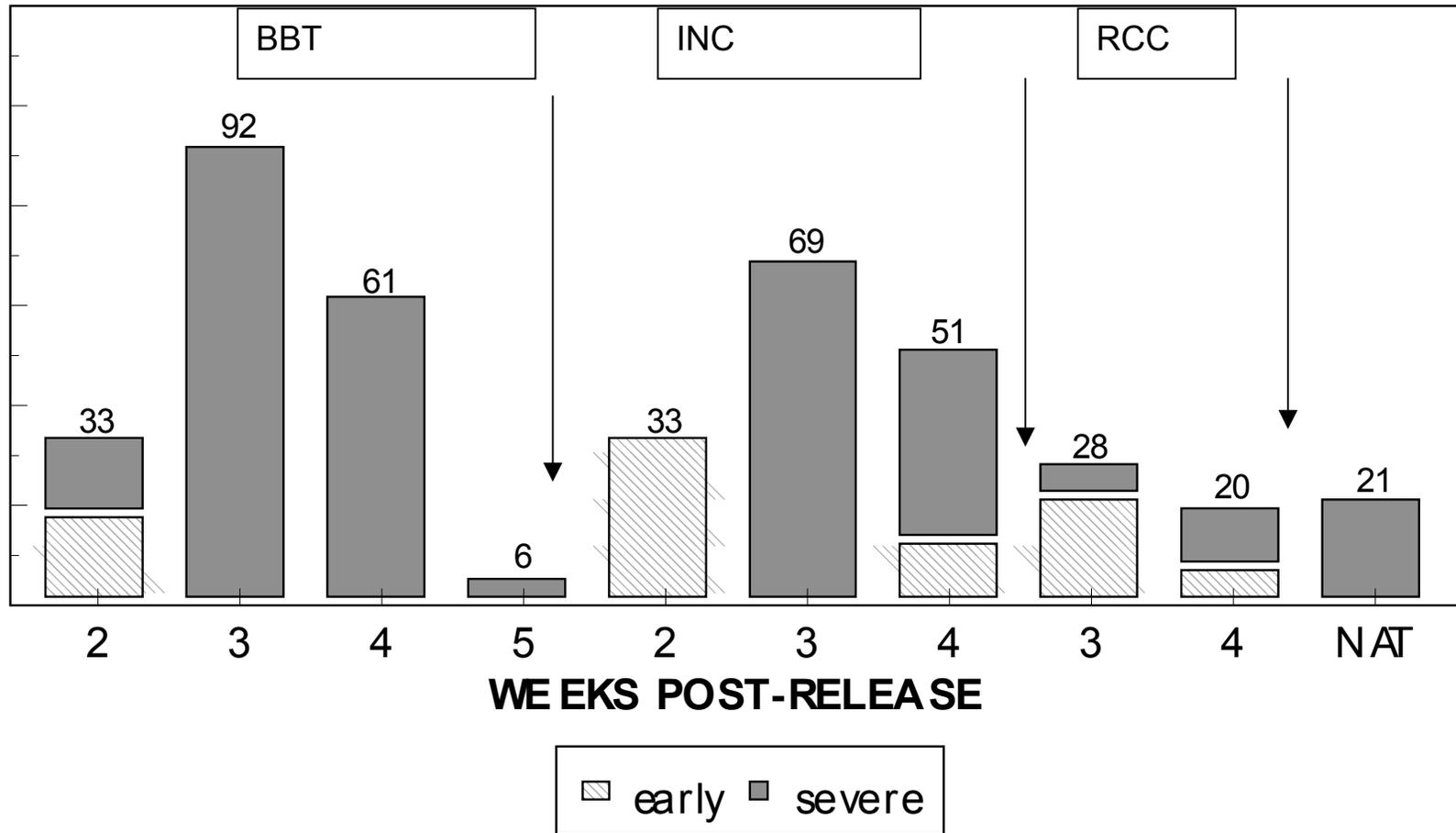
Sample Site / Date	Gill	Kidney	Liver	Intestine
2IC-s2 Lesion	ND	ND	ND	2 / 4 pnc
Parasites				3 / 10 Csha
INC-s3 Lesion	0 / 5	1 / 5 ihp 5 / 5 gmn	0 / 5	8 / 9 ste 4 / 6 pnc 11 / 16 ent
Parasites	5 / 5 meta	1 / 5 meta 5 / 5 myxo	0 / 5	11 / 16 Csha
INC-s4 Lesion	0 / 4	1 / 4 ihp 4 / 4 gmn	0 / 4	6 / 7 ste 7 / 8 pnc 6 / 16 ent
Parasites	4 / 4 meta 1 / 4 Csha **	4 / 4 myxo	3 / 4 **	8 / 16 Csha 1 / 16 helm
RCC-s3 Lesion	ND	ND	ND	4 / 5 ste 2 / 6 pnc 1 / 14 ent
Parasites				4 / 14 Csha 2 / 14 helm
RCC-s4 Lesion	ND	ND	ND	5 / 5 ste 8 / 9 pnc 2 / 15 ent
Parasites				3 / 15 Csha 4 / 15 helm

ND Not done

** Trophozoites within blood vessel of secondary lamellae.

Most of these abnormalities associated with *C. shasta* trophozoite infection in the same tissue section.

Figure 9. Incidence and severity of Ceratomyxosis in June and July samples.



Results - November Release

Sample Site Characteristics- Sample collections of yearling Fall-run chinook (Broodyear 1994) occurred 2 days prior to the November 13-15 ,1995 release and from the Klamath River below the confluence of Indian Creek and at the Hwy 96 bridge at Orleans. In addition to the 904,107 yearling chinook reared and released from IGH, another 172,338 yearlings raised at the Fall Creek satellite facility were released during the 13-15 November period. Approximately 8% of the 1 million release group were adipose fin marked and coded-wire tagged (CDFG planting receipt KS-94-P3).

When water from the hatchery’s supply was sent to the spawning facility in October, flows to production raceways declined and dissolved oxygen levels in the lower section went below 3 ppm . This occurrence was associated with an acute mortality incident. Flows to the raceways were increased and on October 16 oxygen concentration of the raceway effluent was measured at 5 ppm (pers. comm. Mel Willis, CDFG pathologist, Redding, Nov. 1995). During the pre-release examination, dissolved oxygen averaged 3.8 ppm in lower sections of the raceways and 9.1 ppm in the intake water. In the 3 units tested, there was a 5.5 - 6.7 ppm drop in oxygen concentration from the top to the bottom of the raceways. This large oxygen demand indicated too high of a biomass in the raceways. Water temperatures at the 3 in-river collection sites ranged from 9.5 - 10.5°C and dissolved oxygen was ≥ 11.0 ppm (Table 15). Total gas saturation was below harmful levels at all sites and ranged from 100.7 - 102.0 %.

Table15.

Water quality measurements at Fall collection sites. Water temperature and dissolved oxygen (D.O.) measured at 0.3 meter depth. Total dissolved solids (TDS, ppm), pH, and total percent gas saturation (%SAT.) measured at the surface.

	TEMP(° C)	D.O. (ppm)	pH	% SAT.	TDS
IGH-f0	11.3	3.8 **	7.2	100.7	90
INC-f1	10.3	11.0	8.1	101.4	110
ORL-f2	10.5	11.6	8.2	102.0	80
ORL-f3	9.5	ND	7.8	102.0	60 +

** Measurement from lower raceway section. Intake concentration was 9.1 ppm. There was a 5.5 - 6.7 ppm drop from top to bottom in the chinook raceways.

+ Heavy rains on this date.

Morphometries and Organosomatic Data- There was no significant difference between fork lengths of the pre-release group (mean 157 ± 2 mm) and the 3 in-river capture groups (Table 16). The hatchery was conducting an informal feed comparison between a standard semi-dry salmon diet and a floating diet with the yearling chinook production in 1995. Chinook in 2 raceways were fed the floating pellets (C and D units) while the other 4 chinook raceways received the sinking pellet (E-H). Condition factors of the 2 diet groups were similar (1-way ANOVA, P> 0.05) as were the subjective visceral fat scores. Data from the 2 diet groups were pooled for all analyses. By the 2nd week post-release, condition factor had declined and remained low in the ORL-f3 group (Kruskal-Wallis ANOVA on ranks, P< 0.001).

Table 16. Fork length, weight, and condition factor (wt.(g) / total length (mm)³) of November release group. Data reported as mean (\pm SEM).

	Fork length (mm)	Weight (g)	Condition Factor
IGH-f0	157 (\pm 2)	46.2 (\pm 2.3)	0.9807 (\pm 0.0128) ^a
INC-f1	147 (\pm 6)	39.8 (\pm 5.3)	0.9339 (\pm 0.0273) ^a
ORL-f2	153 (\pm 3)	38.0 (\pm 2.4)	0.8267 (\pm 0.0117) ^b
ORL-f3	157 (\pm 2)	41.4 (\pm 1.9)	0.8674 (\pm 0.0072) ^b

ab Significantly different ($P < 0.001$, Kruskal-Wallis ANOVA on ranks).

Scale loss was observed in 20 - 29 % of the fish sampled at IGH and the two ORL collections, however, only 4 fish had loss in excess of 20% of the body surface. Seven of the eleven INC-f1 fish had some level of scale loss. Grey swollen kidneys, caused by Bacterial Kidney Disease (BKD), were observed in 2 to 6 fish of each down-river capture group.

Energy Reserves- Visceral fat tended to decline post-release while plasma triglyceride concentration (TG) was more variable. The ORL-f3 fish showed a significant decline in energy reserves. In comparison, energy reserves of the ORL-f2 fish were similar to pre-release levels. The percentage of fish observed to have visceral fat in the peritoneal cavity or on the pyloric ceca declined from 100% in the fed IGH-f0 fish to 38 % in the ORL-f3 sample group (Table 17). Plasma TG values for both the INC-f1 and ORL-f3 groups were significantly lower than either the IGH-f0 or ORL-f2 groups (Table 17). Mean TG ranged from 37.2 to 90.7 mg / dL. Only the ORL-f3 group had fish with TG levels below the 15 mg / dL analyzer cutoff. Liver glycogen showed a similar pattern as plasma triglyceride (Table 18). Both the INC-f1 and ORL-f3 liver glycogen values were significantly lower than the IGH-f0 and ORL-f 2 groups. The HSI of all out-migrant groups were similar to the IGH-f0 fish. Liver weights of the sample groups were similar with the mean weight ranging from 0.419 - 0.453 g. Mean liver percent moisture (\pm SEM) for the INC-f1, ORL-f2, and ORL-f3 was 73% (\pm 2%), 76% (\pm 2%), and 75% (\pm 1%), respectively. Statistical tests were not performed due to the small sample size, however, no obvious trend was observed in this data. These moisture values are similar to that reported for whole bodies of Atlantic salmon smolts (Shearer et al., 1994). Greater than 91 % of each out-migrant group were observed to have food in the intestinal tract.

Plasma glucose was measured as both an indicator of a fish's response to capture stress and as an indicator of liver glycogen (primary source of blood glucose). The ORL-f3 fish showed a significantly lower blood glucose response than the other sample groups (Table 18). Both the mean (59 mg / dL) and low percent (22 %) of sample with a normal stress glucose response level differed from the other sample groups. Different capture conditions and time from capture to bleeding limits the significance of this observation.

Table 17. Plasma triglycerides (mg / dL) and percent of sample observed to have visceral fat (% VFAT > 0) of November release group. Plasma triglycerides data reported as mean (\pm SEM) and prevalence of samples below analyzer range of 15 mg / dL (% <15).

	Triglyceride		% < 15	%VFAT > 0
IGH-f0	90.7 (\pm 4.5) n = 12	a	0	100
INC-f1	40.9 (\pm 7.3) n = 11	b	0	64
ORL-f2	89.2 (\pm 11.0) n = 11	a	0	64
ORL-f3	37.2 (\pm 3.5) n = 13	b	15	38

a&b Significantly different ($P < 0.001$, Kruskal-Wallis ANOVA on ranks).

Table 18. November release group liver glycogen (mg polysaccharide{PS} / 100 mg liver), hepatosomatic indices (HSI), and plasma glucose response after capture stress. Data reported as mean (\pm S.E.M.).

	HSI	Liver Glycogen PS mg/100mg liver	Plasma Glucose mg / dL	≥ 90 mg/dL
IGH -f0	0.991 (\pm 0.032)	2.635 (\pm .485) a	96 (\pm 5) a	8 / 10 (80%)
INC-f1	1.161 (\pm 0.264)	0.555 (\pm 0.101) b	134 (\pm 36) a	5 / 6 (83%)
ORL-f2	1.179 (\pm 0.061)	1.024 (\pm 0.188) a	90 (\pm 7) a	6 / 10 (60%)
ORL-f3	1.089 (\pm 0.070)	0.500 (\pm 0.088) b	59 (\pm 9) b	2 / 9 (22%)

a&b Significantly different ($P < 0.001$, Kruskal-Wallis ANOVA on ranks).

Smolt Development - The November release group appeared to have undergone smolt development and showed some tendency for increased hypoosmotic regulation following their release. While no statistically significant differences in gill ATPase activities were detected (ANOVA, $p = 0.105$) between the sample groups, the ORL-f2 group had both the highest mean and maximum activity (Table 19). High intra-assay variability (coefficient of variation 22 - 56 %) probably influenced the statistical analysis and limits the precision of this indicator for estimating a population's state of development. There was no correlation between the fork length of the fish and its gill ATPase activity ($r^2 = .002$). All fish held for 24 hrs in saltwater at IGH and Orleans (NOV 28) survived the challenge, however, 39 % of the IGH fish and 21 % of the ORL - f2 fish showed poor ionic control with plasma sodium levels below 174 mmol / L (Table 19). There was some correlation between larger fork length and lower plasma sodium levels in the IGH challenge ($r^2 = 0.33$) but not at ORL ($r^2 = 0.03$). The salinity for the 3 challenge containers at IGH was 26 ppt. The ORL challenge container salinities were 31, 33, and 35 ppt. It was

interesting that despite the higher salinities (target salinity was 29 - 30 ppt) at ORL, only 3 of 14 challenged fish showed poor hypoosmotic regulation.

Table 19. **Smolt development.** Gill adenosine triphosphatase activity (ATPase = $\mu\text{mole ADP} / \text{mg protein} / \text{hr}$), percent survival in a 24 hr. 26 - 35 ppt saltwater challenges (% SW Surv.), and percent of osmoregulatory-stressed survivors with plasma sodium greater than 174 mmol/L (% >174) data. ATPase activity of the 11 - 28 fish samples reported as Average \pm SEM and maximum value.

	ATPase Mean \pm SEM	ATPase Maximum	%SW Surv.	% >174
IGH-f0	2.203 (\pm 0.258)	3.933	100	39
INC-f1	1.461 (\pm 0.258)	2.468	ND	ND
ORL-f2	2.253 (\pm 0.202)	5.166	100	21
ORL-f3	1.843 (\pm 0.208)	3.587	ND	ND

ND Not done.

Blood cells and Immune defenses- The erythrocyte concentration, as indicated by hematocrit values, declined in the yearlings after their November release. While the hematocrit of the ORL-f3 group was significantly greater than the previous two in-river collection groups (Kruskal-Wallis, $P < 0.001$), it was lower than the IGH-f0 sample group (Table 20). Only 2 fish captured post-release were judged to be anemic ($\text{HCT} < 25$, pale gills). In contrast, the leukocrit of the ORL-f3 group was significantly lower than all other sample groups. It is tempting to speculate that the 21 % incidence of high *R. salmoninarum* antigen detected in this group was related to this lowered white blood cell count. High antigen levels generally are associated with a disease state. The phagocytic response observed in the kidney cell preparations was not significantly different among the groups (Table 21). Both the number of active phagocytes (Phagocytic index = PI) and their phagocytic capacity (PC) were similar among the sample groups. Even when fish with high *R. salmoninarum* antigen were compared with low antigen fish, there was no significant difference in the PI or PC (T-test, $P = 0.133$). The 3 μm target sphere used in the Fall smolt phagocyte assay was much easier to count than the 1 μm sphere used with the Spring fish.

Plasma protein concentration was significantly lower in the post-release samples (Table 22). The plasma protein fractions, somewhat analogous to human Beta and Gamma fractions (globulin 5 and 6 / 7), were significantly elevated in the ORL-f2 fish in comparison to the IGH-f0 group (ANOVA $P < 0.05$, Table 22). Despite this globulin protein increase in the ORL-f2 group, the albumin : globulin ratio (A/G) did not change significantly in any post-release group. Unfortunately, only the sample group of any particular plasma sample was recorded and thus no correlation with a fish's *R. salmoninarum* infection can be made with plasma protein data. It is tempting to speculate that smolts with active infections of *R. salmoninarum* were included in the plasma sample set and that the globulin 5-7 increase was in response to this infection. Unlike the profile showed by the spring collection fish, the November release chinook had distinct globulin 4 and 5 fractions. Also, the yearling smolts showed less variability (coefficient of variation < 30) than observed in the spring samples. The pre-albumin fraction of the IGH-f0

sample group was higher than all the other fish and was similar in concentration to albumin. It is likely the cause of the high total plasma protein measurements for the IGH-f0 fish.

Table 20. Hematocrit (HCT), leukocrit (LCT), plasma total protein (T.PRO.), and percent of plasma samples less than analyzer cutoff of 15 g / dL (%<15) of the November release groups. Data reported as mean (\pm S.E.M.) and number of samples per test (n).

	HCT	LCT	T.PRO. g / dL	T.PRO. % < 15
IGH -f0 n = 20	49 (\pm 1) a	1.193 (\pm 0.068) a	3.4 (\pm 0.1) a n = 9	0
INC-f1 n = 11	35 (\pm 2) c	0.917 (\pm 0.095) a	2.9 (\pm 0.1) b n = 11	18
ORL-f2 n = 27	39 (\pm 1) c	1.160 (\pm 0.061) a	3.0 (\pm 0.2) b n = 4	0
ORL-f3 n = 30	42 (\pm 2) b	0.623 (\pm 0.063) b	2.7 (\pm 0.1) b n = 9	11

a-c Significant differences between groups (P<0.001).

Table 21. Phagocytic response of glass-adherent cells (GAC) from the kidneys of the November release group. Phagocytic index (GACs with phagocytized targets / 200 GACs and phagocytic capacity (number of GACs with > 5 targets / 200 GACs) reported as mean (\pm SEM).

	Phagocytic Index	Phagocytic Capacity
IGH -f0 n = 8	0.703 (\pm 0.0.28)	0.296 (\pm 0.0.36)
INC-f1 n = 10	0.711 (\pm 0.043)	0.401 (\pm 0.060)
ORL-f2 n = 12	0.830 (\pm 0.034)	0.378 (\pm 0.065)
ORL-f3 n = 10	0.742 (\pm 0.042)	0.455 (\pm 0.055)

Table 22. Plasma protein fractions (para-albumin, albumin, globulin fractions 1-7) of November release fish. Data reported as mean (\pm SEM) of percent area of electrophoretic bands from 7 fish per group.

	IGH-f0	INC-f1	ORL-f2	ORL-f3
Para-Albumin	30.9 (\pm 1.4) ++	22.0 (\pm 1.1)	16.5 (\pm 2.1)	20.1 (\pm 1.4)
Albumin	31.6 (\pm 1.3)	34.7 (\pm 2.2)	35.8 (\pm 2.5)	38.7 (\pm 1.9)
Globulin-1	9.2 (\pm 0.3)	8.9 (\pm 0.4)	10.1 (\pm 1.3)	10.1 (\pm 0.9)
Globulin-2	5.2 (\pm 0.3)	4.5 (\pm 0.4)	6.1 (\pm 0.8)	5.7 (\pm 0.4)
Globulin-3	6.2 (\pm 0.3)	6.9 (\pm 0.4)	7.5 (\pm 0.6)	6.9 (\pm 0.4)
Globulin-4	8.8 (\pm 0.9)	12.0 (\pm 0.8)	12.1 (\pm 1.3)	10.1 (\pm 0.8)
Globulin-5	4.5 (\pm 0.6)	6.6 (\pm 1.0)	7.7 (\pm 0.5) **	5.3 (\pm 0.3)
Globulin-6/7	3.7 (\pm 0.8)	4.4 (\pm 1.3)	4.3 (\pm 0.3) **	3.1 (\pm 0.4)

++ Significantly greater than all other groups (P <0.05).

** Significantly greater than IGH-0 and ORL

Histopathology and pathogen assays - In the fall of 1995, the yearling chinook production at IGH began to experience mortality associated with BKD (pers. comm. Mel Willis, CDFG pathologist, Redding 10Oct95). The incidence of *Renibacterium salmoninarum* (**RS**) infection, by ELISA detection of RS antigen in the kidney, ranged from 82 - 92 % in the 4 sample groups. Of these antigen positive fish, 69 - 82% showed only low-level infections (Table , SUSPECT rating). While the incidence of high antigen (POSITIVE) fish increased from 8% in the pre-release to 21% in the 4th week post-release sample, there was no significant difference in antigen concentrations among the sample groups (Kruskal-Wallis ANOVA log O.D. ranks , P=0.4572). Histological examination of kidney, heart, and pancreatic tissues revealed granulomatous lesions characteristic of BKD in 23% of all kidney sections examined and in both the heart and pancreatic tissues of two 05DEC fish (Table 23). It appears that \leq 25 % of the release group was suffering from clinical BKD. These fish would not likely survival A much higher percentage of the release group was infected and could later develop the disease.

Table 23. Prevalence (POI) and intensity of infection data for the November group. Metacercaria (Metac) infection data reported as number infected / total sample (%) and calculated number of metacercaria per gram kidney tissue (Metac. / g). *Renibacterium salmoninarum* antigen data reported as % of sample group in the 3 antigen categories: Below Negative Cutoff (BNC), low level or Suspect (SUS), and moderate to high level or Positive (POS).

	Trematode		<i>R. salmoninarum</i> antigen		
	POI Metac.	Metac. / g	BNC	SUS	POS
IGH-f0 n = 62	ND	ND	15 %	77 %	8%
INC-f1 n = 11	0 / 11 (0)	0	18 %	82 %	0 %
ORL-f2 n = 27	2 / 27 (7)	8.0 (\pm 6.6)	8 %	76 %	16 %
ORL-f3 n = 42	15 / 42 (37)	44.7 (\pm 11.5)	10 %	69 %	21 %

Light metacercarial infections were observed in the out-migrants as early as 2 weeks post-release. The severity of infection, as estimated by metacercaria / g kidney and associated histological lesions, was judged to be mild in comparison to chinook collected from the Trinity River. Both the incidence of infection (IOI) and severity of infection increased with time post-release. In the 3rd week sample group, the IOI was 37 % with the mean severity (\pm SEM) of 45 (\pm 12) metacercaria per gram of kidney (Table 23). Metacercaria were seen in histological sections of kidney (IOI: 8/ 33= 24%), gill (IOI: 8/30= 27%), and heart (05DEC: 3 / 11 = 27), however, no significant lesions were associated with the trematode infections (Table 24). No *Ceratomyxa shasta* parasites were seen in 39 intestinal tract sections collected from the out-migrants. Inflammation of the adipose tissue (steatitis) was observed in 21 % (5 of 24) of the intestinal tract sections while 8 % of the sections containing pancreatic tissue showed signs of similar inflammation (Table 24). Because of the close association of adipose tissue, it is unclear if the pancreatitis was related to the steatitis.

Table 24. Histological examination results for November release group. Metacercarial infection (meta), glochidia (gloc), interstitial hyperplasia (int-hp), granuloma (grn), pancreatic inflammatory (pnc), inflammatory cell response (icr) , and steatitis (ste). Data reported as number positive / number examined.

	IGH-f0	INC-f1	ORL-2	ORL-3
Gill				
Parasite	0 / 10	1 / 3 meta	4 / 9 meta	3 / 18 meta
Lesion	0 / 10	0 / 3	0 / 9	1 / 18 gloc 2 / 18 hp
Kidney				
Parasite	0 / 10	0 / 8	2 / 7 meta	6 / 18 meta
Lesion	0 / 10	1 / 8 int-hp	1 / 7 int-hp	8 / 18 hp-grn
Liver				
Parasite	0 / 7	ND	ND	ND
Lesion	0 / 7			
Heart				
Parasite	0 / 7	ND	ND	3 / 11 meta
Lesion	0 / 7			2 / 11 icr**
G.I. Tract				
Parasite	0 / 10	0 / 11	0 / 10	0 / 18
Lesion	0 / 10	2 / 11 pnc 2 / 11 ste	1 / 10 pnc	2 / 18 icr** 3 / 9 ste

ND Not done

** Lesions in pancreatic tissue and heart from same 2 fish which showed signs of BKD

Discussion

The brood year 1994 Fall-run Chinook released from IGH in June of 1995 were affected by a number of factors which reduced their fitness for out-migration success. In both the spring and fall, apparent higher than optimal biomass in the raceways resulted in low dissolved oxygen (DO) concentrations. Low D.O. is reported to induce a general stress response in salmonids and would negatively affect growth (Wedemeyer 1996, Soderbeg 1994). Small size at release was another negative factor for the spring release group. The high proportion of spring release IGH fish less than 80 mm in fork length is in contrast to the consistently larger (≥ 88 mm) fish captured in the river. Sample technique biases (random dipnet "grabs" at IGH raceways compared to capture by rotary screw trap or beach seining) could have biased downriver capture toward larger fish, however, it is generally accepted that larger juveniles migrate more readily and have higher survival rates than "presmolt" fish. Coded wire tagged smolt groups of various fork lengths have shown that smaller chinook (< 90 fish / lbs {or smaller than 4.5 - 5.0 g ,77 - 79 mm FL}) out-migrant at a slower rate, were captured down-river at significantly lower numbers (indicating poor survival), and returned at 3.5 - 7x lower rate than larger cohorts (M. Wallace, Oct 10, 1997 letter to Iron Gate Hatchery). Wallace and Collins (1997) reported that

mean fork length of chinook juveniles captured in the Klamath estuary during the early summer of 1993 and 1994 ranged from 85.4 - 87.6 mm. All efforts to achieve larger size by late May, such as heating egg incubation water and rearing fish on warmer surface water from the reservoir, should be explored for improving out-migrant success. The larger November yearling chinook did not show any significant differences in fork length between pre-release and out-migrant groups.

It is assumed that the majority of the unmarked chinook collected during the spring out-migration at BBT and INC were IGH fish. This assumption is based on the fact that they were captured with marked fish which made up the bulk of the collection (59%) and were of similar morphology to these marked IGH fish. It is tempting to speculate that the chinook juveniles collected on both July 6 or 11 at Red Cap Creek were natural fish as no marked fish were collected from either sample. Also the Red Cap fish had better condition factors and much lower incidence and severity of *Ceratomyxa shasta* infection than other groups collected during the same period.

Condition factor of the IGH-s0 group was relatively low for fed underyearling hatchery fish (mean of 0.800). For comparison, mean condition factors of June release groups in 1992, 1993, and 1994 were 0.8254, 0.7876, and 0.9551 respectively (FHC pre-release organosomatic data, unpublished). Statistically significant differences were only detected between 3 lower condition factor groups (IGH-s0, BBS-s2, and BBT-s5) and the high condition factor INC-s2 group (mean 0.917). The IGH-s0 fish were also significantly lower than the INC-s4 and RCC-s3 groups. In spite of the rapid decline in plasma triglyceride and visceral fat stores, the relative weight of the chinook captured at INC and BBT in weeks 3 and 4 post release tended to increase with time as did the associated condition factors. No clear explanation for this trend is apparent. Four factors, which could affect relative fish weight, were considered as cause(s) for the increase in condition factors:

- a) Artifact due to stress of capture and loss of osmotic balance / water gain - Logistics resulted in > 1hr between capture and necropsy in several groups, however, high condition factors were also measured in fish necropsied within 10 minutes of capture.
- b) Presence of food in fish (added weight) at capture - No relationship was observed in the percentage of fish with food in the gastro-intestinal tract and condition factor.
- c) Osmotic imbalance due to severe Ceratomyxosis - The highest condition factors were detected in groups with lower incidence of infection.
- d) Small sample bias - As the average number of fish collected at any one site was 15, it is quite possible that weight measures do not adequately represent out-migrants in the river at any given time .

Condition factors of the RCC fish were larger than cohort BBT out-migrants in weeks 3 and 4 post-release. It is possible that the majority of RCC fish could have been natural chinook given their higher condition factors, low incidence of both *C. shasta* infection and severity, and absence of adipose-clipped fish in both RCC collections. In the November release fish, condition factors were high at the hatchery and declined during the out-migration period.

It is unclear why HSI of the spring release increased from $\leq 1.1\%$, in both the IGH-s0 and all 2 week sample groups, to over 2% in INC and BBT fish collected at weeks 3- 5 post-release. HSI values for the RCC fish were intermediate with the mean HSI ranging from 1.9% to 1.5%. Neither glycogen content or severity of *C. shasta* infection (possible cause of liver edema) correlated with HSI. The precision of the spring HSI data was probably affected by the small liver weights (means ranged from 0.04 g {IGH-s0} to 0.208 g {BBT-s5}) and error in using a single tare weight for a group's sample tubes. The high degree of variation in liver weights compared with body weights suggests measurement error. The coefficient of variation for spring liver weights ranged from 15 - 48% (mean of 32%) while the coefficient of variation in body weights ranged from 11 - 28% (mean 21%). The HSI values of the November group did not significantly change for the 3 week period following release and stayed in the 1.0 - 1.2% range.

The spring out-migrants showed variable plasma glucose (GLU) response to capture stress. Both GLU and liver glycogen content would be influenced by the duration of the stress response between capture and collection of the blood sample. Feeding history has also been shown to influence GLU response in juvenile chinook. Barton et al. (1988) reported that chinook fasted for 20 days had significantly lower GLU response to stress than fed cohorts. The stress-related hyperglycemia (increased GLU) is initiated by epinephrine's effect on converting liver glycogen to glucose (glycogenolysis). Plasma glucose in stressed juvenile chinook will typically increase from a basal 50 mg/dL to over 80 mg/dL within an hour (Sharpe et al. 1998). In hatchery chinook juveniles, we often observe GLU values of ≥ 90 mg/dL within 15 minutes of capture stress. In the pre-release sample, fish were netted out of a raceway and sampled for blood in less than 15 minutes. This rapid sampling was probably responsible for the relatively low GLU values as liver glycogen was quite high for the IGH-s0 fish. If BBT out-migrants are compared, the week 2 fish showed a typical hyperglycemia response. This ability dropped significantly in the BBT-s3 and s4 groups but returned again in the BBT-s5 fish. The liver glycogen reserves in these BBT-s5 fish were also similar to the BBT-s2 groups. Limited rearing in thermal refugia may help explain how these BBT-s5 fish maintained liver glycogen when the Klamath River was over 19 °C. Any glycogen sparing effect of refugia rearing was not apparent in the INC and RCC fish. Chinook captured at both sites tended to have low glycogen and GLU, however, the data was variable. Differences in the time between blood collection and capture probably affected the data from these spring groups, however, histological examination showed little to no glycogen vaculization in the hepatocytes. One performance consequence of low liver glycogen would be a poor escape respond to multiple stresses (such as consequentive predator attacks). The November yearlings showed a less dramatic drop in liver glycogen and plasma GLU than the spring smolts. Given the similar glycogen and GLU values of both the spring and fall pre-release fish, it likely that that cooler November water temperatures were responsible for the difference in carbohydrate reserves between the respective out-migrant groups.

Many of the energy measurements (plasma TG, HSI, liver glycogen, plasma GLU) for the spring fish are suspect due to a number of factors involved in sample collection, small size of the fish, and laboratory methods. Plasma TG is influenced by the time between the blood sample and feeding, lipid content of diet, and degree of lipolysis activity in the adipose tissue. Future monitoring studies will use the whole body lipid content (% lipid) as a more robust estimator of energy reserves. The unknown feeding history of the fish (and time of captivity for BBT fish) collected down river makes comparison of TG values difficult, however, the general trend was for low plasma TG in fish collected downriver during the spring. In contrast, the

November fish maintained relatively higher plasma TG following release. Visceral fat also tended to remain longer in the November out-migrants compare with the spring release fish. The ORL-f2 was somewhat of an anomaly in their high energy measurements. Both TG and liver glycogen were similar to the pre-release sample and significantly higher than the INC-f1 and ORL-f3 groups. Lipid reserve indicators, such as visceral fat quantity (VFAT) and plasma triglyceride (TG), declined rapidly after the spring IGH release. The elevated water temperatures experienced by the spring release group would have acted to increase their energy demands during out-migration (Brett 1976). A high proportion of each in-river spring collection group had circulating TG levels below the 15 mg/dL detection limit of the TG assay. Given the high energy demands of both out-migration and elevated water temperatures, smolt survival could be enhanced by producing chinook juveniles in the spring with higher body fat.

The BBT-s2 fish may have been a "lead" sub-group of the entire June release as they showed advanced smolt development with gill ATPase activities significantly higher than the later BBT collection groups. Unfortunately, no SW challenges were conducted on this group to help corroborated the ATPase data. Hart et al. (1981) reported that juvenile migrant spring chinook had significantly higher levels of gill ATPase than non-migrant cohorts. The poor SW challenge survival observed in the BBT-s3 and BBT-s4 fish was probably related to both high river temperatures and their *C. shasta* infections. The November yearlings appeared to have undergone smolt development. In particular, the ORL-f2 had high ATPase values and successfully osmoregulated in high saline (31-35 ppt) challenges. Due to the high intra-assay variability of the 1995 ATPase assays, the data should only be viewed for general trends and not as highly accurate estimates of an individual's enzyme activities.

The effect of Ceratomyxosis was apparent in several of the blood measurements taken from the spring out-migrant fish. Hemorrhage associated with the disease resulted in the severe anemia and low plasma protein values observed in the 3rd and 4th week chinook at both BBT and INC. The increased numbers of circulating white blood cells (leukocrits) and the changes in plasma protein composition reflect the immune system's response to this infection. The increase in globulin 3 fraction and decrease in "albumin-like" fractions (drop in A/G ratio) indicates that the fish were shifting production to acute phase proteins. Several acute phase proteins such as alpha-2 macroglobulin, ceruloplasmin, and alpha-antitrypsin have been identified in salmonids (Yano, 1996). While spring fish phagocyte assays were difficult to interpret, there was some indication of reduced phagocyte numbers in the *C. shasta* infected BBT fish. Peters et al. (1991) reports that stress initially increases the number and activity of rainbow trout phagocytes, however, these phagocytes soon die and are not completely replaced by new cells. Similarly, cortisol reduced the ability of plaice to produce an inflammatory cell response (MacArthur et al. 1984). Following acute stress, both resistance to infection and the ability to mount an immune response declined in chinook juveniles (Maule et al. 1989). Thus stress situations eventually reduce the non-specific and specific immune defenses of fish.

The November release fish showed no significant signs of immunodefense problems. The drop in leukocrit experienced by the ORL-3 group may have been related to their *R. salmoninarum* infections or another stress-induced (high cortisol) leukopenia. The similar phagocyte indices between the November group indicates that the non-specific immune system was not compromised during the out-migration. Unlike the spring out-migrants, few yearlings had low plasma proteins or were anemic.

Disease was a significant mortality factor in the Broodyear 1994 IGH chinook population in 1995. The spring out-migrants suffered a very high incidence of severe Ceratomyxosis and the fall release group had a 20 - 25 % incidence of clinical Bacterial Kidney Disease. It is highly likely that a majority of the chinook infected with *C. shasta* perished in the summer of 1995. Direct mortality due to the disease is not the only outcome of Ceratomyxosis. The hemorrhagic anemia associated with advanced Ceratomyxosis would weaken the fish and increase its chances of predation. Mesa (1994) reports how chinook juveniles with clinical BKD infections suffer a higher rate of predation than non-infected cohorts. Entry into the ocean would not aid the infected fish as Ceratomyxosis is reported to continue after entry of infected salmonids into saltwater (Kent et al. 1994). Given both the high BBT trap mortality and incidence of parasite infection, it appears that Ceratomyxosis significantly reduced survival of the 1994 broodyear IGH chinook smolts. The detection of natural chinook with severe Ceratomyxosis on June 21 suggests that both hatchery and natural populations were affected by this disease even though water temperatures in mid-June were well within acceptable ranges for juvenile salmonids. Most salmonids are susceptible to varying degrees of Ceratomyxosis (Johnson 1980). Many workers describe steelhead and salmon stocks from enzootic waters being generally more resistant to Ceratomyxosis than naive stocks (Zinn et al. 1977, Ching & Munday 1984). The genetic component to this resistance has been demonstrated in a reciprocal crossing experiment with F1 trout from resistant and susceptible stocks (Ibarra et al. 1992). This innate resistance could be overwhelmed by long-term exposure to infectious water or intraperitoneal injection of the parasite (Ibarra et al. 1991). It is unclear what defensive mechanisms inhibit the multiplication and dissemination of the parasite in resistant stocks. Fryer (1987) reports that naturally infected rainbow trout did not produce specific antibody to the parasite and that exclusion from the lower intestinal epithelium may be an important resistance mechanism. Hendrickson et al. (1989) reports that the infective stage of *C. shasta* is found in the Sacramento, San Joaquin, Pit, and Klamath river systems. In the Klamath basin, juvenile salmonid infection with *C. shasta* occurs in the Klamath R. but not in the Trinity River (FHC monitoring data 1991 - 1998, Hendrickson et al. 1989). The parasite has a limited geographic range in spite of the occurrence of infected adult salmonids migrating into non-infective waters. The lifecycle of *C. shasta* apparently involves an alternate host found in specific waters. Bartholomew et al. (1992) reported that an actinosporean released from the oligochaete *Nais bretscheri* (found within the algal community attached to freshwater mussels in the Willamette River) could infect trout with *C. shasta*. Natural infections are reported to occur when water temperatures are above 7 °C and in the lower Klamath River began in April and ceased after December 1987 (Hendrickson et al. 1989). No *C. shasta* parasites were detected in the November release group examined in our study.

In response to the Ceratomyxosis data gathered in 1995, Mel Willis (CDFG Fish Pathologist) conducted a challenge experiment with IGH chinook in July 1996 (August 1, 1996 memo, Appendix 1). Chinook were held in a *C. shasta* infective water source at Crystal Lake State Fish Hatchery for over 40 days (Noble 1950). The temperature of this water source does not exceed 16 °C. There was no mortality or clinical signs observed in the chinook group. A cohort group of rainbow trout juveniles (Shasta strain susceptible to Ceratomyxosis) held at the same site developed clinical symptoms and experienced mortalities. Histological examination of several chinook at the end of the challenge did not reveal any *C. shasta* parasites. IGH steelhead challenged at the same site for 85 days were also resistant to infection and disease. When water temperatures are under 16 - 17 °C, it appears that Klamath R. salmonids are more likely to be resistant to Ceratomyxosis. Increased water temperatures have been shown to

inversely effect the survival of salmonids infected with *C. shasta* (Ching & Munday 1984, Udey et al. 1975). The mean time to death (MTD) dropped from 45 - 51 days at 5 - 6 °C to only 27 days at 17 °C (Ching & Munday 1984). Coho juveniles held at 23.3 °C had a geometric mean time to death of 12.5 days post -exposure which increased to 38.5 days at 17.8 °C (Udey et al. 1975). The percent mortality of exposed coho held at 15 °C or below was ≤ 21 % but jumped to 53 % at 17.8 °C and 84 % at 20.5 °C. A further example of the key role water temperature plays in the host : parasite relationship was also demonstrated by Udey et al. (1975). At temperatures below 6.7 °C, exposed Coho were completely resistant to Ceratomyxosis (no deaths or spore detected). This inability to induce disease or sustained infection (spore production) in Coho was likely due to host defense mechanisms and not temperature inhibition of parasite. Susceptible rainbow trout at the same temperature did die of Ceratomyxosis. The advantage of lower water temperatures support any efforts to accelerate growth of the IGH chinook (e.g. heating egg incubation water, using shallower reservoir water in late spring for juvenile rearing) so as to release 70 – 80 mm FL smolts in late May.

The inflammation observed in the adipose and acinar cell regions covering the pyloric caecae could be in response to a number of stimuli. Viral infections, such as Pancreas disease and IPN virus, are reported to induce such lesions in Atlantic salmon and rainbow trout (McLoughlin 1997), however, other clinical signs of viral infection were absent from our study chinook nor has virus been isolated in out-migrants from the basin (1991 - 1998 FHC data, unpubl.). A similar case could be made for bacterial or parasitic infections. Again, no consistent associated pathogen infection could be linked to this inflammation. The feeding of rancid fish diets can induce an inflammatory infiltration of the peripancreatic tissue (Roberts et al 1979), however, other histopathological changes to the liver, heart, muscle, and swimbladder were not seen in the our study. The enzymatic mobilization of lipids from the mesenteric adipose cells could result in the associated release of arachidonic acid from the cell membrane phospholipids. Arachidonic acid can be converted to a number of potent chemotactic factors for phagocytes such as leukotrienes and salmonids are reported to generate such agents (Secombes 1996). Once phagocytes are recruited to the region they would release other chemotactic factors (cytokines) which would further enhance the inflammatory response. The observation of leukocytes migrating from blood vessels in the adipose / pancreatic regions of smolts collected early in the study lends some evidence to an initial release of a chemotactic factor(s) . While high spring water temperature appears to increase both the incidence and severity of this lesion, a few November chinook also had this inflammation. Elevated cortisol levels due to stress could also effect the severity of the inflammation. Manso et al. (1995) report that hydrocortisone exacerbated pancreatitis in rats. It is unclear whether pancreatic functions are significantly reduced or if this condition has a negative effect on smolt survival. We have not located any references to either steatitis or pancreatitis in salmonid smolts to help interpret how “abnormal” these lesions are for smolts.

A myxosporean parasite infection of the spring out-migrant kidney was associated with varying degrees of glomerulonephritis. The genera of the parasite could not be ascertained as no spore stages were observed in the samples. Meyer and McPherson (1985) describe similar myxosporean in adult Rogue R. Chinook suffering from chronic glomerulonephritis. These authors did not observe spore stages and were also unable to identify the parasite. No infections were observed in the November release group. Kidney damage could negatively affect the smolt’s ability to successfully migrate into seawater. The kidney is involved in divalent

ion excretion while the fish is in saltwater. Future studies will need to examine whether this infection progresses into a disease state and if such infections affect a fish's performance. While the spring smolts had low level *Renibacterium salmoninarum* infections they were not experiencing BKD. In contrast, the yearlings had already experienced mortality due to BKD in the fall of 1995 prior to their November release (M. Willis, CDFG pathologist, Pers. Comm.). Cultural stress in the form of overcrowding and low D.O. (< 3ppm in early October) would have aggravated the BKD problem. Both ELISA and histological data indicates that a least 25 % of the November group was suffering from BKD and would likely perish of the disease. *Renibacterium salmoninarum* is the causative agent of Bacterial Kidney Disease (BKD) and is an obligate pathogen of salmonid fishes worldwide (Evelyn 1993). The bacterium is known to survive and multiply within fish macrophages (Young and Chapman 1978), however, survival is lower within activated phagocytes (Campos-Perez et al. 1997). Transmission can be both horizontal (fish - fish) and vertical (female - progeny). BKD is a chronic disease characterized by granulomatous lesions in the kidney. It is unclear if the low *R. salmoninarum* antigen yearlings would progress into a disease state. BKD has been demonstrated to negatively affect the ability of smolts to adapt to seawater (Moles 1997) and the disease continues after the infected salmon moves into saltwater. *Renibacterium salmoninarum* infected salmonids have been reported from the ocean fishery off Washington and Oregon (Banner et al. 1986). Fish weakened by the disease would also be more susceptible to predation. *R. salmoninarum*-infected juvenile chinook demonstrate signs of stress (increased plasma cortisol and lactate) as the disease progress and incur higher predation rate than uninfected cohorts (Mesa et al. 1998).

There was some evidence in this study supporting the hypothesis that cooler water refugia are significant for smolt survival in the Klamath River during the spring and summer. The observation that chinook remained in the downstream plume of Indian Creek in spite of the heavy rafter traffic indicates the strong preference for this limit habitat by the smolts. However, physiological measurements did not demonstrate an obvious difference between the chinook captured at INC and BBT on any given week of the study. As mentioned above, the RCC fish were likely natural and should not be compared with the hatchery fish. When several energy indicators such as plasma TG, liver glycogen, visceral fat quantity, and condition factor are compared between the INC and BBT fish, only liver glycogen appears to be higher in the "refugial" INC group. Likewise, gill ATPase values were similar between the 2 groups. The prevalence of *C.shasta* infection was approximately 20 % higher in the BBT out-migrants, however, both groups suffered from Ceratomyxosis. The most noteworthy spring sample group were the BBT-s5 fish. After several weeks of observing out-migrants in extremely poor shape, both the physical condition (e.g. good energy reserves) and health (low prevalence of clinical Ceratomyxosis) markedly improved in the smolts captured during the week of July 18. River temperature was above 20 °C during this period thus demonstrating that high temperature at the capture site and poor fish health are not always related to each other. It is tempting to speculate these out-migrants had been holding in a cool-water refugia and were now rapidly moving out of the system to the estuary. If long-term exposure to elevated temperature induces a chronic stress response in the smolts, it is expected that crucial performance characteristics such as immunodefences, metabolic scope of activity, and smolt development could be significantly impaired (Maule et al. 1989, Barton and Schreck 1987). Extensive mark and re-capture studies could help elucidate this habitat issue but will be logistically quite difficult. Controlled laboratory experiments on the physiological response of Klamath R. chinook juveniles to elevated water temperatures could provide managers with information necessary for making water flow and hatchery decisions in the basin.

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APPENDIX 1.