

FY96 Investigational Report :
Health and Physiology Monitoring of Coleman NFH
Fall-run Chinook Smolts (FCS-BCW-95-COL)
Component of 1996 Marked Out-migrant Study.

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Summary: During March - May 1996, the California - Nevada Fish Health Center (FHC) sampled juvenile Fall-run chinook salmon (FCS) at Coleman National Fish Hatchery (CNFH) prior to their release and at 3 sites downriver . A limited number of natural chinook juveniles were also sampled from the Upper Sacramento River. Fish were examined for disease, energy reserves, smolt development, plasma proteins, and an organosomatic analysis. There were 3 FCS release groups (14MAR, 29MAR, 23APR) of approximately 4 million each, of which 300,000 were marked with coded wire tags. The most significant disease detected was Infectious Hematopoietic Necrosis Virus (IHNV), which caused high mortalities in some FCS raceways and advanced the release time of the 1st two groups. The two early release groups were smaller (mean 66.5 and 70.5 mm FL) than the normal "smolt" release size. In spite of severe IHNV infection, marked fish of the 3rd release group had the highest Delta recovery. This data could indicate that large size influences recovery (migration survival) more than group health status. Infected fish, swimming with healthy cohorts, were recovered 183 km from CNFH. There were no marked changes in physiological measurements during the short time span between CNFH release and re-capture at Glen-Colusa Irrigation pumps (2-4 days) or Knight's Landing (6-7 days). Energy measures declined slightly in fish captured at Knight's Landing and Chipp's Island (Delta), however, they appear sufficient for the migration. Two weeks post-release may be the point where the smolt's energy balance, while still substantial, is starting to decline rapidly. Smolt development appeared to be more advanced in the 3rd group and improved in lower river captures. The natural chinook had lower condition factors (leaner) than 2 of the release groups yet had plasma triglyceride levels comparable to the fed hatchery fish. While no significant infectious diseases were detected in these fish, degenerative changes were observed in the livers of parr caught near Redding. Monitoring of the health and physiology of the marked groups will allow for better analysis of tag recovery data.

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List of Abbreviations: California Department of Fish and Game = CDFG, California - Nevada Fish Health Center = FHC, Coleman National Fish Hatchery = CNFH, Fall-run chinook salmon = FCS, Glenn-Colusa Irrigation District Pumps = GCID, Infectious Hematopoietic Necrosis Virus = IHNV, Gill Sodium - Potassium Adenosine Triphosphatase = Na-K-ATPase= ATPase, , Sacramento San Joaquin - Fish & Wildlife Office, Stockton = SSJ-FWO.

Introduction

Coleman National Fish Hatchery (CNFH) is located on Battle creek , a tributary of the Upper Sacramento River. The hatchery rears 3 stocks of Upper Sacramento River chinook salmon (fall = **FCS**, late-fall, and winter-run chinook) and steelhead trout. Epizootics of Infectious Hematopoietic Necrosis Virus (IHNV) infection in CNFH juvenile FCS have occurred since the 1940's. These IHNV outbreaks have resulted in the loss of thousands of fish and the release of IHNV-infected smolts. Other significant pathogens found in Sacramento R. chinook populations include *Renibacterium salmoninarum* (BKD agent) and a number of parasites (*Ceratomyxa shasta*, *Nanophyetus salmincola*, *Phyllobothrum salmonis*, *Chloromyxum sp.*, and the myxosporean responsible for Proliferative Kidney Disease"PKX"). The performance of an out-migrant chinook juvenile ("smolt") and its ultimate survival is influenced by its general health, age and size, immunodefence status, energy reserves, and development of osmoregulatory mechanisms ("smoltification").

The California - Nevada Fish Health Center (FHC) performed a series of health and physiological sample collections with both CNFH and natural FCS in support of a 1996 mark and recapture study with brood year 1995 FCS from CNFH. The original study proposal called for 3 releases in April of approximately 330,000 marked fish within a group of 4 million fish per release group (December 20, 1996 letter from J. Smith and T. Nelson (USFWS) to R. Brown, DWR). The study's primary objective was to evaluate distribution, migration rate and survival of Upper Sacramento R. FCS using recapture data at various recapture sites along their migration route. Data on the health and performance of migrating hatchery and natural smolts should be an important component of a juvenile monitoring and hatchery evaluation program. It provides managers with information to compare groups within the production and determine factors for impaired survival. The study was partially funded by the State Department of Water Resources (DWR) and administered by the USFWS Sacramento- San Joaquin and N. Central Valley Fish and Wildlife Offices.

Methods.

Sample sites- Juvenile chinook, from each of the 3 releases, were sampled for disease and physiological tests at the following sites diagramed in figure 1:

- 1) CNFH just prior to the release of their respective cohort group (**P**reliberation = **P** , confluence of Battle Creek and Sacramento River at rm 273)
- 2) CDFG rotary screw trap located at **G**len-Colusa Irrigation District pumps 2-4 days post-release (**G**, rm 206)

Figure 1.

- 3) CDFG rotary screw traps located below Knight's Landing Bridge, 6-7 days post-release (**K**, rm 90)
- 4) Interagency Ecological Program (CDFG / USFWS) lower Delta mid-water trawl catches at Chipps Island (**C**, rm 1).

Natural juvenile chinook were collected for histological examination in the Upper Sacramento R. adjacent to the Redding Posse Grounds (rm 298) on 23FEB96 and for physiological tests from a CDFG rotary screw trap located on the river near Ash creek (rm = 277) on 01APR96.

Field Collections- 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 heparinized tubes. The blood sample was centrifuged (10,000 RPM, 10 min.) for measurement of hematocrit, leukocrit, and collection of plasma. Plasma and a gill sample (in SEI buffer) were frozen on dry ice and held at -80° C. After an internal organosomatic examination, various tissues were removed for either *R. salmoninarum* antigen assay, viral assay, or histology. To determine the total body % moisture (indirect measure of total body lipid content, see below), 6- 12 fish from each collection site were euthanized, weighed, measured, and frozen intact within pre-weighed aluminum foil pouches. To estimate the degree of capture stress, capture time (time removed from trap or raceway) and euthanization time were recorded for each fish. Surface water temperature and dissolved oxygen content (ppm) were measured at each collection site.

Organosomatic analysis- Fish were evaluated by a modified organosomatic assay (Goede and Barton 1987, 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, degree of silvering, eyes, gill, visceral fat, presence of food in the gastrointestinal tract, and any hemorrhagic lesions on the 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 ($K (x 10^{-5}) = \text{weight (g)} / \text{length (mm)}^3$), however, only total length condition factors are reported. The criteria for the severity scores are listed in Table 1.

Table 1 Organosomatic analysis criteria scores

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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
Silver	0 = fully silver, no parr marks viable (rotate fish in light) 1 = partial silver, parr marks viable (caudal region)

2 = little to no silvering - full parr marks

- Eye 0 = no abnormalities
 1 = missing 1 eye, diminutive, external abrasion, some opacity
 2 = exophthalmic 'pop-eye', cataract, bubbles, parasites
 3 = hemorrhage, rupture
- 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 caeca or peritoneal cavity
 1 = < 50 % coverage of caeca and/or cavity fat dia. < caeca vol.
 2 = >50 % but not covering caeca and/or cavity fat dia. = caeca vol.
 3 = caeca and cavity completely filled with fat, organs obscured by fat
- Food (+) = food in any part of GI tract
 (-) = no food seen in GI tract
- Hemor. Notes about any hemorrhagic organs- abnormal size / color
Organs N = no , Y = Yes

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Saltwater challenge: A 30 ppt saltwater static aquarium (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. Test fish are quickly captured and placed in the SW buckets (6 fish/bucket). After 24 hrs, the surviving fish are quickly captured, euthanized in a saltwater-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 (Kodak DT60 clinical chemistry analyzer).

Histology - Gill, intestinal tract, pyloric caeca, 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- Kidney tissue was removed and either frozen for later *R. salmoninarum* antigen analysis by an Enzyme Linked Immunosorbent Assay (ELISA) or smears prepared for microscopic observation of the bacteria by fluorescent antibody technique (FAT). The small size of the early release groups required that FAT smears be tested while kidneys from larger smolts could be sampled for the ELISA. For ELISA, kidney tissue was diluted 8x (w/v) in PBS-0.05% tween20, homogenized, boiled for 15 min., centrifuged, and the supernatant tested for antigen. The optical density (O.D. =

absorbance at 405 nm) of the labeled antibody- antigen reactions were averaged between sample replicates, a blank well O.D. subtracted, 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 FAT), and 3) Positive = sample O.D. > -0.7 . Commercial polyclonal antisera ,to whole cell preparations, was used for both assays.

Two fish pools of kidney were used for the viral assays. The samples were homogenized in sterile Hank's Buffered saline at a 10X dilution (w/v), centrifuged at 5,000xG for 10 min. at 4 °C, and the supernatant diluted 2X with an antibiotic / antimycotic solution. After an overnight decontamination step at 4 °C, replicate 100 μ L inoculum of 20X and 100X sample dilutions were placed onto 24 hour-old cultures of *epithelioma papulosum cyprini* cells (EPC) grown in 48-well plates (Fijan et al. 1983). The plates were overlaid with a MEM-7.5%Fetal Bovine Serum media and incubated for 14- 18 days at 15 °C. EPC cultures were examined microscopically for signs of cytopathic effect (CPE) on day 1, 3, 5, 10, 14 or 18 post-inoculation and a subset of positive samples were confirmed by a polyclonal dot blot test.

Physiological Assays - Moisture content of some carcasses was determined as an indirect measure of lipid content. There is an inverse relationship between moisture and lipid content of the carcass (Shearer, 1994). The frozen carcasses were dried at 200 °F until a constant weight was obtained (tissue would powder if crushed). The dried sample and its aluminum foil container was removed from the oven and quickly weighed to the nearest 0.0001 g with a Mettler balance. Plasma was assayed for total protein, triglyceride, sodium, and glucose using a Kodak DT60 Clinical Chemistry Analyzer and reagents. Plasma protein electrophoresis was performed with a 7 μ L sample run on a CIBA agarose gel (1M barbital buffer, 90V for 45 min.). The stained gels were scanned and the percent area of each fraction determined with Seprascantm software. Analysis of Variance was performed on percent area values for each fraction (or combined fractions). Gill Adenosine Triphosphatase activity (μ moles ADP / mg protein / hr) was assayed by the method of McCormick and Bern (1989). 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' s body against the concentration gradient to the saltwater.

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 1-2-3tm spreadsheets and SigmaStattm software was used for data manipulation and analysis.

Results

Sample site characteristics / problems - Three release groups, of approximately

4 million fish each including 276,423 - 338,355 marked fish, were pumped for 1-2 days into Battle creek starting on 15MAR96 (group 1), 29MAR96 (group2), and 23APR96 (group 3). North Central Valley FWO biologists determined the post-tagging mortality figures for each tag group by examining the frozen carcasses of all mortalities with a tag detector (Table 2).

Table 2. Tag code, total number marked, cumulative mortality post-mark, total number of marked fish released, and date of release for the 3 release groups of coded wire tagged (CWT) BY95 fall-run chinook juveniles reared at CNFH in 1996 (N. Central Valley FWO data, 17JUN96 memorandum).

tag code	No. tagged	Cumulative No. CWT mortality (%)	No. CWT released	Release Date
0501020114	339,325	970 (0.3%)	338,355	14MAR96
0501020115	329,368	8,741 (2.7%)	320,627	29MAR96
0501020201	333,466	57,043 (17.0%)	276,423	23APR96

Based on fork lengths and external features, the 01APR Ash creek trap chinook were assumed to be natural, however, 2 CNFH groups had been released and entered the river several miles downstream (confluence of Battle creek rm 273, Ash creek = 277 rm). Generally, water temperature and dissolved oxygen concentrations at the sample sites were within normal ranges for chinook smolts (Table 3).

There were different sampling biases involved in each collection. The pre-liberation samples at CNFH were generally collected by random dipnet "grabs" in the middle sections of both marked and unmarked rearing units. This type of collection is less likely to obtain moribund animals than if sampling in the lower third of a raceway. The 26MAR96 viral sample from the marked group was mistakenly collected in the lower tail region of the rearing unit. At the GCID trap, CDFG biologists generously sorted the fish in the livebox and selected ad-clipped fish for us. As fewer marked fish were encountered at the Knight's landing trap, we took an unbiased "net full" from the livebox plus whatever marked fish were later measured by the trap biologist. The number of marked fish in each sample group is listed in table 3.

Table 3. Sample site characteristics for the pre-release (P), GCID (G), Knight's Landing (K), and Chipp's Island (C) collections for groups 1 - 3 and natural chinook (NAT).

Sample grp./ date	Temperature °C	Dissolved Oxygen. (ppm)	Adipose marked (%)
P1 14MAR	9.3	9.5	NA
G1 18MAR	11.8	ND	29 / 30 (97)**
K1 21MAR	13.7	9.1	1 / 44 (2)
NAT 01APR	10.0	ND	NA
P2 26MAR	9.4	ND	NA
G2 01APR	ND	ND	21 / 34 (62)**
K2 04APR	13.1	9.9	9 / 25 (36)
P3 22APR	9.4	10.5	NA
G3 25APR	ND	ND	15 / 27 (56)**
K3 30APR	16.6	9.1	2 / 44 (5)
C1 02APR	13.8	7.6	5 / 38 (13) 4 fish = P1 group 13 days post-rel.
C2 06MAY	17.9	7.5	24 / 45 (53) 10 fish = P3 group 19 days post-rel.

ND Not done
NA Not applicable as identity known.
** CDFG Biologists at trap selected ad-clipped fish.

The effect of the different capture and handling conditions on physiological measurements is not precisely known. We do not know the length of time a given fish had been in the rotary screw trap livebox (up to 24 hrs). Capture stress could alter several blood and energy measurements in comparison to the fish's pre-capture value (Mazeud et al. 1977). We were generally able to euthanize the fish and collect samples within 30 - 45 min. of sorting at the trap. A similar uncertainty of stress effects is associated with the Chipp's Island trawl. A given fish could be impinged upon the net for up to 30 minutes (20 minute trawl + recovery). Scale loss and weak swimming behavior were commonly observed in the trawl sample fish. Many of fish captured in the river or delta were unmarked and their identity (CNFH, Feather R. SFH, natural) is not known.

Viral Disease - Moribund FCS, from raceways 1,4,5, and 7, were diagnosed with IHNV infection beginning on February 28, 1996. Infected fish were collected from an additional 15 raceways (including the marked fish groups) on March 6. The coded-wire tag study cooperators were contacted in early March with the Fish Health Center's

recommendation to release the first tag group early. This action was proposed to reduce the horizontal transmission of the virus among the fish by releasing them from the confined raceway environment. The cumulative percent mortality of the first release group, for the 2 week period prior to release, ranged from 1.40% to 0.06% (Fig 2). The marked fish % mortality was 0.17 % for this same period. If one considers a percent mortality of 0.2 - 0.5 % to be "significantly elevated" and > 0.5 % to be "high", than 2 of the 9 units were experiencing *high* mortality prior to release. At the 14MAR release, no IHNV-infected fish were detected in the "unbiased" grab samples taken from both marked and unmarked units (Table 4). Out-migrant samples collected at GCID 4 days later (all marked) were also negative for IHNV. Infected fish were detected at the Knight's landing trap 7 days post-release.

The second release group was also planted into Battle creek early than planned due to IHNV concerns. The prevalence of IHNV infection for this group at release was judged to range from 13 % to 33 % (Table 4). While 5 of the 8 raceways of this group experienced "elevated mortality", none were judged to be "high" (Fig. 2). Infected out-migrants were collected at both GCID and at Knight's landing. The 13% prevalence of infection of the 04APR Knight's landing sample is a better estimate of prevalence than the GCID sample ("29%") due to the collection bias at GCID.

A decision to tag the infected fish in raceway 14 and hold the tagged group until 23 April was based on a desire to maintain the original study design. The Fish Health Center recommendation to tag another FCS raceway (Raceway 19) was not accepted because of concerns about the smaller fish size (in comparison to the previous tag groups). Following the tagging operation, mortality became quite high and was 10.7 % for the 2 weeks prior to release (Fig. 2). This value is 3X higher than cohort raceway from which the fish had been tagged. Seven of the 11 raceways in this release group were judged to have " high" mortality. The magnitude of mortality was far greater in the 3rd release group than the previous groups . In spite of the higher incidence of sick fish, the 3rd release had the highest survival index to Chipp's Island based on recoveries from this site (P. Brandes, USFWS Stockton, pers. comm.). Infected fish were detected in both the GCID (53 % prevalence) and Knight's landing sample (14 % prevalence of infection). As mentioned above, the sampling techniques bias the accuracy of the prevalence data. No infected samples were collected in the 06MAY Chipps island trawl.

Figure 2.

Table 4. Prevalence of IHNV in 2-fish pooled samples of kidney. Recorded as number positive / total sample pools.

sample group	date	prevalence (%)	No. fish	Comments
P1	14MAR CWT unmarked	0 / 15 (0)	38	mid-pond grab sample mid-pond grabs - several units
		0 / 29 (0)	58	
G1	18MAR	0 / 29 (0)	58	selected by trap crew, all marked
K1	21MAR	4 / 20 (20)	40	grab from livebox - no marked fish
P2	26MAR CWT unmarked	2 / 15 (13)	30	net grab near tailscreen mid-pond grabs- several units
		13 / 40 (33)	80	
G2	01APR	7 / 24 (29)	48	selected by trap crew
K2	04APR	2 / 16 (13)	32	grab from livebox- no marked fish
P3	22APR CWT UNMARKED	11 / 15 (73)	30	mid-pond grab mid-pond grabs- several units
		2 / 15 (13)	30	
G3	25APR	8 / 15 (53)	30	some (+) samples from mark fish
K3	30APR	3 / 22 (14)	44	grab from livebox- 1 mark =neg.
C2	06MAY	0 / 15 (0)	30	4 CNFH marked fish in sample

Histological specimens No significant pathogens or lesions were detected in the pre-release samples (Tables 5-7). Fat vacuoles (fatty change = FATC) were seen in the livers of several fish from both the pre-release and the 21MAR Knight's Landing sample (release 1). The degree of this abnormality was considered mild and did not resemble clinical liver lipid disease. Marked cytoplasmic vacuolization was common in the hepatocytes (88% prevalence) of the natural parr collected at the Redding Posse ground on 23 FEB96 (Table 9). Lipofuscin deposits were present within the vacuolated hepatocytes of these parr. Some fish collected at each Knight's landing sampling had varying degrees of inflammation associated with the acinar cells of the pancreas (prevalence of pancreatitis 8 - 42 %). Inflammation of the adipose tissue associated with the intestinal tract (steatitis) was also observed in 2 fish collected at Knight's landing. Yellow nodules were seen grossly on the pyloric caecae of several fish collected at GCID, Knight's landing, and Chipps island (Tables 6,7,8). Histologically,

these nodules were deposits of lipofuscin pigment associated with acinar cell clusters. Lipofuscin is a breakdown product of phospholipid oxidation. Although endemic to the Sacramento R., the enteric parasite *Ceratomyxa shasta* was not observed in any specimen collected in the river or delta (Hendrickson et al 1989). Low numbers of metacercaria (presumptive identification *Nanophyetus salmincola*) were seen in the kidney of only a few specimens collected at Knight's landing and in the 06MAY Chipps sample. The trematode, *Nanophyetus salmincola*, is endemic to Battle creek and is commonly observed in CNFH fish. No significant disease problem has been associated with this parasite at CNFH. A pre-sporogonic stage of a myxosporean was seen in the kidney tubules and glomeruli of fish collected at Knight's Landing, Chipps island, and in a natural parr collected at Posse Grounds on 29FEB. It is quite possible that most of the (unmarked) fish infected with this myxosporean were natural chinook. It has not been seen in fish held at the hatchery or those collected at GCID. One CNFH-P3 tagged fish collected in the 06MAY Chipps sample was infected with this myxosporean (13 days post-release). Three of the fish with this parasite were suffering from an associated inflammation of the glomerulus.

Tables 5 - 9. Histological examination of tissues from chinook smolts. Parasites include metacercaria stage (METAC) of *Nanophyetus salmincola*, *Ichthyophtharis multiphilis* (ICH), and pre-sporogonic myxozoans (MYXO) found within the lumen of kidney tubules and glomeruli. Lesions observed include glomerulonephritis (GLMN), kidney interstitial hyperplasia (INHP), mild fatty change of hepatocyte (FATC), pancreatitis (PANC), adipose inflammation or steatitis (STEA), kidney tubule pigment deposits (TPD), and lipofuscin deposits (LPFD). Prevalence of infection or disease data presented as number positive / total examined (%)

Table 5.
RELEASE 1
21MAR

		Pre-release 14MAR	GCID Trap 18MAR	Knights Landing
Gill	Parasite	0 / 5	0 / 9	METC 1 / 12 (8)
	Lesion	0 / 5	0 / 9	0 / 12
Kidney	Parasite	0 / 5	0 / 9	METC 1 / 12 (8) MYXO 3 / 12 (25)
	Lesion	0 / 5	0 / 9	GLMN 2 / 12 (17)
Liver	Parasite	0 / 5	0 / 2	0 / 11
	Lesion	FATC 2 / 5 (40)	0 / 2	FATC 2 / 11 (18)
Intestine Pyloric cecum	Parasite	0 / 6	METC 1 / 9 (11)	0 / 24
	Lesion	0 / 6	0 / 9	PANC 3 / 24 (13) STEA 1 / 24 (4)

Table 6.

RELEASE 2		GCID Trap 01APR	Knights Landing 04APR	
Gill	Parasite	0 / 6	ICH	1 / 9 (11)
	Lesion	0 / 6		0 / 9
Kidney	Parasite	0 / 6	METC	1 / 10 (10)
			MYXO	2 / 10 (20)
	Lesion	0 / 6	GLMN	1 / 10 (10)
Liver	Parasite	0 / 5		0 / 8
	Lesion	0 / 5		0 / 8
Intestine Pyloric cecum	Parasite	0 / 6		0 / 15
			PANC	1 / 13 (8)
	Lesion	0 / 6	STEA	1 / 14 (7)

Table 7.

RELEASE 3		Pre-release 22APR	CID Trap 25APR	Knights Landing 30APR		
Gill	Parasite	0 / 4		0 / 4	0 / 5	
	Lesion	0 / 4		0 / 4	0 / 5	
Kidney	Parasite	0 / 5		0 / 5	0 / 6	
	Lesion	INHP	1 / 5	TPD	1 / 5	MYXO
Liver	Parasite	0 / 4		0 / 5	0 / 6	
	Lesion	FATC	2 / 4 (50)	0 / 5	0 / 6	
Intestine Pyloric cecum	Parasite	0 / 4		0 / 7	0 / 17	
	Lesion	0 / 4		LPFD	3 / 7***	PANC

nd

Not done

Pyloric cecum observed to have a yellow spot during necropsy

Table 8.

		CHIPPS1 02APR	CHIPPS2 06MAY
Gill	Parasite	0 / 9	0 / 11
	Lesion	0 / 9	0 / 11
Kidney	Parasite	MYXO 2 / 8	MYXO 2 / 8 (25) METC 1 / 8 (13)
	Lesion	GLMN 2 / 8	0 / 8
Liver	Parasite	0 / 12	0 / 8
	Lesion	FATC 1 / 12 (8)	FATC 1 / 8 (13)
Intestine Pyloric cecum	Parasite	0 / 12	0 / 25
	Lesion	STEAL 2 / 4 LPFD 2 / 12 (17)**** PANC 2 / 12 (17)	PANC 2 / 25 (8)

**** lesions appeared as yellow spots on pyloric cecum

Table 9. **Natural** chinook smolts collected in the Upper Sacramento R .

		Posse Gr. 29FEB	Ash Crk 01APR
Gill	Parasite	0 / 7	0 / 11
	Lesion	0 / 7	0 / 11
Kidney	Parasite	MYXO 1 / 8 (13)	0 / 15
	Lesion	0 / 8	0 / 15
Liver	Parasite	0 / 8	0 / 14
	Lesion	FATC 7 / 8 (88) LPFD 6 / 8 (75)	FATC 4 / 14 (29)
Intestine Pyloric cecum	Parasite	0 / 8	0 / 15
	Lesion	STEAL 1 / 8 (13)	PANC 2 / 5 (40) STEAL 2 / 4 (50)

R. salmoninarum - Bacterial kidney disease did not appear to be a significant problem in any of the fish tested for this pathogen. Neither the bacterium (by DFAT) nor high concentrations of its antigens (as indicated by high ELISA Optical Densities reading , O.D.- POSITIVE) were detected in fish sampled at the hatchery or at the 04APR Knight's landing sample (Table 10). By ELISA, 20 % of the 3rd pre-release group had low - level antigen concentrations (SUSPECT) indicative of an early, sub-clinical infection.. Because of the need for kidney tissue for either viral or histological assays, it was necessary to make 2-fish ELISA pools of kidney collected at Chipps island on 06MAY. Later information about the origin of the marked fish revealed ("Murphy's Law") that of the 4 CNFH chinook (code = 05-01-02-02-01, 23 APR release) included in the ELISA sample, each had been paired with either a Feather R. hatchery (FRH) or Merced R. Fish Facility (MRFF) chinook. While the median O.D. value was higher in the CNFH chinook at Chipps island in comparison to the 22APR pre-release sample, the O.D. values were still considered to be low (SUSPECT) . Only 2 ELISA samples (FRH + unmarked and MRFF + FRH) from the 06MAY Chipps island sample had elevated O.D. values indicative of active infections.

Table 10. Prevalence of *Renibacterium salmoninarum* infection in kidney tissue from juvenile chinook as detected in fluorescent antibody test (F.A.T.) or Enzyme Linked ImmunoSorbant Assay (ELISA).

	FAT	BNC	SUS	POS	MEDIAN O.D	MEDIAN LOG O.D.
P1	0 / 20	ND	ND	ND	ND	ND
P2	0 / 20	ND	ND	ND	ND	ND
K2	0 / 20	ND	ND	ND	ND	ND
P3	ND	19 / 30 (63%)	11 / 30 (37%)	0 / 30 (0%)	0.005	-2.3010
CHIPP2 all fish	ND	3 / 19 (16%)	15 / 19 (79%)	1 / 19 (5 %)	0.0295	-1.5301
CNFH**	ND	0 / 4 (0)	4 / 4 (100)	0 / 4 (0)	0.0310	-1.5133

** 2-fish kidney samples resulted in CNFH fish mixed with FRH and MRFF fish.

Organosomatic analysis / morphometries - Gross tissue abnormalities, such as scale loss, anemia (pale gills), and hemorrhagic foci on internal organs, were noted in many of the sampled fish (Table 11). It is presumed that those fish with internal hemorrhagic lesions on the liver or visceral fat were suffering from IHNV infections. The deciduous nature of smolt scales and the abrasion associated with net capture were probable factors in the scale loss. This occurrence was particularly severe in all fish captured in the Chipps Island trawls.

Mean erythrocyte numbers of all sample groups, as estimated by hematocrit (HCT), were within normal limits for juvenile chinook (Table 11). There was no statistical difference detected among the pre-liberation and out-migrant sample HCT values from release groups 1 and 2. The hematocrits of the third Knights Landing sample group (K3) increase significantly over both the pre-release and GCID sample groups ($P=0.0047$, K-W ANOVA). Although the CWT fish in release 3 had a higher incidence of IHNV infection than the unmarked fish, their HCT values were similar. While similar to P3 groups, natural chinook HCT values were smaller than either P1 or P2 ($P<0.001$, ANOVA). The hematocrits of the smolts captured in the Chipps Island trawl were significantly higher than the pre-liberation or other in-river capture groups. The statistical increase of K3 and Chipps Island HCT may indicate some trend for erythrocyte increases with time post-release or a stress-induced hemoconcentration effects.

Fork lengths (FL) were similar for fish captured in each of the respective release group series : pre-release, GCID, Knights Landing (Table 12). The nine marked P3 fish captured 13 days post-release at Chipps Island were slightly larger (avg. $82.9 \text{ mm} \pm 1.5$ Std error) than the pre-release sample group (avg. 79.3 ± 1.4). Although sampled 15 days apart, there was no significant difference ($P<0.05$, t-test) between the FL of the 1st and 2nd pre-release groups (Table 12). The marked P3 group was longer than either the 1st or 2nd release groups. Natural chinook, collected on 01APR, had similar FL as the P1 group sampled at CNFH 16 days early, yet were significantly shorter than the CNFH P2 group sampled only 4 days prior. The C1 fish were smaller than the C2 group.

For release 2 and 3, condition factor ($K = \text{Wt g} / \text{Total length mm}^3 \times 10^5$) tended to decline after release (Table 12). The condition factor of the first release groups (P1-G1-K1) did not differ significantly from each other and were similar to the natural fish sampled on 01APR. It appears that unmarked fish in the K2 group were representative of coded-wire fish. Nine marked fish captured in the K2 sample had similar condition factors (avg 0.6692) as the entire sample group (avg 0.6862). Condition factors of both Chipps Island samples were similar to each other and lower than the pre-release and GCID samples

Table 11. Mean (Standard Error) hematocrit and tissue abnormalities (% of sample group).

		Hematocrit	Tissue abnormalities (%)
P1	14MAR	45.2 (0.7) a n=30	None
G1	18MAR	43.7 (1.6) a n=18	1 / 29 (3) hemorrhagic organs
K1	21MAR	42.9 (2.1) a n=17	3 / 32 (9) scale loss 1 / 32 (3) hemorrhagic organs
NAT	01APR	39.7 (1.8) n=13	None
P2	26MAR	44.4 (1.1) b n=17	None
G2	01APR	43.4 (2.5) b n=21	2 / 23 (9) exophthalmus / anemia 1 / 23 (4) scale loss
K2	04APR	46.6 (1.2) b n=20	6 / 25 (24) scale loss
P3	22APR	40.0 (1.5) n=20 c	2 / 20 (10) exophthalmus
	CWT	40.7 (0.5) n=20 c	None
	UNMARKED		
G3	25APR	43.6 (1.4) c n=15	5 / 21 (24) scale loss
K3	30APR	45.3 (1.6) d n=20	3 / 20 (15) scale loss
C1	02APR	53.9 (1.7) e n=16	26 / 26 (100) scale loss
C2	06MAY		
	All Fish	46.1 (1.3) f n=23	36 / 36 (100) scale loss
	P3 marked	44.3 (3.6) n=7	

a - f Sample group values tested for significance ($P < 0.05$) by either T-test or 1-way ANOVA. Different letters within a release group or between the Chipps island samples signify statistically differences.

Table 12. Weight , length, and condition factor (Wt / total length ³ x 100,000) of fall-run chinook. Mean (Standard error).

		Total Length (mm)	Fork Length(mm)	Body Weight (g)	Condition Factor (K)
P1	14MAR	71.5 (0.6)	66.5 (0.6) a	2.99 (.09)	0.7770 (.012) a
G1	18MAR	71.7 (0.7)	66.6 (0.7) a	2.75 (.09)	0.7402 (.097) a
K1	21MAR	72.5 (0.9)	66.7 (0.9) a	2.95 (.11)	0.7628 (.008) a
NAT	01APR	64.4 (0.7)	59.5 (0.7)	2.02 (.06)	0.7106 (.004)
P2	26MAR	76.3 (1.0)	70.5 (0.8) b	3.72 (.15)	0.8283 (.013) b
G2	01APR	76.5 (0.8)	69.8 (0.8) b	3.25 (.10)	0.6365 (.047) c
K2	04APR	73.2 (1.0)	67.7 (1.0) b	2.69 (.12)	0.6862 (.006) c
P3	22APR				
	CWT	85.6 (1.6)	79.3 (1.4) c	5.46 (.26)	0.8400 (.010) c
	UNMARKED	81.5 (1.7)	75.6 (1.6) c	4.36 (.25)	0.8192 (.010) c
G3	25APR	86.4 (0.9)	80.5 (0.9) c	4.83 (.17)	0.7618 (.008) d
K3	30APR	87.8 (0.9)	80.7 (0.8) c	5.28 (.18)	0.7724 (.008) d
C1	02APR	79.7 (0.7)	73.9 (3.9) d	3.5 (0.2)**	0.7099 (.019) e
C2	06MAY				
	All Fish	90.6 (0.8)	83.8 (0.8) e	5.4 (0.2)**	0.7363 (.011) e
	Rel. 3 CWT	90.0 (1.8)	82.9 (1.5) e	ND	ND

a - e Sample group values tested for significance (P<0.05)by either T-test or 1-way ANOVA. Different letters within a release group or between the Chipps island samples signify statistically differences.

** Weights taken from frozen carcasses

Energy reserves - The short time span between release at CNFH and recapture at GCID (2-4 days) and Knight's landing (6-7 days), did not allow for marked changes in energy measurements. The subjective visceral fat scores showed some tendency to drop slightly with distance (and time) post-release (Table 13). The prevalence of fish with no obvious visceral fat deposits (%VFAT<0) increased with distance from CNFH. In particular, smolts examined from the 2 Chipp's island trawls had the least amount of visceral fat. Food was observed in the intestinal tract of all the fish sampled at both GCID and Knight's Landing as well as 92 % of the fish sampled in both Chipps island trawls.

Plasma triglyceride levels (TG) were variable within each sample group. Except for the 26MAR release group (P2), the TG values of the GCID and Knight's landing samples were not significantly different from their respective pre-release status (Table 14). High sample group variation affected the statistical analysis. The high TG values seen in the P2 fish may have been a "post-prandial" shift due to feeding prior to the sample. It is not understood why the 01APR G2 group had such low TG levels. Interestingly, the marked CNFH fish from the 06MAY Chipps sample had much higher TG values than the 30APR K3 sample taken only 7 days prior. This trend did not occur with the Release 2 fish in the 04APR K2 and 02 APR Chipps sample. Although the amount of visceral fat was much lower, natural fish had TG values similar to the fed hatchery fish sampled prior to release.

Plasma glucose values (GLU) indicated that the tested fish (P1, P3-G3-K3) had adequate glycogen stores to respond to the stress of capture. The mean (SEM) values were; P1 = 87.8 (2.3), P3 = 91.3 (7.0), G3 = 98.5 (16.0), and K3 = 113.7 (20.5). The general stress response relationship of increased plasma GLU with time held post-capture was observed in all groups. Over 80 % of the fish held for over 10 minutes prior to sacrifice had GLU > 80 mg/dL. We normally observe a range of 30 - 70 mg/dL in hatchery chinook juveniles rapidly sampled after netting.

Total body lipid content, indirectly measured by % moisture content of the carcass, did not appear to change in the post-release groups (Table 13). The low mean value from the 21MAR K2 group may have been an operator error in weighing or excessive heating resulting in burning of lipid. The 18MAR G1 sample was mistakenly comprised of the caudal peduncle region only (mean 43.7 %) and cannot be compared to the measurements of the entire carcasses (range 76 - 82 %). Both Chipps Island samples had somewhat higher % moisture contents than the hatchery or in-river sample groups indicating a reduction in body lipid.

Table 13. Mean visceral fat (V-FAT) score (Std error), number of fish with no obvious visceral fat deposits (%VFAT<0), and mean (SE) percent moisture of carcass (indirect total lipid measure).

		Mean V- FAT score (SE) % VFAT<0	%Moisture
P1	14MAR	1.0 (0.0)	0 / 41 (0) 79.3 (1.6) n=10
G1	18MAR	1.0 (0.1)	1 / 29 (3) ND**
K1	21MAR	1.2 (0.1)	6 / 32 (19) 53.6 (3.5) n=12
NAT	01APR	0.2 (0.1)	1 / 6 (17) ND
P2	26MAR	1.0 (0.0)	0 / 30 (0) 78.7 (0.4) n=10
G2	01APR	1.2 (0.1)	2 / 23 (9) 79.4 (0.5) n=6
K2	04APR	0.7 (0.1)	6 / 20 (30) 79.3 (0.3) n=10
P3	22APR		
	CWT	1.7 (0.2)	0 / 30 (0) 77.5 (1.0) n=5
	UNMARKED	1.3 (0.1)	0 / 30 (0) 77.5 (0.5) n=5
G3	25APR	1.0 (0.1)	1 / 30 (3) 76.4 (0.3) n=6
K3	30APR	1.0 (0.2)	6 / 30 (6) 76.1 (1.2) n=9
C1	02APR	0.2 (0.1)	5 / 24 (21) 81.5 (0.4) n=8
C2	06MAY	1.0 (0.1)	12 / 36 (33) 80.1 (1.4) n=10

ND

Not done

**

Mean moisture content of caudal region only was 43.7 (1.6), n=5.

Table 14. Plasma triglyceride and total protein concentrations in chinook smolts collected at CNFH prior to release (P1-3), Glenn-Colusa Irrigation Trap(G1-3), Knight's Landing traps (K1-3), Chipp's Island Trawl (C1 &2), and in natural chinook seined at Ash creek. Data recorded as mean (Std. error) and sample number (n).

		TRIGLYCERIDE (mg / dL)			TOTAL PROTEIN (g / dL)	
P1	14 MAR	120.7 (12.9)	a	n = 11	3.9 (0.1)	n=12
G1	18MAR	88.9 (13.9)	a	n = 12	3.5 (0.1)	n = 5
K1	21MAR	141.3 (31.5)	a	n = 12	2.6 ***	n = 3
NATURAL 1APR		153.0 (32.8)		n = 6	ND	
P2	26MAR	207.0 (10.9)	a	n = 12	3.1 (0.1)	n = 13
G2	01APR	69.9 (6.5)	b	n = 15	3.3 (0.2)	n = 8
K2	04APR	108.5 (19.7)	c	n = 10	2.4 (0.3)	n = 9
P3	22APR Marked	93.2 (13.0)	a	n = 10	2.9 (0.3)	n = 5
	Unmarked	89.6 (9.8)	ab	n = 9	3.6 (0.1)	n = 6
G3	25APR	54.0 (4.5)	b	n = 10	3.1 (0.1)	n = 10
K3	30APR	61.7 (11.9)	ab	n = 10	2.9 (0.1)	n = 9
C1	02APR	80.0 (7.6)		n = 5 ***	ND	
C2	06MAY all fish	138.9 (24.3)		n = 12	3.1 (0.1)	n = 3
	CNFH CWT	186.0 (38.2)		n = 6	3.2	n = 1

** Two of the 3 samples were below assay detection limit of 2.0 g /dL - later samples were spiked with protein if below this level.

*** 2-4 fish pools

ND Not done

a - f Sample group values tested for significance (P<0.05)by either T-test or 1-way ANOVA. Different letters within a release group signify statistically differences.

Smolt development - All pre-release groups had good survival in the 24 hr. SW challenges (Table 15). Plasma sodium regulation improved with increasing fish size (age). The percentage of challenge survivors, with plasma sodium values above the upper normal limit of 170-174 mmol/L (Blackburn and Clarke 1987), decreased from 50 % in the 64 mm FL chinook of P1 to none of the 80 mm FL P3 chinook (unmarked). It is likely that the fish with high sodium values would not survive more extended exposures to 30 ppt seawater. The condition factor of all SW challenged fish were significantly less than their FW cohorts from the organosomatic sample (T-test, $P < 0.001$). As the lengths of the 2 groups were similar, this data indicates that all SW challenge groups experienced significant weight loss due to dehydration.

Gill sodium- potassium- adenosine triphosphatase activity (ATPase) tended to increase with time (distance) post-release for a release group (Table 16). Chinook collected in the Chipp's Island trawls and the 3rd release group at Knight's Landing had the highest mean ATPase values. The fork length of an individual did not correlate with its ATPase value ($r\text{-sq} = 0.146$). The high degree of variation (coefficient of variation 32 - 55 %) seen in the ATPase values of each sample group affected the statistical relationships. Statistical difference **was not** detected ($p < 0.05$, ANOVA) in the following sample groups: 1) pre-release groups (P1-P2-P3), 2) all collection sites within the 1st release group (P1,G1,K1), or 3) between the 2 Chipps island samples. It is not clear why G2 produce so many low ATPase values. Because of the possibility of sampling error (improper freezing, excessive time post-mortem, etc.), the statistical significance of this sample group to its cohorts (P2, K2) is questionable. It is much more likely that no biologically significant changes in ATPase activities occurred in the 66 - 71 mm FL smolts of the 1st and 2nd release groups during the 6-7 days of out-migration. Chinook from the 3rd release group, collected at Knight's landing, had higher ATPase activities than their GCID or pre-release cohorts ($P < 0.01$). No evaluation of natural chinook ATPase values could be made due to the wide range of values of the 3 gill samples collected on 01APR.

Table 15 Saltwater challenge of CNFH chinook smolts prior to release. Groups of 12 -18 fish were challenged in aerated, 27 - 31 ppt saltwater for 24 hrs and their plasma tested for sodium content (Na+). Data reported as number positive / number tested (%) for survival and percent of plasma samples greater than 174 mmol/L, mean (std. error) of plasma sodium concentration (mmol/L), Fork length (mm), and condition factor (Weight (g) / (Total length (mm))³).

	P1 14MAR	P2 26MAR	P3 CWT 22APR	P3Umrk
% Survival	12 / 12 (100)	11 /12 (92)	11 /12 (92)	6 / 6 (100)
% Plasma Na+ >174	3 / 6 (50)	5 /11 (45)	3 / 10 (30)	0 / 4
Mean Plasma Na+	180.6 (3.8) n= 6 **	173.3 (5.1) n= 11	172.9 (5.8) n= 10	162.5 (4.3) n= 4
Mean fork length (mm)	64.3 (1.0)	68.6 (0.8)	71.3 (4.9)	80.5 (1.4)
Condition Factor (K)	0.7336 (.0169)	0.7362 (.0340)	0.6424 (.2022)	0.7129 (.0530)

** 4 samples were 2-fish pools from P1 sample group

Table 16.

Gill Sodium- Potassium Adenosine Triphosphatase (Na-K-ATPase) activity in chinook smolts collected at CNFH prior to release (P1-3), Glenn-Colusa Irrigation Trap(G1-3), Knight's Landing traps (K1-3), Chipp's Island Trawl (C1 &2), and in natural chinook seined at Ash creek (Rm=275). Data recorded as mean (Std. error), sample number (n), and maximum value.

		GILL ATP (μ moles ADP / mg protein / hr)			Fork length (mm)
P1	14 MAR n = 9	1.1642 (0.1925)	a	max = 1.8136	66.0 (0.9)
G1	18MAR n = 11	1.1434 (0.1721)	a	max = 2.090	65.9 (0.8)
K1	21MAR n = 11	1.5401 (0.2568)	a	max = 3.838	67.4 (1.8)
NATURAL	1APR n = 3	1.3999 (0.7163)		max = 2.832	61.7 (4.1)
P2	26MAR n = 12	1.8240 (0.4120)	b	max = 5.910	71.3 (1.5)
G2	01APR n = 9	0.7568 (0.2103)	c	max = 2.060	71.2 (1.2)
K2	04APR n = 12	1.2272 (0.3683)	bc	max = 4.7186	66.5 (1.0)
P3	22APR n = 12	1.0920 (0.1513)	d	max = 1.9174	77.6 (1.9)
G3	25APR n = 12	1.4726 (0.2393)	d	max = 3.2402	81.1 (0.8)
K3	30APR n = 11	2.7795 (0.3662)	e	max = 6.1021	79.7 (0.8)
C1	02APR n = 9	2.2147 (0.3768)	f	max = 4.3417	71.8 (1.1)
C2	06MAY n = 11	2.0578 (0.2088)	f	max = 3.1367	85.1 (1.6)

a - f Sample group values tested for significance ($P < 0.05$)by either T-test or 1-way ANOVA. Different letters within a release group or between the Chipps island samples signify statistically differences.

Leukocrit - Leukocrit (Lct) data was variable and, in many instances, undetected in the microcentrifuged blood samples. A number of sample group Lct values were not analyzed or reported due to difficulty in measuring the buffy coat. We have often observed little to no buffy coat from blood samples of young salmon. Apparently, the concentration of white blood cells is lower than in yearling salmon. There was a marked decline in Lct from pre-release (unmarked) to Knight's Landing sample in the 3rd release group (Table 17). Unmarked P3 fish were compared to the K3 group as only 2 of the 19 K3 samples were marked fish. The Lct values of the 2 Knight's landing samples (K1 and K3) were not statistically different. Similar to their plasma protein measurements, the marked P3 fish had a wide variation in Lct. In previous years, the mean Lct of Coleman FCS at release has ranged from 0.527 - 0.579.

Table 17. Mean Leukocrit (Standard deviation) of FCS-BCW-95-COL.

K1	21MAR	n = 16	0.780	(0.124)
P2	26MAR	n = 16	0.559	(0.065)
P3	22APR	n = 17	0.616	(1.020) a
	CWT		1.020	(0.077) b
	UNMARKED	n = 18		
K3	30APR	n = 19	0.735	(0.089) a

a - b Sample group values tested for significance ($P < 0.05$) by either T-test or 1-way ANOVA. Different letters signify statistically differences.

Plasma protein analysis - The plasma total protein concentration (TP) declined significantly (ANOVA, $P < 0.05$) between the pre-release and Knight's landing samples in all 3 release groups (Table 14). In the case of the K2 sample, 8 of the 9 TP samples were from marked FCS. The 2nd Chipp's island sample group's TP (including 1 marked CNFH P3 fish) was similar to the 2nd and 3rd pre-release levels and did not follow the same trend of decline as the Knight's landing groups. No samples were analyzed from the C1 or natural groups due to insufficient volumes. The 1st pre-release group had the highest TP of the 3 pre-release sample groups. Marked P3 fish showed a great degree of variation in TP values (coeff. of variation = 23 %).

Electrophoresis of the plasma samples yielded up to 11 fractions made up of 1-3 pre-albumin fractions, albumin, and 4 - 7 globular protein fractions (Table 18). To accommodate the variable appearance of some fractions, the percent area of several fractions were combined for statistical analysis. These fractions were: 1) all pre-albumin fractions, 2) globular protein fractions 4 and 5, and 3) globular fractions 6 and 7. The coefficient of variation for the percent area of each specific fraction ranged from 6 - 44 %. Pre-albumin and albumin tended to show an inverse relationship to each other. In a pattern similar to TP levels, pre-albumin fractions declined significantly between the 1st

and 2nd pre-release (P1, P2) and the corresponding Knight's landing sample groups (K1 & K2). Fraction 1 (alpha 1) was significantly elevated in the Knight's landing fish (K1 & K3) in comparison to their respective pre-release cohorts (P1 & P3). Fractions 4 & 5 (alpha 2 and/or beta) were elevated in the P1 fish in comparison to the K1 sample group. The 3rd GCID sample group also showed this elevation in comparison to both the pre-release (P3) and Knight's sample (K3). No significant differences in percent area were detected in the fraction 6 / 7 (gamma) between any of the sample groups.

Table 18. Plasma protein fractions separated by agarose gel electrophoresis. Data presented as mean (SE) %area of profile curve of pre-albumin (PA) fractions, albumin (ALB), fraction 1,2,3,4 & 5, and 6 & 7 .

Group	PERCENT AREA OF CURVE						
	PreAlb	ALB	F1	F2	F3	F4 / 5	F6 / 7
P1	31.8 b (3.1)	31.1 a (1.6)	7.8 b (0.6)	4.9 (0.8)	5.7 (0.5)	13.9 a (0.4)	4.8 (0.4)
K1	39.1 a (1.0)	21.3 b (1.7)	13.5 a (0.8)	5.6 (0.5)	5.7 (0.5)	11.3 b (0.7)	3.6 (0.4)
P2	28.6 b (1.7)	33.4 (2.3)	8.5 (0.6)	7.4 a (0.3)	5.7 (0.4)	12.5 (0.8)	3.9 (0.4)
G2	32.9 a (3.2)	27.9 (1.7)	11.1 (1.6)	5.6 b (0.6)	7.5 (0.9)	11.4 (0.5)	3.6 (0.4)
K2	37.3 a (1.2)	29.0 (0.9)	7.6 (0.9)	5.2 b (0.4)	5.8 (0.4)	11.8 (0.8)	3.3 (0.4)
P3	42.4 a (1.8)	27.5 b (1.0)	6.9 b (0.6)	4.4 (0.5)	5.1 (0.7)	10.8 b (0.9)	2.8 (0.4)
G3	20.0 b (2.5)	39.3 a (2.1)	8.5 b (0.8)	5.3 (0.3)	7.5 (0.5)	16.0 a (1.0)	3.5 (0.6)
K3	28.7 b (3.6)	27.4 b (2.0)	13.7 a (2.0)	6.4 (1.0)	6.8 (0.7)	12.6 b (0.6)	3.8 (0.4)

a - b Sample group values tested for significance ($P < 0.05$) by either T-test or 1-way ANOVA. Different letters within a release group signify statistically differences.

DISCUSSION

IHNV infection, in the FCS juveniles at CNFH, was judged to be more severe in 1996 than during the previous 5 years. Overall mortality in the hatchery was higher and sick fish could be detected in the majority of the rearing units. The disease became progressively worse following the initial detection in late February and handling stress seemed to exacerbate it in the 3rd tag group. It appears that at least some fish, in the later stages of the infection (with clinical signs such as exophthalmus "pop-eye"), can actively migrate along with apparently healthy cohorts. We could collect such fish 2-3 days post-release at GCID or 6-7 days post-release at Knight's Landing. Because of the short time span, it is likely that the clinically-ill fish at GCID were in advanced stages of the infection at release. Disease progression after release could also explain the sick fish captured at Knight's landing. Our sampling method at the downriver sites did not allow for a valid estimation of prevalence of infection, however, it is interesting that the 3 "grab" samples at Knight's landing had similar prevalences (20%, 13 %, and 14%). Given the size of the release groups, there could have been substantial numbers of infected fish in the river. In April 1996, we examined the virus shedding characteristics of clinically-ill FCS. Fish were placed into 100 mL sterile water and a 10 mL water sample taken after 1, 10, and 30 minutes. After the 30 min. sample, the fish was euthanized and samples of its mucus and kidney were taken. The fish rapidly shed 50 - 2500 Plaque forming units (PFU = " virus particle") / mL into the water and their external mucus contained 1000X more virus (mean 5.1×10^6 PFU / mL) . It is unclear whether these quantities of virus represent a threat to other fish. The limited work done with the "Coleman" strain of IHNV indicates that a long exposure (60 min.) to a high "dose" of virus (10,000 or more PFU / mL) is required before much mortality occurs to the test group (R. Hedrick, University of California, Davis, pers. comm.). The only physiological indicator of sub-clinical infection detected in the pre-release groups was the highly variable plasma protein concentrations of marked P3 group. No histological, hematological, or specific protein fraction changes were observed in sampled fish which could be attributed to early IHNV infection.

It is important to note that given the large population per raceway (avg. 450,000 fish), an IHNV- affected raceway would contain hundreds of thousands of "uninfected" fish. This phenomenon is shown in the low -to - moderate prevalence of IHNV infection detected in affected units when " grab " samples are taken. It is not unusual to only detect IHNV infections from the moribund "tailscreen" sub-population in a raceway. This distribution of sick animals is difficult to manage - does one destroy 100,000's of uninfected fish to cull out the infected fish? One management option to reduce the impact of releasing infected fish could be to cull out any weak fish from the raceway prior to release. Early release of infected groups may have reduced horizontal transmission, however, it appears that the migratory behavior (or survival) of chinook less than 70 - 75 mm FL was less than the standard "90 fish / lbs" smolt (such as the P3 group). Recovery of marked fish at Chipps island steadily improved with each release (group survival value of 0.21 for the P1 fish, 0.28 for P2, and 0.38 for P3 - preliminary data from SSJ-FWO, 04NOV960). As demonstrated by the marked P3 fish, the trend for larger smolt size and increased out-migrant survival appeared to outweigh health status at release. Marked chinook (presumptively P1 group) were observed 1-3

days after the 14MAR release within several small streams near Red Bluff but were not detected after several weeks (T. Moore, Chico St. Univ., pers. comm.). Portions of this release group may have attempted to rear in the upper river for some period of time before migrating. A more definitive answer to the validity of early release may come from adult return data.

No other infectious agents appeared to be significant health factors in the examined fish. Although various internal parasites were observed, none were judged to be causing significant disease. The absence of *Ceratomyxa shasta* infection in out-migrants was noteworthy as severe infections of this intestinal parasite are commonly observed in adult chinook collected in the winter and spring for CNFH broodstock. Lipofuscin deposits, observed within the hepatocyte vacuoles in 6 of 8 natural parr livers from the 23 FEB Posse Ground collection, indicate that oxidative damage had occurred to this organ. It is unclear what caused the abnormal fatty change in the liver or the associated oxidative damage to the cells. Natural smolts collected on 01APR near Ash creek did not show such lipofuscin deposits. The Posse Ground site is just downstream from Keswick Dam and the mouth of Spring Creek, while the Ash Creek site is some 70 m downstream of the Posse Grounds. The degenerative changes to portions of the pancreas (acinar cell & adipose cells) seen in some out-migrant fish may reflect the effect of rapid temperature change or lipid mobilization. Grossly these lesions appear as yellow spots on the pyloric caeca and are foci of lipofuscin deposits. We have observed these yellow spots from chinook smolts collected in the Klamath River and very rarely from chinook juveniles at CNFH. It does not appear that these lesions would affect the overall health of the fish. A higher percentage of the out-migrants showed inflammatory cell infiltration into the adipose tissues (steatitis) similar to that observed in hatchery smolts collected in the Klamath River during the warm water temperatures in spring. The severe scale loss observed in chinook captured in the Chipp's Island trawl suggests a high post-capture mortality in those fish released after measurements. Damage to the skin would affect the osmotic integrity of the fish and decrease its performance capabilities (Gadomski et al. 1994).

It is assumed that the majority of unmarked chinook collected at GCID and Knight's landing were CNFH fish from the same release group. While not all fish collected downriver were marked (K1-3 = 2%, 5%, 36 % CWT collected), the fork lengths and external characteristics of the marked and unmarked fish were quite similar. Also, these collections occurred during the peak catch for each release group.

The energy reserves of the CNFH smolts appear to be sufficient for their out-migration down the Sacramento River. Energy reserve indicators such as % moisture (lipid), plasma triglyceride, visceral fat, blood glucose response to stress changed little during the 7 day post-release period. The increase in plasma TG (circulating lipid) seen in the 06MAY Chipps island chinook, compared to their CNFH cohorts collected 6 days earlier at Knights Landing, may reflect the mobilization of body lipids. This occurrence corresponds with the change in % moisture (drop in body lipid) and indicates that 2 weeks post-release may be the point where the fish's energy balance, while still substantial, is in a negative state. In contrast to the drop in post-release condition factor seen with the 2nd and 3rd release groups, the condition factor of the 1st release

group did not change significantly as the fish moved downstream. It is possible that incomplete smolt development influenced this difference. Water temperature and time post-release for each site collection was nearly identical for each group. While showing less visceral fat reserves than hatchery fish, the plasma TG of natural chinook was significantly higher than the out-migrant hatchery smolts at Knight's landing (Kruskal-Wallis 1-way ANOVA on ranks, $H= 8.51$, $P=0.0366$) and similar to the fed pre-release sample groups. This data indicates that the natural chinook obtained adequate energy from dietary sources.

In spite of wide size differences (FL range 61 - 92 mm), individual fish from each of the 3 release groups demonstrated the ability to osmoregulate in 30ppt saltwater. Fork length did not always explain the ability of a given fish to maintain its plasma sodium near the normal maximum of 170 mmol /L (Blackburn and Clark 1987). We used 174 mmol/L as a cutoff to take into account technique variation. The osmoregulatory ability for the overall release group did appear to improve in the 3rd release group over the previous 2 groups. While fish size was one obvious difference between these groups, age or exposure to different environmental cues could also be factors in the smolt development differences. The gill ATPase activities appeared to increase after 1- 2 weeks post-release. Both Chipp's Island samples and the K3 group had significantly higher activities than their respective pre-release cohorts. Similar to the SW challenge response, fork length did not explain a fish's chloride cell ATPase activity. Gill ATPase may not be a valid indicator of smolt development for fish in the hatchery. Environmental cues such as novel water chemistries and cortisol responses to stress may both act to accelerate the number and activity of chloride cells following release from the hatchery (Nishioka et al. 1985, McCormick et al. 1991).

No definitive changes in the immune defense indicators (Lct, Total plasma protein and electrophoretic profile) were identified in the sampled groups. The drop in circulating white blood cell numbers (as estimated by Lct) seen in the smolts captured post-release was probably due to cortisol effects and is similar to that observed in the Trinity River (FHC 1992- 1995 data, unpubl.). It is possible that fewer lymphocytes will result in a reduction in host immunodefences (Maule et al. 1989). The evaluation of the electrophoresis data was hindered by several factors including:

- 1) uncertainty of the composition for each plasma protein fraction (inability to locate reference literature on this subject).
- 2) use of plasma which unlike clotted serum contains fibrinogen and could introduce variation in the later globular protein fractions (F3-7).
- 3) the relationship of the substantial pre-albumin fractions and albumin (do they contain breakdown products due to processing effects?) .

The electrophoretic profile of human serum run on agarose gel from (+) anode to (-) cathode is: pre-albumin, albumin, alpha 1 proteins, alpha 2 proteins, beta proteins , fibrinogen, and (nearest the sample origin), gamma proteins (CIBA product information). Pre-albumin proteins are reported to act as carriers for thyroxine and retinol (Burtis and Ashwood 1986). The liver is the site of production for the majority of plasma proteins. Albumin is the most abundant plasma protein in humans and can account for 30 - 60 % of the total protein. It has 3 primary functions; 1) carrier protein for many ligands such as hormones, fatty acids, drugs, calcium, and bilirubin , 2) major

determinant of plasma oncotic pressure, and 3) acts as a source for endogenous amino acids. Globular proteins of significance to fish are listed below:

Alpha 1	antitrypsin	neutralize phagocytic elastase
Alpha 2	haptoglobin	capture of free hemoglobin
	macroglobulin	inhibits proteases
	ceruloplasm	oxidize iron, binds copper
Beta	transferrin	binds cations such as iron
	lipoprotein	carriers of fatty acids
	fibrinogen	clotting protein group
	C3	part of complement system
Gamma	immunoglobulin	in fish = IgM
	lysozyme	proteolytic enzyme
	c-reactive protein	agglutinates bacterial cells

Further studies will be required to positively identify the components of the 11 fractions separated by agarose electrophoresis. Tentative assignments are:

pre-albumin	
albumin	
F1 - F2	alpha 1
F3-F4-F5	alpha 2 and beta
F6-F7	gamma

Recommendations for future studies:

- 1) Examine horizontal transmission of IHNV when smolts are exposed to different concentrations of virus for varying amounts of time. This information would aid in judging the risk of releasing infected fish on other juvenile chinook in the river.
- 2) If an IHNV outbreaks occurs, track the prevalence of infection in the affected unit and compare to the mortality rate. Is mortality an accurate indicator of infection ?
- 3) Determine whether the prevalence of infection changes in the population following release by conducting regular collections at downriver sites.
- 4) Examine physiological changes in hatchery smolts collected 1 - 2 weeks post-release. Compare to natural chinook smolts of similar age.
- 5) Examine natural chinook smolts for tissue abnormalities (histology) and the presence of IHNV.

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