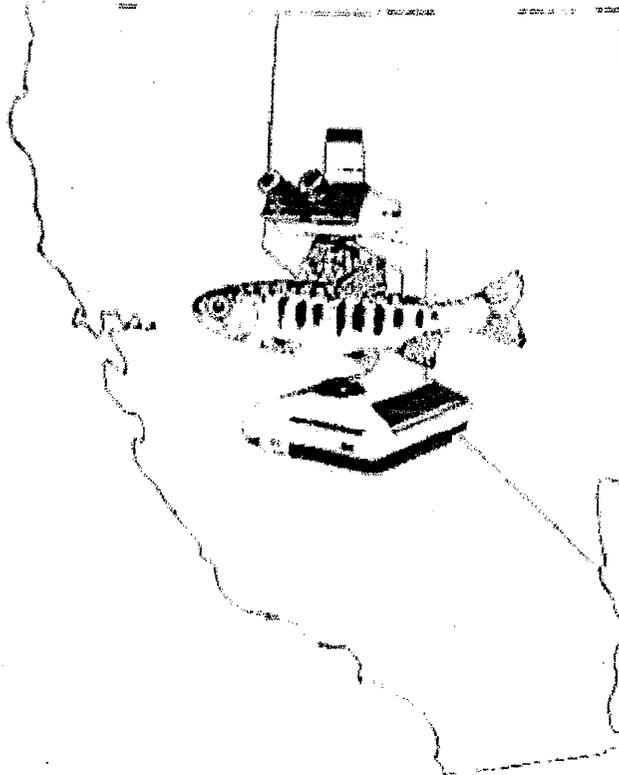


**FY97 Investigational Report :**

**Effects of diet composition, feeding rate, and water temperature on liver lipid disease, growth, smolt development, and adult return in Coleman NFH Late-Fall Chinook Salmon (broodyear 1996).**



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**Summary:** Broodyear 1996 Late-Fall Chinook juveniles were reared under various diet, feeding rate, and temperature conditions and evaluated for health, liver lipoid disease, growth, and adult return. Significant results include:

- 1) Late Fall Chinook can be fed rations at rates substantially less than the manufacturer's recommendations without impaired growth, health, or physiological development. Growth rate was not strictly correlated with feeding rate and was highly variable among the study groups.
- 2) Liver lipoid disease occurred, to varying degrees, in all diet groups and did not significantly affect general health, growth, physiological development, out-migration rate, or smolt - adult survival. Lower water temperatures (< 17C), food deprivation, and smoltification were all associated with a shift from lipid-filled hepatocytes to those filled with a glycogen / lipid mixture. We could not accurately distinguish, by gross observation, between livers having hepatocytes laden with only fat (liver lipoid) and those with a mixture of glycogen and fat.
- 3) Chinook fed the Lowfat diet (23 - 27 % less lipid than Control BioMoist diet) had higher mortality and poor food conversion in comparison to both the controls and variable feed rate groups.
- 4) Adult returns were almost 2X greater in the Control group compared to the variable rate group. The controls were approximately 15 % larger than the variables at release.

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## INTRODUCTION

Coleman National Fish Hatchery is located on lower Battle Creek, a tributary of the Sacramento River. The hatchery rears and releases two stocks (fall and late-fall) of chinook salmon *Oncorhynchus tshawytscha*. Adult fall chinook are spawned in October and November and progeny are released in April. Late-fall chinook adults are spawned from December through February and progeny are reared from March until their release the following January. This protracted hatchery residence of late-fall chinook is surmised to have lead to development of liver lipoid disease in 15-40% of hatchery-reared juveniles. Additionally, higher than normal return rates of late-fall chinook "mini-jacks" (fish that return after one year or less of ocean residence) was cause for some concern regarding hatchery fish culture practices. It was suspected that diet may be influencing both development of fatty livers and age at maturity.

The California-Nevada Fish Health Center, in coordination with Coleman National Fish Hatchery, and the Northern Central Valley Fish and Wildlife Office initiated a broodyear 1996 late-fall chinook diet study to evaluate the effects of feeding rate and feed lipid composition on the development of liver lipoid disease and, as a secondary evaluation, the age at maturity and smolt to adult survival of the study groups. These studies should assist in the development of fish husbandry practices that improve the health and physiology of hatchery-origin late-fall chinook, and ultimately, increase smolt to adult survival while minimizing potential natural/hatchery fish interactions. This report summarizes the effects of diet on fatty liver disease as well as other physiological parameters important to the successful culture of anadromous salmonids. The Northern Central Valley Fish and Wildlife Office initiated the tagging and marking of study fish to evaluate the rate-of-return and age-at-return of study fish. This portion of the evaluation is ongoing and will be completed after all study fish are supposed to have returned and data is analyzed.

The study consisted of three treatments: 1) Control treatment-- Fish in this treatment were fed at standard manufacturer's recommendations throughout the study period; 2) Lowfat treatment-- Fish were fed a lowfat formulation of the control feed, but at the same rate as fish in the control treatment; 3) Variable feed rate-- Fish were fed at a reduced ration level as compared to the control and lowfat treatment fish. In addition, three related secondary studies were initiated, but these fish were not as completely scrutinized nor were they tagged or marked for later evaluation following release. These studies were initiated to better understand what factors may be involved in the development of liver lipoid disease and to evaluate the feeding of alternative diets produced by other manufacturers. These studies were: 1) Effects of Temperature on Physiological Parameters of Fish Fed Reduced Rations and Reduced Lipid Diet-- This study evaluated the effects of rearing fish from the primary study for fifty-eight days in a reduced temperature environment; 2) Effects of Food Deprivation on Fish Physiology-- This study evaluated the physiological effects of starvation in fish from the control

treatment of the primary study;

3) Evaluation of Commercial Feeds-- This study evaluated the effects of feeding moist feeds produced by three different manufacturers.

## METHODS

### Physiological Effects of Feeding Reduced Rations and Reduced Dietary Lipid

*Fish culture*--Study fish were transferred from incubators to multiple 2.3 m<sup>3</sup> nursery tanks in April. Two "replicates" for each treatment group (**control**, **variable**, **lowfat**) were comprised of fish from egg takes 2 & 3 (replicate 1) and egg takes 5 & 6 (replicate 2). Multiple tanks for each treatment replicate were combined and transferred to a single 29.9 m<sup>3</sup> raceway. Fish were vaccinated against *Yersinia ruckeri* and transferred to 171.7 m<sup>3</sup> raceways in June for replicate 1 (rep1) and July for rep 2. Fish populations in each treatment replicate were adjusted to approximately 60,000 fish in August. All treatment fish were adipose fin-clipped and implanted with a distinct coded-wire-tag (CWT) in November and December. Fish were pumped to Battle Creek for release on 16-17 January.

Fish were fed seven days/week between 8:00am and 3:30pm. Feed quantities for **control** and **lowfat** replicates were calculated at equal rates based on manufacturers recommendations. Feed quantities for **variable** replicates were calculated based on a rate of 0.5% less than rates for **control** and **lowfat** replicates for the months of April and May, 1.0% less during the period June through October, and 0.5% less for the months of November and December. Feeding rates (%body weight/day) were determined by monthly sample counts (number of fish/pound) and predicted weight gain based on water temperature and a Fulton's condition factor ( $(\text{weight}/(\text{total length})^3) \times 10^5$ ) of 1.10. Due to the greater amount of feed required by fish in **control** and **lowfat** treatment replicates, they were generally fed more frequently than **variable** replicates. Raceways were cleaned by manual brushing two to four times weekly.

*Feed production*--Three "batches" of feed were produced by **Bioproducts**<sup>TM1</sup> (Warrenton, OR.; Dennis Roley) for the feed study. The **lowfat** feed and standard **Biomost Grower**<sup>TM</sup> and **Biomost Feed**<sup>TM</sup> was produced from the same batch to eliminate variation in components and conditions during production. A 25% reduction in lipid content, but equal energy (kcal/g) was the desired **lowfat** product. This was accomplished by reducing the amount of *marine* fish/krill oils and replacing with anchovy meal to increase energy content. Molasses replaced sugar and 3% Betanite was added to reduce fractility and improve lubrication during the extrusion process. The resulting **lowfat** feed provided 3.0 kcal/g as compared to 3.1 kcal/g for the standard diet. Proximate analysis revealed a 23% lipid reduction in the **lowfat**

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<sup>1</sup>Manufacturer's name has since changed to Bio-Oregon<sup>TM</sup>

Bio moist Grower™ and a 27% reduction in the **lowfat** Bio moist Feed™ (Appendix A). Fatty-acid profiles (Corning Hazleton, Inc; Madison, WI.) of **lowfat** and standard feeds indicated a 30% reduction of some marine fish/krill fatty acids in the **lowfat** feed (Appendix B).

*Growth rate determination*--A representative sample of fifty fish from each treatment replicate were measured for total length (mm) and weighed to the nearest 0.1g on a monthly basis for determination of growth rate (mm/day) and Fulton's condition factor ( $(\text{weight}/(\text{total length})^3) \times 10^5$ ). A representative sample was obtained by taking fish from three areas in the rearing unit; head, middle, and tail.

*Organosomatic assay*--Ten to twenty fish from each treatment replicate were collected by mid-pond sample for modified organosomatic assay on 20JUN, 29JUL, 27AUG, 26SEP, 4NOV 1996, and 6JAN, 1997. 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 C). 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 was measured, 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. Leukocrits are 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. Plasma was analyzed for total protein (Sigma Procedure No.541; Ektachem EL340 Microplate Reader) and plasma triglycerides (Kodak Ektachem DT-60II). The abdominal cavity was then opened and internal observations were made (organ abnormalities, visceral fat score). The liver was removed and weighed to the nearest 0.0001g on a Mettler Analytical Balance for hepatosomatic index (HSI) calculation ( $\text{HSI} = \text{liver weight}/\text{body weight} \times 100$ ). A random sample of these livers and other tissues were processed for histological evaluation. Tissues were put in Davidson's fixative (Humason 1979) for twenty-four hours followed by 70% ethanol, processed for 5 µm paraffin sections, and stained with hematoxylin and eosin. Tissue abnormalities and parasite infections were evaluated by light microscopy.

*Histological evaluation of liver*--Liver sections evaluated histologically were classified into one of four groups:

Normal                      Less than 30 % of section containing abnormal hepatocytes.  
Hepatocytes considered abnormal if > 10 % of cytoplasm contains

vacuoles (micro- or macrovesicular).

High Glycogen  
"HIGH GLYC"

Greater than 30% of section contains hepatocytes with microvesicular vacuoles or vacuoles with poorly defined walls containing granular material. Staining with Periodic-Acid Schiff's (PAS) stain reveals this granular material to be carbohydrate.

Fatty Change/  
High Glycogen

Greater than 30 % of section contains hepatocytes with either micro- or macrovesicular vacuoles. Hepatocytes tend to be of a normal size. Some macrovesicular vacuoles have well-defined walls and clear interiors (lipid extracted during slide processing).

Fatty Change

Greater than 30 % of section contains hepatocytes with PAS (-) macrovesicular vacuoles having well-defined walls and clear interiors. Hepatocytes tend to be hypertrophic.

*Salt-water challenge*--Study fish were challenged in 30ppt saltwater for twenty-four hours on 4Nov and 6Jan. Six to ten fish from each treatment group (3-5 from each rep) were collected by mid-pond sample and placed in 30L buckets of ambient aerated water with 900g of Instant Ocean™ (salt) added. After twenty-four hours, buckets were checked for mortality and salinity and dissolved oxygen was measured. 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.

*Assessment of fatty liver in outmigrants*--Seventy broodyear '96 late-fall chinook outmigrants were collected at Knight's Landing Rotary Screw Traps by California Department of Fish and Game biologists in November - January and sent frozen to the FHC. Another 20 were examined by the FHC staff on 21JAN. The carcasses were measured for fork length, dissected, and the degree of fatty liver change recorded for each fish (0= none, 1 = pale red/tan, or 2 = white). It is likely that some frozen livers could have been rated as "0" instead of "1". No Coded Wire Tags were read, however, fish were placed into the 3 release groups based on the following criteria :

- 1) 07Nov, 10Dec, and early January were release periods
- 2) Three days was the minimum time allotted to a given fish released from CNFH to reach the trap.
- 3) Given the above criteria, fish captured from 08Nov - 13Dec were placed in "NOV" group, 14Dec - 31Dec were placed into the "DEC" group, and captures

after 05JAN = "JAN" group.

The possibility of a later-released straggler being captured along with the predominate release group may account for 5 - 8 fish in the December and January group.

*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 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.

#### Effects of Temperature on Physiological Parameters of Fish Fed Reduced Rations and Reduced Lipid Diets

*Fish culture*--Approximately 850-1000 fish were transferred from replicate 1 treatment groups in 171.7m<sup>3</sup> raceways into three individual 2.3m<sup>3</sup> nursery tanks on 30JUL until completion of the investigation on 26SEP (58days). Temperature of nursery tanks was maintained at 10°C. Raceway temperature was reflective of ambient water temperature (mean 17.1°C in AUG and 15.6°C in SEP). Fish held at 10°C were fed at the same rate as their ambient temperature cohorts.

*Evaluation*--Fish were evaluated and data was analyzed according to aforementioned organosomatic assay, histological assay, and statistical analysis procedures.

#### Effects of Food Deprivation on Fish Physiology

On August 26, 1996, sixty fish were moved from the **Control** group (raceway 3) into a 2.3m<sup>3</sup> rectangular nursery tank. The tank received approximately 40 gpm and the temperature ranged from 13°C to 20°C during the twenty-four day experiment. No food was given to this group during the study period. On September 18, 1996, the thirty-eight remaining fish were sampled for blood chemistry, histological analysis, and an organosomatic analysis. Twenty fed cohorts (**Control**  $\approx$  1.9% BW/day, raceways 3 and 8) were sampled as above on 25SEP.

#### Evaluation of Commercial Feeds

*Fish culture*--Approximately 1600 fish from egg take #9 were split into three 2.3m<sup>3</sup> nursery tanks for evaluation of three commercially available soft-moist feeds produced by different manufacturers (Rangen Inc., Buhl, Idaho; Nelson's Sterling Silver Cup, Murray, Utah; Bioproducts, Warrenton, Oregon). Manufacturer-provided proximate analysis' are found in Table 1. Fish were fed at the same rate for each treatment group (RANGEN, BIOMOIST, NELSON).

analysis procedures.

**Table 1.** Manufacturer provided proximate analysis of soft-moist feeds.

Diet	Protein	Fat	Fiber	Ash	Moisture	Metabolizable energy
Rangen	44	18	<5	<8	<20	NA
Biom moist	43.5	14.5	1	7	24	3055kcal/kg
Nelson	50	18	1	9	<18	3600kcal/kg

## RESULTS

### Physiological Effects of Feeding Reduced Rations and Reduced Dietary Lipid

*Fish culture*--Treatment group replication was compromised due to mathematical errors, sample count and inventory errors, and feeding rate calculation methodology. Sample count and inventory errors were likely the result of the large numbers of fish comprising each replicate and difficulties in determining accurate fish numbers. Feeding rate calculation methodology was the source of most replication discrepancies and was caused by the following factors (Table 2):

1. Replicates were composed of fish from different egg takes which resulted in dissimilarities in age and size of replicates;
2. Feeding rates were based on manufacturers recommendations which are largely based on fish size and water temperature.

These discrepancies likely could have been avoided by setting the rates for each month based on historical records rather than consulting manufacturer's recommendations and using fish for replicates that were initially similar in age and size.

**Table 2.** Feed rates of treatment replicates at specific dates. Feed rate is calculated as percent body weight/day.

Trtmnt/rep	30Apr	30May	30June	30July	30Aug	30Sept	30Oct	30Nov	30Dec
CTRL/R1	3.96	4.61	4.07	3.11	1.89	2.04	1.76	1.39	1.14
CTRL/R2	4.14	3.8	3.87	3.19	1.90	1.86	1.88	1.48	1.12
VFR/R1	3.44	4.07	3.08	2.02	0.97	1.02	0.89	0.73	0.59
VFR/R2	4.24	3.49	2.85	1.97	2.00	0.95	0.79	0.68	0.54
LF/R1	4.03	4.96	3.94	3.27	1.91	2.07	1.79	1.45	1.13
LF/R2	4.39	4.31	3.7	2.86	2.26	2.01	1.76	1.88	1.10

Monthly feed conversion rates (amount of food fed for the month/fish weight gain for the month) were the lowest (best) for **variable** treatment replicates for five of the six months of the study (Table 3). **Lowfat** treatment replicates had the highest (worst) monthly feed conversion rates for five of the six months of the study.

**Table 3.** Monthly conversion rates of treatment replicates. Conversions in **bold type** represent the lowest (best) rates for each month.

Month	Control rep1	Control rep2	Variable rep1	Variable rep2	Lowfat rep1	Lowfat rep2
June	1.50	1.37	1.25	<b>1.05</b>	1.50	1.32
July	1.44	1.65	1.12	<b>1.03</b>	1.61	1.21
August	<b>1.17</b>	1.19	1.28	1.19	1.32	1.40
September	1.39	1.05	<b>0.93</b>	1.19	1.61	1.13
October	1.52	1.79	<b>0.98</b>	1.05	1.89	1.20
November	1.22	1.63	<b>0.99</b>	1.12	1.26	4.03

*Growth*--Statistically significant differences (one-way ANOVA; P<0.05) in mean total

fish length were observed both between replicates and between treatments throughout the study period until just before release (9JAN sample) when statistical analysis indicated that fish from **control** and **lowfat** treatment replicates were similar in length while fish from the **variable** treatment were significantly smaller (Table 4). Additionally, significant differences in mean total length of **variable** replicates were detected during the analysis of the 9JAN data. Interestingly, analysis of condition factor data for the 9JAN sample revealed different results: significant differences in condition factors were observed both between **control** and **lowfat** treatments and between replicates while condition factors of **variable** treatment replicates were statistically similar. All treatment replicates exhibited a dramatic decrease in condition factor during the period between the 6NOV and 9JAN sample dates.

**Table 4.** Sample Mean, SE mean, and [coefficient of variation] of total length (TL;mm) and condition factor (Kfact) measurements for treatment replicates sampled on specific dates. Condition factor mean and SE mean values are multiplied by a factor of 10<sup>5</sup>.

Date	<u>CTRL/R1</u>		<u>CTRL/R2</u>		<u>VFR/R1</u>		<u>VFR/R2</u>		<u>LF/R1</u>		<u>LF/R2</u>	
	TL	Kfact										
17June	67.9 <sup>f</sup>	0.968 <sup>a</sup>	61.7 <sup>g</sup>	0.975 <sup>b</sup>	64.8 <sup>h</sup>	0.944 <sup>c</sup>	58.4 <sup>i</sup>	0.918 <sup>d</sup>	61.2 <sup>a</sup>	0.976 <sup>b</sup>	57.1 <sup>f</sup>	0.934 <sup>a</sup>
	0.72	0.0079	0.92	0.0097	0.74	0.0087	0.63	0.0127	0.69	0.0125	0.86	0.0102
	[7.5]	[5.8]	[10.5]	[7.0]	[8.0]	[6.4]	[7.6]	[9.7]	[7.9]	[8.9]	[10.5]	[7.7]
2Aug	93.2 <sup>c</sup>	1.006 <sup>a</sup>	90.8 <sup>d</sup>	1.058 <sup>b</sup>	88.7 <sup>e</sup>	0.998 <sup>a</sup>	83.1 <sup>f</sup>	0.982 <sup>a</sup>	90.9 <sup>g</sup>	1.007 <sup>a</sup>	86.3 <sup>h</sup>	0.984 <sup>a</sup>
	0.99	0.0104	1.12	0.0150	0.86	0.0082	1.37	0.0089	1.10	0.0089	1.10	0.0092
	[7.4]	[7.3]	[8.7]	[9.9]	[6.8]	[5.7]	[11.5]	[6.3]	[8.5]	[6.2]	[8.9]	[6.6]
6Nov	149.3 <sup>d</sup>	1.032 <sup>a</sup>	136.9 <sup>e</sup>	1.034 <sup>a</sup>	119.8 <sup>f</sup>	0.941 <sup>b</sup>	109.4 <sup>g</sup>	0.941 <sup>b</sup>	133.3 <sup>h</sup>	0.979 <sup>c</sup>	139.7 <sup>i</sup>	1.003 <sup>a,c</sup>
	3.09	0.0096	3.65	0.0099	1.84	0.0082	1.98	0.0101	3.11	0.0084	3.29	0.0102
	[14.5]	[6.5]	[18.7]	[6.7]	[10.8]	[6.1]	[12.7]	[7.5]	[16.3]	[6.0]	[16.5]	[7.1]
9Jan	164.2 <sup>f</sup>	0.948 <sup>a</sup>	166.7 <sup>f</sup>	0.976 <sup>b</sup>	143.0 <sup>g</sup>	0.861 <sup>c</sup>	138.8 <sup>h</sup>	0.860 <sup>c</sup>	161.5 <sup>f</sup>	0.920 <sup>d</sup>	163.1 <sup>f</sup>	0.935 <sup>c</sup>
	3.38	0.0072	3.86	0.0104	2.52	0.0088	2.28	0.0072	3.32	0.0096	3.50	0.0089
	[14.4]	[5.3]	[16.2]	[7.5]	[12.3]	[7.2]	[11.5]	[5.9]	[14.4]	[7.3]	[15.0]	[6.6]

a, b, c, d, e, f, g, h, i Different superscript letters indicate significant differences at  $P \leq 0.05$ . Differences were detected using one-way ANOVA or Kruskal-Wallis ANOVA on Ranks and comparisons were made using the Student-Newman-Keuls Method.

Growth rates were highly variable between both treatments and replicates; consequently, no statistical analysis were performed on growth rate data. As expected, growth rates of **variable** treatment replicates tended to be lower than either **control** or **lowfat** treatment replicates (Table 5; Figures 1 and 2). Changes in feeding rates did not necessarily result in similar changes in growth rates. In fact, the highest rate of growth (0.62mm/day) observed during the study for either **variable** replicate occurred for the period 6NOV-9DEC when feed rates were extremely low. Interestingly, growth rates between treatment replicates tended to oscillate; that is, when comparing replicates within treatments, a high (relative) rate of growth during a period was typically followed by a period of less growth and vice versa. This trend was less noticeable in **lowfat** replicates. Unexpectedly, the lowest growth rates observed in **variable** replicates were after temperatures had started to decrease rather than during the warmer summer months when metabolic demand should be higher. This decrease in growth rate of **variable** replicates was followed by the highest rates observed for this treatment group.

**Table 5.** Growth rates (mm/day) of treatment replicates for specific date intervals. Values in **bold** type represent the treatment/replicate exhibiting the highest rate of growth for the corresponding interval. Mean temperature ( $^{\circ}\text{C}$ ) data is provided as a comparison of growth rates at different temperatures.

Trmnt/Rep	Growth Interval							
	5/17-6/3	6/4-7/3	7/4-8/2	8/3-9/5	9/6-10/1	10/2-11/6	11/7-12/9	12/10-1/9
CTRL/R1	0.41	0.41	0.65	<b>0.45</b>	0.55	<b>0.72</b>	0.20	0.26
CTRL/R2	<b>0.46</b>	<b>0.51</b>	0.62	0.31	0.6	0.54	0.33	<b>0.61</b>
VFR/R1	0.39	0.45	0.49	0.44	0.12	0.35	0.24	0.50
VFR/R2	0.42	0.38	0.53	0.3	0.43	0.13	<b>0.62</b>	0.29
LF/R1	0.35	0.43	0.61	0.43	0.55	0.36	0.48	0.40
LF/R2	0.35	0.39	<b>0.68</b>	0.41	<b>0.76</b>	0.54	0.30	0.43
Meantemp	13.7	16.8	18.6	18.4	15.4	11.0	9.6	7.9

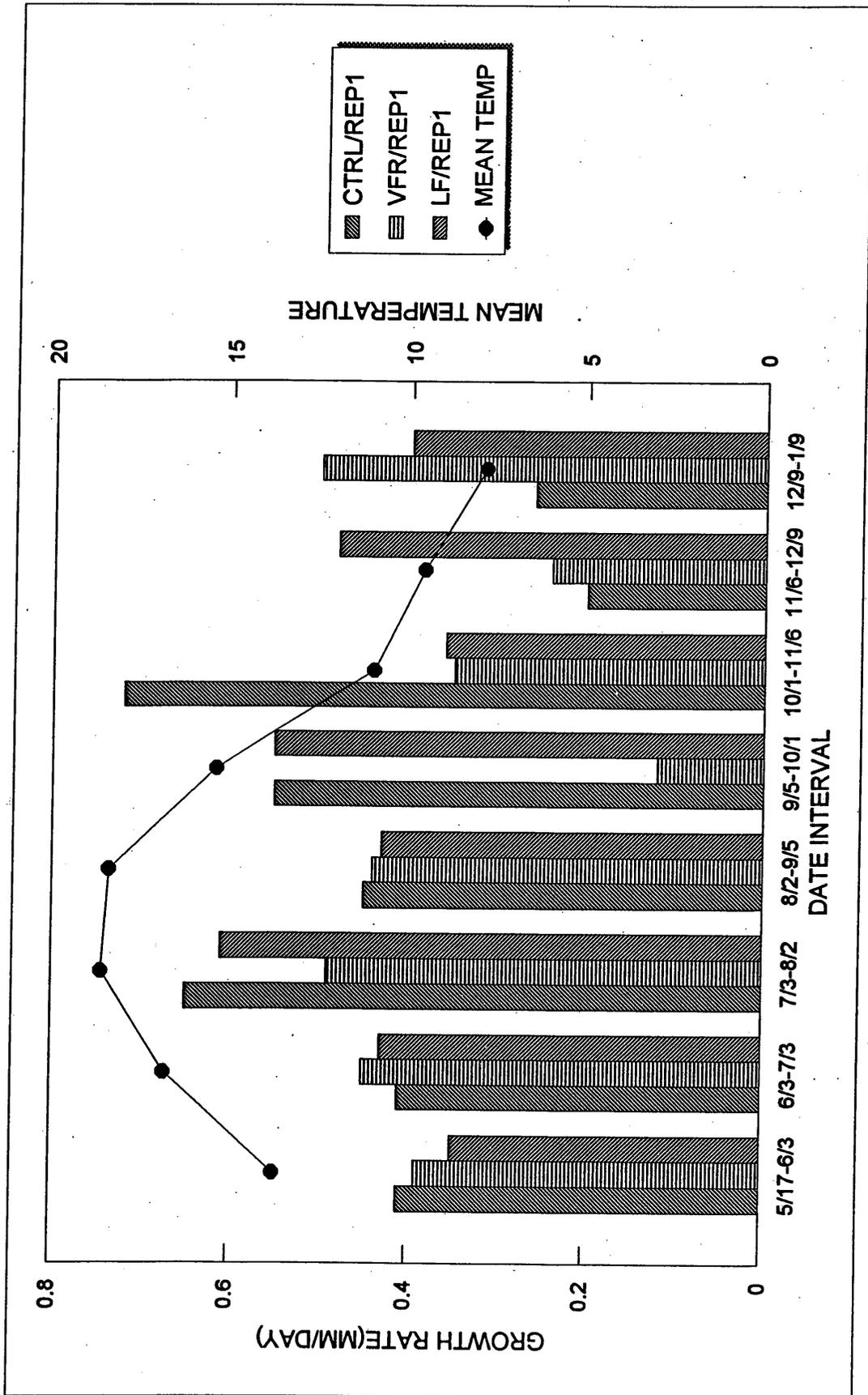


Figure 1. Growth rates for control, variable, and lowfat replicate 1 treatments for specific date intervals.

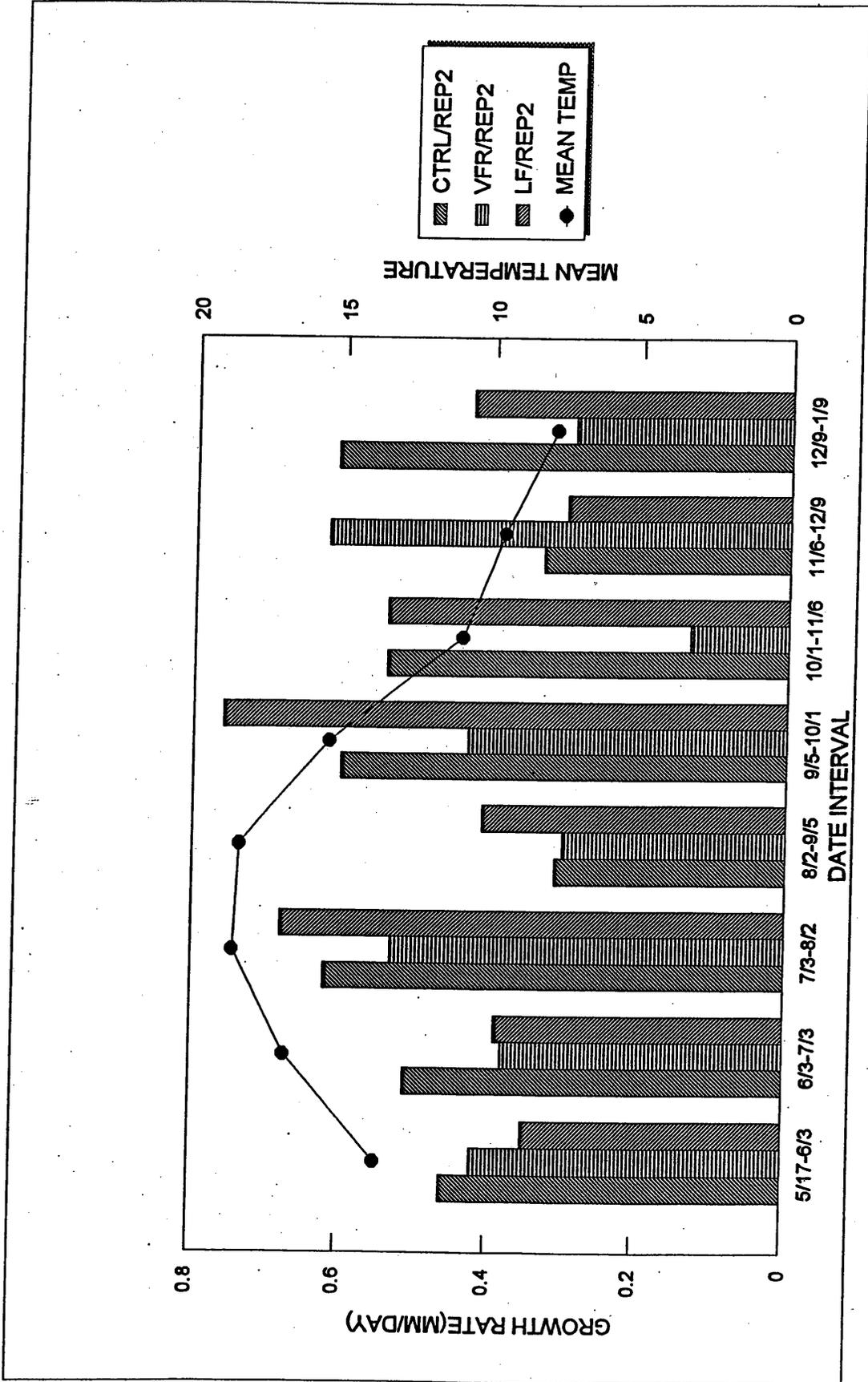


Figure 2. Growth rates for control, variable, and lowfat replicate 2 treatments for specific date intervals.

**Mortality**--As expected, the highest cumulative monthly mortality rates observed for all treatments occurred in June and were likely the result of stress-induced disease associated with increasing water temperatures, increasing densities, and increased handling (Figures 3 and 4). Similar trends in monthly mortality rates were observed for treatment replicates, but rates of replicate 2 treatments were typically higher than replicate 1 mortality rates. The highest monthly mortality rates were observed in both **lowfat** treatment replicates. Comparisons of replicate 1 treatments indicated that the **lowfat** treatment exhibited the highest monthly mortality rate in 100% (8/8) of the months monitored and replicate 2 comparisons indicated that the **lowfat** treatment exhibited the highest rate in 75% (6/8) of the months monitored. Additionally, these same comparisons revealed that both **variable** treatment replicates exhibited the lowest monthly mortality rate in five out of the eight months ( $\approx 63\%$ ) monitored.

**Pathogen detection**--External parasites and columnaris disease were problems with this stock during the summer months, however, no significant infections were detected at the times of release in November - January (Table 6). While 18 % of the kidney samples tested for *Renibacterium salmoninarum* antigen (ELISA) had Optical density values indicative of an active infection, no statistical trend was observed between groups tested in the summer, fall, or at release (1-way ANOVA). No clinical sign of BKD were observed in the sampled fish.

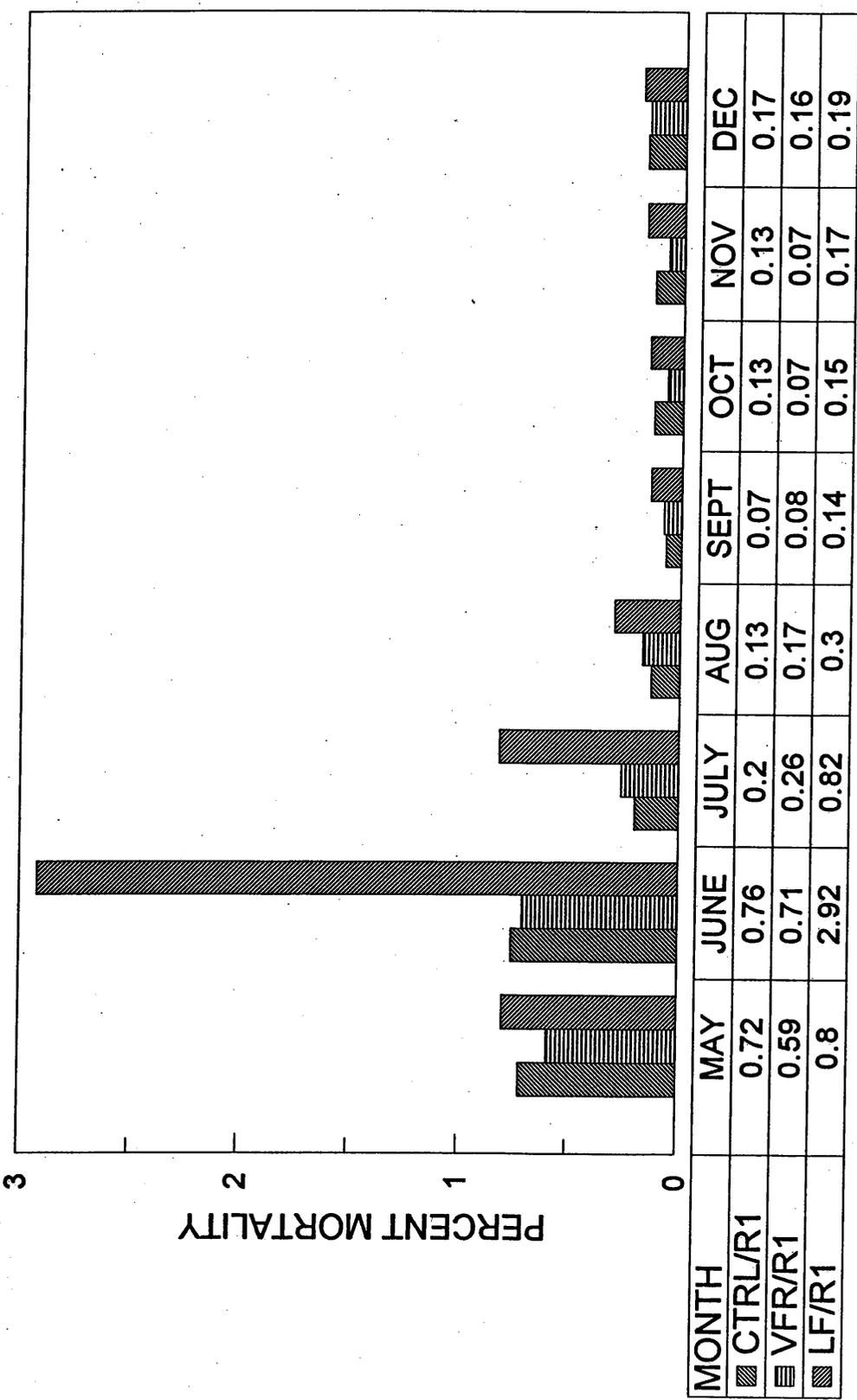


Figure 3. Cumulative monthly percent mortality of replicate 1 treatment groups.

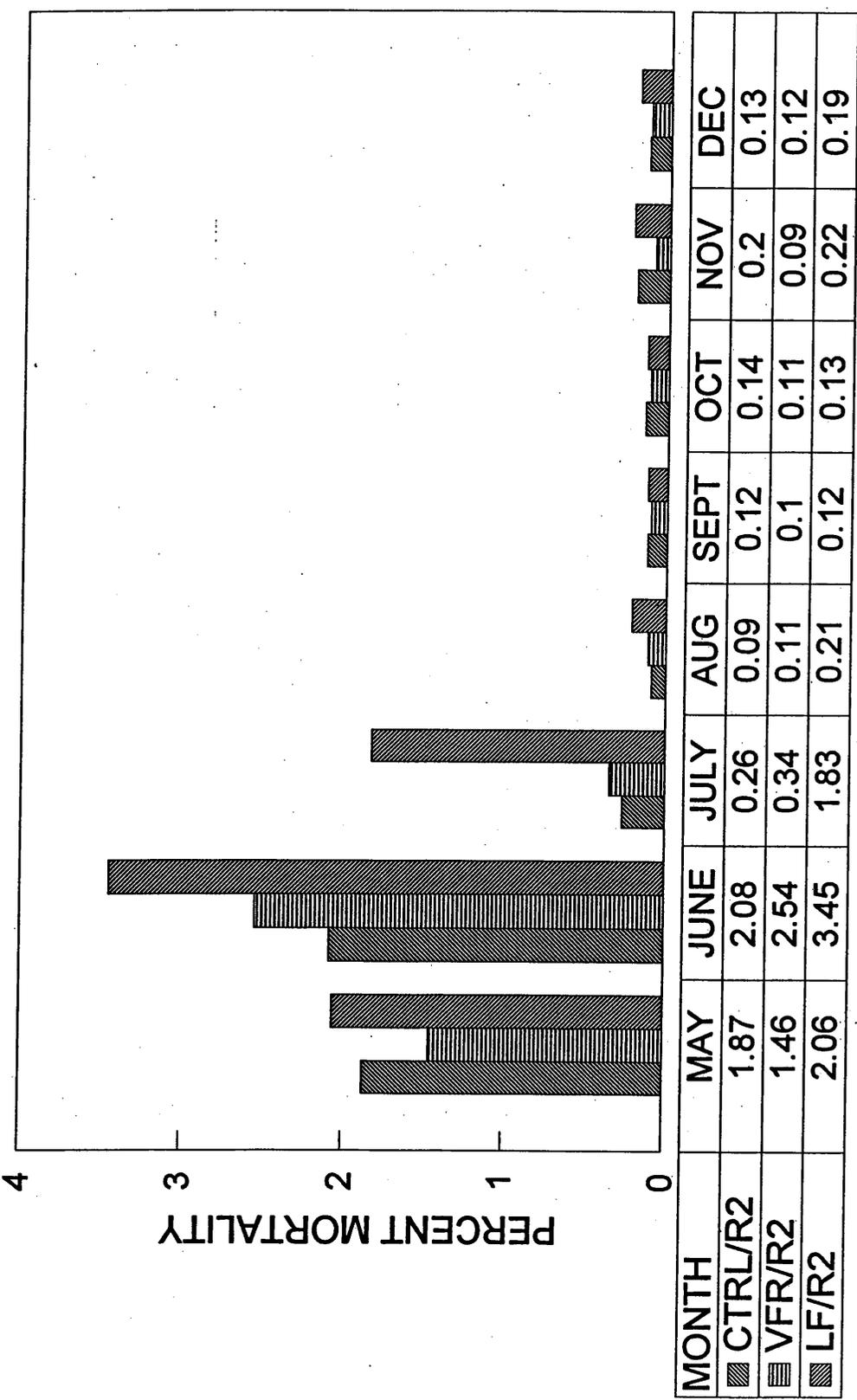


Figure 4. Cumulative monthly percent mortality of replicate 2 treatment groups.

**Table 6.** Pathogen summary of brood year 1996 late fall chinook juveniles; Battle Creek stocks (LFS-BCW-96-COL), Coleman NFH. Incidence of infection for: Infectious Hematopoietic Necrosis Virus (IHNV) in 3-5 fish pools of kidney tissue (KD) or whole fry (WF); blood parasites and erythrocyte viral inclusions (BPV) by blood smear (BS); *Renibacterium salmoninarum* (RSAL) by Enzyme Linked Immunosorbent Assay (ELISA) of 2 fish pools of kidney samples (KD); parasites (PARA) by individual wet mounts of skin(SK), gill (GL), P.A.S. or Giemsa stained kidney imprints (KIMP), kidney squash (KSQ), gut smears (GUT), and 10 fish pools of heads (HD); cultured systemic bacteria (BACTE) in individual samples. Recorded as number positive/sample total (percent positive).

	POOLS	INDIVIDUAL	No. FISH SAMPLED	DATES(s) DETECTED
IHNV-KD	0 / 23 (0)	NA	92	
IHNV-WF	0 / 57 (0)	NA	234	
BPV-BS	ND	0 / 10 (0)	10	
<b>RSAL-ELISA-KD **</b>			168	
Antigen Positive	15 / 84 (18)			
Antigen Suspect	66 / 84 (78)			JUN-RELEASE
Below Neg. Cutoff	3 / 84 (4)			
<b>PARA-SK/GL</b>			145	
<i>Ichthyophthirius sp.</i>	ND	13 / 145 (9)		APR-AUG
<i>Ichthyobodo sp.</i>	ND	0 / 145 (0)		
<i>Epistylis / Ambiphya</i>	ND	17 / 145 (12)		NOV-RELEASE
<i>Trichodina sp.</i>	ND	4 / 145 (3)		DEC-RELEASE
<b>PARA-KIMP / HISTO</b>				
ROSETTE AGENT	ND	0 / 30 (0)	30	
<b>PARA-KSQ / HISTO</b>				
<i>Nanophyetus sp.</i>	ND	27 / 30	(90) 30	JUL-RELEASE
<b>PARA-GUT</b>				
<i>Hexamita sp.</i>	ND	15 / 55 (27)	55	JUN-RELEASE
<b>PARA-HD</b>	0 / 6 (0)	ND	60	
<b>BACTE-KI</b>			200	
<i>Yersinia ruckeri</i>	ND	3 / 200 (1)	asymptomatic fish	APR
<i>Serratia sp.</i>	ND	4 / 200 (2)	1 of 2 = OTC resist.	JUL
Motile Aeromonid **	ND	17 / 200 (9)		MAY-RELEASE
<i>Staphylococcus</i>	ND	3 / 200 (1)		OCT
<i>F.columnaris</i> ***	ND	5 / 8 (63)		MAY- OCT

NOTE: Fc lesions & tail rot observed in sick fish throughout summer, associated with filamentous GNR October isolation from RBPP test fish held at BOR lab. Coagulative yolk mortalities common in April & May.

ND Not done.

\*\* ELISA showed 69 of 84 samples to have *R. salmoninarum* antigen levels greater than 2 Std. Dev. above normal tissue reference, however, only 3 fish showed high enough levels of antigen as to support a positive designation. No clinical BKD observed in this stock

\*\* Presumptive identification to either *A. hydrophila* or *Pseudomonas sp.*

+++ Isolated on TYE agar from moribund fish spleen inoculum.

*Organosomatic assay*--Overall, mean organosomatic parameters (HSI, hematocrit, leukocrit, VFAT) of fish from each treatment replicate assayed on different dates were within the normal ranges for hatchery-origin chinook salmon juveniles (Table 7). Liver lipid abnormalities were observed in all groups at varying frequencies, but not at each sample date. Percentage of fish from the **control** and **lowfat** treatments observed with severe fatty liver disease (score=2) on 6JAN is similar to that observed in previous years (J. Scott Foott; CA-NV Fish Health Center, unpublished data). Because of the low numbers of fish sampled (20-30) as compared to the number of fish in the populations ( $\approx 70,000$  fish/replicate), sample size may have been lower than that necessary to overcome potential discrepancies between sample variance and population variance. Analysis of hematocrit and leukocrit values did not reveal any trend for diet-related effects. Additionally, no trend in HSI values was detected. Mean VFAT score of fish from the **control** treatment was significantly greater than mean VFAT score of both the **lowfat** and **variable** treatments on the 20JUN assay date while the assay on 29JUL revealed significantly higher mean VFAT scores for **control** and **variable** treatments as compared to the **lowfat** treatment. No significant differences in VFAT scores were detected again until the final assay on 6JAN when mean VFAT score of **control** treatment fish was significantly higher than fish from the **variable** treatment.

*Histological evaluation of livers*--The histological and gross appearance of the livers was not well correlated with each other. Many livers rated in the organosomatic analysis as a #2 (pale cream - white) were composed of hepatocytes with both extensive glycogen and lipid deposits (GLYC/FAT). Many livers rated as normal (0) in the organosomatic analysis had a HIGH GLYC histological rating.

The first observation of severe fatty liver (rating #2) was made in fish from all three diet groups on 29JUL and could be seen in histological specimens. The number of **lowfat** and **variable** treatment fish with fatty liver dropped over time (Figure 5 and 6), however, this trend was less clear in the **control** fish (Figure 7). These **control** fish had the highest number of fatty change livers in the September sample. Diet treatment did not appear to eliminate livers with either fatty change or mixed glycogen/fatty change. Lipolysis associated changes in hepatocytes during the September-January period may be partially explained by physiological changes correlated with smoltification. We are unsure of the effects of cooler water temperatures between the September and January sample on lipolysis/lipogenesis in liver tissues.

# Liver histology rating - Low Fat Feed

normal, high glycogen, glycogen/fat vacuoles, fatty change

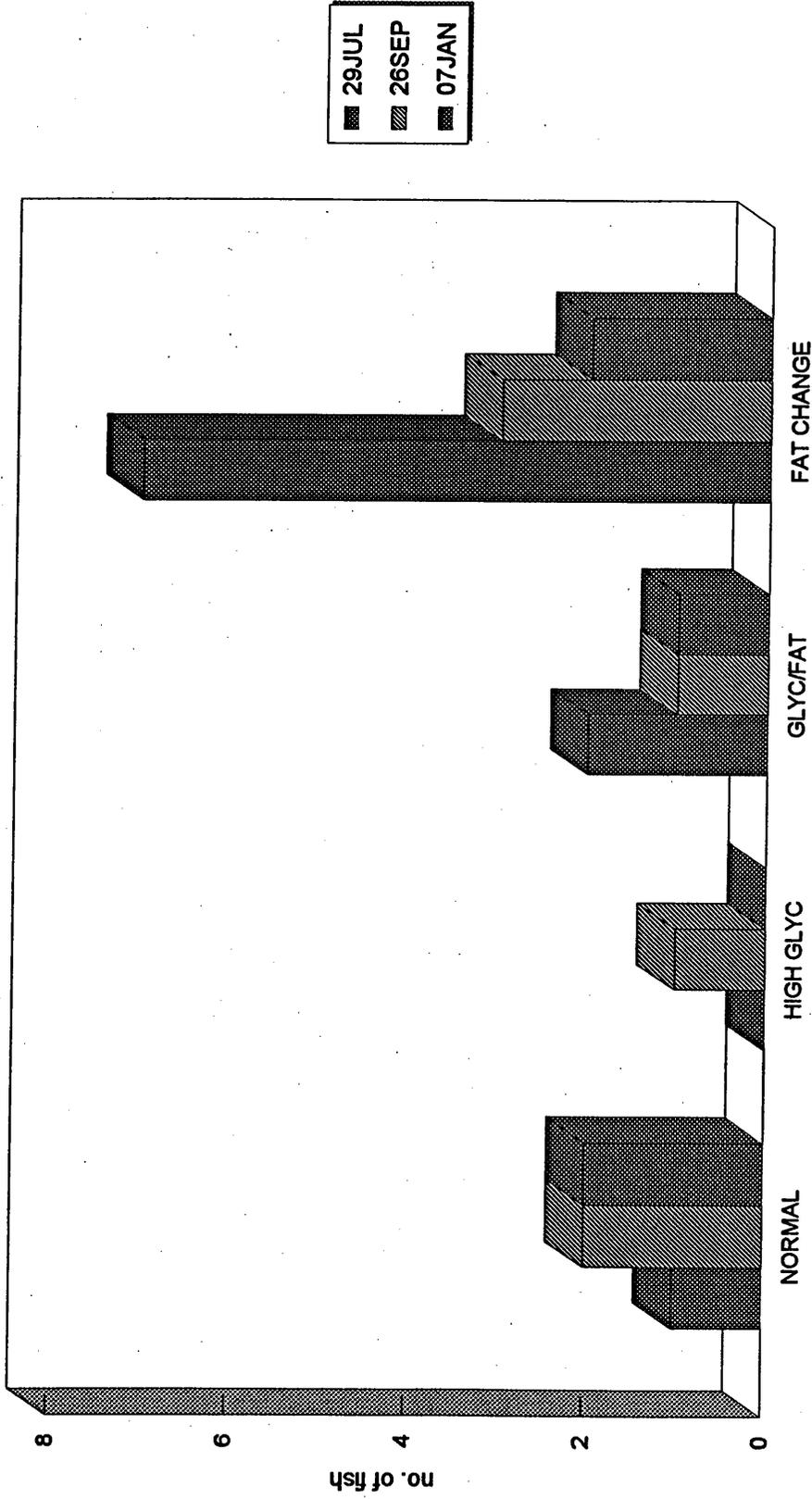


Figure 5. Histological rating of liver sections from late-fall chinook fed the lowfat diet. Livers were collected from five to ten fish in JUL, SEP, and JAN. Liver sections were rated as normal, high glycogen (HIGH GLYC), mix glycogen and fatty change (GLYC/FAT), or fatty change (FAT CHANGE).

# Liver histology rating - Variable Feed

normal, high glycogen, glycogen/fat vacuoles, fatty change

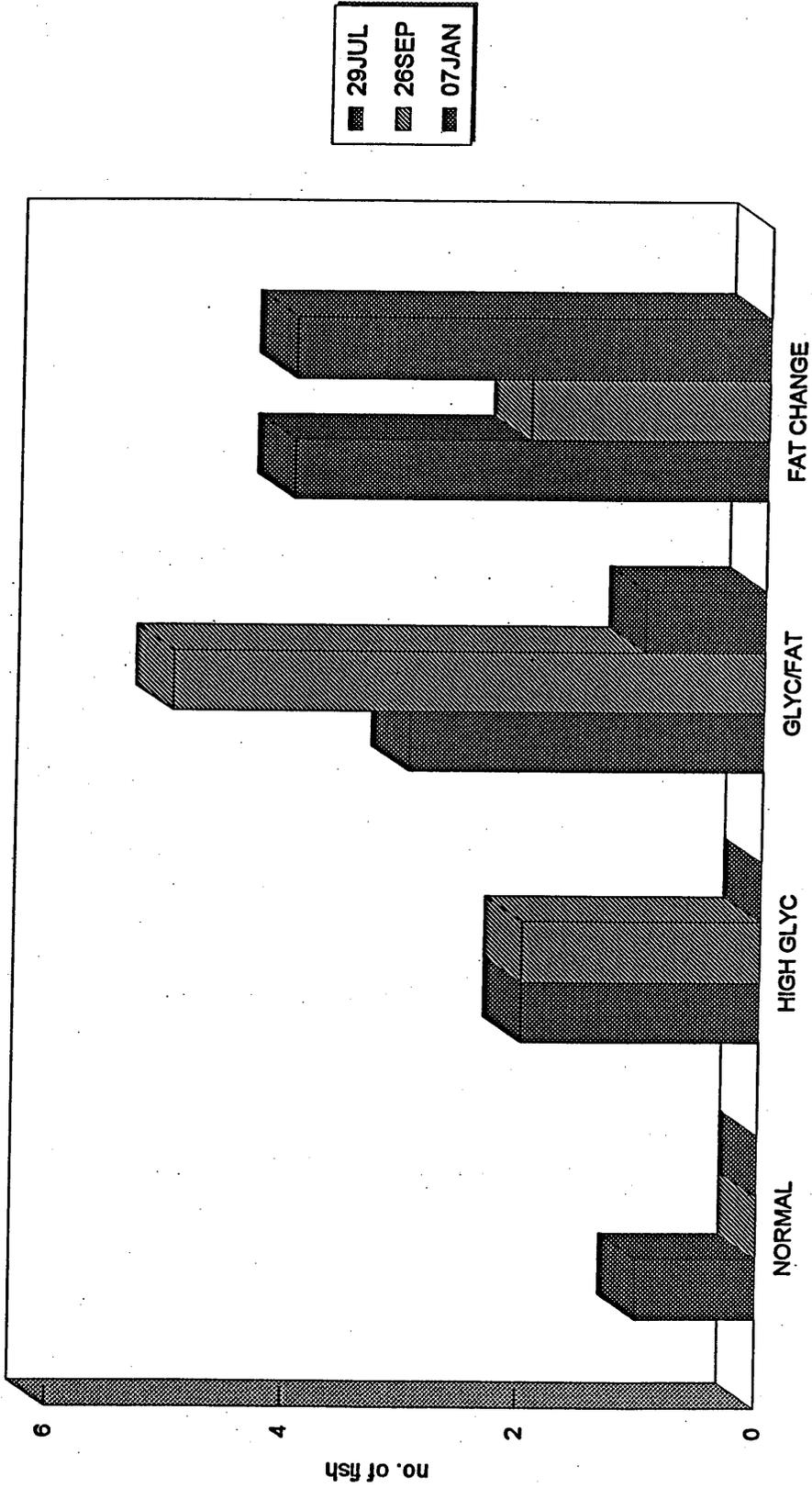


Figure 6. Histological rating of liver sections from late-fall chinook fed the variable diet. Livers were collected from five to ten fish in JUL, SEP, and JAN. Liver sections were rated as normal, high glycogen (HIGH GLYC), mix glycogen and fatty change (GLYC/FAT), or fatty change (FAT CHANGE).

**Liver histology rating - Control Feed**  
 normal, high glycogen, glycogen/fat vacuoles, fatty change

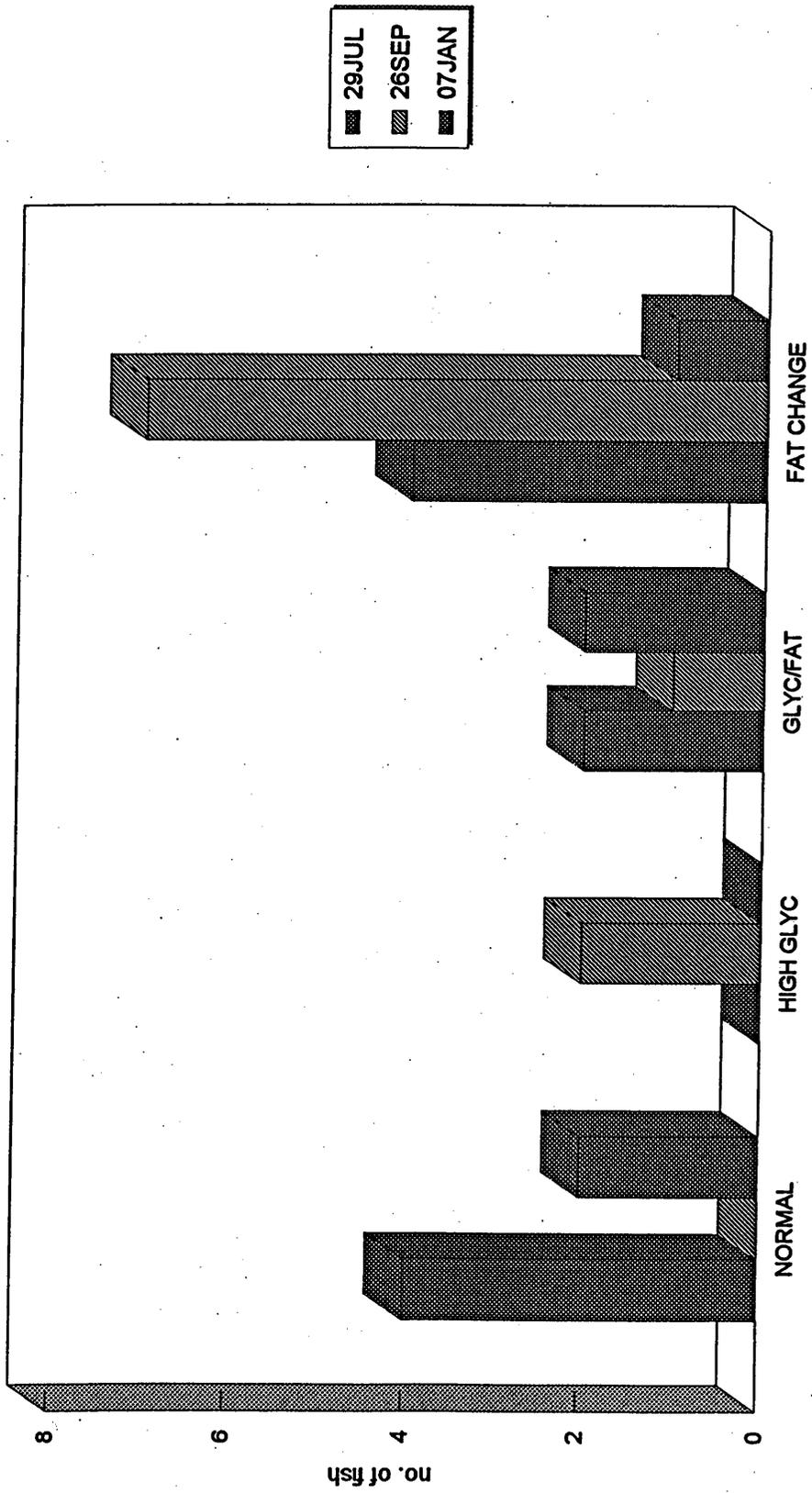


Figure 7. Histological rating of liver sections from late-fall chinook fed the control diet. Livers were collected from five to ten fish in JUL, SEP, and JAN. Liver sections were rated as normal, high glycogen (HIGH GLYC), mix glycogen and fatty change (GLYC/FAT), or fatty change (FAT CHANGE).

**Table 7.** Sample mean values for fork length (FL;mm), condition factor (KFACT), hepatosomatic index (HSI), hematocrit, leukocrit, visceral fat (VFATSCORE), and percent of fish with severe fatty-liver (%LIVER=2) of sampled fish from organosomatic assays at selected dates.

TREATMENT	FL(mm)	K-FACTOR	HSI	HEMATOCRIT	LEUKOCRIT	VFATSCORE	%LIVER=2
<b>20JUN</b>							
CTRL	62.4	0.8260	1.61 a <sup>1</sup>	39.8 a,b	0.4050 a	2.45 a	0%
VFR	59.6	0.8840	1.65 a	40.3 b	1.0550 b	1.45 b	0%
LF	55.0	0.7552	1.64 a	37.3 a	0.8050 b	1.10 b	0%
<b>29JUL</b>							
CTRL	82.1	1.0194	1.57 a	34.4 a	0.8640 a	3.00 a	10%
VFR	82.7	0.9811	1.89 a	33.5 a	1.4093 b	3.00 a	25%
LF	83.1	1.0094	1.89 a	34.3 a	1.2200 b	2.10 b	15%
<b>27AUG</b>							
CTRL	100.9	1.0055	1.48 a	41.3 a	No data	3.00 a	15%
VFR	92.90	0.8991	1.21 a	41.3 a	No data	3.00 a	20%
LF	95.40	0.9113	1.34 a	42.4 a	No data	2.95 a	30%
<b>26SEP</b>							
CTRL	119.2	1.0000	1.50 a	41.2 a	0.8210 a	3.00 a	30%
VFR	104.3	0.9444	1.28 a	41.2 a	1.1308 a	3.00 a	10%
LF	106.4	0.9305	1.32 a	43.6 a	1.0139 a	3.00 a	0%
<b>4NOV</b>							
CTRL	145.5	1.0555	1.25 a	48.4 a	0.9450 a	2.80 a	10%
VFR	111.7	0.9094	1.39 b	44.0 b	0.9943 a	2.30 a	10%
LF	111.4	0.9220	1.25 a	42.5 b	0.5752 b	2.70 a	20%
<b>6JAN</b>							
CTRL	155.8	0.8268	1.31 a	49.0 a	1.1639 a	3.00 a	47%
VFR	130.1	0.7354	1.12 a	42.0 b	1.3759 a	2.10 b	0%
LF	153.2	0.7563	1.25 a	45.5 a,b	1.3186 a	2.60 a,b	20%

<sup>1</sup> Different letters indicate significant differences at P<0.05.

**Smolt Development**--No significant differences (1-way ANOVA,  $P=0.08$ ) in Gill ATPase activity was observed among the 3 diet groups tested just prior to their January 1997 release and those fish collected at Knight's Landing on 21JAN (Table 8). All Knight's Landing fish were adipose fin clipped, however, no coded wire tags were read and the diet group identity of the individuals is not known. While not statistically significant, the variable rate diet group (VR) had both the lowest mean ATPase (2.44) and percentage of "smolt" activities (20%). These VR fish were also the smallest of the January test fish (mean FL = 129 mm). In 1997, the FHC set tentative ATPase activity ranges for the different developmental stages: freshwater parr (0.1 - 3.1  $\mu$ moles ADP/mg protein/hr), developing "smolt" (3.2 - 6.6  $\mu$ moles ADP/mg protein/hr), and saltwater 2 year olds (>6.7  $\mu$ moles ADP/mg protein/hr). These ranges were based on the 1997 method modification run on samples from 50 mm Fall-run parr and 2 yr. chinook held in saltwater at Bodega Marine Laboratories. This index is used to highlight the wide range of values normally encountered in similar sized smolts and the difficulty of relying on descriptive statistics for analysis. The ATPase activity of the 05NOV sample group was also similar to the January fish. This data indicates that smolt development was underway in November. It is likely that the 10JAN sample from outmigrants collected at Knight's Landing (rm 183) had higher activity than that measured, due to being transported frozen for 3 hrs on blue ice. This freezer block had been held at  $-80^{\circ}\text{C}$  prior to travel on 10 JAN, however, it could have warmed above the usual  $-50$  to  $-70^{\circ}\text{C}$  of commercial dry ice.

**Table 8.** Gill ATPase activity ( $\mu$ moles ADP/mg protein/hr), fork length of fish sampled for gill (FL), and percentage of sample (%SMOLT) which showed activity above the typical parr level (>3.2 to 6.7  $\mu$ moles ADP / mg protein / hr  $\pm$  2 STD). Groups include the date of release fish (cwt-REL), control diet (CON), variable rate (VR), lowfat diet (LF), and adipose-clipped fish captured at Knight's Landing. Measurement data reported as mean  $\pm$  standard error of the mean.

Group	ATPase activity	FL(mm)	%SMOLT
<b>05NOV</b> n= 10			
cwt-REL	3.17 $\pm$ 0.39	110 $\pm$ 4	58 %
<b>07JAN</b> n=5 each			
CON	3.83 $\pm$ 0.44	164 $\pm$ 12	80 %
VR	2.44 $\pm$ 0.36	129 $\pm$ 7	20 %
LF	3.35 $\pm$ 0.59	152 $\pm$ 8	40 %
cwt-REL	4.68 $\pm$ 0.71	164 $\pm$ 12	80 %
<b>10JAN</b> n=5			
Knight's Landing	3.84 $\pm$ 0.46	141 $\pm$ 12	60 %

Twenty-four hour saltwater challenges (28 - 30 ppt) showed two important trends (Table 9). First, the November test fish from all diet groups fared better than the January test fish. Date of challenge had an apparently stronger effect than diet or fish size. The mean plasma sodium values of the November fish tended to be lower (mean range 169 - 174 mmol/L) compared with the January fish (172 - 189 mmol/L). Sodium levels above the upper normal range of  $170 \pm 4$  mmol / L were also more prevalent in the January groups (Blackburn and Clarke 1987). The selection of 174 mmol /L as the upper normal plasma sodium cutoff took into account assay variability. Plasma sodium was not correlated with fish size ( $r^2 = 0.004$ ) in the 100 - 200 mm fork length range in the challenges. Beckman et al. (1996) reported that growth rate and the resultant growth hormone levels had a stronger effect on smoltification than size alone. Second, the plasma sodium level did not correspond well to either plasma osmolarity ( $r^2 = 0.304$ ) or condition factor ( $r^2 = 0.04 - 0.33$  in January group). The osmolarity value is reflective of all solutes (proteins, metabolites, ions) in the plasma. The reduction in condition factor (7 - 18 % drop in  $\{K\} = \text{Weight} / \text{Total Length}^3 \times 10^5$ ) of challenged fish compared with their cohorts in the FW organosomatic analysis groups suggests a moderate level of dehydration. These condition factor values were not as low as those we see in moribund SW fish.

**Table 9.** Twenty-four hour, 27-30 ppt saltwater challenge data for late-fall chinook (LFS-BCW-96-COL) reported as mean ( $\pm$  SE) plasma sodium (Na<sup>+</sup>), plasma osmolarity, condition factor ( $K = \text{weight (g)} / \text{length (mm)}^3 \times 10^5$ ) Six fish groups taken from raceways rearing fish on control diet (CON), variable feeding rate (VR), low-fat diet (LF), and coded wire tagged fish in the time of release study (cwt-TR). There was 100% survival in each of the challenges.

Group	Fork length (mm)	Plasma Na <sup>+</sup> (mmol/L)	Plasma Na <sup>+</sup> % greater than 174mmol/L	Plasma osmolarity (mOsm/kg)	Condition factor (k)
<b>05NOV</b>					
CON	126 ( $\pm$ 2)	172 ( $\pm$ 7)	33%	not done	0.953 ( $\pm$ .013)
VR	112 ( $\pm$ 3)	170 ( $\pm$ 4)	33%		0.875 ( $\pm$ .016)
LF	111 ( $\pm$ 2)	169 ( $\pm$ 3)	33%		0.842 ( $\pm$ .021)
cwt-TR	107 ( $\pm$ 3)	174 ( $\pm$ 5)	50%		0.849 ( $\pm$ .02)
<b>09DEC</b>					
cwt-TR	137 ( $\pm$ 2)	189 ( $\pm$ 6)	70%	410 ( $\pm$ 8)	0.888 ( $\pm$ .019)
<b>07JAN</b>					
CON	159 ( $\pm$ 7)	175 ( $\pm$ 4)	67%	353 ( $\pm$ 9)	0.862 ( $\pm$ .007)
VR	120 ( $\pm$ 10)	189 ( $\pm$ 5)	83%	391 ( $\pm$ 7)	0.746 ( $\pm$ 0.32)
LF	126 ( $\pm$ 9)	185 ( $\pm$ 5)	83%	364 ( $\pm$ 9)	0.827 ( $\pm$ .015)
cwt-TR	123 ( $\pm$ 8)	172 ( $\pm$ 7)	50%	354 ( $\pm$ 4)	0.797 ( $\pm$ .032)

*Plasma Protein Data*--Plasma protein measurements, taken from five fish per diet group prior to the January release and again from twelve fish captured at Knight's Landing on 21JAN, did not reveal any statistically significant differences (1-way ANOVA, P= 0.27); however, the variable treatment fish tended to have the highest total protein values (Table 10). The albumin values are suspected to be artificially high due to the assay method (Bromcresol green) and would also act to inflate the A/G ratio (Table ). Albumin and A/G are reported for group comparison purposes only. No trend with fatty liver score (0,1,2) was observed and values were within reported ranges for smolts.

**Table 10.** Mean plasma protein (g/dL), albumin (g/dL), and calculated A/G ratio of diet study fish (variable rate {VR}, low fat {LF}, control {CN} diet group) sampled on 6Jan97 prior to release and on 21JAN97 at the Knight's Landing Rotary Screw traps (rm 183). Data reported as mean  $\pm$  SE.

	Total protein	Albumin	A/G ratio
<u>6JAN</u>			
VR	3.3 $\pm$ 0.2	1.6 $\pm$ 0.0	1.1 $\pm$ 0.2
LF	4.1 $\pm$ 0.1	1.8 $\pm$ 0.1	0.8 $\pm$ 0.1
CN	3.7 $\pm$ 0.2	1.7 $\pm$ 0.1	0.8 $\pm$ 0.1
<u>21JAN</u>			
Knight's	3.9 $\pm$ 0.2	1.7 $\pm$ 0.1	0.8 $\pm$ 0.1

*Assessment of fatty liver in outmigrants*--The prevalence of severe fatty liver (#2) ranged from 15 - 30 % of the collection group in comparison to 19 - 35 % prevalence seen in the corresponding release group (Table 11). Fish examined fresh at the trap on 21JAN had a similar fatty prevalence as the frozen fish. There was poor correlation with fish size and fatty liver score ( $r^2 = 0.04$ ). It appears that fatty liver did not significantly inhibit migration 183 rm to Knight's Landing (i.e. no absence of affected fish in capture group).

**Table 11.** Prevalence of fatty liver in hatchery-origin outmigrants sampled at Knight's Landing.

Release group	%Liver=0	%Liver=1	%Liver=2	Prerelease %Liver=2
<u>NOV</u> n=15	53	33	14	35
<u>DEC</u> n=25	64	32	4	33
<u>JAN</u> n=30	33	47	20	19
<u>21JAN</u> n=20	25	45	30	19



10). After thirty-one days of exposure to reduced temperature (10°C), the number of fish exhibiting fatty change of hepatocytes declined in all treatment groups. Conversely, the number of fish exhibiting abnormal hepatocyte structure tended to increase over time in treatment groups held at ambient temperatures (16°C). Interestingly, there was a tendency towards increased glycogen storage in hepatocytes of fish exposed to reduced temperatures which further corroborates the aforementioned observations of increasing HSI and decreasing lipid stores.

**Table 12.** Sample mean values for fork length (FL;mm), condition factor (Kfact), hepatosomatic index (HSI), hematocrit, leukocrit, visceral fat (VFAT), and percent of fish with severe fatty-liver (%fatliver) of sampled fish held at different temperatures from organosomatic assays at selected dates.

Treatment	Temp °C	FL	Kfact	HSI	VFAT	%fatliver
<u>28AUG</u>						
Control	15.5	100.9 d <sup>1</sup>	1.0055 d	1.4781 n	3.0 n	15
Variable	15.5	92.9 n	0.8991 n	1.2145 d	3.0 d	20
Lowfat	15.5	95.4 d	0.9113 n	1.3410 d	3.0 n	30
Control	10.5	88.2 d	0.9033 d	1.5048 n	2.9 n	0
Variable	10.5	85.7 n	0.8884 n	1.6853 d	2.5 d	0
Lowfat	10.5	79.2 d	0.8966 n	1.5989 d	2.7 n	10
<u>26SEP</u>						
Control	15.5	119.2 d	1.0000 d	1.4952 n	3.0 n	30
Variable	15.5	104.3 n	0.9444 d	1.2833 n	3.0 n	10
Lowfat	15.5	106.4 d	0.9305 d	1.3248 n	3.0 n	0
Control	10.5	98.9 d	0.8937 d	1.4744 n	3.0 n	10
Variable	10.5	97.3 n	0.8905 d	1.3260 n	3.0 n	15
Lowfat	10.5	94.3 d	0.8377 d	1.2479 n	2.9 n	5

<sup>1</sup> The letter "d" signifies differences at P<0.05. The letter "n" signifies no statistical

difference. Comparisons were made between similar treatments at different temperatures, but not between treatments or dates.

## Control - 31 day temperature study

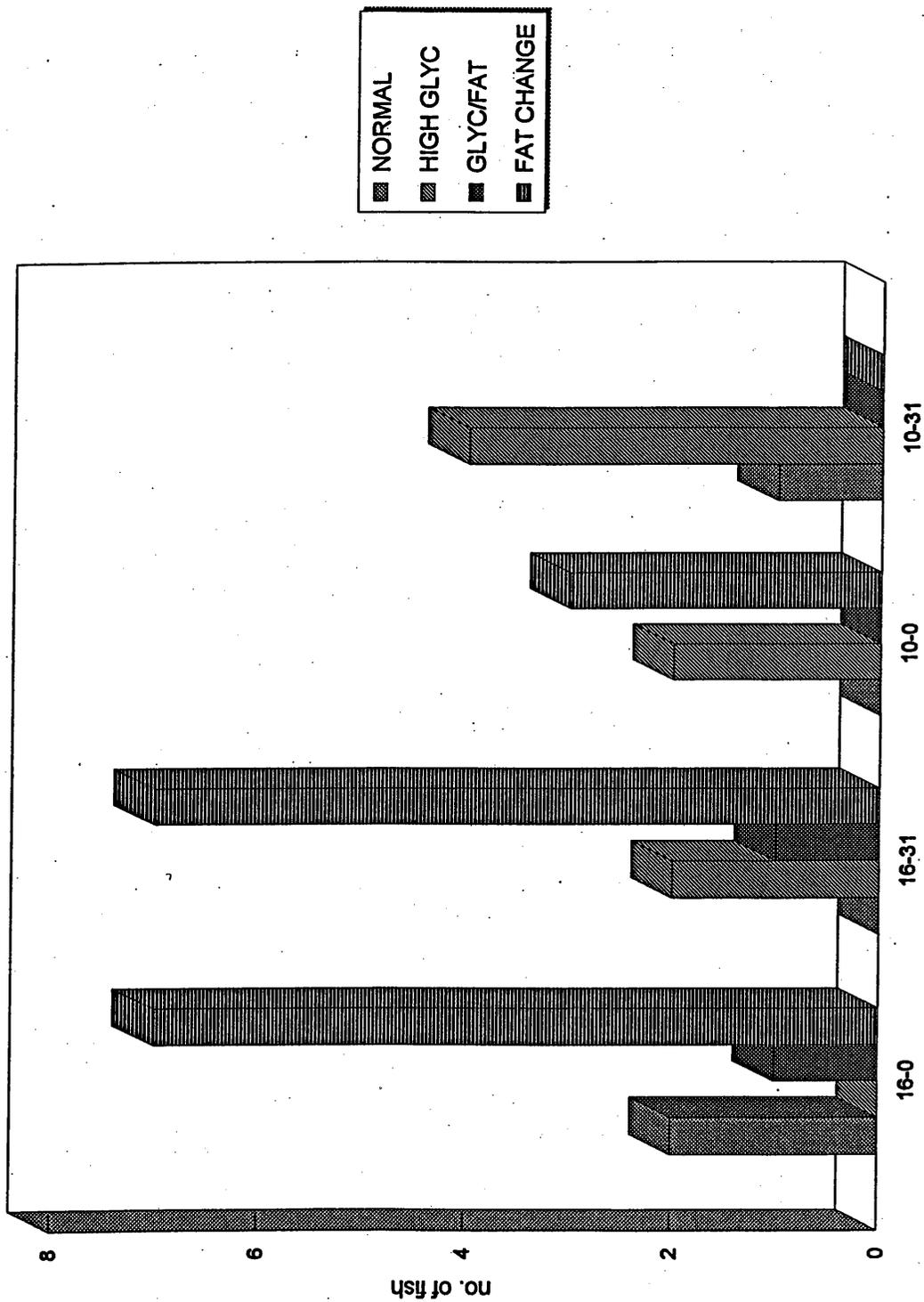


Figure 8. Histological rating of livers from late-fall chinook fed the control diet and held at 16°C and 10°C. Livers were collected from eight to ten fish on day 0 and day 31 (16-0=16°C, day 0). Liver sections were rated as normal, high glycogen (HIGH GLYC), mix glycogen and fatty change (GLYC/FAT), or fatty change (FAT CHANGE).

# Low Fat - 31 day temperature study

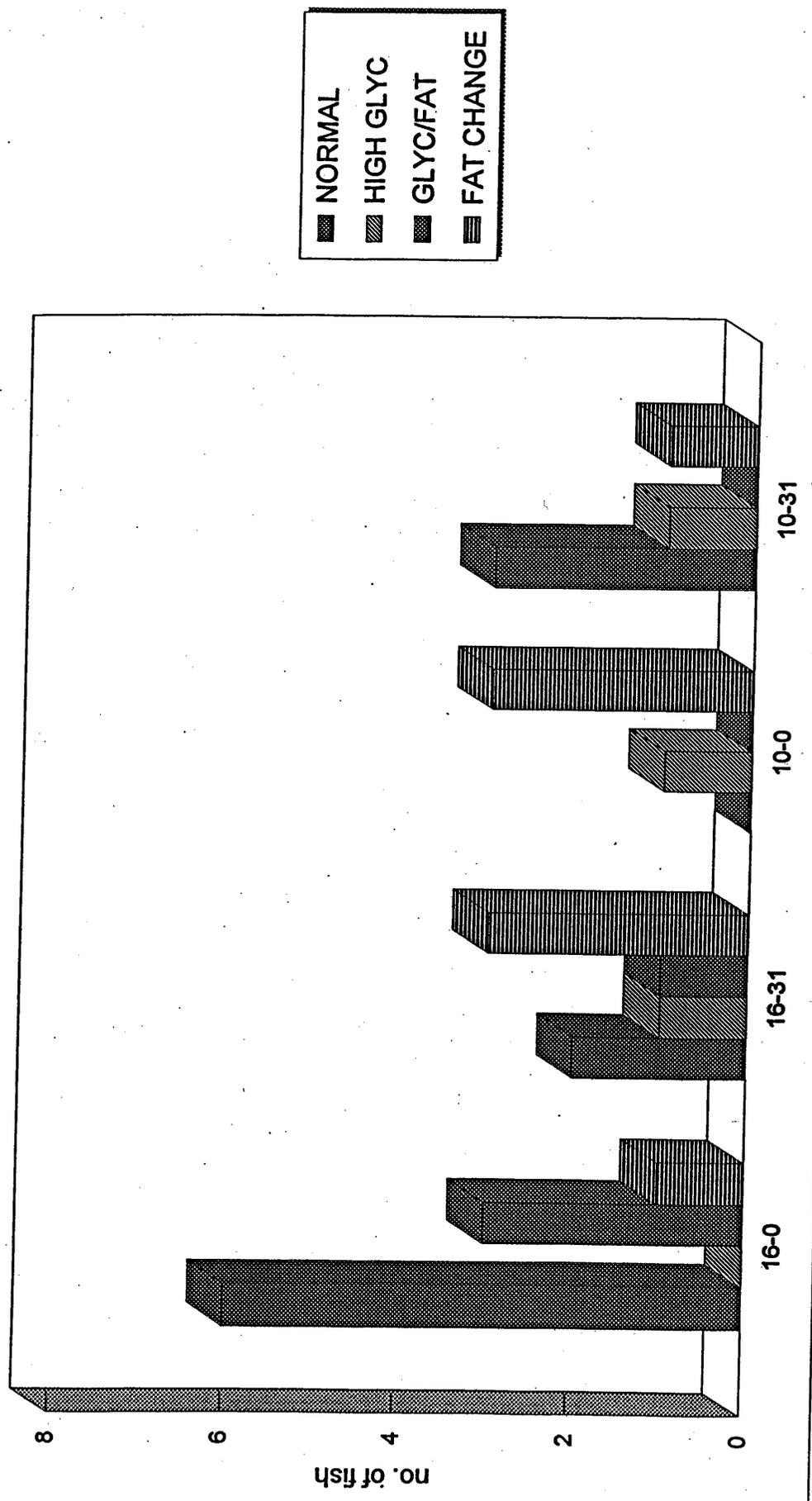


Figure 9. Histological rating of livers from late-fall chinook fed the lowfat diet and held at 16°C and 10°C. Livers were collected from eight to ten fish on day 0 and day 31 (16-0=16°C, day 0). Liver sections were rated as normal, high glycogen (HIGH GLYC), mix glycogen and fatty change (GLYC/FAT), or fatty change (FAT CHANGE).

## Variable Feed Rate - 31 day temperature study

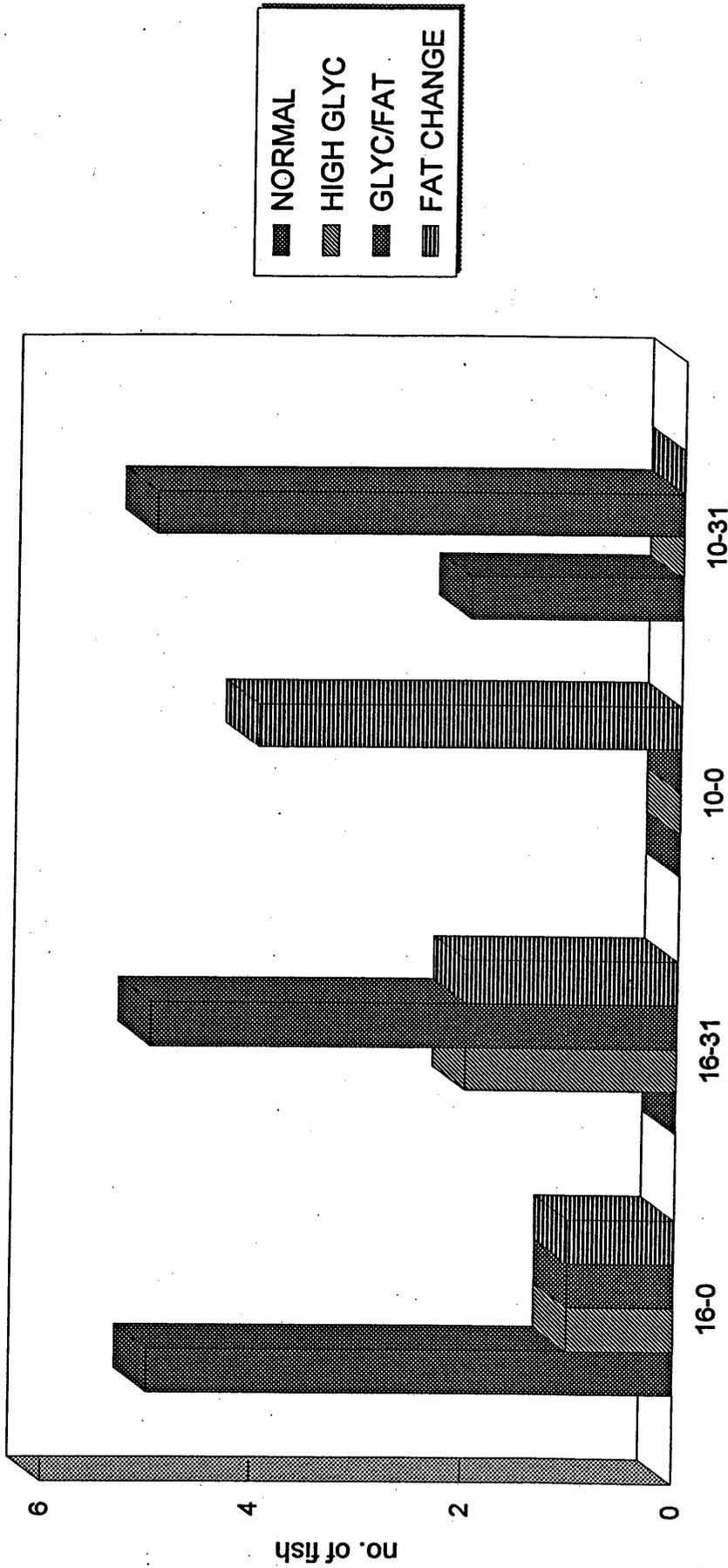


Figure 10. Histological rating of livers from late-fall chinook fed the variable diet and held at 16°C and 10°C. Livers were collected from eight to ten fish on day 0 and day 31 (16-0=16°C, day 0). Liver sections were rated as normal, high glycogen (HIGH GLYC), mix glycogen and fatty change (GLYC/FAT), or fatty change (FAT CHANGE).

### Effects of Food Deprivation on Fish Physiology

**General**--Fish in the food deprived group incurred a 37% cumulative mortality over the twenty-four day experiment. External lesions characteristic of *Flavobacterium (Flexibacter) columnare* infection were observed in the dead and moribund fish. Scale loss was seen in all food deprived chinook sampled on 18SEP with over 60% having scales missing from more than 20% of the body. The scales of this group were quite deciduous and easily shed upon handling of fish. The **control** group did not experience this abnormality. The food deprived group was significantly smaller in length, weight, and condition factor than the fed controls (Table 13). There was a 16% difference in the mean length measurements of the two groups.

**Organosomatic assay**--The subjective visceral fat score was high (3) for all fish examined in both groups (Table 13). While the mean plasma triglyceride value was similar for both groups, the food deprived fish displayed a greater variation (12 - 145 mg /dL). We observed triglyceride values ranging from 32 - 68 mg / dL in fed broodyear 1994 late-fall chinook (CA-NV Fish Health Center, unpublished data). Plasma protein values were within the range we commonly observe in juvenile chinook, however, the glucose response to capture stress (bled 10 - 15 minutes after capture) was low in all the food deprived fish (Table 14). Normally, we see greater than 90 mg / dL glucose following handling stress in fed hatchery chinook. There was no significant difference in leukocrit values between groups. It is unclear why hematocrits were elevated in the food deprived group.

**Table 13.** Mean  $\pm$  SE values for fork length, condition factor, hepatosomatic index, visceral fat, and percent of fish with severe fatty liver (score=2) of fish deprived of food (Food deprived) or fed normally (Fed controls).

Organosomatic parameter	Food deprived	Fed controls
Fork length (mm)	101 $\pm$ 2	119 $\pm$ 4 ++
Weight (g)	10.5 $\pm$ 0.6	22.6 $\pm$ 2.1 ++
Condition factor ( $K \times 10^5$ )	0.81 $\pm$ 0.016	1.00 $\pm$ 0.012 **
Visceral fat score	3.0 $\pm$ 0	3.0 $\pm$ 0
Fatty liver score=2	9/20 fish (45%)	6/20 fish (30%)
Hepatosomatic index	1.313 ( $\pm$ 0.103)	1.495 $\pm$ 0.052 ++

++ Significantly larger (Mann-Whitney Rank Sum Test,  $P < 0.001$ ).

\*\* Significantly larger (T Test,  $P < 0.05$ ).

**Table 14.** Mean  $\pm$  SE values for hematocrit (HCT), leukocrit (LCT), plasma triglyceride (TRG), plasma glucose (GLU), and plasma total protein (TP) for fish deprived of food or fed normally (Fed controls).

Group	HCT	LCT	Plasma TRG	Plasma GLU	Plasma TP
Food deprived	47 $\pm$ 1 ++	0.95 $\pm$ 0.1	73 $\pm$ 24	73 $\pm$ 4	2.4 $\pm$ 0.1
Fed controls	41 $\pm$ 1	0.82 $\pm$ 0.1	73 $\pm$ 9	ND	ND

++ Significantly larger (Mann-Whitney Rank Sum Test, P =0.0046).

ND Not done

The hepatosomatic index (HSI) was significantly less in the food deprived fish (Table 13). Pale livers were seen in both groups (30 & 45%). However, when examined histologically, the majority of food deprived fish had normal hepatocyte structure in contrast to the 70% incidence of fatty change seen in controls (Table 15). No other significant lesions were observed in the gastrointestinal tract, visceral adipose tissue, kidney (the *Nanophyetus salmincola* metacercaria seen in the kidney and gill are common trematode parasites for CNFH fish), heart, or gills. Further evidence of fatty liver reversibility to a normal state, under conditions of reduced energy intake, came from a histological liver specimen from a late-fall chinook held until late February 1997 (used for swimming performance tests which were unsuccessful due to the poor design of the test apparatus). The liver showed a high degree of apoptosis (self-destruction of cells) of the fat-infiltrated hepatocytes and had islets of regenerating hepatocytes. This fish had been given only a low maintenance diet for up to fifty days prior to sampling.

**Table 15.** Summary of results of histological examination for fish deprived of food (Food deprived) or normally fed (Fed controls). Results reported as number positive/total examined(%).

Tissue examined	Food Deprived	Fed Controls
<u>Liver</u>		
normal	8 / 9 (89%)	0 / 10 (0%)
high glycogen	1 / 9 (11%)	2 / 10 (20%)
glycogen / fat	0 / 9 (0%)	1 / 10 (10%)
fatty change	0 / 9 (0%)	7 / 10 (70%)
<u>Intestine, visceral fat abnormalities</u>	0 / 9 (0%)	ND
<u>Kidney metacercaria</u>	5 / 10 (50%)	ND
<u>Gill metacercaria</u>	2 / 10 (20%)	ND
<u>Heart metacercaria</u>	2 / 6 (33%)	ND

#### Evaluation of Commercial Feeds

*Growth rate*--Growth rates of fish fed different commercial diets were highly variable between months and no trend was evident (Table 16). In general, all feeds seemingly provided adequate and similar levels of metabolizable energy for growth. This finding is further corroborated by analysis of final mean total length on 5SEP which indicated no significant differences between groups fed different commercial diets (Table 17). One notable trend observed was the significantly lower condition factor of **NELSON** treatment fish which may be indicative of lower body lipid levels. Unfortunately, body composition analysis' were not conducted.

**Table 16.** Growth rates (mm/day) of fish fed different commercial diets for specific date intervals. Values in **bold** type represent the diet group exhibiting the highest rate of growth for the corresponding interval. Mean temperature (°C) data is provided as a comparison of growth rates at different temperatures.

Feed type	Growth interval				
	5/31-6/17	6/18-7/3	7/4-7/16	7/17-8/2	8/3-9/5
Rangen	<b>0.54</b>	0.49	0.30	0.56	<b>0.63</b>
Biom moist	0.45	0.68	0.35	<b>0.63</b>	0.47
Nelson	0.35	<b>0.92</b>	<b>0.47</b>	0.62	0.45
Mean temp	16.9	15.7	17.7	17.6	18.4

**Table 17.** Sample Mean of total length (TL;mm) and condition factor (Kfact) measurements for treatment replicates sampled on specific dates. Condition factor mean values are multiplied by a factor of 10<sup>5</sup>.

Feed type	17JUN		3JUL		16JUL		2AUG		5SEP	
	TL	Kfact	TL	Kfact	TL	Kfact	TL	Kfact	TL	Kfact
Rangen	51.1	0.9026	58.9 a <sup>1</sup>	0.9138a	62.8	0.9563	72.4 a	1.0077 a	93.8 a	1.0590 a
Biom moist	49.7	0.8923	60.5 a	0.9228a	65.1	0.9556	75.8 b	1.0089 a	91.7 a	1.0740 a
Nelson	47.6	0.8923	62.6 b	0.9112a	68.7	0.9319	79.2 c	0.9710 b	94.5 a	1.0120 b

<sup>1</sup> Different letters indicate significant differences at P<0.05. Comparisons were made between treatments on each date (3JUL, 2AUG, and 5SEP), but not between dates.

*Feed utilization*--Conversion rates of fish fed different commercial diets were generally similar and no trend was evident (Table 18). No explanation is apparent for the abnormally high (poor) conversion rates observed for the month of September.

**Table 18.** Monthly feed conversion rates of fish fed different commercial diets. Values in bold represent the lowest (best) rates for each month.

Feed type	June	July	August	September
Rangen	1.13	1.20	1.23	<b>1.58</b>
Biom moist	1.11	<b>1.09</b>	<b>1.17</b>	1.96
Nelson	<b>0.91</b>	1.12	1.27	2.46

*Organosomatic assay*--Overall, mean organosomatic parameters (HSI, hematocrit, leukocrit, VFAT) of fish from each diet treatment assayed on different dates were within the normal ranges for hatchery-origin chinook salmon juveniles (Table 19). Liver lipid abnormalities were observed in all groups at varying frequencies, but not at each sample date. Notable differences between different diet treatments were observed during the 26SEP assay and included the significantly higher mean HSI value of fish from the **BIOMOIST** treatment as compared to **NELSON** and **RANGEN** treatments and the significantly larger mean VFAT score of **BIOMOIST** treatment fish as compared to fish from the **NELSON** treatment, which further substantiates supposition of reduced body lipid levels in fish fed the **NELSON** diet.

**Table 19.** Mean values for fork length (FL;mm), condition factor (KFACT), hepatosomatic index (HSI), hematocrit, leukocrit, visceral fat (VFATSCORE), and percent of fish with severe fatty-liver (LIVERSCORE) of sampled fish from organosomatic assays for commercial feed evaluations.

FEED TYPE	FL(MM)	K-FACTOR	HSI	HEMATOCRIT	LEUKOCRIT	VFATSCORE	LIVERSCORE
<b>28AUG</b>							
RANGEN	84.8	0.9999	1.55 a <sup>1</sup>	No data	No data	2.50 a	0%
BIOMOIST	81.9	1.0340	1.91 a	No data	No data	2.10 a	0%
NELSON	88.5	0.9348	1.75 a	No data	No data	2.10 a	0%
<b>26SEP</b>							
RANGEN	88.9	0.9909	1.04 a	40.8 a	0.9544 a	2.75 a,c	0%
BIOMOIST	95.7	0.9626	1.26 b	41.2 a	1.1493 a	3.00 a	10%
NELSON	99.0	0.9286	1.07 a	38.8 a	1.3525 a	2.65 c	5%

<sup>1</sup> Different letters correspond to significant differences (P<0.05). Comparisons were made between treatments on the same date, but not between dates.

*Histological evaluation of livers*--No trend is evident from results of histological evaluation of livers except that all diets produced fish with abnormal hepatocyte structure (Figures 11 and 12). Unfortunately, sample sizes of four to seven fish are not adequate to make determinations of differences in fish fed different diets.

*Mortality*--The highest cumulative monthly mortality rates for all treatments occurred in July and were likely the result of stress-induced disease caused by increased water temperatures and increasing densities (Figure 13). *Flavobacterium (Flexibacter) columnare* was presumptively identified as the causative organism for this disease-associated mortality. Interestingly, fish fed **RANGEN** and **NELSON** diets had mortality rates for June, July and August that were more than double the rate of fish fed the **BIOMOIST** diet. This trend was reversed for the month of September.

**Biodiet, Rangen, Nelson feed, 27 AUG - liver histology**  
 normal, high glycogen, glycogen/fat vacuoles, fatty change

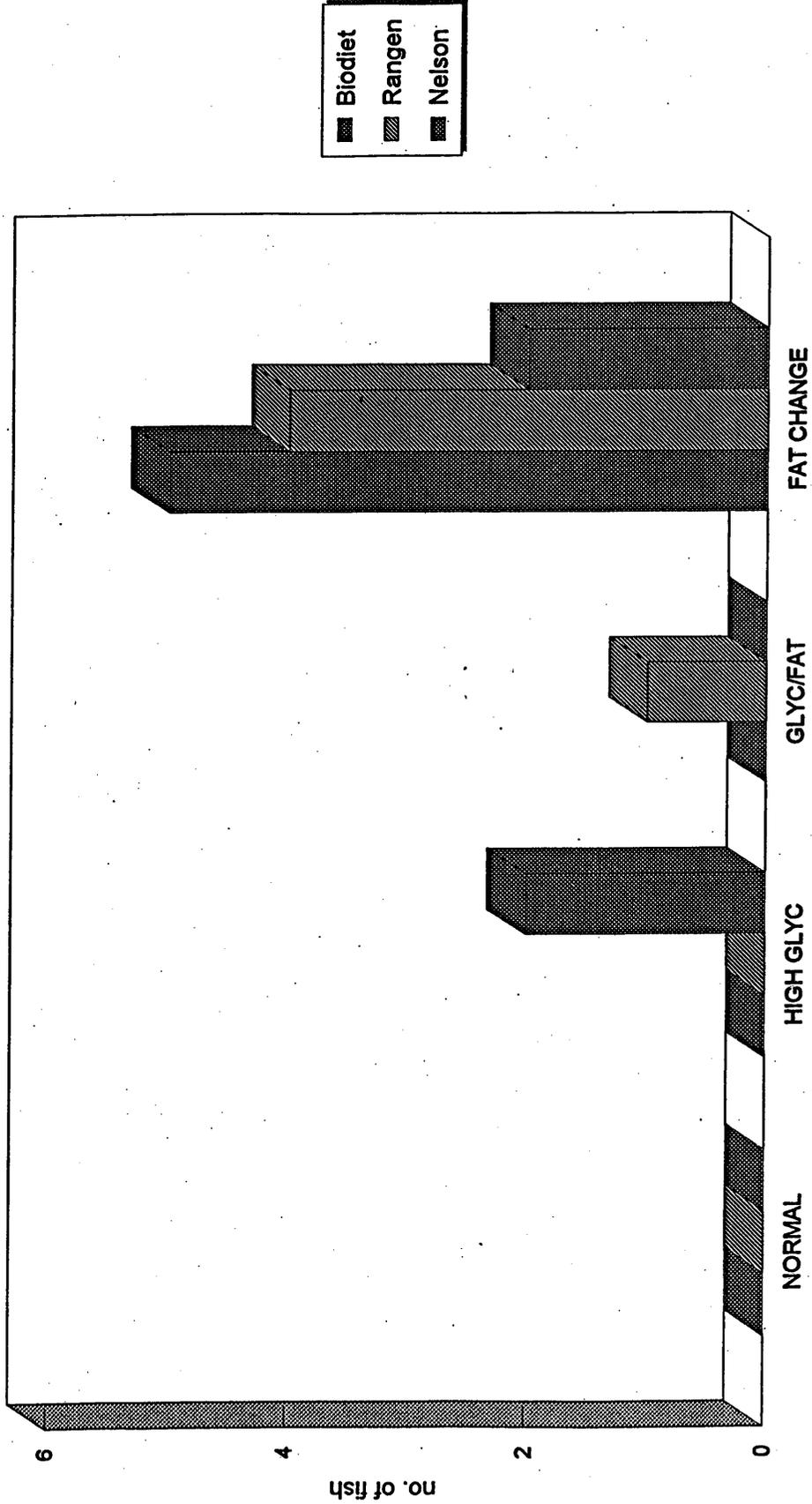


Figure 11. Histological rating of liver sections from late-fall chinook fed BIOMOIST, RANGEN, or NELSON diets. Livers were collected from five to eight fish on 27AUG. Liver sections were rated as normal, high glycogen (HIGH GLYC), mix glycogen and fatty change (GLYC/FAT), or fatty change (FAT CHANGE).

# Biodiet, Rangen, Nelson feed, 26 SEPT - liver histology

normal, high glycogen, glycogen/fat vacuoles, fatty change

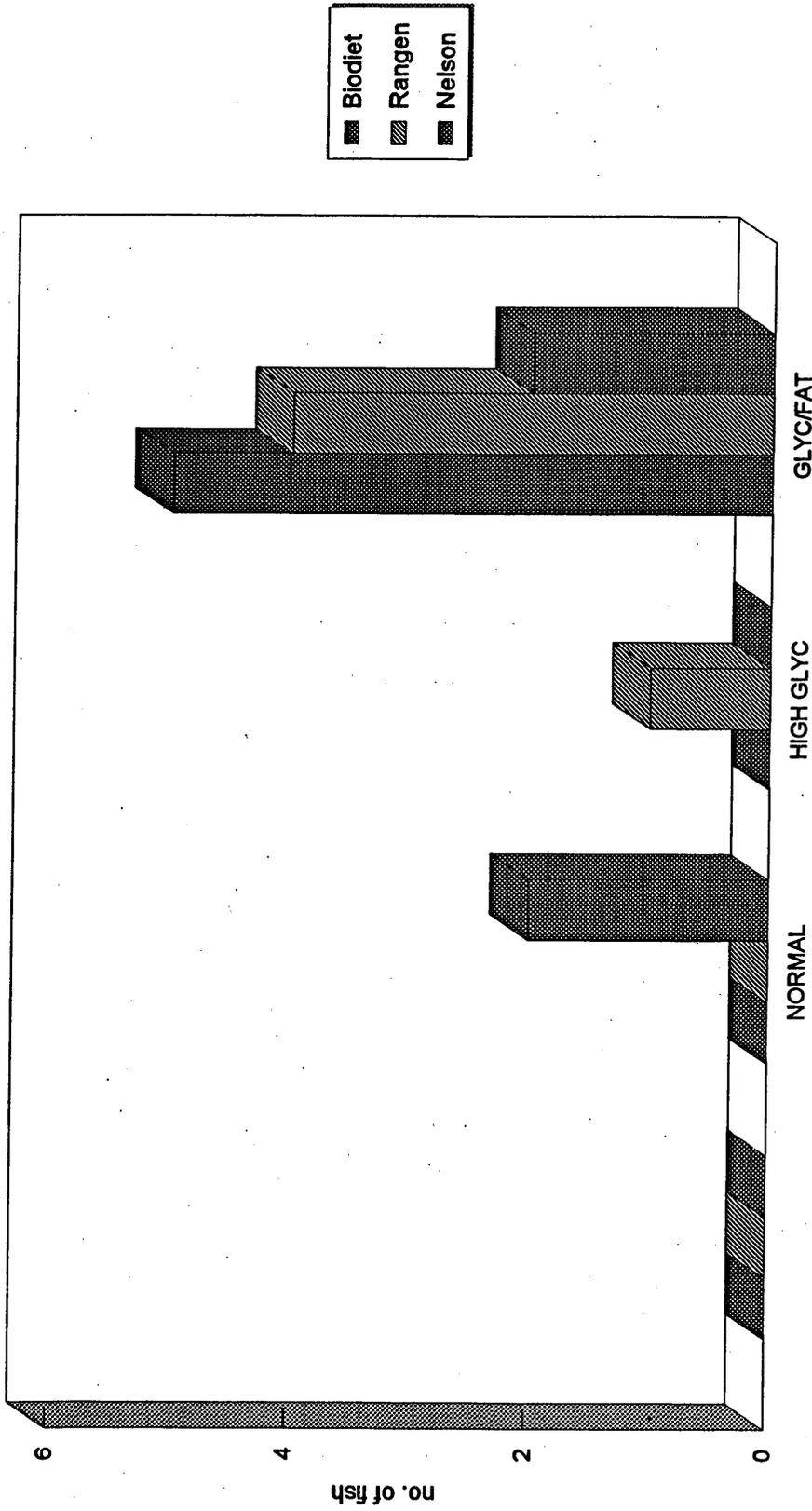


Figure 12. Histological rating of liver sections from late-fall chinook fed BIOMOIST, RANGEN, or NELSON diets. Livers were collected from five to eight fish on 26SEP. Liver sections were rated as normal, high glycogen (HIGH GLYC), mix glycogen and fatty change (GLYC/FAT), or fatty change (FAT CHANGE).

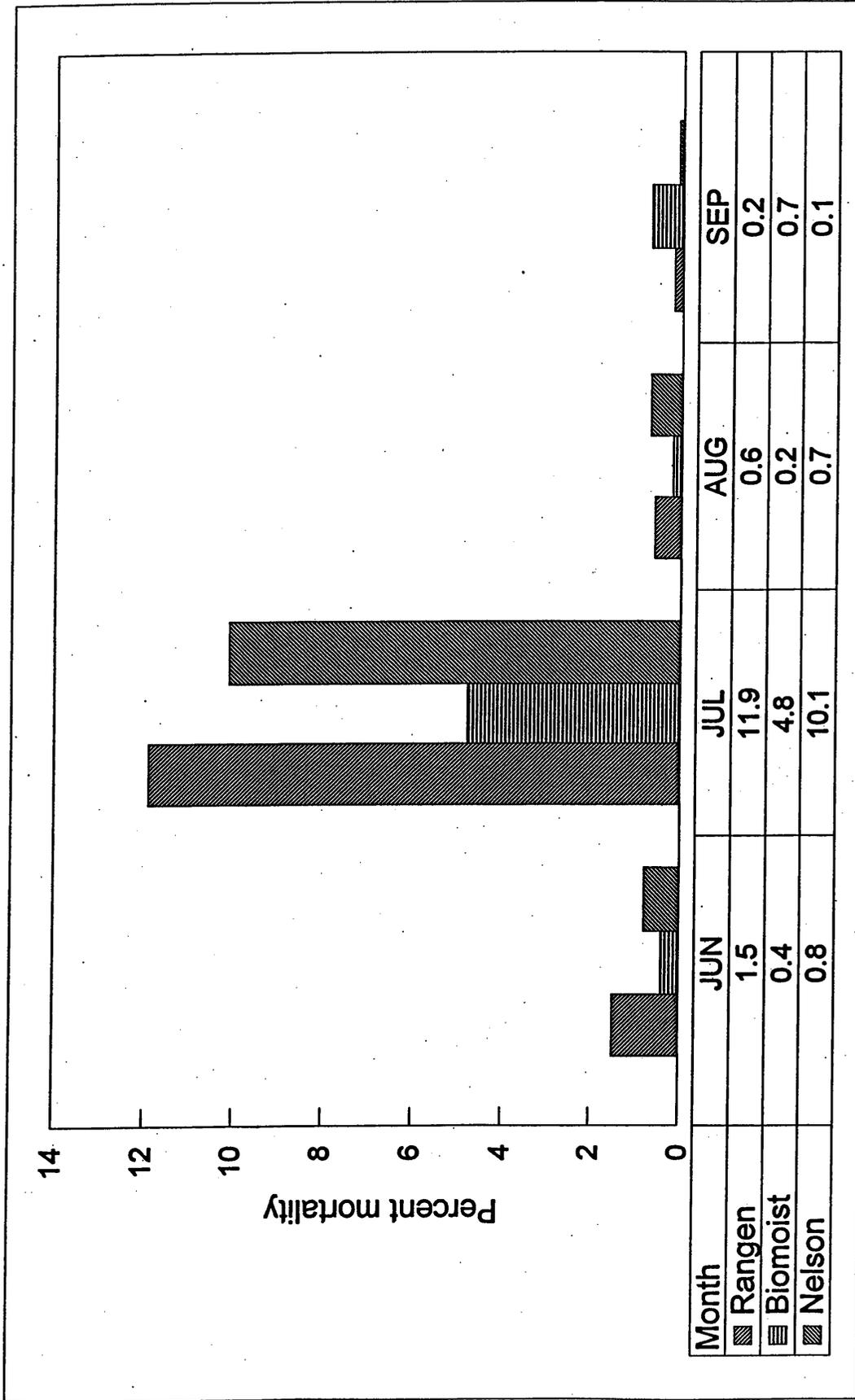


Figure 13. Cumulative monthly percent mortality of late-fall chinook fed the RANGEN, BIOMOIST, or NELSONSON diets.

## DISCUSSION

### Physiological Effects of Feeding Reduced Rations and Reduced Dietary Lipid

As in year's past, liver lipid abnormalities were observed in late-fall chinook despite dietary changes designed to address this concern. Interestingly, histological evaluation of livers sampled in September and January indicated a decrease in fatty change and a tendency towards glycogen storage in liver hepatocytes in fish from all treatment groups. This trend indicates potential reversal of severe liver lipid abnormalities with either decreasing temperature or the onset of smoltification. Subjective evaluation of livers during organosomatic assays on 6JAN revealed distinct differences in the frequency of liver abnormalities in fish from the three diet treatments: 47% of fish from the **control** treatment exhibited severe abnormalities, 20% of fish from the lowfat treatment exhibited severe abnormalities, and 0% of fish from the variable treatment were observed with severe liver abnormalities. Although differences were not significant, mean hepatosomatic indices of fish from the three diet treatments seemed to corroborate this trend. We are unable to distinguish the difference between excessive glycogen storage and excessive lipid storage during subjective evaluation of livers.

No diet related trends were evident from analysis of hematocrit, leukocrit, and plasma protein. Additionally, no trend in these parameters was evident for fish exhibiting severe liver lipid abnormalities. We are unsure of the potential physiological effects associated with excessive liver lipid storage, but to this point we have been unable to establish a connection between liver lipid abnormality and impaired physiology.

No diet related trends were significant in regard to smoltification as evidenced by analysis of saltwater challenge data and gill ATPase levels, but **control** treatment fish did exhibit both higher gill ATPase levels and greater ability to osmoregulate in saltwater on the 7JAN assay date. Non-detection of statistically significant differences is more than likely a result of small sample size (5gill ATPase, 6-10 saltwater challenge fish) rather than actual observation of no difference. Additionally, detection of correlation between fish size and smoltification was also hampered by sample size. Analysis of saltwater challenge and gill ATPase data from all diet treatments and subsequent subjective scoring of livers indicated no apparent correlation between osmoregulation competence and liver lipid abnormalities. These findings are similar to our observations of past years' data (CA-NV FHC, unpublished data).

Final size at release was influenced by ration level, but not necessarily influenced by diet lipid levels; at least not by the end of the study period. Interestingly, somatic growth rates were not necessarily correlated with feeding rates. The observed monthly oscillation between presumed lipid storage and somatic growth is not easily interpreted. Additionally, we are unable to explain the unexpected increased lipid storage and relatively low growth during higher temperature periods. We would expect to see increased utilization of energy stores during warmer periods resulting from increased metabolic demand. However, it is plausible that an evolutionary adaptation concept of

insulin production and subsequent lipid storage during presmolt development (which in late-fall chinook, occurs during the warmer summer months) followed by increased plasma insulin-like growth factor-I (IGF-I) and subsequent somatic growth stimulated by decreasing photoperiod may partially explain this anomaly. This adaptation of increased lipid storage during warm summer months may be required for natural fish consuming a natural diet which may not provide the necessary energy required by the metabolic demands of smoltification and outmigration during winter months. We cannot explain this result satisfactorily.

One notable observation was the relatively large increase in growth rates of **variable** treatment fish during the months of November and December which occurred when feeding rates were at their lowest level. Presumably, this period of rapid somatic growth corresponds with physiological change associated with smoltification. Additionally, this increased somatic growth was coupled with increased mobilization of lipid reserves as evidenced by decreasing condition factor and decreasing subjectively scored visceral fat levels. All treatment groups exhibited their highest somatic growth rates between September and their release in January and was coupled with decreasing condition factor which may be related to physiological changes indicative of smoltification. Interestingly, this time of the year is typically when feeding rates are at their lowest levels. Beckman et al. (1996) observed results that suggest somatic growth rate and stimulation of the growth hormone/IGF-I axis was more physiologically important for the smolt transformation process than ultimate fish size. Similarly, Ewing et al. (1980) observed a correlation between growth rate and changes in gill ATPase activity. With this in mind, perhaps more emphasis should be placed on providing adequate, and potentially enhanced, nutrition during developmentally critical periods such as during early development and finally, during the parr-smolt transformation process. Perhaps life-stage diets could be produced that provide components that enhance development during critical stages yet do not cause abnormalities such as liver lipoid disease. Additionally, although manufacturer's recommended feeding rates are established to maximize growth in weight, perhaps an innovative approach needs to be taken with respect to establishing anadromous fish feeding rates that enhance smolt quality and subsequent smolt-to-adult survival. Intuitively, we suspect that development of both life stage diets and innovative feed schedules would be species and site specific.

One concern with developing different diet formulations was partially evident in our observations of decreased health and survival of fish fed the **lowfat** feed as evidenced by elevated mortality rates. We surmise that by reducing the marine fish/krill fatty acid content of the **lowfat** feed there was a concurrent reduction in some unknown factor, perhaps a micronutrient, that is critical to development of immunocompetence in salmonids. Interestingly, recent work by Barrows and Lellis (1997) with rainbow trout indicates that some unknown factor(s) is present in krill meal that reduces or eliminates fin erosion. Additionally, McKenzie et al. (1994) observed a repressed response of adriatic sturgeon (*Acipenser naccari*) to hypoxic stress when fed a diet composed of saturated fatty acids (hydrogenated coconut oil) as compared to sturgeon fed a diet rich

in long-chain unsaturated fatty acids (menhaden oil). With this in mind, development of life stage diets should consider the quality/type of components used in feed processing as well as ratios of major nutrient groups.

Feeding late-fall chinook at levels substantially less than manufacturer's recommended levels seemingly did not adversely affect physiology or immunocompetence. Additionally, adequate growth and deposition of energy reserves, although reduced, was not adversely affected. This determination further avows the potential for development of innovative feed scheduling.

#### Effects of Temperature on Physiological Parameters of Fish Fed Reduced Rations and Reduced Lipid Diets

Our observations of lipid reserve mobilization and reversal of hepatocyte fatty change in fish exposed to reduced temperatures seem to point to a relationship between elevated temperature and development of excessive lipid deposits in hepatic and mesenteric tissues of fish fed high energy diets and are contrary to current knowledge of fish metabolism. This finding stimulates our interest in future studies where external factors such as potential photoperiod differences can be controlled. Currently, we are unable to explain our results satisfactorily.

#### Effects of Food Deprivation on Fish Physiology

Food deprivation appeared to have a negative effect on immune defenses. In spite of similar circulating white blood cell numbers (estimated by leukocrit), the food deprived group experienced an outbreak of columnaris. Controls, reared on the same water source, remained healthy.

It appears that the food deprived group used their energy reserves in the order reported for other salmonids (Akiyama, T. and T. Nose, 1980; Arndt et al. 1996). First, liver glycogen and adipose lipid associated with the muscle bundles was used. Next, reserves used would have been visceral lipid, and lastly muscle proteins. Evidence for the assertion that liver glycogen was used preferentially by the food deprived fish for standard metabolism are: 1) the reduction in HSI; 2) poor response in plasma glucose to stress and; 3) low prevalence of glycogen-laden hepatocytes seen in histological sections of food deprived fish. The presence of large quantities of visceral fat in a fish with a reduced condition factor indicates that muscle lipid reserves were also utilized before its visceral fat. We cannot speculate on the degree of protein catabolism in the food deprived group as protein determinations were not done on the muscle, however, the drop in liver mass was not the sole determinant for the low condition factors. When liver weight was subtracted from body weight, the adjusted body weight values still generated a significant difference (T-test,  $P < 0.001$ ) in condition factor. The presence of visceral fat in food-deprived Atlantic salmon was also observed by Arndt et al. (1996). It is assumed that triglycerides in the myosepta (connective tissue) of both dark and white muscle were mobilized for standard metabolism during the experiment (Zhou et al. 1995).

Despite a (presumed) history of fatty change in the starting population's livers , the food deprived fish appeared able to mobilize lipid stores for energy and their livers reverted to a normal hepatocyte structure. If this trend is a valid observation, then late-fall chinook with fatty liver can successfully used their lipid reserves during their smolt migration and have their livers return to normal in a period of months. It is possible that the examined group was biased toward healthy individuals if fish with severe fatty liver had died prior to the 18SEP sample. This is highly unlikely as we subjectively observed a 47% incidence of severe liver abnormality in this group.

#### Evaluation of Commercial Feeds

All feed types seemingly provided adequate nutrition for growth. No diet eliminated development of liver lipid abnormalities, but subjective scoring indicated that fish fed the **BIOMOIST** diet had higher visceral fat levels, higher HSI's, and more fish exhibiting severe liver abnormality. This potentially points to some differences between diets, but histological analysis did not reveal this same trend. However, analysis of mortality rates indicate some trend for differences in development of immunocompetence in fish fed different manufacturer's formulations. This is similar to our observation of the effects of feeding the **lowfat** formulation. Again, we reiterate our concerns regarding the quality/type of components used for feed production.

## CONCLUSIONS AND RECOMMENDATIONS

- ▶ *Late-fall chinook can be fed rations at rates substantially less than manufacturer's recommendations without impairing health, physiological development, or growth.*  
Recommendation: Develop innovative feed schedules that enhance nutrition at critical periods in fish development, yet do not promote (minimize) abnormal lipid storage. For example, fish could be fed at normal or enhanced rates during the first two months and the last two months of rearing while significantly reducing feed rates (1-2% less than manufacturer recommendations) during the warm summer months.
- ▶ *Severe liver lipid abnormality may not necessarily impair normal fish physiological function; at least not during hatchery residence.*  
Recommendation: Although we did not observe physiological impairment in fish exhibiting severe liver lipid abnormalities, intuitively we remain concerned that liver function may be impaired by excessive lipid storage and recommend development of strategies to eliminate or minimize its occurrence.
- ▶ *Liver lipid disease may reverse during smoltification.*  
Recommendation: None given.
- ▶ *Components used during feed production are important for normal development of immunocompetence in late-fall chinook.*  
Recommendation: Commercial feed protein and lipid sources should be taken into account when evaluating different feed formulations. This may be especially important when developing life stage diets.
- ▶ *Temperature appears to play a role in lipolysis and lipogenesis in liver and mesenteric tissues of late-fall chinook.*  
Recommendation: Although we realize that rearing all Coleman NFH late-fall chinook on chilled water is not practical or possible, we recommend further investigation of the effects of temperature on lipid storage.
- ▶ *Coleman NFH late-fall chinook appear to undergo smoltification in November and may be reverting back to parr by January.*  
Recommendation: Further investigation of Coleman NFH release protocol and smolt development is needed. Additionally, we recommend development of innovative feed schedules that promote growth during critical smolt development. The extensive late-fall chinook tagging program provides the opportunity to evaluate feed rate changes on smolt outmigration and adult contribution rates

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Late-fall chinook salmon diet study, coded-wire tagged adults recovered at CNFH and in the ocean fishery for 1998-99.

Brood Year	Study Groups	Tag Code	Date Released	Number Released	Length at Release (inches)	% Hatchery Return	% Ocean Contribution
1996	Control	054125	01/16/97	57,739	6.30	1.415	1.015
	Control	054239	01/17/97	53,936	6.40	1.159	1.353
	Low Fat	054123	01/16/97	53,302	5.90	1.041	1.195
	Low Fat	054241	01/16/97	46,137	6.00	1.261	0.936
	Variable Rate	054124	01/16/97	52,286	5.20	0.738	0.618
	Variable Rate	054240	01/17/97	61,902	4.90	0.609	0.614

**DRAFT**

