FY2000 Investigational Report:

Health Monitoring of Hatchery and Natural Fall-run Chinook Salmon Juveniles in the San Joaquin River and Delta, April – June 2000.

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Summary - No clinical signs of disease, virus, or obligate bacterial pathogens were detected in any of the 242 juvenile fall-run chinook salmon examined from the San Joaquin River and Estuary between 29 March and 7 June 2000. Light infections of the PKX myxosporean (the causative agent of Proliferative Kidney Disease) were detected in a few hatchery and natural fish. Merced River Hatchery release dates appear to correspond with increased smoltification and resulted in rapid out-migration.

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Introduction

Declining chinook populations in California's Central Valley is prompting an intense restoration effort of this valuable resource and a key element of the state's aquatic biodiversity. Health and fitness of juvenile salmon out-migrants ("smolts") are major determinants of their performance and survival. Infectious disease can influence survival due to both direct mortality and reduced physical performance (predator avoidance, saltwater adaptation, etc.). Contaminants and elevated water temperature are identified in the CALFED process as stressors for salmonids in the San Joaquin River (SJR) and Estuary. Both of these stressors have the potential for immunosuppressive effects. While hatchery-wild fish interaction is a controversial topic in natural resource management, a comparison of the pathogens present in both populations is needed to either support or refute the charge that hatchery fish spread disease to natural populations. The criteria used to define a quality hatchery fish is being reviewed and debated among hatchery and fish biologists. It will be important to profile the physiological condition of natural populations compared with hatchery fish.

No comprehensive fish pathogen survey of juvenile fall-run chinook in the SJR and Estuary has been reported to date. Rich and Loudermilk (1991) performed a smolt quality study that included a number of physiological and blood chemistry measurements on both hatchery and natural fish. The California-Nevada Fish Health Center has performed smolt health surveys and detected pathogens in the Klamath and Trinity Rivers of Northern California (Foott and Walker 1991, Walker and Foott 1992, Foott et al. 1999).

Sample sites were chosen from existing US Fish & Wildlife Service and California Department of Fish & Game monitoring projects. Field sample methods included beach seine, Kodiak trawl, and midwater trawl. Sample sites included Merced River Hatchery (MRH), seine and trawl sites on the SJR, and the Chipps Island trawl in the Sacramento/San Joaquin Estuary (Figure 1). The different sample methods and locations were chosen to sample a range of smolt development stages.

No clinical signs of disease, viral or obligate bacterial pathogens were detected in any of the juvenile fall-run chinook salmon examined. Light infections of the PKX myxosporean (the causative agent of Proliferative Kidney Disease) were detected in both hatchery and natural fish. Hatchery release dates appear to correspond with increased smoltification and resulted in rapid out-migration. Some differences in body composition and energy storage were seen between hatchery and natural fish.

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Methods

Sampling

Sampling occurred over 11 weeks, 29 March through 7 June 2000, in the SJR and Suisun Bay of the Sacramento/San Joaquin Estuary (Estuary). Sample methods included beach seine, Kodiak trawl and midwater trawl (Table 1) and were tied to ongoing monitoring studies conducted by the Interagency Ecological Program. SJR seine, conducted by the US Fish & Wildlife Service – Sacramento/San Joaquin Fisheries Resource Office (USFWS), consisted of nine sites on the SJR from river miles 41 to 83. Broodyear 1999 juvenile fall-run chinook salmon were sampled at the California Department of Fish & Game (CDFG) Merced River Hatchery (MRH) by dip net from 4 raceway sections representing the range of fish sizes (ages). Trawl sites were located below Mossdale on the SJR and near Chipps Island in the Estuary. Both trawls were operated cooperatively by the USFWS and CDFG. All fish sampled at a site within a sample week (Mon-Sun) were combined (Table 2). Sample groups are identified as: natural fish, pre-release MRH fish, in-river MRH smolts, Estuary MRH smolts, or in-river unmarked smolts.

Table 1. Sample site, location, method, and cooperator who provided capture effort on the San Joaquin River (SJR), Merced River (MR) and Suisun Bay in the Sacramento/San Joaquin Estuary (SB).

Site	Location (river mile)	Sample Method	Cooperator
Seine	SJR 41-83	Beach Seine	USFWS
MRH	MR 52	Dip Net	CDFG
Mossdale	SJR 54	Kodiak Trawl	USFWS & CDFG
Estuary	SB 18	Midwater Trawl	USFWS

Figure 1. Sample sites in the San Joaquin River drainage. Adapted from the National Atlas of the United States.

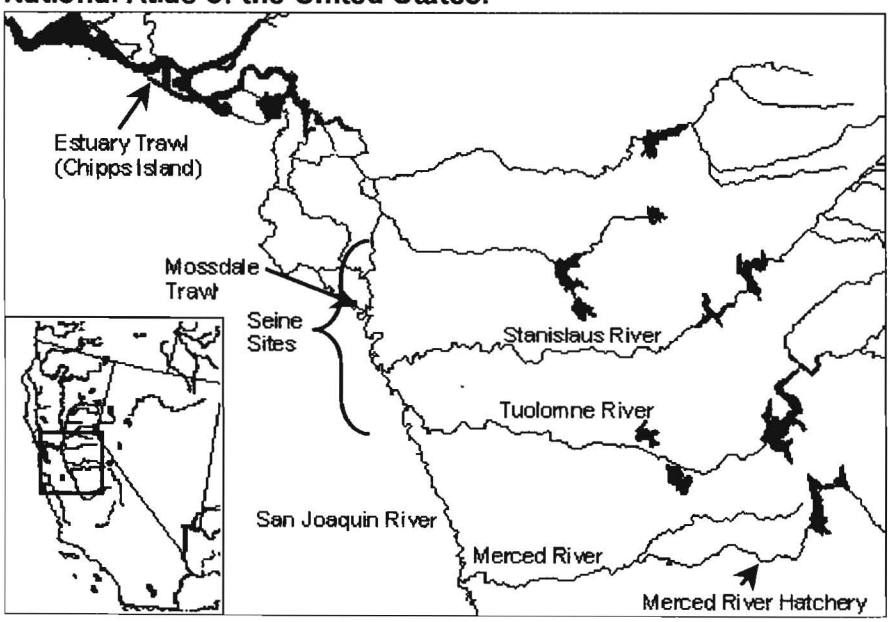


Table 2. Number of natural and MRH chinook juveniles sampled by site and sample week. Blank entries indicate no sampling effort. Origin of fish indicated as natural, MRH, or mixed (unmarked).

Week Beginning	27 Mar	3 Apr	10 Apr	17 Apr	24 Apr	1 May	8 May	15 May	22 May	29 May	5 Jun
Sample Week	1	2	3	4	5	6	7	8	9	10	11
Seine	28	6	13								
Mossdale			24	30	0	0	0		0	12	0
Estuary						5	5	1	2		
Pre-release			60								
		natural					MRH			mixed	

In-river natural fish

Sampling was conducted by seine (29 March, 4 April and 10 April) and Mossdale trawl (13 April). Seventy-one natural fish were sampled during these first 3 weeks. There is a remote possibility that some hatchery fish could have been captured during the collection of natural fish; however, all unmarked fish captured prior to 15 April were considered of natural production. No adipose clipped or dye marked fish were seen during this period.

Pre-release MRH

Sixty fish were sampled at MRH on 11 April just prior to the first of 3 releases. Of the 1.26 million Merced River Hatchery Fall-run chinook salmon released into the SJR system, 86% were adipose fin clipped or dye marked (Tim Heyne, CDFG, e-mail communication). Releases occurred mid-April, early May, and mid May over a week or more.

In-river MRH

Sampling was conducted at Mossdale 6 times between 18 April and 24 May. Adipose clipped fish were targeted, and coded-wire tags (CWT) were used to verify the fish came from MRH. Thirty MRH origin fish were sampled, and all were captured on 18 April (week 4). Catch in the Mossdale trawl was variable and sample days did not correspond well to peak catch days (figure 2). Due to the time requirements to process fish and typical peak catches in the early morning, sampling was limited to the first 5 of 10 or 20 tows made at Mossdale in a day.

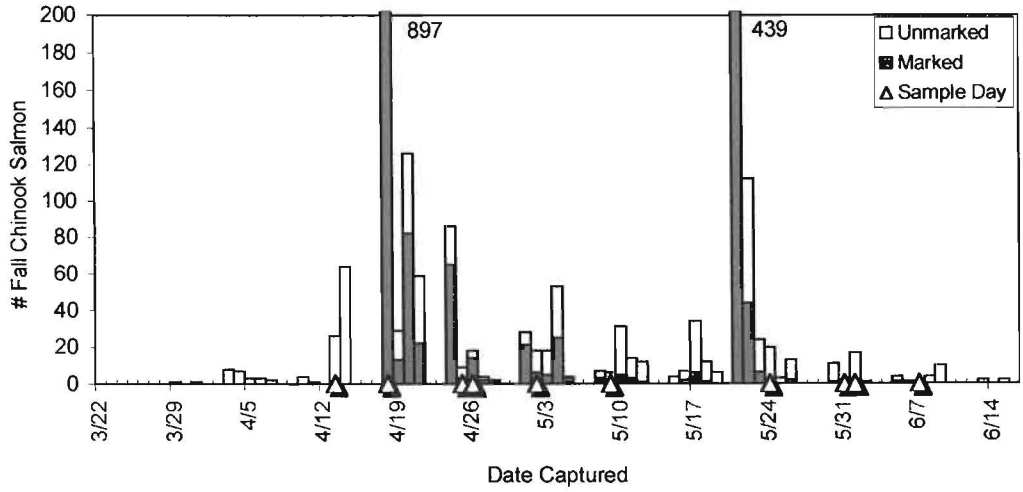
Estuary MRH smolts

Trawls in the Estuary were sampled 5 times between 4 May and 24 May. Sixty-nine marked smolts were sampled of which 13 were identified by CWT as MRH origin.

In-river unmarked smolts

Due to the poor catch of MRH smolts, twelve unmarked chinook salmon were sampled at Mossdale on 29 May (week 10), and these fish were considered of mixed origin but representative of late season out-migrants.

Figure 2. Total catch at Mossdale trawl sample site and fish health sampling days. Catch is not standardized for effort of 0-20 tows per day. Fish health samples were taken from the first five tows each day.



Sample Processing

At MRH, groups of 15 fish were netted and rapidly necropsied for various samples. Captured fish were held in buckets of ambient temperature water. At field sites, up to 30 fish were captured and held in aerated ice chests filled with water from the sample site. The water in the ice chests was refreshed and cooled by adding blue ice blocks as needed. Fish were euthanized in small groups by an overdose of tricaine methanesulfonate solution, rapidly examined for external organosomatic parameters, weighed, measured for fork length, and bled from the caudal peduncle into 75 mm heparinized micro-hematocrit tubes. The blood sample was centrifuged for measurement of hematocrit and collection of plasma. Blood value measurements were likely affected by the stress of capture. Trawl and seine capture were judged to be more stressful than dip netting and rapid euthanization. Plasma and a gill sample (see ATPase assay) were frozen on dry ice and later stored at –70 °C. After an internal organosomatic examination, various tissues were removed for histological examination, a sample of anterior kidney was inoculated onto bacteriological media, and the anterior ⅓ of the kidney was removed for both *Renibacterium salmoninarum* Direct Fluorescent Antibody Technique (*Rs*-DFAT) and virology. The head of natural fish and pre-release MRH fish were frozen to later test for *Myxobolus cerebralis* spores (the causative agent of Whirling Disease). Heads of marked fish were given to the USFWS for CWT reading.

Organosomatic analysis

Fish were evaluated by a modified organosomatic assay (Goede and Barton 1990, Foott 1990). The organosomatic assay is a method for ordered observation and reporting of the gross morphology of selected organs of each individual. Features evaluated included skin condition (scale loss), eye, gill, visceral fat, and any gross abnormalities of internal organs. Hematocrit (% packed erythrocyte volume) was determined from centrifuged micro-hematocrit tubes containing ammonium heparin. Fulton condition factor was calculated from the fork length (K=weight (g)/length (mm)^3 x 10^5). Due to the motion of the boat, weights and therefore condition factors taken in the

Estuary were suspect and not used for analysis. The criteria for the severity scores are listed below in Table 3.

Table 3. Explanation of 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
Eye	0 = no abnormalities
	1 = missing 1 eye, diminutive, external abrasion, some opacity
	2 = exophthalmia ("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 vol. < caeca vol.
	2 = >50 % but not covering caeca and/or cavity fat vol. = caeca vol.
	3 = caeca and cavity completely filled with fat, organs obscured by fat

Pathogens

Bacteriological cultures were grown on Brain Heart Infusion Agar, and identification was done using standard biochemical tests. *Rs*-DFAT was performed on individual kidney imprints on microscope slides using a fluorescent-labeled polyclonal anti-*Renibacterium salmoninarum* antibody, and examined with a fluorescent microscope at 600x. Kidney samples from two fish were pooled for virology in 2 ml centrifuge tubes containing 0.5 ml of antibiotic solution. Viral samples were held at 5°C, shipped cool to the California-Nevada Fish Health Center within 48 hours, and cultured on EPC and CHSE-214 cell lines for a minimum of 14 days. Gill, intestinal tract, pyloric ceca, kidney, and liver tissues were examined by histology. Tissues were rapidly removed from the fish after death, fixed in Prefer Fixative (Anatech, Ltd), processed for 5 μm paraffin sections, and stained with hematoxylin and eosin. Tissue abnormalities and parasite infections were evaluated by light microscopy. Screening for *Myxobolus cerebralis* was done by trypsin/pepsin digest (*Mc*-TPD) of cranial elements and examination by phase contrast microscopy at 400x.

All bacteriological and viral samples taken were assayed, and only relevant *Rs*-DFAT and histological samples were completed (Table 4). Due to the negative status of hatchery pre-release samples and the short time period between release and recapture, only those *Rs*-DFAT samples from natural and MRH pre-release fish were processed. The majority of marked fish sampled in the Estuary were from Sacramento River hatcheries. Histology slides from all SJR origin and a few non-SJR were read.

Table 4. Total number of fish collected in the field and processed in the lab for each pathogen assay. Totals included some CWT marked fish from the Sacramento River basin.

Assay	Virology	Bacteriology	*Rs*-DFAT	Histology	*Mc*-TPD
# sampled	242	212	242	136	131
# processed	242	212	143	96	131

Physiological Assays

Gill Sodium-Potassium-Adenosine Triphosphatase activity (ATPase) was assayed by the method of McCormick and Bern (1989). Briefly, gill lamellae were dissected and frozen in sucrose-EDTA-Imidazole (SEI) buffer on dry ice. The sample was later homogenized, centrifuged, supernatant sonicated, protein content assayed, and the ATPase activity determined by the decrease over time in optical density (340 nm) as NADH was converted to NAD+. This activity was reported as μmole ADP / mg protein / hr as one mole of NAD is produced for each mole of ADP generated in the reaction. Gill Na-K-ATPase activity is correlated with osmoregulatory ability in saltwater and primarily located in the "chloride cells" of the lamellae. This enzyme system transports salts from the fish's blood against the concentration gradient into the saltwater.

Body lipid content was assayed using a modification of the Bligh and Dyer (1959) chloroform:methanol (C:M) method. Fish carcasses with head and tail removed were frozen on dry ice in the field and stored frozen at –70°C. Samples were homogenized, lipid was extracted in a 2:1 C:M solution, and water was added to separate the sample into chloroform and methanol layers. An aliquot of the chloroform layer was removed, evaporated in a pan and the lipid residue was weighed to within 0.0001g. The visceral and muscle lipid (triglyceride) stores were of primary interest in this assay and elimination of the head (with large phospholipid content) and tail would not significantly alter this measurement of energy lipid stores.

Plasma albumin to globulin (A:G) ratio was determined using colormetric methods on blood plasma samples. In the field, blood was centrifuged and plasma was frozen on dry ice. The sample was later analyzed for albumin using a kit from Sigma Diagnostics (procedure no. 631), and total protein using a kit from Pierce (no. 23225). Both kits were adapted for use with a microplate reader. Plasma globulin concentrations were calculated by subtracting the albumin fraction from total protein concentrations.

Data analysis / Statistics

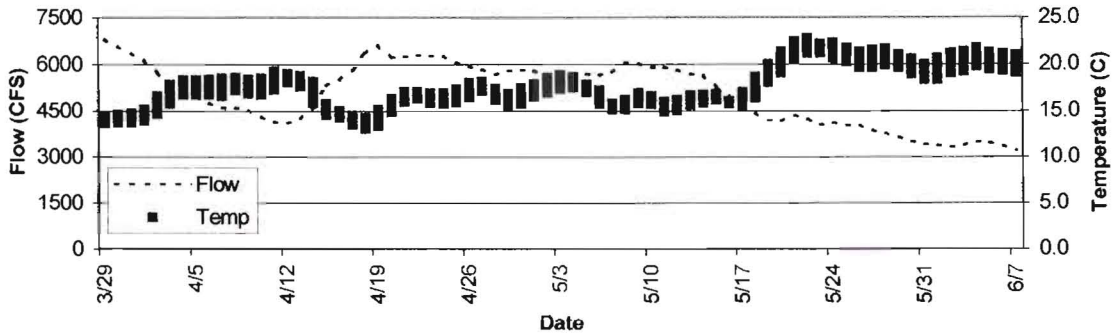
Group data was tested for normality and either analyzed by the parametric (T-test, 1-way ANOVA) or non-parametric tests (Mann-Whitney rank sum test, Kruskal-Wallis ANOVA on ranks). If significant differences among the groups were detected in the ANOVA tests, Tukey test (parametric) or Dunn's method (non-parametric) were performed to identify which group(s) were different. Pearson product-moment correlation coefficient was used to analyze the relationship between lipid content and condition factor. An alpha (type I "false difference" error) value of $P \leq 0.05$ was chosen for all tests. Microsoft Excel 2000™ spreadsheets were used for data manipulation and SigmaStat™ software was used for analysis.

Results

River conditions

Hourly river temperatures ranged from a minimum of 13°C on 18 April to a maximum of 23°C on 22 May. Daily flows ranged from 3200 CFS on 7 June to 6725 on 29 March (Figure 3).

Figure 3. Average daily river flow and daily temperature ranges (bars) at Vernalis on the San Joaquin River during the study period. Data obtained from the California Data Exchange Center records of US Bureau of Reclamation (temperature) and California Department of Water Resources (flow) gauges.



Pathogens

No viral or obligate bacterial fish pathogens were detected in any of the sample groups. Other bacterial isolates from these fish included: *Aeromonas sp.* (not *A. salmonicida)*, *Pseudomonas sp.*, gram-positive cocci (most likely *Staphylococcus*, *Micrococcus*, and *Streptococcus*), and gram-positive rods (most likely *Bacillus sp.*). Due to poor field sampling conditions, these bacteria were considered environmental contaminates. No *Myxobolus cerebralis* spores were detected in natural fish or pre-release MRH samples.

Blood plasma proteins

Total protein and albumin measurements were elevated in some samples far beyond the range commonly observed in salmonid blood (Total protein range = 1.3 - 10.8 g/dL). These elevated measurements were likely due to evaporative loss during field processing. Due to the low confidence in sample viability all data was regarded as suspect and not reported.

In-river natural fish

These unmarked fish were captured by beach seine (weeks 1-3) and in the Mossdale trawl (week 3). One percent of the fish examined had eye or gill abnormalities. Scale loss, probably due to sampling trauma, was seen in 15% of fish caught by beach seine and 100% of fish captured in the Mossdale trawl. No abnormal organs were observed in internal examination. Hematocrit values ranged from 35% to 59% with no fish showing signs of anemia. Tissues from 49 natural chinook out-migrants were examined by histology. No significant lesions were observed in the gill, intestinal tract, liver, and kidney sections. One fish captured 13 April in the Mossdale trawl was infected with the PKX myxosporean kidney parasite. One fish captured 4 April in the beach seine was infected with an unidentified Nematode parasite in the intestine.

There were no significant differences between natural fish sample groups for percent body lipid or ATPase activity (Table 5). The small size of the week 2 seine group and all lipid groups (Table 2) limited the power of the statistical tests and reduced confidence in non-significant results. Fish caught by seine during week 3 had higher condition factors (P=0.003) than other natural fish groups (Figure 4).

All natural fish were included as one group for comparison with later sample groups. Condition factors were significantly greater than pre-release MRH (P<0.001) and in-river MRH smolts (P=0.019, Figure 4). Lipid content was lower than pre-release MRH (P<0.001) and in-river MRH smolts (P<0.001, Figure 5). The correlation of body lipid content to condition factor (Figure 6) was not strong in natural fish (P=0.141) or MRH fish (P=0.117). The ATPase activity levels of natural fish were greater than pre-release MRH (P=0.017) and lower than the in-river unmarked group (P<0.001, Table 6).

Table 5. Physiological data for naturally produced fish by sample week captured by beach seine and Kodiak trawl. Data presented as Mean ±SE and number of samples.

Sample Group	Seine	Seine	Seine	Trawl
Sample Week	1	2	3	3
Fork Length (mm)	59.4 ±1.2 n=28	66.0 ±3.1 n=6	65.2 ±1.9 n=13	70.5 ±0.9 n=24
Condition Factor ($K=Wt(g)/FL(mm)^3 \times 10^5$)	1.13 ±0.02 n=28 c	1.07 ±0.04 n=6 c	1.22 ±0.03 n=13 a,b,d	1.10 ±0.02 n=24 c
Lipid Content (%)	3.1 ±0.3 n=9	3.4 ±0.2 n=2	3.4 ±0.4 n=4	2.8 ±0.3 n=8
ATPase Activity (mmol ADP/mg protein/hr)	4.91 ±0.59 n=18	7.36 ±1.04 n=5	3.86 ±0.62 n=12	5.61 ±0.83 n=14

a,b,c, and d denote significant (P<0.05) difference from:
a: Seine week 1
b: Seine week 2
c: Seine week 3
d: Trawl week 3

Table 6. Physiological data for natural and Merced River Hatchery origin fall-run chinook salmon sampled at Merced River Hatchery, in the San Joaquin River, or in the Sacramento/San Joaquin Estuary. Data presented as Mean ±SE and number of samples.

Sample Group	In-river natural	Pre-release MRH	In-river MRH	In-river unmarked	Estuary MRH
Sample Week	1-3	3	4	10	6-9
Fork Length (mm)	64.8 ±0.9 n=71	68.3 ±1.5 n=55	82.7 ±0.8 n=30	93.3 ±1.9 n=12	86.2 ±1.6 n=13
Condition Factor ($K=Wt(g)/FL(mm)^3 x10^5$)	1.13 ±0.01 n=71 b,c	1.05 ±0.01 n=31 a	1.07 ±0.01 n=30 a	1.06 ±0.01 n=11	Not Done
Lipid Content (%)	3.1 ±0.2 n=23 b,c	5.2 ±0.3 n=12 a	5.2 ±0.5 n=10 a	3.5 ±0.5 n=4	4.5 ±1.1 n=5
ATPase Activity (mmol ADP/mg protein/hr)	5.1 ± 0.4 n=49 b,d	2.6 ±0.4 n=12 a,c,d,e	5.3 ±0.5 n=16 b,d	9.4 ±0.6 n=11 a,b,c	7.2 ±1.0 n=12 b

a,b,c,d, and e denote significant ($P<0.05$) difference from:
a: In-river natural
b: Pre-release MRH
c: In-river MRH
d: In-river unmarked
e: Estuary MRH

Figure 4. Mean (±SE) condition factor for natural (○) and hatchery origin (▲) fish captured by beach seine, Kodiak trawl, or prior to hatchery release.

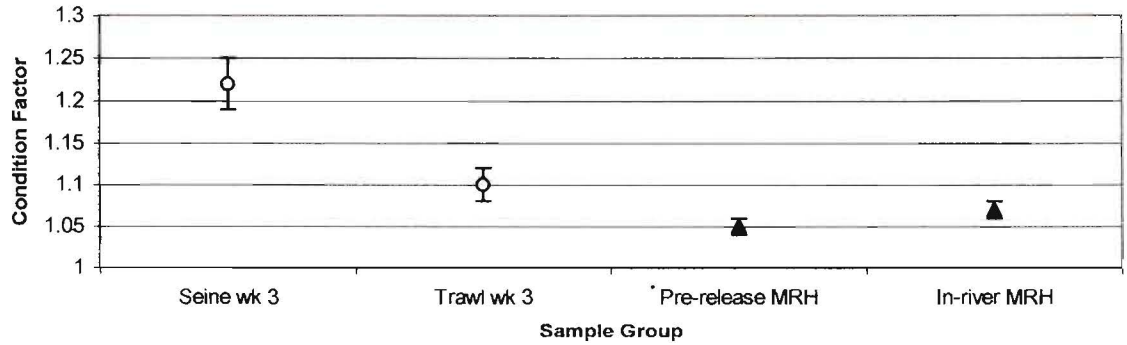


Figure 5. Mean (±SE) lipid level for natural (○), hatchery origin (▲), and unmarked (◊) fish captured in the San Joaquin River, Sacramento/San Joaquin Estuary, or sampled at Merced River Hatchery prior to release.

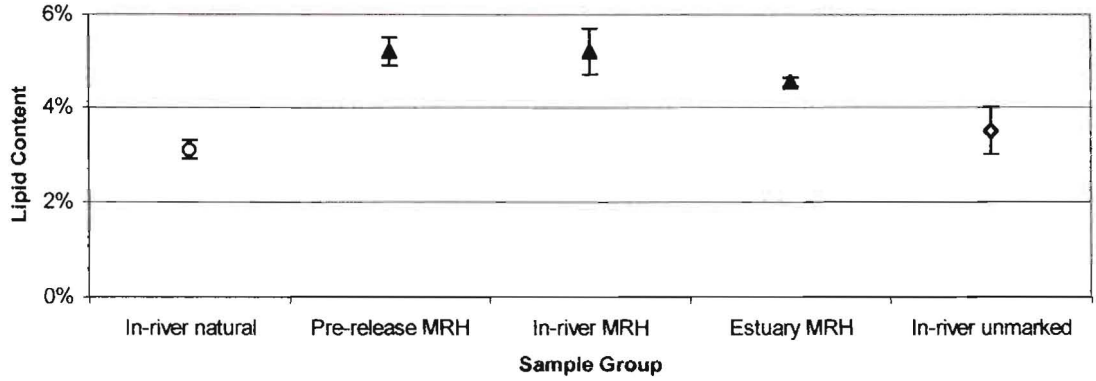
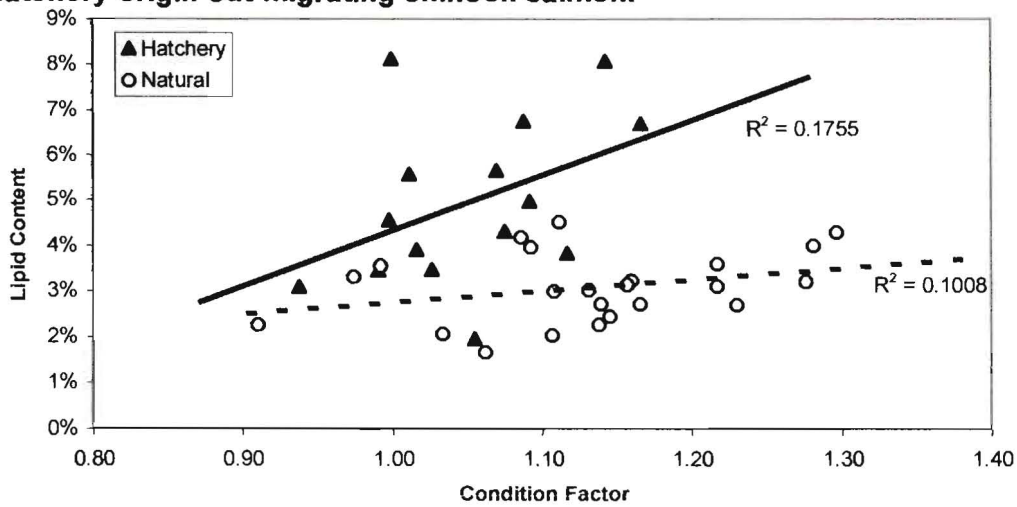


Figure 6. Relationship between lipid level and condition factor in natural and hatchery origin out-migrating chinook salmon.



Pre-release MRH

This group was sampled at MRH during week 3 (11 April) prior to the first hatchery release. Two percent of fish (1 fish) examined had a single small cyst on the gill. The cause of this cyst was not investigated, but *Ichthyophthirius multifillis* ("Ich") was suspected. No abnormal organs were observed in internal examination. Hematocrit values ranged from 26% to 43% with no fish showing signs of anemia. A less stressful sample method (dip net and rapid euthanization) and different hematocrit centrifuge were used with this group and may explain these lower values compared to other sample groups. Tissues from 12 juvenile chinook were collected for histological examination. No significant lesions or parasites were observed in the gill, intestinal tract, liver, and kidney sections.

This group had significantly lower condition factors ($P<0.001$, Figure 4) and greater body lipid content ($P<0.001$, Figure 5) than natural fish. ATPase activities were lower than natural ($P=0.017$), in-river MRH ($P=0.047$), in-river unmarked ($P<0.001$), and Estuary MRH ($P<0.001$) smolts (Table 6).

In-river MRH smolts

This was the only marked MRH origin group sampled in-river, and all were captured during week 4 (18 April) within 4 days of release. Three percent of fish examined had gill or eye abnormalities. Scale loss, due to sampling trauma, was seen in 100% of these fish. No abnormal organs were observed in internal examination. Hematocrit values ranged from 37% to 57% with no fish showing signs of anemia. Tissues from 20 chinook smolts were collected for histological examination. No significant lesions or parasites were observed in the gill, intestinal tract, liver, and kidney histology sections.

This group had lower condition factors ($P=0.019$, Figure 4) and higher lipid content ($P<0.001$, Figure 5) than natural fish. ATPase activities were lower than in-river unmarked smolts ($P<0.001$) and higher than pre-release MRH fish ($P=0.047$, Table 6).

In-river unmarked smolts

This was an unmarked group likely of mixed hatchery and natural origin sampled in week 10 (1 June). Scale loss, due to sampling trauma, was seen in 100% of these fish. No abnormal organs were observed in internal examination. Hematocrit values ranged from 42% to 65% with no fish showing signs of anemia. Tissues from 8 chinook out-migrants were sampled for histology. While poor fixation limited the quality of the tissue sections, no parasites or significant tissue abnormalities were observed in gill, kidney, intestinal tract, or liver samples.

This group had significantly higher ATPase activity levels than natural ($P<0.001$), pre-release MRH ($P<0.001$), and in-river MRH ($P<0.001$) sample groups (Table 6).

Estuary MRH smolts

These 13 fish were captured in sample weeks 6-9 (4 May – 24 May), and CWT data indicated a range of 3 to 27 days post release. Scale loss, due to sampling trauma, was seen in 100% of these fish. No abnormal organs were observed in internal examination. Hematocrit values ranged from 35% to 56% with no fish showing signs of anemia. Tissues from 4 MRH-tagged chinook out-migrants collected from the Chipps Island trawl were collected for histological examination. No significant lesions were observed in the gill, intestinal tract, liver, and kidney sections. One fish captured on 10 May (9 days post release) was lightly infected with the PKX myxosporean kidney parasite.

No trends were observed in MRH origin smolts recovered in the Estuary based on days post release or sample date. The small sample groups (Table 2) limited the power of the statistical tests and reduced the confidence of negative findings. All MRH origin fish caught in the Estuary were combined for comparisons to other sample groups. ATPase activities of Estuary fish were significantly higher ($P<0.001$) than pre-release MRH values (Table 6).

Discussion

This study was designed to examine any spatial and temporal differences in the health of out-migrating fall-run chinook salmon. Inconsistent catch of hatchery fish at our sample sites made both analyses difficult. The data demonstrated some expected trends and some interesting hatchery verses natural smolt relationships.

We expected to see changes in the health and physiology of the juvenile salmon during the decreasing flows and increasing water temperatures typical of late spring. River temperatures reached, but did not exceed 23°C, the temperature shown statistically to reduce survival of migrating smolts by 50% (Baker et al. 1995). Normal physiological changes associated with smolting and migration were observed, and no decline in health was detected in our sample groups. Early infections of PKX (the causative agent of Proliferative Kidney Disease) were detected in hatchery and natural fish, but no clinical signs of disease were seen.

Energy Reserves

Lipid levels and condition factor were used as indicators of energy reserves. High lipid levels in hatchery origin fish were expected given their high-energy diets. The higher condition factors observed in natural fish compared to hatchery fish may be worth further investigation. It was not clear if this extra weight was muscle mass (protein), water or some other constituent. If this is a consistent trend, it may indicate growth strategies (lipid deposition vs. muscle mass growth) differ between hatchery and natural fish.

Smolt development

Hatchery release dates appeared to correspond with increased smoltification and resulted in rapid out-migration. Based on gill ATPase activities, the most advanced smolt development was seen in the in-river unmarked sample group during week 10 (1 June) which were similar to the Estuary samples in weeks 6 - 9. Rich and Loudermilk (1991) observed a similar peak in gill ATPase activity in SJR chinook juveniles during early June. The increased gill ATPase activity of the out-migrant MRH chinook (<5 days post-release) in comparison to levels seen 8 days earlier in the hatchery could be related to several factors. Exposure to "novel" water, such as would occur to a smolt migrating through new water chemistry profiles, has been demonstrated to stimulate the hormone thyroxine (Hoffnagle and Fivizzani 1990, Nishioka et al. 1985). Elevated thyroxine precedes other smolt developmental changes such as silvering and increased gill ATPase activity (Folmar and Dickhoff 1980). As seen in Trinity hatchery chinook smolt, the fastest migrants tend to show elevated gill ATPase levels (unpublished California-Nevada Fish Health Center data, 1993 and 1994 spring and fall release studies). Hart et al. (1981) observed that migrant spring chinook had significantly higher ATPase activities than similar sized non-migrants. The timing of the first release from MRH coincided with an increase in natural fish migration (Figure 2). In-river MRH smolts had similar gill ATPase activities to natural fish, and moved quickly through the system.

Histology

One objective of this study was to evaluate liver and kidney abnormalities such as coagulative hepatocellular necrosis, neoplasia, hepatocytomegaly, and hydrophic degeneration of kidney tubules, which can be induced by chronic exposure to contaminants (Hinton and Lauren 1990, Meyers and Hendricks 1985). No lesions were seen in 96 histological specimens, nor was there any other evidence of obvious contaminant effects.

The PKX (myxosporean) infections observed were in early stages and not associated with significant tissue damage. Proliferative kidney disease (PKD), contracted in freshwater, is reported to continue in chinook salmon after their entry into saltwater (Hedrick and Aronstein 1987). The effect of PKD on saltwater survival may not be severe if the fish does not become anemic. These authors reported a 90% survival in the saltwater groups over the course of the 120-day experiment. PKD has been identified in the Merced River (Hedrick et al. 1986), and these light infections were expected. *Ceratomyxa shasta,* another myxosporean parasite, was not observed in the intestinal tract by histology although it is known to occur in the SJR basin (Hendrickson et al. 1989).

Eosinophilic granular cells (EGCs) were quite prominent in the lamina propria layer of the intestine and pyloric caeca from approximately half of each sample group. These immunodefensive cells are found in many organs, particularly those in direct contact with the environment such as gill, skin, and digestive tract. They are often associated with parasitic infections and contain both peroxidase and lysozyme (Sveinbjornsson et al 1996, Sire & Vernier 1995). Earlier assumptions that EGCs acted as mast cells have been found to be incorrect as histamine is not present (Sire & Vernier 1995). While not unusual to see in adult chinook, we have not observed such high numbers of intestinal EGCs in juvenile chinook from the Sacramento and Klamath R. systems. No lesions or parasites were associated with the EGCs found in the natural and MRH fish. Genetic differences may play a role in the different presentation of EGCs in San Joaquin chinook.

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