

**HEALTH AND PHYSIOLOGY OF BROODYEAR 1996
COLEMAN NATIONAL FISH HATCHERY FALL CHINOOK
(*ONCORHYNCHUS TSHAWYTSCHA*)**



Daniel Free and J. Scott Foott
U.S. Fish and Wildlife Service
California-Nevada Fish Health Center
24411 Coleman Hatchery Road
Anderson, CA 96007

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SUMMARY

During March through May 1997, the California-Nevada Fish Health Center performed a series of health and physiological sample collections prior to release from Coleman National Fish Hatchery (CNFH) and 183 river miles downstream (Knight's Landing RM90) in support of a 1997 mark and recapture study with brood year 1996 fall chinook (FCS) juveniles from CNFH. Fish from three of the four release groups were examined for disease, smolt development, energy reserves, immunodefences, and organosomatic parameters. As in years past, IHNV infection was the most significant factor affecting FCS production at CNFH. Three of the four release groups had infection rates as high as twenty percent prior to release. Ten-day percent mortality rates ranged as high as 10.7% in some raceways. Our data indicates that an increase in mortality over 0.5% in a seven-day period may indicate infection rates exceeding ten percent. We observed nine to sixteen percent incidence of IHNV infection rates 183 river miles downstream at Knight's Landing. Further efforts to minimize IHNV at CNFH and investigations of in-river horizontal transmission are warranted. Determination of smolt status through analysis of twenty-four hour saltwater challenge data and gill ATPase activity indicated that fish released on 31 March and 14 April were likely more developed as smolts than fish released on 6 May. Analysis of smolt indices of fish collected downstream at Knight's Landing indicated that they were more developed as smolts than their prerelease cohorts. Energy reserves of CNFH smolts appears to be sufficient for their migration down the Sacramento River. Comparisons of energy reserves of prerelease groups with their Knight's Landing cohorts indicates significant mobilization of lipid reserves 11-16 days post-release. Monitoring of the health and physiology of coded-wire-tagged groups will allow for better analysis of tag recovery data.

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INTRODUCTION

Coleman National Fish Hatchery (CNFH) is located on lower Battle Creek, a tributary of the Upper Sacramento River. The hatchery rears and releases three stocks of upper Sacramento River chinook salmon (fall = FCS, late-fall, and winter run chinook, *Oncorhynchus tshawytscha*) and steelhead trout (*O. mykiss*). 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 River chinook populations include *Renibacterium salmoninarum* (BKD agent) and a number of parasites (*Ceratomyxa shasta*, *Nanophyetus salmincola*, *Phyllobothrium salmosis*, *Chloromyxum* sp., and the myxosporean responsible for Proliferative Kidney Disease). 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 at both CNFH and 183 river miles downstream (Knight's Landing RM90) in support of a 1997 mark and recapture study with brood year 1996 FCS from CNFH. The original study design was to fractionally mark and coded-wire-tag (CWT) fish from each of twenty-eight raceways for a total fraction of one million marked fish from a total population of twelve million. These fish would be released in three replicate groups to evaluate migration rate and survival of Upper Sacramento River FCS at various recapture sites along their migration route to the Pacific Ocean. 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 as 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 and administered by the USFWS' Sacramento-San Joaquin and Northern Central Valley Fish and Wildlife Offices.

METHODS

Sample Collections

Juvenile chinook, from three of four releases, were sampled for organosomatic, disease, and physiological parameters at the following sites (Figure 1):

- 1) CNFH just prior to the release of their respective cohort group (confluence of Battle Creek and Sacramento River at river mi. 273); Prerelease I, II, and III.
- 2) California Department of Fish and Game screw traps located below Knight's Landing Bridge 11-16 days postrelease (river mile 90); Knight's Landing I and II were scheduled to approximately coincide with the peak migration timing of Prerelease I and II.

In addition, all fall chinook production was monitored for IHNV throughout their rearing at Coleman NFH. This was done to identify raceways of fish infected with IHNV in conjunction with CWT tagging operations in order to minimize the disease exacerbating effects of handling associated with the marking effort. This monitoring effort also provided information for early release decisions. In general, weekly collections of moribund fish were made approximately five to ten days ahead of tagging operations. These collections were processed according to the viral assay methods described later in this report.

Organosomatic Assay

Approximately thirty fish were collected at CNFH from three of four release groups and Knight's Landing rotary screw traps for a modified organosomatic assay on 31 March (Prerelease I), 14 April (Prerelease II), 16 April (Knight's Landing I), 25 April (Knight's Landing II), and 30 April (Pre-release III). A group of fish released early on 9 April due to high IHNV-related mortality was not sampled. The organosomatic assay is an autopsy-based method for ordered observation of external and internal tissues and organs, hematological parameters, and size criteria for evaluation of the health status of a group of fish (Goede and Barton 1987, Foott 1990). A numeric "severity" score (0, 1, 2, 3) is assigned to each tissue where zero represents normal status and three represents severe abnormality (Appendix A). Generally, five fish were netted from a bucket and euthanized with an overdose of benzocaine. External characteristics (fins, eyes, skin, gills) were scored, length and weight measurements were taken, and the caudal peduncle was severed for collection of blood in heparinized capillary tubes. Blood was centrifuged at 10,000 RPM for ten minutes after which hematocrits (% packed erythrocyte volume) and leukocrits (% white blood cell volume) were measured and plasma was collected and stored at -80°C for later analysis of total protein, albumin, and plasma osmolality. Leukocrits were calculated by measuring the "buffy coat" (BC) above the red blood cell band with an eyepiece micrometer (Bausch and Lomb binocular dissection scope, one eyepiece unit=0.017mm) at 30x magnification. The leukocrit is equal to: BC length(mm) divided by total liquid length(mm) multiplied by 100. The abdominal cavity was then opened and internal observations were made (organ abnormalities, visceral fat score). Tissues from five to ten fish were processed for histological evaluation.

Pathogen Assays

Kidney tissue was removed and frozen for later *R. salmoninarum* antigen analysis by an Enzyme Linked Immunosorbent Assay (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 O.D. subtracted, log transformed, and the data analyzed by ANOVA. Three semi-quantitative categories are used to view the non-transformed data: 1) BNC = sample O.D. **Below the Negative kidney Control** value, 2) Suspect = sample O.D. above BNC and less than 0.2 (subjective value where there is the likelihood of confirming infection by direct fluorescence antibody testing), and 3) Positive = sample O.D. >0.2 .

Two fish pools of a triangular subsection of each fish were processed for viral assays. The subsection was produced by a vertical incision through the head at the insertion of the operculum and a diagonal incision from the dorsal fin to the ventral aspect of the operculum. This subsection contained gill, epithelium, anterior kidney, and liver. Because skin was included in the subsection, we cannot differentiate between an “external” infection of the integument and a systemic infection. The samples were homogenized in sterile Hank's Buffered saline at a 10X dilution (Wt / vol), centrifuged at 10,000RPM 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 uL 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 and pretreated with 7%PEG (Fijan et al. 1983). The plates were overlaid with a MEM-7.5%FBS 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 antibody dot blot test.

Incidence of IHNV Infection Compared with Mortality Rate

A ninety-two fish sample was collected at seven day intervals from a raceway (#22) of approximately 440,000 fall chinook juveniles. Collection began soon after the initial observation of IHNV associated mortality on 20March and continued every seven days up to their release on 9April. An “unbiased” sample was obtained by collecting approximately thirty fish with a cast net from the front, middle, and rear of the raceway to produce the ninety-two fish sample. The ninety-two fish sample size was chosen to produce an eighty-five percent Group Sensitivity confidence interval (GSENS) (Thornburg 1996). This sensitivity was derived from the formula: $GSENS=1-(1-\text{apparent prevalence})^n=1-(1-0.02)^{92}=0.85$ and describes the probability that a test-positive animal is collected in a population with a given prevalence of infection. Samples were prepared in the same manner as the previous description for viral assays. Cumulative mortality was tracked on a weekly basis.

Incidence of IHNV Infection in Out-migrant CNFH Smolts Collected at Knight's Landing

Ninety to Ninety-two fish samples were collected by California Department of Fish and Game and CA-NV FHC six, ten, and fifteen days post-release at Knight's Landing (183 river miles downstream of CNFH) for determination of IHNV infection incidence in out-migrants. These fish were presumably (adipose fin-clipped fish from CNFH were present in each sample) from two releases of approximately four million fish each from which IHNV infection prevalence had been previously determined. Samples were prepared in the same manner as the previous description for viral assays.

Physiological Assays

Plasma was assayed for total protein (Sigma Procedure No.541; Ektachem EL340 Microplate Reader), albumin (Sigma Procedure No.631; Ektachem EL340 Microplate Reader), and osmolality (Wescor Vapro™ mod.#5520 vapor pressure osmometer). Gill Adenosine Triphosphatase activity ($\mu\text{moles ADP} / \text{mg protein} / \text{hr}$) 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 energetically transports salts from the fish against the concentration gradient to the saltwater.

Energy reserves (lipid) were assayed by chloroform/methanol extraction of fish with head, gut contents, and fins removed using a modification of the methods described by Bligh and Dyer (1959). Fish were prepared by removing head, fins, and gut contents, weighed, homogenized in a Waring Blender in a 1.5:1 deionized water:sample ratio, and a subsample of homogenate (target: 1-2g of fish tissue) was removed and placed into 50ml conical tube for drying. Subsample tissue weight can be calculated from homogenate weight by:

$$\frac{(\text{sample weight})}{(\text{sample weight} + \text{DI water weight})}(\text{homogenate subsample weight}).$$

The homogenate subsample was then dried at 100°C for twenty-four hours, a 10x wet tissue weight volume of a two part chloroform: one part methanol (CM) solution was added and allowed to extract for thirty minutes, deionized water at 40% of CM solution volume was added to separate chloroform + lipid from methanol fraction, mixture was centrifuged at 4000RPM for five minutes, known volume of top chloroform + lipid supernatant was drawn-off and added to pre-weighed aluminium pan, chloroform was evaporated, and pan was reweighed to obtain lipid (extract) weight. Percent lipid was calculated by:

$$[(\text{extract(g)}/\text{wet tissue subsample(g)}) \times (\text{chloroform volume(ml)} \times \frac{2}{3} / \text{supernatant volume(ml)})] \times 100.$$

Example:

10g fish homogenized in 10 ml DI water; 2grams homogenate subsample removed---How much fish tissue in homogenate subsample?

$$(\text{10g fish}) / (\text{10g} + \text{10ml}) * \text{homogenate subsample weight} = \text{subsample tissue weight}$$

$$(\text{10}/\text{20}) * \text{2} = \text{1g}$$

tissue subsample weight = 1.0g

10X CM volume = 10ml; chloroform volume = $\frac{2}{3}(10) = \mathbf{6.7ml}$

extract volume = 5ml

extract weight = 0.1g

$$[(\text{0.1g}/\text{1.0g}) \times (\text{6.7ml}/\text{5ml})] \times 100 = \mathbf{13.4\% \text{ lipid (wet weight)}}$$

All extraction procedures were performed in a fume hood while wearing Nitrex gloves and a full-face respirator with organic filter.

Histology

Gill, intestinal tract, pyloric ceca, 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.

Salt-water Challenge

A subsample of fish from each of the three release groups were challenged in 30ppt saltwater for twenty-four hours on 31 March, 14 April, and 29 April. Ten fish from each release group (representatives from each raceway) were collected by mid-pond sample and placed in 30L buckets of aerated, ambient temperature water with 900g of Instant Ocean[™] (salt) added. After twenty-four hours, buckets were checked for mortality, salinity, and dissolved oxygen. Living fish were euthanized with an overdose of a 30ppt saline MS-222

solution and immediately weighed and measured. The caudal peduncle was severed for blood collection in heparinized capillary tubes and plasma was separated by centrifugation (10 minutes @ 10,000RPM). Plasma was stored at -80°C for later sodium analysis using a Kodak DT-60/DTEII Module and osmolality determination using a Wescor Vapro™ mod.#5520 vapor pressure osmometer.

Statistical Analysis

Data was tested for normality and either analyzed by parametric (T-test, 1-way ANOVA) or non-parametric tests (Mann-Whitney rank sum test, Kruskal-Wallis ANOVA on ranks). If significant differences among groups were detected in ANOVA tests, Student-Newman-Keuls or Dunn's 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

Release Statistics

A total of 12,441,975 broodyear 1996 FCS smolts were released from CNFH on four dates from 1 April to 6 May (Table 1). Approximately eight percent (1,002,015) of these were implanted with a coded-wire-tag and adipose fin-clipped for identification. Recovery of coded-wire tags from fish in outmigration samples, ocean fisheries, and after adult return to the hatchery will provide important information regarding the survival and contribution of these release groups. We sampled fish from three of the four release groups prior to release.

Table 1. Broodyear 1996 FCS release summary. Information was obtained from the U.S. Fish and Wildlife's Northern-Central Valley Fish and Wildlife Office.

| Release date | Number marked | Number unmarked | Mean fork length (mm) | Sampled? |
|--------------|------------------|-------------------|-----------------------|----------|
| 1 April | 324,977 | 3,618,122 | 71.1 | Yes |
| 9 April | 101,323 | 1,948,233 | 68.5 | No |
| 15-16 April | 466,902 | 4,800,757 | 67.6 | Yes |
| 6 May | 108,813 | 1,072,848 | 72.3 | Yes |
| Total | 1,002,015 | 11,439,960 | --- | --- |

Organosomatic Assay

Gross tissue abnormalities, such as fatty liver, necrotic gill spots, exophthalmia, hemorrhaged thymus, and skin damage were noted in all groups with the exception of Prerelease II (Table 2). Incidence of skin damage in the two Knight's Landing samples may indicate trap-related injuries.

It is presumed that the hemorrhaged thymus and exophthalmia observed in the Knight's Landing I sample was due to IHNV infection. Fatty liver was observed in some fish from Prelease I and III and is likely the result of the high energy diet these fish receive during hatchery residence.

Mean hematocrits of all sample groups were within the normal range for juvenile chinook (Table 2). Both Knight's Landing samples had significantly higher ($p < 0.05$) hematocrits than their corresponding prerelease samples (Table 2). This may indicate hemoconcentration in fish from the Knight's Landing samples due to the stress of capture and holding at the trap or splenic RBC release. A comparison between prerelease groups indicated a significantly lower ($p < 0.05$) mean hematocrit for fish from Prerelease II than Prerelease I or III (Table 2). Reasons for this observation are unknown, but hemodilution

may indicate a higher incidence of IHNV infection in Prerelease II fish sampled for hematocrit as compared to Prerelease I and III.

Table 2. Mean (standard error) values for hematocrit, total sample number, and brief description of tissue abnormalities observed in sampled fish from pre-release and Knight's Landing organosomatic assays.

| Organo ID / Date | Hematocrit | Tissue abnormalities |
|-------------------------|-------------------|--|
| PrereleaseI/31MAR | 42.4 (0.74) | 1/20 w/necrotic gill spots, 1/20 w/fatty liver |
| Knight I/16APR | 49.7 (3.37) | 13/20 w/skin damage, 3/20 w/necrotic gill spots, 1/20 w/ exophthalmia, 2/20 w/hemorrhaged thymus |
| PrereleaseII/14APR | 39.5 (0.89) | 0/20 |
| Knight II/25APR | 43.3 (0.88) | 2/20 w/skin damage |
| PrereleaseIII/30APR | 43.4 (0.74) | 4/20 w/fatty liver, 2/20 w/necrotic gill spots |

No significant differences in mean fork lengths or condition factor were detected for fish sampled during the three prerelease examinations (Table 3). Fish from the first release group (Prerelease I) were similar in size when captured sixteen days post-release at Knight's Landing. Fish from the second release group (Prerelease II) were significantly ($P < 0.05$) shorter than their cohorts captured eleven days post-release. Fish sampled downstream at Knight's Landing had significantly ($P < 0.05$) lower condition factors than their respective prerelease cohorts (Table 3).

Table 3. Mean (standard error) values for total length(mm), fork length(mm), weight(g), and condition factor (k) of sampled fish from pre-release and Knight’s Landing organosomatic assays.

| Organo ID/date | Total length (mm) | Fork length (mm) | Weight (g) | Condition factor |
|-----------------------|--------------------------|-------------------------|-------------------|-------------------------|
| PrereleaseI/31MAR | 80.6 (0.712) | 75.3 (0.700) | 4.6 (0.145) | 0.8629 (0.009) |
| Knight I/16APR | 84.3 (0.842) | 77.5 (0.771) | 4.8 (0.131) | 0.7913 (0.011) |
| PrereleaseII/14APR | 79.9 (1.452) | 74.3 (1.429) | 4.7 (0.282) | 0.8827 (0.011) |
| Knight II/25APR | 86.0 (0.991) | 79.7 (0.964) | 4.8 (0.161) | 0.7471 (0.009) |
| PrereleaseIII/30APR | 77.8 (0.902) | 72.7 (0.989) | 4.1 (0.154) | 0.8692 (0.008) |

Observed mean leukocrit values were highly variable between prerelease and Knight’s landing samples. Prerelease I leukocrit values were significantly ($P=0.0259$) higher than Prerelease II or III (Table 4). Knight’s Landing I leukocrit values were significantly ($P=.00416$) lower than their prerelease I cohorts. Reasons for variability in leukocrit values are unknown, but capture stress associated with Knight’s Landing samples may confound results.

Table 4. Mean (standard error) leukocrit values for sampled fish from prerelease and Knight’s Landing organosomatic assays.

| Organo ID / date | Number of samples | Leukocrit value |
|-------------------------|--------------------------|------------------------|
| PrereleaseI/31MAR | 24 | 1.139 (0.082) |
| Knight I/16APR | 19 | 0.740 (0.106) |
| PrereleaseII/14APR | 22 | 0.863 (0.108) |
| Knight II/25APR | 20 | 1.476 (0.368) |
| PrereleaseIII/30APR | 18 | 0.814 (0.071) |

Significant Mortality Events

Two notable elevated mortality events were observed in brood year 1996 FCS. The first mortality event occurred shortly after ponding and coagulated yolk was identified as partially responsible for this elevated mortality. Fifteen-day mortality rates as high as 3.5% were observed in some groups with chronic mortality that continued approximately thirty days post-ponding. The second elevated mortality event was caused by IHNV infection. This event began on about 20 March, occurred randomly throughout production raceways, and continued through the final release of affected raceways on 15 April. Mortality rates during this event ranged as high as 10.7% in some raceways (Figure 2).

ELISA-R. salmoninarum (Bacterial kidney disease)

As in the previous years, bacterial kidney disease was not a problem in brood year 1996 FCS. Assays for *R. salmoninarum* antigens did not reveal concentrations in any sampled fish that were high enough to warrant a positive designation (Table 5).

Table 5. Summary of ELISA results. Each grouping represents the number of fish (percent) that had optical densities of the antibody-antigen reaction that categorized the sample as being negative, suspect, or positive for *R. salmoninarum*.

| Below negative control | Antigen suspect | Antigen positive |
|-------------------------------|------------------------|-------------------------|
| 24/31 (77) | 7/31 (23) | 0/31 (0) |

Incidence of IHNV Infection Compared with Mortality Rate

The cumulative weekly mortality in raceway 22 increased from 0.3% to 3.5% over the four week period and coincided with an increased IHNV infection prevalence from 2% to 22% (Figure 3). The vast majority of fish collected were asymptomatic for IHNV infection. Previous decisions on early release of raceways due to IHNV infection were triggered when mortality was greater than or equal to one percent and beginning to double. It appears that cumulative weekly mortality rates of 0.5% or greater may indicate a substantial incidence of IHNV infection (10% or more) in the population. Early release of juveniles due to IHNV should take these findings into account.

Incidence of IHNV Infection in Out-migrant CNFH Smolts Collected at Knight's Landing

The Prerelease I viral sample indicated a twenty percent prevalence of infection (POI) in this group. Outmigrants from this release group captured six, ten, and fifteen days post-release 183 river miles downstream at Knight's Landing had a POI that ranged from nine to sixteen percent (Figure 4). The majority of fish sampled were asymptomatic for IHNV infection. This pattern was not observed in the evaluation of Prerelease II in which a twenty percent POI at CNFH did not correlate with any IHNV infected fish ten days post-release at Knight's Landing.

Histological Examination

While several trematode and protozoan parasites were observed in 29% of the fish examined in the study, no infection was deemed significant (Table 6). It is common for CNFH fish to have asymptomatic infections of *Nanophyetus salmincola* metacercaria.

Necrotic changes were seen in some regions of the heart (ventricular myocardium), kidney interstitium, and liver parenchyma of chinook collected at Knight's Landing and in one fish from the first pre-release examination. It is curious to speculate that these necrotic foci were related to an early stage of IHNV infection. The excessive glycogen deposits observed in the liver hepatocytes of chinook sampled prior to release was not seen in cohorts collected 11-16 days post-release at Knight's Landing. These glycogen stores are reflective of the excess dietary energy that fish enjoy at the hatchery and cause the liver to be slightly pale and enlarged. Liver and muscle glycogen is one of the first energy stores utilized by salmonids during times of food-deprivation (Arndt et al. 1996). Moderate inflammatory changes were seen in the pancreatic tissue (adipose and acinar cells) of several fish collected at Knight's Landing. We have observed a much higher incidence and severity of this lesion in Klamath River chinook smolts during their June and July outmigration. The cause(s) of this condition is not understood, however, high water temperatures ($> 17^{\circ}\text{C}$) seem to increase its severity.

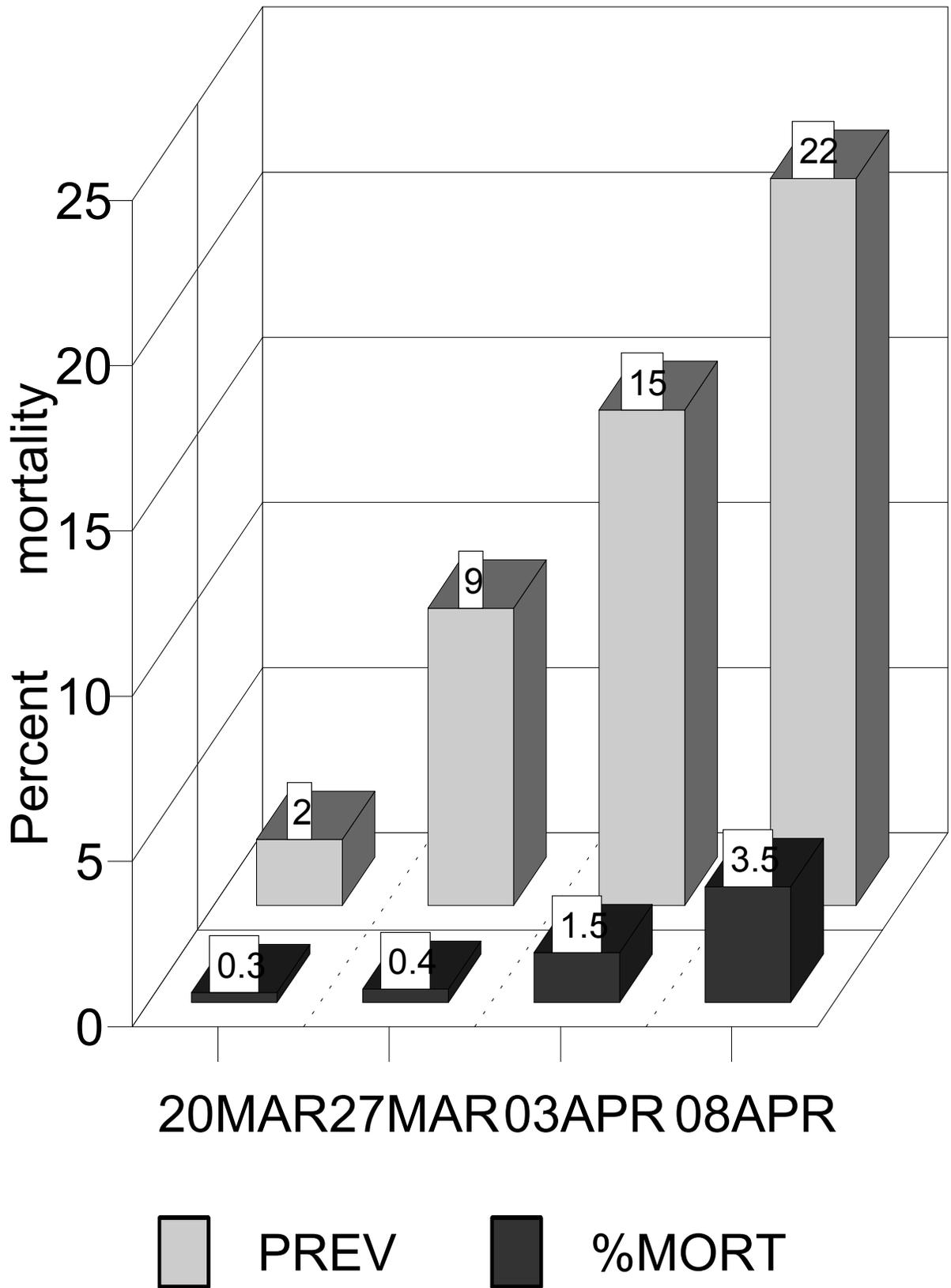


Figure 3. IHNV prevalence and percent seven-day mortality of the raceway 22 population.

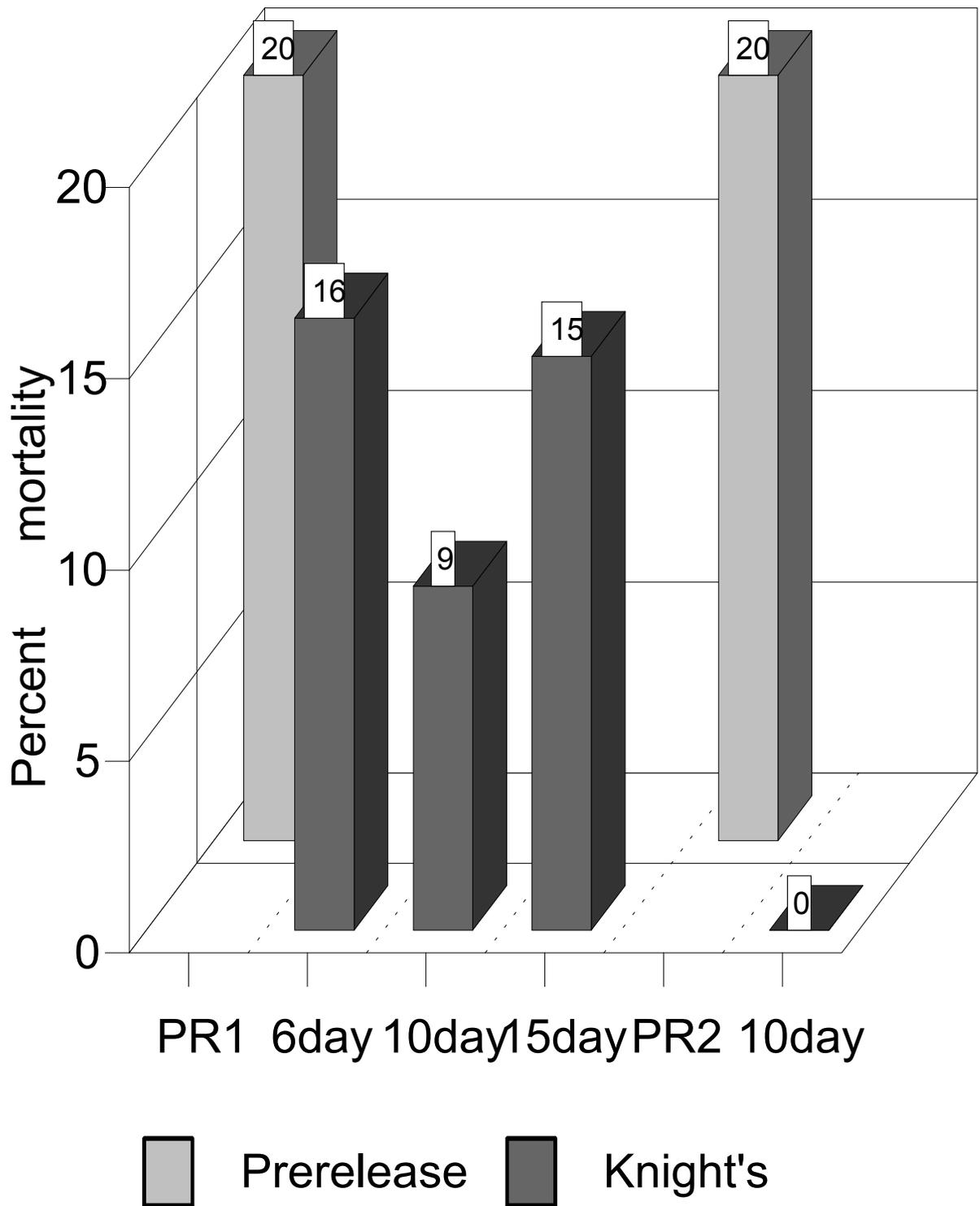


Figure 4. IHNV prevalence observed in prerelease samples and six to fifteen days post-release downstream at Knight's Landing

Table 6. Histological examination of tissues from chinook smolts collected prior to release (PR1 & PR2) and at Knight's Landing (KL). Parasites include metacercaria stage (METC) of *Nanophyetus salmincola*, *Ichthyophtharis multiphilis* (ICH), and pre-sporogonic myxozoans (MYXO) found within the glomeruli and lumen of kidney tubules. Lesions and abnormalities observed include large glycogen deposits (HGLY), necrosis (NECR) pancreatitis (PANC), adipose inflammation or steatitis (STEA), epithelial hypertrophy (EHPT), and lipofuscin deposits (LPFD). Prevalence of infection or disease data presented as number positive / total examined.

| | PR1 31MAR KL 25APR | PR2 14APR | KL 21APR |
|----------------------------------|-----------------------------|------------|------------|
| Gill Parasite | 1 / 8 METC | 0 / 5 | 0 / 9 |
| Lesion | 3 / 8 EHPT | 0 / 5 | 1 / 9 NECR |
| Kidney Parasite | 0 / 10 | 0 / 5 | 2 / 9 METC |
| Lesion | 0 / 10 | 2 / 5 NECR | 3 / 9 NECR |
| Liver Parasite | 0 / 11 | 1 / 5 METC | 0 / 9 |
| Lesion | 11 / 11 HGLY 1 / 11 NECR | 4 / 5 HGLY | 3 / 9 NECR |
| Heart Parasite | 0 / 2 | Not done | 0 / 4 |
| Lesion | 0 / 2 | | 2 / 4 NECR |
| Intestine Pyloric cecum Parasite | 0 / 9 | 0 / 5 | 0 / 9 |
| Lesion | 0 / 9 | 0 / 5 | 2 / 9 PANC |
| | | | 2 / 7 METC |
| | | | 2 / 7 LPFD |

Smolt development

Twenty-four hour saltwater challenge survival for the three release groups was 82%, 90% and 92% for Prerelease I, Prerelease II, and Prerelease III, respectively (Table 7). These survival values may not be indicative of osmoregulation competence or smolt development. A better indicator may be mean plasma sodium levels and the percentage of fish with values less than 174 mmol/l (Clarke and Blackburn 1987). Other indicators are plasma osmolality and the magnitude of dehydration (as indicated by a decrease in condition

factor) of saltwater challenge fish as compared to their freshwater cohorts. From the results of these different assays and comparisons, it may be assumed that fish from Prerelease II had a greater ability to osmoregulate in 30ppt saltwater than fish from Prerelease I or III. The observed poor osmoregulatory ability of fish from Prerelease III is also corroborated by evidence of significant dehydration in this group (11.1%) as compared to either Prerelease I or II (3.7% and 4.8%, respectively) (Table 7). One variable that should be taken into account is the smaller size of fish (mean FL=69.5mm) from the Prerelease III challenge as compared to the size observed in the population (mean FL=72.7mm). The results of the challenge with the smaller fish may not accurately portray smolt development of the population as a whole, but we are unable to detect correlation between fork length and plasma sodium levels. Also, the effects of active outmigration on the aforementioned measured variables are unclear, but there is evidence in the literature that suggests that certain physical and biological cues are experienced by outmigrants that are not experienced by fish residing in the hatchery and these cues may trigger further smolt development (McCormick et al. 1991, Nishioka et al. 1985).

Table 7. Mean (standard error) plasma Na⁺(mmol/l), percent survival, percent with plasma Na⁺ >174mmol/l, mean plasma osmolality(mmol/kg), mean fork length(mm), and mean condition factor(k) of samples of fish from prerelease groups challenged with 27-30ppt saltwater for 24hrs.

| Variable measured | Prerelease I 31MAR | Prerelease II 14APR | Prerelease III 30APR |
|---------------------------------|-------------------------------|--------------------------------|---------------------------------|
| Number of samples | 11 | 20 | 12 |
| Plasma Na ⁺ (mmol/l) | 178.4 (6.7) | 172.5 (5.8) | 183.8 (3.2) |
| Percent survival | 82% | 90% | 92% |
| %Plasma Na ⁺ >174 | 50% | 38% | 88% |
| Osmolality(mmol/kg) | 370 (18.2) | 396 (22.2) | 421 (23.8) |
| Fork length(mm) | 73.5 | 73.5 | 69.5 |
| Condition factor(FW) | 0.8629 | 0.8827 | 0.8692 |
| Condition factor(SW) | 0.8311 | 0.8402 | 0.7725 |

Another measure of smolt development is gill Na-K-ATPase activity (μ moles ADP / mg protein / hr). The results of this assay somewhat confound the results of the saltwater challenge results. The results of this assay indicate that fish from Prerelease I had higher gill Na-K-ATPase activities than fish from Prerelease II and III which may indicate greater smolt development (Table 8). Also, fish from both Knight's Landing samples had higher gill Na-K-ATPase activities than their respective release groups. Fish from the Knight's Landing II sample had gill Na-K-ATPase activities that were significantly ($P=0.0049$) higher than the Prerelease II sample. Fish from the Knight's Landing I sample had gill Na-K-ATPase activities that were higher than the Prerelease I sample, but the difference was not statistically significant ($P<0.05$). It could be argued that gill Na-K-ATPase activity *increased* over time post-release, but this is confounded by the larger size of the fish from both Knight's Landing samples as compared to their respective prerelease samples (Table 8). It could also be argued that fish sampled at Knight's Landing are actively outmigrating and more developed as smolts, therefore the population being sampled may be different than the population sampled during prerelease examinations.

One additional observation that may indicate increased smolt development is the lower condition factor values observed in fish from the two Knight's Landing samples as compared to the prerelease samples (Table 8). A decrease in condition factor has been identified as one change that occurs during smolt development (Wedemeyer 1996). It is also interesting to note that fish collected for the Knight's Landing II sample had the highest mean gill Na-K-ATPase activity and also the lowest mean condition factor, further attesting to possible correlation between condition factor and smolt development (Table 8). Again, these results are confounded by sampling strategies and environmental differences between the two sample sites.

Table 8. Mean (standard error) gill Na-K-ATPase activity(μ molesADP/mg protein/hr), percent of fish with gill Na-K-ATPase activity characteristic of smolts, mean fork length(mm), and mean condition factor (k) in a subsample of fish from pre-release and Knight's Landing organosomatic assays.

| Organo ID/Date | Na-K-ATPase | Fork length (mm) | Condition factor |
|-----------------------|--------------------|-------------------------|-------------------------|
| PrereleaseI/31MAR | 6.1 (0.34) | 74.8 | 0.8704 |
| Knight I/16APR | 6.8 (0.82) | 76.9 | 0.8202 |
| PrereleaseII/14APR | 4.4 (0.58) | 76.6 | 0.8777 |
| Knight II/25APR | 7.5 (0.62) | 79.6 | 0.7258 |
| PrereleaseIII/30APR | 4.1 (1.31) | 74.4 | 0.8778 |

Energy Reserves

Evaluation of the energy reserves of FCS smolts by direct observation (visceral fat scores) and body composition analysis (%lipid) revealed a significant ($P < 0.05$) decrease post-release (Table 9). Visceral fat score (VFAT) of fish from Prerelease III were significantly higher ($P = 0.0156$) than VFAT scores of Prerelease I or II which is not correlated with observed percent lipid values (Table 9). Reasons for this are unknown, but may be explained by the subjective nature of the VFAT scoring and the fact that the body composition analysis technique measured percent lipid of the whole fish; not just visceral fat.

Table 9. Median visceral fat (VFAT) score, number of fish in sample with visceral fat scores < 1, and percent lipid in sampled fish from prerelease and Knight’s Landing organosomatic assays.

| Organo ID / Date | VFAT score | #VFAT<1 | %Lipid |
|-------------------------|-------------------|-------------------|---------------|
| PrereleaseI/31MAR | 2 | 0/30 | 11.8% |
| Knight I/16APR | 1 | 1/30 | 7.99% |
| PrereleaseII/14APR | 2 | 1/23 | 11.9% |
| Knight II/25APR | 1 | 0/20 | 8.70% |
| PrereleaseIII/30APR | 2 | 0/20 | 10.6% |

Blood Chemistries

Mean plasma total protein concentration was significantly ($P<0.05$) lower for Prerelease II as compared to Prerelease I or III (Table 10). Reasons for this are unclear, but hemodilution effects of IHNV infection may provide partial explanation. Also, hemodilution was observed in hematocrit analysis of this same group (Table 2). Total protein values observed downstream at Knight’s Landing did not follow this trend. Plasma albumin and plasma osmolality values reported in Table 10 are suspect due to concerns regarding albumin assay methodology and osmometer malfunction and are provided for documentation only.

Table 10. Mean (standard error) plasma total protein (g/dl), mean plasma albumin(g/dl), and mean (standard error) plasma osmolality (mmol/kg) of fish from prerelease and Knight's Landing organosomatic assays.

| Organo ID / date | Total protein(g/dl) | Albumin(g/dl) | Osmolality(mmol/kg) |
|-------------------------|----------------------------|----------------------|----------------------------|
| PrereleaseI/31MAR | 3.53 (0.13) | 2.05 | 300 (1.89) |
| Knight I/16APR | 3.32 (0.34) | 1.96 | 285 (5.13) |
| PrereleaseII/14APR | 3.19 (0.14) | 1.56 | 308 (5.37) |
| Knight II/25APR | 3.64 (0.12) | 2.26 | 307 (3.25) |
| PrereleaseIII/30APR | 3.66 (0.10) | 1.79 | 322 (4.19) |

DISCUSSION

As in years past, IHNV infection was again the most significant factor affecting FCS production at Coleman NFH. Three of the four release groups had infection rates as high as twenty percent prior to release. A group of approximately 1.9 million fish released on 9April was liberated earlier than scheduled due to high rates of IHNV infection. A decision was made on 10April to hold the 6May release group due to small size and the likelihood of reduced survival, relatively large number of marked and CWTed fish, and perceived absence of IHNV infection. The primary argument for early release of this group was based on the possibility of future IHNV infection and the lack of downstream Sacramento River fish passage protection if it was necessary to release prior to the scheduled 6May release. The results of the decision to hold this group were deemed successful based on the absence of IHNV infection at release, the target size of >70mm mean fork length was achieved, and valuable outmigrant information was obtained.

Mortality rates in IHNV infected units may not accurately portray prevalence of infection and much of the infected population is often asymptomatic at the time of release. Past year's release protocols recommended the release of fish once mortality rates exceeded one percent and were beginning to double. Our data indicates that an increase in mortality over 0.5% in a seven-day period may indicate infection rates exceeding ten percent. Also, our observations of nine to sixteen percent incidence of infection rates 183 river miles downstream at Knight's Landing indicates that infected fish survive for extended periods. This finding is contrary to the previous assumption that infected fish die soon after release. We are unsure if horizontal transmission is occurring post-release. Laboratory IHNV challenges of Coleman NFH juvenile chinook has indicated that infection can occur as a result of a brief one minute dip in water containing a low concentration of virus (10^2 PFU/ml), however, IHNV associated mortality over a 19-23 day period was low (unpublished CA-NV FHC studies conducted at UC Davis Fish Pathology wet lab, 1997). Releasing fish after mortality rates exceed one percent may result in the release of hundreds-of-thousands of infected fish which likely results in severe reductions in contribution rates of these releases and presents an undetermined risk to natural fish populations in the Sacramento River.

One confounding factor that should be accounted for when making decisions regarding early release of fish due to IHNV is the size of the fish and their apparent smolt status. One must weigh the consequences of not releasing infected fish and the subsequent epizootic occurrence or the release of juveniles that may not rapidly outmigrate which may lead to increased natural/hatchery fish interactions and decreased survival. Further efforts to minimize IHNV at Coleman NFH and investigations of in-river horizontal transmission are warranted.

No other pathogens were observed to significantly effect the health of broodyear 1996 FCS. One notable concern is the relatively high post-ponding mortality (3.5%) of FCS partially attributable to coagulated yolk. Although Coleman NFH typically observes egg eye-up rates exceeding ninety percent, elevated rates of coagulated yolk related mortality

may reduce the benefits of high eye-up rates. The cause(s) of this coagulated yolk has not been determined.

Determination of smolt status for broodyear 1996 FCS from prerelease samples through analysis of twenty-four hour saltwater challenge data and gill ATPase activity indicated that fish released on 31March (Prerelease I) and 14April (Prerelease II) were likely more developed as smolts than fish released on 6May (Prerelease III). Comparisons between Prerelease I and Prerelease II are somewhat more troublesome due to conflicting results of saltwater challenge and gill ATPase assays. Longer duration saltwater challenges may provide greater insight into osmoregulatory competence of release groups. Analysis of smolt indices of fish collected downstream at Knight's Landing indicated that they were more developed as smolts than their prerelease cohorts. We are unsure if this is a result of physiological changes that occurred post-release or if the population sampled at Knight's Landing was comprised of those fish in a particular release group that were the most developed as smolts.

Evaluation of the immune defense status of the sampled groups was problematic due to conflicting results from the different assays (leukocrit, hematocrit, total plasma protein). In addition, IHNV infection may have confounded results. Individual fish were not followed in regard to IHNV infection so this information cannot be used to "tease" out immune defense status of each individual fish. Therefore, mean assay values may not represent the population as a whole if there was a higher percentage of IHNV infected fish in a given assay group. Comparisons between Knight's Landing and prerelease groups is also troublesome due to differential sampling strategies associated with the two sample sites. Fish sampled during prerelease examinations were netted and euthanized within a time window of approximately sixty minutes. Fish sampled at Knight's Landing may have occupied the trap live box for up to twenty-four hours, fish were netted and sorted at the trap, transported to shore, and euthanized greater than sixty minutes post-netting. We cannot differentiate between capture stress and associated changes in blood parameters or blood parameter values that are indicative of different immune defense status.

Energy reserves of Coleman NFH smolts appears to be sufficient for their migration down the Sacramento River. Comparisons of energy reserves (VFAT score and body composition analysis) of prerelease groups with their Knight's Landing cohorts indicates significant mobilization of lipid reserves 11-16 days post-release. A better indicator of the adequacy of energy reserves might be obtained from energy reserve analysis of fish from Sacramento Delta trawls.

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APPENDIX A

Appendix A 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 |
| Silver | 0 = fully silver, no parr marks visible (rotate fish in light) 1 = partial silver, parr marks visible (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 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 |
| Hemor. Organs | Notes about any hemorrhagic organs- abnormal size / color N = No, Y = Yes |
