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**Coastal Zone and Estuarine Studies**

**Effects of Starvation on  
Presmolt Coho Salmon**

by  
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EFFECTS OF STARVATION

ON

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## INTRODUCTION

Excessive mortality of juvenile salmonids migrating downstream in the Columbia River were identified in the early 1960's by Cleaver (1968); these losses approximated 70% of fall chinook salmon juveniles, Oncorhynchus tshawytscha, released from two conservation hatcheries on the lower river. In the 1960's hatcheries produced 5% of the total salmon production in the Columbia River; now, hatcheries produce approximately 50%. One of the major expansions since the 1960's has been the production of coho salmon, O. kisutch, (Wahle and Smith 1979); e.g., juvenile production has increased from 7 to 30 million fish from 1960 to 1976. Today, over 80% of the coho salmon in the Columbia River Basin originate at hatcheries. Although production of coho salmon juveniles has increased at the conservation hatcheries, the return of adults has not increased in proportion to juvenile production (production peaked in 1970, and has been at a high level but general decline since that time (Gunsolus 1978).

However, 20 years later the basic problem remains--excessive losses of downstream migrants. The sources of these mortalities have not been isolated and quantified. Ebel (1970) demonstrated that survival could be improved by transporting fish around Bonneville Dam, which suggested that passage through dams was a major mortality factor. Ellis and Noble (1960) reported losses of fall chinook salmon of 12 to 29% in 64 km of the Klickitat River but did not identify the source of mortality. At the present time there is justification for a hypothesis that starvation is one of the causes of mortality of juvenile salmon in the Columbia River; the rationale for this hypothesis follows:

1. Adult coho salmon production is declining in spite of increased hatchery production of juveniles; there is evidence that the limiting factor is not oceanic productivity. Favorite and Laevastu (1979) state that the "apparent carrying capacity" of the North Pacific Ocean in respect to salmon can easily sustain a ten times higher standing stock of salmon than at present.

2. Royal (1972) found that food and space were limiting factors in the Columbia River for the production of steelhead trout; his findings suggest that the limiting factors are associated with the juvenile portion of the life cycle of salmonids in fresh water.

3. Only 20% of the coho salmon released from hatcheries are ready to "smolt" (NMFS, Project 817, 1979). This indicates that the remaining 80% as presmolt (or transitional) fish are not physiologically prepared to migrate downstream, and are delayed for some time.

4. Dawley et al. (1979) measured the mean passage time of juvenile migrants from release at hatcheries to a capture point at Jones Beach River Kilometer (Rkm 74). The mean passage time for coho salmon is approximately 20 days, but there is evidence that these are only the "smolting fish," i.e., "the observation that migrants captured at Jones Beach nearly always exhibited high  $\text{Na}^+\text{-K}^+$  ATPase activities reinforces the concept that active seaward migration and elevated gill  $\text{Na}^+\text{-K}^+$  ATPase activities are concurrent events," (NMFS, Project 817, 1979). This suggests that smolting fish are found migrating downstream, presmolting fish are not.

5. Sanborn (1975) found low benthic productivity in the Columbia River from Rkm 168 (mouth of the Willamette River) to the estuary in a predominately fine-sandy habitat. Craddock et al. (1976) indicate that zooplankton levels in the lower Columbia River are relatively abundant but

seasonal. This suggests that there could be a restricted and selective food supply available to migrating coho salmon.

6. There is a relatively high incidence of empty stomachs from salmonids and other species of fish captured from Rkm 168 (Durkin et al. 1977) to the lower river (Durkin et al. 1979; Blahm et al. 1979; McConnell et al. 1978). Although these food utilization studies were primarily aimed at benthic finfish, the pelagic coho salmon inadvertently captured revealed an incidence of empty stomachs as high as 19 to 60%. The diet of fish with food in their stomachs consisted of larger food items like aquatic insects, not zooplankton.

7. Presmolting coho salmon released from hatcheries are usually over 1 year old (1<sup>+</sup>). Wahle and Smith (1979) indicate that larger juveniles are being released now than in the 1960's. The larger coho salmon apparently move out of the river through a narrow period of time from 5 May to 25 May (Durkin and Sims 1975); presmolts remain in the river as selective feeders. It appears that coho salmon (1<sup>+</sup>) prefer larger insects or small fish (small fall chinook salmon) as prey and do not feed effectively nor with any degree of success (based on stomach contents) on smaller zooplankton.

The rationale exists that starvation could be a potential threat to downstream migrants, and proof is needed that starvation is a source of mortality. There is a need to recognize starvation in presmolting coho salmon. Juvenile fish that are forced to exist on a starvation diet may have serious alterations in health state. An alteration in the health state of these active fish could result in an impairment of disease resistance mechanisms, a susceptibility to predation, or manifestation of other factors which could account for the demise of the fish but not

necessarily reflect the cause. A review of the literature illustrated that the effects of starvation (on presmolting juvenile salmonids from the Columbia River) has not been investigated. Consequently, a laboratory study, funded in part by the NMFS Northwest Regional Office, Environmental and Technical Services Division, Portland, Oregon, was initiated with the following objectives:

1. Conduct a literature review of the effects of starvation on fish with special emphasis on presmolting coho salmon.
2. Establish how the problem of starvation could apply to the specific problem of mortalities found in the lower Columbia River.
3. Determine what changes occur during starvation of presmolt coho salmon in selected hematological and chemical variables and condition factor by time, temperature, size, and treatment (fed vs unfed).
4. Investigate the effects of starvation on swimming performance of presmolting coho salmon.
5. Compare the susceptibility of fed and unfed presmolt coho salmon to natural predators.

#### METHODS AND MATERIALS

The research was conducted in three phases. Phase I was initiated in August 1978; the goal was to establish time and temperature limitations for the later phases. A literature review was conducted concurrent with Phase I to determine what hemotological, chemical, and physical factors should be included in Phase II and III.

Phase II was initiated in January 1979 to investigate condition factor changes, and deviations in selected hemotological and chemical variables in presmolting coho salmon juveniles. Variables measured or calculated included: condition factor, hematocrit, hemoglobin, red blood cell count,

white blood cell count, and differential white cell counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), blood urea nitrogen (BUN), total protein, albumin, globulin, the globulin fractions, and the albumin/globulin (A/G) ratio. Time, temperature, size, and treatment (fed-unfed) were the independent variables imposed.

In Phase III of the study, one size range of presmolting coho salmon was tested at optimum temperature to determine the swimming performance of fed vs unfed fish over time. Predation tests, utilizing presmolting coho salmon as prey, were also conducted during this phase--June through October 1979.

#### Test Facility

All phases of this study were conducted at Prescott, Oregon, on the National Marine Fisheries Service test facility described in detail by Snyder et al. (1971). The testing facilities are housed on two covered barges (33.5 by 10.4 m) moored on the Columbia River at Rkm 115.8. Test water is pumped from the Columbia River and is heated, cooled, and filtered as required. The covered barges housed the specialized equipment and work space necessary for the conduct of this series of experiments. A diagrammatic view of the fish holding and testing areas is shown in Figure 1.

#### Experimental Design

The goal of Phase I was to determine if a measurable change in condition factor would occur with fish held for 6 weeks on restricted food intake, at temperatures typical of those occurring during downstream migration. Twenty presmolt juvenile salmon were placed in each of six test tanks with three replicated water temperatures: 12°, 15°, and 18°C. Fish

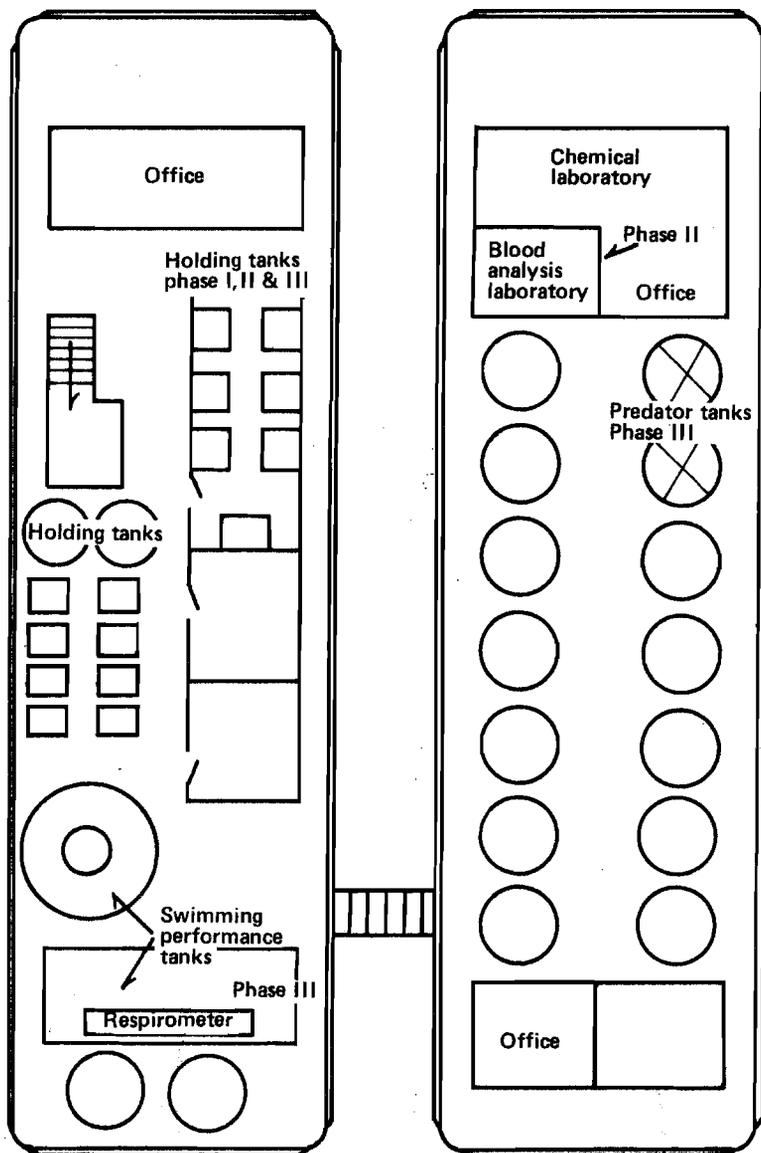


Figure 1.--Diagrammatic view of the fish holding and testing areas on two covered barges moored on the Columbia River at Prescott, Oregon (River Kilometer 115.8) which serves as a fisheries research test facility for the National Marine Fisheries Service, Northwest and Alaska Fisheries Center, Seattle, Washington, and was the site for the research conducted in this study.

in one set of the paired tanks were fed a maintenance diet, the others were unfed. The fish were observed twice each day, and the mortalities were documented and removed. At the end of the fifth and sixth weeks, 20 fish (or the number of surviving fish) were anesthetized, weighed, and measured for length.

Phase II was designed to examine length of holding time, water temperature, fish size, and treatment (fed-unfed) differences through alterations in condition factor, hematology, serum chemistry, and survival. The experimental design for Phase II is shown in Table 1.

The effects of starvation on swimming performance and susceptibility to predation were investigated in Phase III. In the swimming performance tests, fed and unfed coho salmon juveniles were subjected to similar and constant water velocities each week for 6 weeks at 18°C to test fatigue levels. Twenty fed and unfed coho salmon juveniles were offered as prey to predators each week for 6 weeks at 18°C to test susceptibility to predation.

#### Test Fish

When the Phase I portion of the study was started, the fish available were spring chinook salmon subyearlings. The chinook salmon were obtained from the U.S. Fish and Wildlife Service hatchery at Little White Salmon, Washington and were transported to Prescott, Oregon on 21 August 1978. The spring chinook salmon averaged 92.5 mm (SD 7.5) and 9.8 g (SD 2.0) at the start of the study.

Two sizes of presmolting coho salmon were obtained for the second study phase. Large coho salmon were obtained from the Lower Kalama Hatchery (Washington Department of Fisheries); small coho salmon were

Table 1.--Experimental design of study to determine effects of starvation on presmolt coho salmon, in three phases.

Phase I (Pilot Study)

Temperature	Fed			Unfed		
	Week 0	Week 5	Week 6	Week 0	Week 5	Week 6
12°C	XX	X	X	XX	X	X
15°C	XX	X	X	XX	X	X
18°C	XX	X	X	XX	X	X

X = 10 fish sample--measured condition factor and mortality

Phase II Measured Characteristics of Starvation

Week Temperature	Fed												Unfed														
	Large						Small						Large						Small								
	0	1	2	3	4	5	6	0	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	
12°C	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
15°C	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
18°C	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

X = 5 fish pooled sample--measured or computed variables included Condition Factor, RBC, White Blood Cell count and differential, Hematocrit, Hemoglobin, MCV, MCHC, MCH, BUN, Total Protein, Albumin, Globulin (and components), and Albumin/Globulin Ratio.

Phase III Swimming Endurance and Predation

A. Critical fatigue levels

Temperature 18°C	Week	Fed						Unfed						
		0	1	2	3	4	5	6	1	2	3	4	5	6
		X	X	X	X	X	X	X	X	X	X	X	X	X
		X	X	X	X	X	X	X	X	X	X	X	X	X
		X	X	X	X	X	X	X	X	X	X	X	X	X

X = 6 fish sample

B. Predation (W/coho salmon as prey)

Temperature 18°C	Week	Fed						Unfed						
		0	1	2	3	4	5	6	1	2	3	4	5	6
		X	X	X	X	X	X	X	X	X	X	X	X	X

X = 20 fish sample

obtained from the Kalama Falls Hatchery (WDF). The fish were transported to Prescott in February 1979. Fish from the upper hatchery averaged 104 mm (SD 5.8) and 12.3 g (SD 2.2); fish from the lower hatchery averaged 128 mm (SD 5.3) and 21.5 g (SD 2.7). The fish were from the same brood.

Coho salmon for the swimming performance portions of Phase III were obtained from the Lower Kalama Hatchery in June, 1979. The fish averaged 71 mm (SD 7.2) and 5.1 g (SD 1.2) at the initiation of the study.

Prey fish (presmolt coho salmon) for the Phase III predator/prey studies were obtained from the Kalama Falls Hatchery July, 1979; the fish were small, averaging 58 mm (SD 6.5) and 2.9 g (SD 0.5).

Initially the predators chosen for Phase III were brood stock cutthroat trout (Salmo clarki) that were obtained from the Beaver Creek Hatchery (Oregon Department of Fish and Wildlife). The fish were large (all over 1000 g), but did not prove to be satisfactory predators; they were replaced by "wild" cutthroat trout obtained through purse seining in the Columbia River estuary (at Rkm 22.5). The wild cutthroat (20 were used for the predation tests) ranged in length from 282 to 457 mm.

#### Fish Hauling And Handling

All fish utilized during the study were transported to the facility in a 1,500-liter tank. Hatchery water and supplemental oxygen were used during transportation. Hauling time did not exceed 3 hours for any of the fish utilized. Transported fish were placed in a redwood holding tank for approximately 1 week to provide for proper acclimation (Brett 1952). In all cases, fish were handled as little as possible in the conduct of each study.

### Testing and Holding Tanks

Fish were initially held in 1.8-m diameter redwood tanks supplied with water at a flow rate of 75 liters/min; water depth was maintained at 1.2 m. Fish were transferred to test tanks (constructed of wood, Plexiglass,<sup>a/</sup> and stainless steel) that were 80x60x60 cm filled to a capacity of 175 liters. Water was supplied to each test tank at a rate of approximately 1 liter/min; a complete interchange of water occurred about every 3 hours.

### Experimental Conditions

Water throughout the experiment was maintained at  $\pm 0.5^{\circ}\text{C}$  of test temperatures. Lights were provided in the holding and testing areas. Oxygen was maintained at 90-95% saturation through a once-through gravity system employed at the facility, and through the placement of air-stones in each holding and test tank. Although holding tank loading was optimum throughout the experiment, water quality was normally checked twice each week. During feeding, observation, and testing, personnel movements were minimized.

### Acclimation And Conditioning

When necessary, temperature acclimation was accomplished at a rate of  $1^{\circ}\text{C}$  every 24 hours as recommended by Brett (1952). All fish were fed maintenance diets until acclimation was finalized. Then during the experiment groups of large fish were fed 4.4, 5.4, and 6.4% body weight at

<sup>a/</sup> Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

temperatures of 12°, 15°, and 18°C, respectively; small fish in groups were fed 5.8, 7.2, and 8.4% body weight at temperatures of 12°, 15°, and 18°C. Fish were fed a diet of Clarks dry food twice each day.

Presmolt coho salmon were conditioned to the respirometer just before the testing of swimming performance. Fish to be tested were netted from test tanks with fine mesh dip nets, placed in plastic containers filled with water, carried to the respirometer, and carefully poured into the testing chamber. Fish were allowed to orient themselves for 10 min before being subjected to flowing water (approximately 0.5 m per second). The cutthroat trout from the estuary were not fed 1 week before small coho salmon were placed into the tank. The cutthroat were fed small coho salmon only once prior to the start of the predation tests.

#### Water Quality

Water quality parameters germane to the study were analyzed once each week by the staff of the NMFS Prescott Facility. Routine analysis included water temperature, turbidity, pH, oxygen, nitrogen gas (N<sub>2</sub>), carbon dioxide, total dissolved solids, and ammonia. Water quality was optimum for the holding of salmonids throughout this study.

#### Preparation Of Blood Samples

Blood analysis was conducted during Phase II of this study. Two groups of five fish each were anesthetized in a solution of tricaine methanesulphonate (MS222) buffered with sodium bicarbonate (Wedemeyer and Yasutake 1977). The fish were measured for fork length and weighed, the caudal peduncle of each fish was severed and blood was collected from five fish with heparinized microhematocrit tubes and pooled (Newcomb 1974, Amend & Smith 1974) in a small vial and mixed by gentle inversion. Two blood

smears were prepared from two fish randomly selected from each five fish sample; slide imprints were made from the kidney, liver, and spleen of the two fish. Two subsamples were collected in capillary tubes from the pooled vial for cell counts and chemistry, and two aliquots were removed for hemoglobin measurements. Two microhematocrit tubes were then filled, plugged, centrifuged, and used to measure packed cell volume; the two hematocrit tubes were then chilled and the plasma used for chemical analysis.

#### Hematology

Hemoglobin was determined by the cyanmethemoglobin method (Blaxhall and Daisley 1973). Blood counts (red cell) were done using Rees-Ecker diluting fluid (Klontz 1979), and a Hemocytometer. Mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) were calculated from red cell counts, hemoglobin and hematocrit measurements according to formulas provided by Wintrobe (1932). Morphological hematology was conducted on the blood films (circulating system) with a basic classification of cells, Table 2.

#### Clinical Chemistry

Blood Urea Nitrogen (BUN) was determined by the Hycel direct serum urea nitrogen method (Coulombe and Farreau 1963).

Total plasma protein was determined by the Biuret method (Kingsley 1939). Serum albumin and globulin were measured and separated by the serum electrophoresis cellulose acetate technique (Kohn 1958). Electropherograms were made of all pooled subsamples. Proteins were separated by incident division using established nomenclature defined for protein electrophoresis, i.e., albumin and globulin where globulins are divided

Table 2.--Classification of blood cells used in this study, Phase II; blood smears made from fed and unfed coho salmon (presmolts) (from Klontz 1979).

<u>Erythrocytic series</u>	<u>Granulocytic series</u>	<u>Lymphocytic series</u>
Hemocytoblast	Hemocytoblast	Hemocytoblast
Small lymphoid hemoblast	Large lymphoid hemoblast	Small lymphoid hemoblast
Proerythroblast		Lymphoblast
Erythroblast	Granuloblast	Prolymphocyte
Polychromatocyte	Progranulocyte	Lymphocyte
Reticulocyte	Granulocyte	
Erythrocyte	Juvenile Neutrophil	
	Band Neutrophil	
	Segmented Neutrophil	

into Alpha I, Alpha II, Beta I, and Gamma; the Beta II symbol suggested by Amend and Smith (1974) was utilized for the slowest mobility fraction in place of the standard Gamma symbol.

#### Swimming Performance

Studies to determine the effect of starvation on swimming performance were conducted in a recirculating respirometer (Brett 1964). A tachometer on the pump shaft allowed the selection of repeatable velocities, which were verified with a current meter. Coho salmon presmolts were tested at  $18^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ , which is below their optimum swimming performance level (Brett et al. 1958). Flow was progressively changed from 0.20 mps (5 min) to 0.30 mps (10 min) then to 0.45 mps; after 1 hour in 0.45 mps, flow was increased to 0.60 mps and held until 50% of the fish were impinged. Fish were tested for critical fatigue levels (Flagg & Smith 1979) in three replicates of six fish per replicate for the fed and unfed condition from 0 through 6 weeks. The test was terminated when one-half of the fish were impinged on non-electrified end screens. Time was tabulated for all impingements, but only the time of the third fish impingement (i.e. 50%) was considered in these calculations.

Water was continually added to the respirometer to change the normally closed circuit to a flow-through system. Water temperature and dissolved oxygen were maintained at  $18^{\circ}\text{C}$  and 8.0 ppm during the experiment. Before and during each test, the water velocity was measured with an Ott current meter; velocity levels were adjusted with a manual control lever and checked with the pump shaft tachometer.

After impingement of 50% of the fish, the test was terminated and the fish were anesthetized, weighed, and measured for length. For convenience

the time/velocity results were converted to meters traveled. Fish were held 2 days for observation of delayed mortality.

#### Predation Study

Presmolt coho salmon used in the test were divided into two groups, then freeze branded (Mighell 1969); fed fish on the right side, unfed fish on the left side. Initially and at 1-week intervals, 20 fed and 20 unfed fish were introduced simultaneously into a holding tank containing approximately 20 adult cutthroat trout. After 3 hours, the water level in the predator tank was drawn down, and the surviving fish were removed. The brands provided for positive identifications of the fed/unfed presmolts. The study was based on previous predation studies conducted by Bams (1966) and Coutant (1973).

#### Analysis Techniques

Phase I data were not analyzed statistically but were used as the model for Phase II. All data from Phase II were entered manually from a desk terminal to a computer (Burroughs, B-6800) at the Northwest and Alaska Fisheries Center. A 4-Way Analysis of Variance using time, temperature, size, and treatment (fed-unfed) was conducted on each variable measured in Phase II.

Phase III data were processed differently for the swimming performance and predator prey studies. For convenience, results from the critical fatigue levels of swimming performance were converted into meters traveled by 50% of the sample. Distance traveled (after conditioning) and condition factor of fed and unfed fish were compared by predictive sample reuse (Geisser and Eddy 1979); a method that was used to compare the sum of square of the differences between observed and predicted values.

Predator/prey study results were analyzed by regression analysis.

## RESULTS

### Phase I

#### Condition Factor

Condition factor was computed initially for each fish of the 5-fish pooled sample using the formula  $K = \frac{W}{L^3}$

Where K = Condition Factor (or coefficient of condition)

L = fork length in mm

and W = weight in g

Condition factor remained fairly stable at 12°C, but increased from the initial sampling period then stabilized at 15° and 18°C for fed fish during the study period. Condition factor of starved fish decreased with time and increased water temperature levels (see Table 3a).

#### Survival

Survival was effected at the higher temperature levels during the 5th and 6th weeks for fed and unfed fish (see Table 3b). A 60% mortality occurred at 6 weeks and 18°C.

### Phase II

Twenty-two physical and hematological characteristics measured or computed during Phase II were tabulated by holding time, water temperature, fish size, and treatment (fed, unfed). A four-way analysis of variance was computed for each measured (or computed) characteristic, and significance was determined at the 0.05 level. A table was prepared that summarizes the main interactions between variables studied in Phase II, at the 0.05 level of significance (Table 4). The mean and standard deviation of each significant characteristic was then computed and tabulated for holding time, water temperature, fish size, and treatment (Tables 5, 6, 7, and 8).

Table 3.

a. Weight, length, and condition factor (K) of presmolt spring chinook salmon initially and at 5 and 6 weeks in a fed or unfed condition (Phase I).

Condition	Initial condition			5 Weeks			6 Weeks		
	Wt. (g)	Lgth (mm)	K ( $\times 10^{-5}$ )	Wt. (g)	Lgth (mm)	K ( $\times 10^{-5}$ )	Wt. (g)	Lgth (mm)	K ( $\times 10^{-5}$ )
12°C									
Fed	9.8	94.6	1.17	10.4	95.8	1.18	11.7	98.5	1.17
Unfed	9.6	94.4	1.14	8.2	91.6	1.06	9.9	96.3	1.11
15°C									
Fed	9.2	90.0	1.26	10.9	91.3	1.43	10.0	94.2	1.20
Unfed	9.8	90.8	1.31	7.7	86.0	1.22	9.3	94.5	1.10
18°C									
Fed	10.1	92.9	1.26	10.4	89.7	1.44	13.4	101.4	1.29
Unfed	9.9	92.5	1.25	8.9	91.6	1.16	7.7	92.1	.96

b. Effect of starvation for 5 and 6 weeks on survival (Phase I).

Temperature	No. of fish initially	Mortalities		Condition
		5 weeks	6 weeks	
12°C	20	0 (10) <sup>a</sup>	0 (10)	fed
	20	0 (10)	0 (10)	unfed
15°C	20	1 (10)	1 (10)	fed
	20	2 (10)	0 (10)	unfed
18°C	20	1 (10)	0 (10)	fed
	20	2 (10)	6 (10)	unfed

<sup>a</sup> Number of fish in sample.

Table 4.--Summary of main interactions between time, temperature, size, and treatment (fed vs unfed) and variables measured or computed during Phase II, that are statistically significant at the 0.05 level of probability.

<u>Variable</u>	<u>Holding time</u>	<u>Water temperature</u>	<u>Fish size</u>	<u>Treatment</u>
Condition Factor		X	X	X
Hematocrit	X		X	
Hemoglobin	X	X	X	X
Red Blood Cell Count	X	X	X	
MCV			X	X
MCHC	X	X	X	X
MCH	X			
White Blood Cell Count				X
Band Neutrophil	X	X		X
Segmented Neutrophil			X	X
Prolymphocyte	X			X
Lymphocyte				
Macrophage				X
BUN	X	X	X	
Total Protein	X			X
Albumin	X	X	X	X
Globulin	X			X
Alpha I	X			X
Alpha II	X		X	X
Beta I	X	X	X	X
Beta II	X	X		
Albumin/Globulin Ratio	X	X	X	X

Table 5.--Mean and standard deviation of blood characteristics measured or computed from all fish, large and small, held at three temperatures that were significantly different at the 0.05 level of probability for time in a 4-way analysis of variance conducted on 22 variables from coho salmon during Phase II of this study.

Variable	Unit	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6	
		Fed	Unfed										
Hematocrit	%	37.1	(2.1)	37.1	(2.3)	37.3	(2.1)	40.1	(1.6)	36.6	(3.2)	35.6	(.8)
		38.1	(2.4)	37.1	(2.6)	36.0	(1.6)	35.8	(2.1)	37.0	(2.2)	35.8	(.7)
Hemoglobin	g/100 ml	6.3	(.4)	5.9	(.7)	6.2	(.8)	6.6	(.7)	6.7	(.3)	6.6	(.2)
		6.7	(.2)	6.4	(.4)	6.4	(.5)	6.6	(.5)	6.6	(.4)	6.3	(.3)
RBC	$\times 10^6 \text{ mm}^3$	1.35	(.15)	1.26	(.20)	1.25	(.21)	1.32	(.08)	1.27	(.08)	1.29	(.06)
		1.34	(.12)	1.35	(.16)	1.22	(.12)	1.31	(.15)	1.29	(.14)	1.22	(.05)
MCHC	%	16.9	(.9)	15.9	(1.5)	16.4	(1.3)	16.4	(1.2)	17.4	(1.6)	17.5	(.9)
		17.5	(.6)	17.2	(.7)	17.9	(1.1)	18.5	(.8)	18.6	(.5)	18.8	(.9)
MCH		278	(29)	299	(32)	303	(34)	305	(13)	302	(30)	294	(12)
		294	(20)	277	(17)	297	(16)	274	(16)	277	(25)	268	(24)
Band Neutrophil	$\times 10^3 \text{ mm}^3$	.21	(.14)	.28	(.17)	.22	(.25)	.31	(.36)	.28	(.17)	.42	(.40)
		.22	(.07)	.28	(.17)	.22	(.25)	.31	(.36)	.32	(.11)	.31	(.27)
Prolymphocyte	$\times 10^3 \text{ mm}^3$	.13	(.05)	.93	(.55)	.76	(.67)	.78	(.63)	.63	(.43)	.62	(.57)
		.30	(.34)	.50	(.46)	.58	(.41)	.30	(.14)	.47	(.22)	.37	(.28)
BUN	mg/100 ml	6.7	(2.5)	7.4	(1.5)	9.1	(2.9)	6.3	(3.2)	2.1	(.5)	4.4	(1.6)
		7.7	(4.4)	7.9	(.7)	4.8	(.9)	5.3	(2.0)	2.1	(1.4)	4.1	(1.3)
Total Protein	g/100 ml	2.7	(.5)	3.2	(.3)	3.2	(.4)	3.4	(.2)	3.2	(.2)	3.0	(.3)
		2.7	(.4)	2.8	(.2)	2.8	(.2)	2.4	(.3)	2.5	(.1)	1.6	(.2)
Albumin	g/100 ml	.74	(.16)	.85	(.12)	.81	(.08)	.87	(.05)	.80	(.08)	.75	(.08)
		.74	(.12)	.69	(.09)	.66	(.09)	.52	(.05)	.46	(.08)	.30	(.07)
Globulin	g/100 ml	1.9	(.3)	2.3	(.2)	2.4	(.3)	2.4	(.1)	2.4	(.1)	2.2	(.2)
		1.9	(.3)	2.0	(.2)	2.1	(.2)	1.8	(.2)	2.0	(.1)	1.3	(.2)

Table 5.--Continued.

Alpha I	g/100 ml	.65 (.11)	.86 (.11)	.81 (.16)	.79 (.12)	.92 (.12)	.78 (.14)
		.65 (.11)	.71 (.11)	.67 (.11)	.59 (.10)	.59 (.14)	.40 (.09)
Alpha II	g/100 ml	.45 (.09)	.59 (.12)	.74 (.15)	.63 (.14)	.71 (.14)	.56 (.23)
		.43 (.08)	.50 (.11)	.63 (.08)	.47 (.10)	.56 (.17)	.32 (.09)
Beta I	g/100 ml	.43 (.14)	.45 (.13)	.33 (.08)	.49 (.10)	.42 (.11)	.44 (.09)
		.48 (.22)	.38 (.17)	.24 (.07)	.24 (.07)	.35 (.12)	.23 (.06)
Beta II	g/100 ml	.42 (.10)	.52 (.16)	.56 (.19)	.56 (.11)	.50 (.13)	.49 (.16)
		.38 (.10)	.52 (.16)	.59 (.13)	.57 (.10)	.55 (.13)	.41 (.14)
A/G Ratio	%	37.3 (3.6)	34.8 (.27)	33.1 (2.6)	34.8 (2.6)	32.3 (2.0)	31.3 (1.5)
		37.3 (5.2)	32.6 (2.9)	30.8 (3.6)	26.8 (.8)	22.1 (3.8)	21.8 (3.3)

\* ( ) Standard deviation

Table 6.--Mean and standard deviation of condition factor and blood characteristics measured or computed from all fish, large or small, held for a 6-week period that were significantly different at the 0.05 probability level for temperature in a 4-way analysis of variance conducted on 22 variables from coho salmon during Phase II of this study.

Variable	Unit	12°		15°		18°	
		Fed	Unfed	Fed	Unfed	Fed	Unfed
Condition Factor	X10 <sup>-5</sup>	1.03 (.05)*	.91 (.05)	1.05 (.05)	.88 (.05)	1.04 (.06)	.85 (.06)
Hemoglobin	g/100 ml	6.1 (.7)	6.2 (.3)	6.5 (.5)	6.6 (.4)	6.5 (.5)	6.7 (.3)
RBC	X10 <sup>6</sup> mm <sup>3</sup>	1.28 (.18)	1.25 (.12)	1.32 (.12)	1.32 (.15)	1.28 (.10)	1.30 (.11)
MCHC	%	15.7 (.1.2)	17.4 (.8)	17.1 (.9)	18.0 (.8)	17.5 (1.0)	18.7 (.8)
Band Neutrophil	X10 <sup>3</sup> mm <sup>3</sup>	.27 (.16)	.19 (.08)	.44 (.32)	.36 (.25)	.67 (.82)	.28 (.22)
BUN	mg/100 ml	7.4 (3.8)	5.8 (2.6)	5.3 (2.1)	5.9 (3.5)	5.2 (2.7)	4.2 (2.2)
Albumin	g/100 ml	.84 (.10)	.60 (.20)	.80 (.11)	.57 (.14)	.78 (.11)	.51 (.17)
Beta I	g/100 ml	.48 (.11)	.37 (.20)	.44 (.11)	.30 (.08)	.36 (.10)	.31 (.15)
Beta II	g/100 ml	.50 (.12)	.43 (.13)	.47 (.13)	.52 (.12)	.55 (.19)	.54 (.18)
A/G Ratio	%	34.1 (3.9)	30.1 (6.5)	33.6 (3.0)	29.3 (5.2)	34.7 (2.5)	26.4 (7.5)

\* ( ) standard deviation

Table 7.--Mean and standard deviation of condition factor and blood characteristics measured or computed from all fish held at three temperatures, over a 6 week period that were significantly different at the 0.05 level of probability for size in a 4 way analysis of variance conducted on 22 variables during Phase II.

Variable	Unit	Small		Large	
		Fed	Unfed	Fed	Unfed
Condition Factor	X10 <sup>4</sup>	1.07 (.04)*	.89 (.05)	1.01 (.05)	.88 (.06)
Hematocrit	%	36.6 (2.3)	35.6 (1.2)	38.1 (2.2)	37.6 (2.3)
Hemoglobin	g/100 ml	6.1 (.6)	6.3 (.3)	6.7 (.4)	6.7 (.4)
RBC	X10 <sup>6</sup> mm <sup>3</sup>	1.22 (.12)	1.23 (.09)	1.32 (.11)	1.35 (.13)
MCV	μ <sup>3</sup>	309 (26)	288 (22)	284 (21)	275 (21)
MCHC	%	16.3 (1.5)	17.9 (0.8)	17.2 (0.8)	18.0 (1.0)
Segmented Neutrophils	X10 <sup>3</sup> mm <sup>3</sup>	2.5 (2)	2.4 (2.1)	4.7 (3.9)	2.7 (1.8)
BUN	mg/100 ml	5.8 (5.5)	4.8 (2.1)	6.1 (2.6)	5.9 (.3.4)
Albumin	g/100 ml	.85 (.09)	.55 (.20)	.75 (.10)	.56 (.15)
Alpha II	g/100 ml	.66 (.19)	.49 (.13)	.57 (.16)	.47 (.17)
Beta I	g/100 ml	.43 (.12)	.28 (.09)	.43 (.12)	.37 (.13)
A/G Ratio	%	35.1 (3.)	29.9 (8.3)	32.7 (2.4)	28.2 (4.3)

\*( ) standard deviation

Table 8.--Mean and standard deviation of condition factor and blood characteristics measured or computed from all fish, large and small, held at three temperatures for 6 weeks, that were significantly different at the 0.05 probability level, for treatment (fed vs unfed) in a 4-way analysis of variances conducted on 22 variables from coho salmon.

Variable	Unit	Fed	Unfed
Condition Factor	X10 <sup>-4</sup>	1.04 (.06)*	.89 (.06)
Hemoglobin	g/100 ml	6.4 (.66)	6.5 (.49)
MCV	μ <sup>3</sup>	297 (3.1)	282 (1.1)
MCHC	%	16.8 (1.4)	18.1 (1.1)
WBC	X10 <sup>3</sup> mm <sup>3</sup>	25.1 (0.2)	20.4 (11.2)
Band Neutrophils	X10 <sup>3</sup> mm <sup>3</sup>	.45 (.50)	.28 (.21)
Segmented Neutrophils	X10 <sup>3</sup> mm <sup>3</sup>	3.6 (3.3)	2.51 (1.7)
Prolymphocyte	X10 <sup>3</sup> mm <sup>3</sup>	.71 (.53)	.44 (.36)
Macrophage	X10 <sup>3</sup> mm <sup>3</sup>	.80 (.76)	.61 (.60)
Total Protein	g/100 ml	3.2 (.4)	2.4 (.4)
Albumin	g/100 ml	.80 (.11)	.56 (.11)
Globulin	g/100 ml	2.2 (.3)	1.8 (.3)
Alpha I	g/100 ml	.78 (.15)	.60 (.11)
Alpha II	g/100 ml	.61 (.18)	.48 (.15)
Beta I	g/100 ml	.43 (.12)	.33 (.16)
A/G Ratio	%	33.9 (3.0)	28.6 (6.0)

\* ( ) standard deviation

## Condition Factor

The analysis of variance was computed with the mean of the condition factor for the five fish pooled sample (Table 9).

Condition factor was higher for fed fish (Table 8), small fish (Table 7), and at 12° (Table 6). Fed fish increased in weight throughout the study whereas the weight of unfed fish decreased (Table 10).

## Hematology

Hematocrit was higher for large fish, and decreased over time (37.0 to 35.0%).

Hemoglobin was significant for time, temperature, size, and treatment (Table 4). Hemoglobin was higher for unfed fish, for large fish, increased with temperature, and was relatively constant over time (6.5 g/100 ml).

There was no statistical difference between red blood cell counts of fed and unfed fish. Red blood cell counts were greater for large fish, highest at 15°C and slightly decreased over time (1.35 to 1.26 x 10<sup>6</sup>mm<sup>3</sup>).

Three erythrocyte indices were computed and compared to independent variables; they include MCV, MCHC, and MCH. The MCV was statistically significant at the 0.05 level for treatment fish. MCV was higher for fed fish and for small fish. The MCV ranged in values from 221 to 416 m<sup>3</sup> for all fish in the study. The MCHC was significant for all the independent variables in the study, i.e. holding time, water temperature, fish size, and treatment. MCHC was highest for the unfed fish, the large fish, at 18°C, and the 5th and 6th weeks of the study. Values of MCHC ranged from 13.6 to 21.0% for all fish measured. MCH was significant only for holding time; values were higher at the 5th and 6th week of study (Week 1, 48.6 to Week 6, 52.1).

Table 9.--Mean and standard deviation of condition factor for 5-fish pooled sample taken from coho salmon juveniles, Phase II.

CONDITION FACTOR  
(X10<sup>-5</sup>)

Week	Temp	Rep	Fed						Unfed					
			Small			Large			Small			Large		
			$\bar{X}$	SD	SD	$\bar{X}$	SD	SD	$\bar{X}$	SD	SD	$\bar{X}$	SD	SD
1	12	a	1.15	(.01)	1.06	(.09)	1.00	(.06)	1.06	(.03)	1.06	(.06)	1.06	(.03)
		b	.99	(.08)	1.02	(.03)	1.03	(.07)	1.03	(.04)	1.03	(.07)	1.03	(.04)
15	15	a	1.06	(.05)	1.05	(.07)	1.00	(.06)	.93	(.02)	1.05	(.03)	.98	(.06)
		b	1.07	(.20)	1.19	(.22)	1.05	(.04)	1.01	(.05)	.82	(.11)	.90	(.01)
18	18	a	1.06	(.08)	.95	(.04)	.90	(.01)	.90	(.01)	.92	(.05)	.82	(.11)
		b	1.02	(.11)	.97	(.02)	.90	(.01)	.90	(.01)	.92	(.01)	.90	(.01)
2	12	a	1.09	(.04)	.98	(.04)	.92	(.06)	.92	(.05)	.92	(.06)	.92	(.05)
		b	1.05	(.06)	.84	(.18)	.89	(.07)	.91	(.05)	.89	(.07)	.91	(.05)
15	15	a	1.06	(.08)	.97	(.03)	.83	(.05)	.86	(.03)	.83	(.05)	.86	(.03)
		b	1.05	(.07)	.92	(.03)	.89	(.06)	.89	(.06)	.89	(.06)	.89	(.06)
18	18	a	1.08	(.08)	1.03	(.05)	.86	(.01)	.88	(.83)	.86	(.01)	.88	(.83)
		b	1.04	(.05)	1.05	(.21)	.89	(.03)	.88	(.03)	.89	(.03)	.88	(.03)
3	12	a	1.08	(.04)	.95	(.05)	.97	(.04)	.93	(.05)	.97	(.04)	.93	(.05)
		b	1.12	(.04)	.98	(.03)	.91	(.07)	.94	(.05)	.91	(.07)	.94	(.05)
15	15	a	1.07	(.07)	.97	(.06)	.85	(.13)	.91	(.02)	.85	(.13)	.91	(.02)
		b	1.11	(.03)	1.03	(.06)	.87	(.04)	.87	(.08)	.87	(.04)	.87	(.08)
18	18	a	1.07	(.05)	1.02	(.07)	.93	(.06)	.85	(.02)	.93	(.06)	.85	(.02)
		b	1.10	(.02)	1.05	(.05)	.94	(.02)	.91	(.02)	.94	(.02)	.91	(.02)
4	12	a	1.05	(.05)	.97	(.04)	.88	(.05)	.85	(.05)	.88	(.05)	.85	(.05)
		b	.99	(.11)	1.02	(.03)	.94	(.14)	.93	(.03)	.94	(.14)	.93	(.03)
15	15	a	1.13	(.04)	.96	(.01)	.91	(.05)	.87	(.06)	.91	(.05)	.87	(.06)
		b	1.11	(.05)	1.11	(.12)	.87	(.11)	.89	(.01)	.87	(.11)	.89	(.01)
18	18	a	1.10	(.05)	.95	(.09)	.95	(.10)	.84	(.04)	.95	(.10)	.84	(.04)
		b	1.13	(.06)	1.01	(.04)	.92	(.05)	.80	(.02)	.92	(.05)	.80	(.02)
5	12	a	1.08	(.03)	1.01	(.07)	.90	(.06)	.94	(.07)	.90	(.06)	.94	(.07)
		b	1.05	(.02)	1.01	(.04)	.82	(.02)	.88	(.03)	.82	(.02)	.88	(.03)
15	15	a	1.05	(.01)	1.07	(.11)	.89	(.07)	.82	(.02)	.89	(.07)	.82	(.02)
		b	1.11	(.06)	1.03	(.05)	.84	(.06)	.86	(.06)	.84	(.06)	.86	(.06)
18	18	a	1.14	(.03)	1.05	(.05)	.85	(.04)	.83	(.04)	.85	(.04)	.83	(.04)
		b	1.16	(.10)	1.01	(.02)	.87	(.07)	.80	(.06)	.87	(.07)	.80	(.06)
6	12	a	1.08	(.06)	1.02	(.02)	.85	(.10)	.87	(.01)	.85	(.10)	.87	(.01)
		b	1.03	(.02)	1.03	(.05)	.85	(.07)	.88	(.05)	.85	(.07)	.88	(.05)
15	15	a	1.07	(.08)	1.08	(.01)	.81	(.05)	.92	(.16)	.81	(.05)	.92	(.16)
		b	1.04	(.10)	1.08	(.05)	.93	(.11)	.83	(.02)	.93	(.11)	.83	(.02)
18	18	a	1.12	(.03)	1.04	(.03)	.87	(.02)	.77	(.04)	.87	(.02)	.77	(.04)
		b	1.15	(.07)	1.07	(.08)	.79	(.05)	.77	(.04)	.79	(.05)	.77	(.04)

Table 10.--Mean, standard deviation, and coefficient of variation (CV) of lengths and weights of coho salmon juveniles utilized in starvation experiments during Phase II.

	Fed Fish				Unfed Fish				
	Small		Large		Small		Large		
	Length (mm)	Weight (g)	Length (mm)	Weight (g)	Length (mm)	Weight (g)	Length (mm)	Weight (g)	
Week 0	$\bar{X}$	104	12.3	128	21.5				
	SD	5.8	2.2	5.3	2.7				
	CV	5.6	1.8	4.1	1.2				
Week 1	$\bar{X}$	101	11.1	131	23.7	100	10.5	130	21.6
	SD	7.4	2.6	6.9	4.8	10.8	1.8	7.5	3.9
	CV	7.3	2.3	5.2	2.0	10.8	1.7	5.8	1.8
Week 2	$\bar{X}$	107	13.1	137	25.2	107	9.6	131	20.1
	SD	5.6	2.0	10.6	4.2	6.5	2.2	7.5	3.6
	CV	5.3	1.5	7.7	1.7	6.4	2.3	5.7	1.8
Week 3	$\bar{X}$	109	14.7	136	25.8	99	9.8	133	21.4
	SD	7.1	2.8	5.7	4.0	10.6	1.9	6.4	3.3
	CV	6.5	1.9	4.2	1.5	10.6	1.9	4.8	1.5
Week 4	$\bar{X}$	115	17.1	140	28.2	102	9.8	133	20.5
	SD	7.5	2.8	7.1	3.8	5.6	1.6	6.8	3.1
	CV	6.5	1.6	5.1	1.3	5.5	1.6	5.1	1.5
Week 5	$\bar{X}$	121	19.5	140	28.5	103	9.8	130	18.6
	SD	5.5	2.9	4.7	3.0	6.0	2.0	7.3	3.5
	CV	4.6	1.5	3.3	1.0	5.8	2.1	5.6	1.9
Week 6	$\bar{X}$	123	22.5	142	30.6	103	9.4	132	19.7
	SD	22.2	5.3	6.2	4.1	6.4	1.7	7.3	3.7
	CV	18.1	2.3	4.3	1.3	6.2	1.8	5.5	1.8
Total loss or gain from Week 0		+19	+10.2	+14	+9.1	-1	-2.9	+4	-1.8

Differential counts were made on major white blood cell groups from blood films of two randomly selected fish from each five-fish pool. Visual counts were made of juvenile neutrophils, band neutrophils, segmented neutrophils, prolymphocytes, lymphocytes, and macrophages.

The number of total white blood cells were higher for fed fish and were statistically significant.

Juvenile neutrophils occurred in only 11 of the 120 blood films, were found in fed and unfed fish, and were not statistically analyzed. Band neutrophils were higher for fed fish ( $0.45 \times 10^3 \text{mm}^3$ ), for large fish, and during the 3rd week of the study. Band neutrophils were significantly different (at the 0.05 level) for time, temperature, and treatment.

There was a significant difference in numbers of segmented neutrophils between fed and unfed fish ( $2.5 \times 10^3 \text{mm}^3$  for unfed fish,  $3.6 \times 10^3 \text{mm}^3$  for fed fish); numbers of neutrophils were higher for large fish. Numbers of prolymphocyte cells were significantly different for holding time and between fed and unfed fish ( $0.71 \times 10^3 \text{mm}^3$  for fed fish and  $0.44 \times 10^3 \text{mm}^3$  for unfed fish); prolymphocytes were highest at Week 2 of the study. Lymphocytes were not statistically significant but were the most numerous of the white blood cells. There was a significant difference in numbers of macrophages between fed and unfed fish; numbers were higher in fed fish.

#### Clinical Chemistry

BUN was higher for large fish, at  $12^\circ\text{C}$ , and at Week 1 of the experiment; there was no significant difference between fed and unfed fish.

Total protein was highest for fed fish and during Week 4 of the study. There was a significant difference in levels of albumin and albumin was

highest for fed fish, small fish, at 12°C, and at Week 2; albumin levels in the blood decreased from 0.74 g/100 ml at Week 1 for unfed fish to 0.30 g/100 ml at Week 4.

The amount of total globulin in the blood was significantly different for holding time and between fed and unfed fish; it was highest for fed fish and at Week 5. Weekly means of total globulin ranged from 1.9 g/100 ml at Week 1 to 2.4 g/100 ml at Weeks 3, 4, and 5 for the fed fish, and for the unfed fish reduced from 1.9 g/100 ml at Week 1 to 1.3 g/100 ml at Week 6.

The amount of Alpha I globulin measured in the blood was significantly different between fed and unfed fish and holding time; Alpha I was highest for the fed fish and varied over time. The amount of Alpha II globulin was significantly different for holding time (varied over time), fish size, (highest for small fish), and between fed and unfed fish (highest for fed fish). Beta I globulin was highest for fed fish, large fish, at 12°C, and at Week 1 of the study.

Beta II, the last fraction to show on the electropherogram, was statistically significant for time and temperature. This globulin fraction was highest at 18°C and at Week 6.

The albumin-globulin (A/G) ratio was computed, averaged, and statistically analyzed; it was statistically different for time, temperature, size, and condition. The A/G ratio was highest for fed fish, for small fish, at 12°C, and at Week 1 of the experiment (considering overall means). Weekly averages of the A/G ratio for unfed fish declined from 37% during Week 1 to 21% during Week 6 (Table 5).

### Phase III

#### Swimming Performance

In this portion of the study, the distance swam and condition factor of presmolt coho salmon were measured at 18°C over a 6-week period. Condition factor (K) was computed using the fish's fork length (L) and weight (W) in the formula  $K = W/L^3$  and is shown in Table 11; values (means) ranged from 1.4 to 1.8 for the fed fish and 1.1 to 1.5 for the unfed fish during the 6 weeks of the study. The condition factor was drastically reduced for the unfed fish, as expected, dropping sharply from Week 1 (see Figure 2). Predictive sample reuse regression analysis was utilized to compare the models utilized for the condition factor of the fed and unfed populations. The models considered were  $M_1 : y = a+bx$  no matter whether the fish were fed or unfed, and

$$M_2: y = a_1 + b_1x$$

if the fish were fed

$$y = a_2 + b_2 x$$

if the fish were unfed

Where y represents the condition factor, x represents time, and a and b are constants. Model  $M_1$  asserts that the relationship between condition factor and time is the same for both the fed and unfed populations, and Model  $M_2$  asserts that the relationship is different. The sums of squares are computed for Models  $M_1$  and  $M_2$  and are called  $D_1$  and  $D_2$ , respectively, then

$$D_1 = 0.8904 \text{ and}$$

$$D_2 = 0.3485$$

Thus since  $D_2 < D_1$  the model preferred by the data is  $M_2$  which indicates a different condition factor-time relationship for the fed and unfed populations.

Table 11.--Distance and condition factor of fed and unfed, presmolt coho salmon measured during a 6-week period, July-September, 1979.

Week	Replicate	Fed		Unfed	
		Distance <sup>&lt;1</sup> (meters)	Condition factor (X10 <sup>-5</sup> )	Distance (meters)	Condition factor (X10 <sup>-5</sup> )
0	A	2660	1.27 <sup>&lt;2</sup>		
	B	2911	1.45		
	C	1795	1.52		
	$\bar{X}$	2455	1.41		
1	A	1872	1.88	1800	1.60
	B	1764	1.94	1406	1.55
	C	1979	1.63	1298	1.43
	$\bar{X}$	1871	1.81	1501	1.53
2	A	1657	1.65	1800	1.54
	B	1764	1.60	414	1.53
	C	662	1.48	994	1.52
	$\bar{X}$	1361	1.58	1069	1.54
3	A	1764	1.63	276	1.30
	B	1764	1.57	165	1.43
	C	1728	1.67	165	1.44
	$\bar{X}$	1752	1.61	202	1.39
4	A	939	1.47	55	1.27
	B	1800	1.40	110	1.28
	C	1710	1.52	22	1.15
	$\bar{X}$	1483	1.47	64	1.24
5	A	1728	1.46	82	1.09
	B	1692	1.44	55	1.10
	C	1728	1.41	82	1.06
	$\bar{X}$	1716	1.44	73	1.09
6	A	1800	1.46	55	1.12
	B	1872	1.43	82	1.21
	C	1800	1.42	55	1.17
	$\bar{X}$	1824	1.44	64	1.17

<1 Mean distance of a 50% impingement of a 6-fish sample.

<2 Mean condition factor of the 6-fish sample.

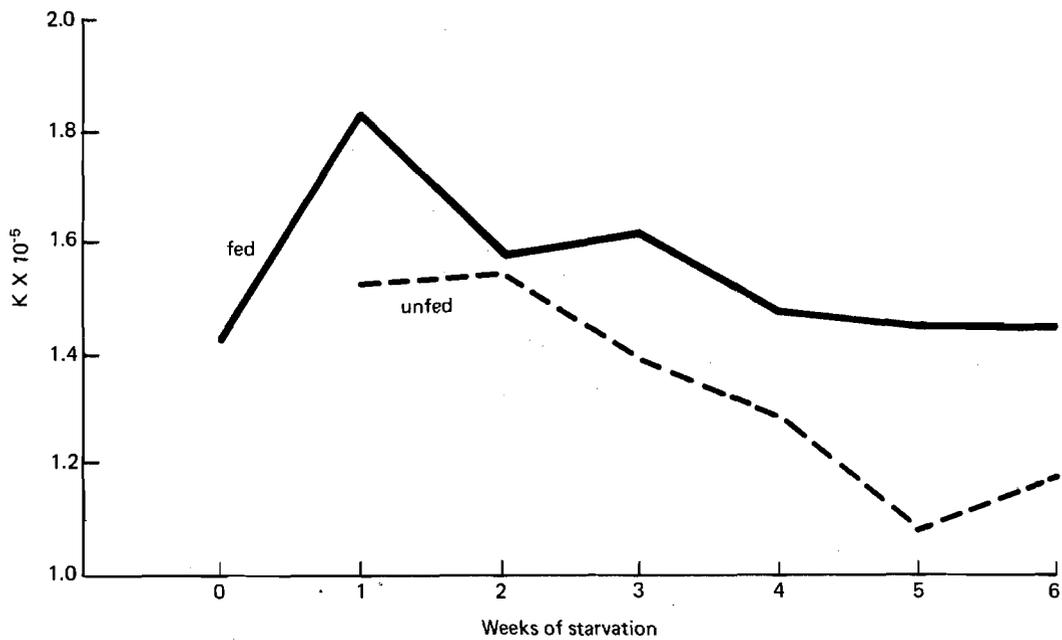


Figure 2.--Mean condition factor of 18 fed and unfed coho salmon utilized in the swimming endurance tests during Phase III, fish were held and tested at 18°C in Columbia River water.

Swimming endurance of starved and fed fish was reduced during the 1st week of the study, for starved fish swimming ability continued to decline to Week 4 and remained at this low level through Week 6 (Table 11). Fed fish generally increased performance from Week 2 to Week 6. Again, predictive sample reuse regression analysis was utilized to select and compare the model suitable for the fed and unfed regression lines for swimming endurance depicted in Figure 3. The following models were considered:

$$M_1 : y = a + bx + cx^2$$

no matter whether the fish were fed or unfed

and

$$M_2 : y = a e^{bx}$$

if the fish are unfed

$$y = a + bx$$

if the fish are fed

Where  $y$  represents the condition factor,  $x$  represents time, and  $e$  is a mathematical constant 2.718.

Model  $M_1$  asserts that the relationship between distance and time is the same for both the fed and unfed populations, and Model  $M_2$  asserts that the relationship is different. The sums of squares are computed for models  $M_1$  and  $M_2$  and are called  $D_1$  and  $D_2$ , respectively, then:

$$D_1 = 21,393,514.66 \text{ and}$$

$$D_2 = 4,813,009.20$$

Since  $D_2 < D_1$ , the selected model is  $M_2$ . The Model  $M_2$  suggests that there is a different distance-time relationship for the fed and unfed population.

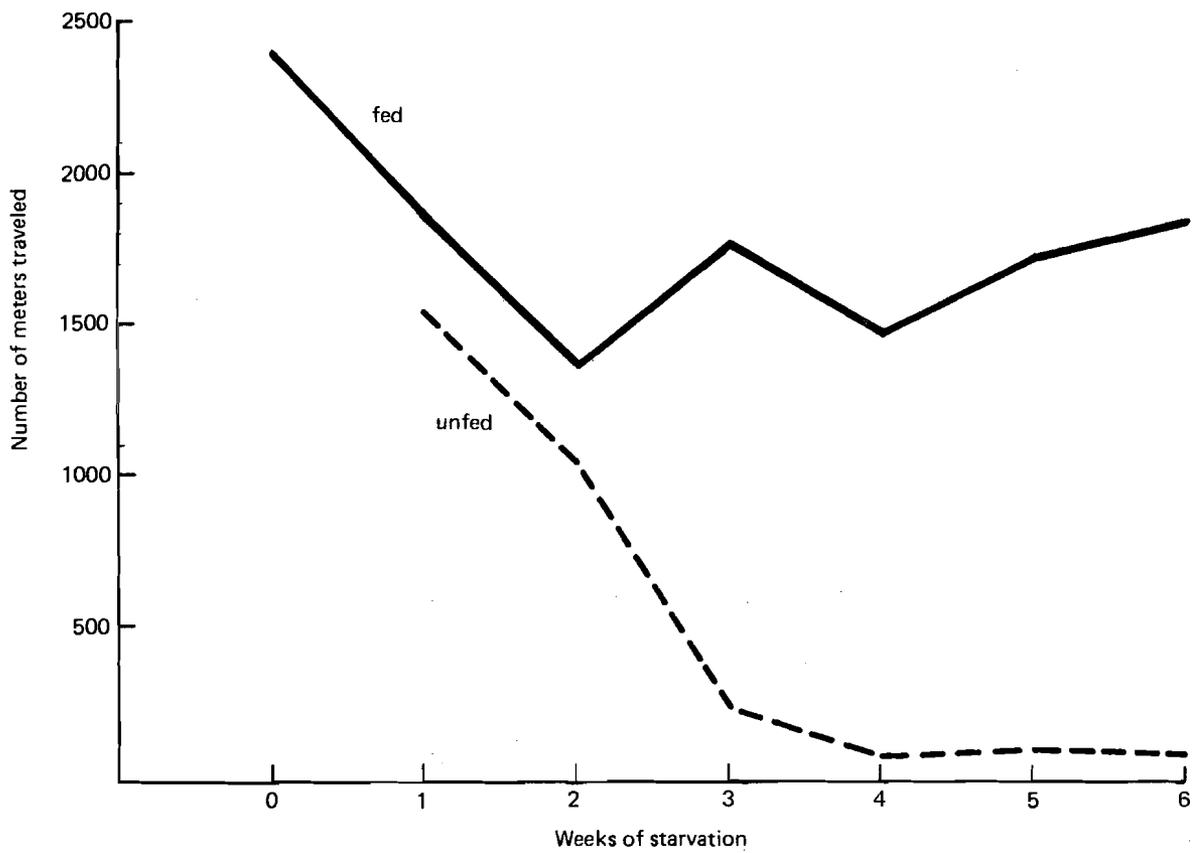


Figure 3.--Effect of starvation on the swimming endurance at the 50% impingement level of pre-smolt coho salmon, Columbia River, 1979.

## Predation

Numbers and percentages of fed and unfed coho salmon presmolts that survived introduction into a predator tank during the 6-week study are shown in Table 12.

Using the regression analysis model  $y = bt$  where  $y = y^*-1$  ( $y^*$  represented the ratio of number of unfed survivors to the number of fed survivors), and  $t = \text{time}$ , a hypothesis was tested that on the average the ratio of unfed to fed is constant over time (or that starvation has no effect on predation); the hypothesis could not be rejected at the 0.05 level of significance, (95% CI for  $b$  was  $-0.1030 \leq b \leq 0.212$ ).

## DISCUSSION

To starve means to perish with hunger or to distress or subdue by famine. A review on the effects of depletion (starvation) on fish was presented by Love (1970). In this review, Love considered mainly the effect of nutritional depletion as a temporal phenomenon with marine species, (where depletion occurs in association with spawning and/or a seasonal drop in food availability) and changes in adult salmon as they migrate from the ocean to natal spawning grounds. Love indicated that starvation produced involuted thymus and spleen; enlargement of the gall bladder; physical weakness; reduction in the concentration of blood serum protein; glycogen decrease; and gross changes in the blood, i.e., specific gravity changes, reduction in red and white cell counts, red cell volume, and reductions of immature cells. Love indicated that the thyroid, brain, heart, and vascular system show little change during starvation.

Physical and chemical changes occurred in the presmolt coho salmon as a result of imposed starvation over a period of 6 weeks. The length and weight relationship, reflected by condition factor was drastically reduced

Table 12.--Results of predator/prey studies where fed and unfed coho salmon (presmolts) were placed in predator tanks, August-October, 1979.

Week	Date Tested	N	Survivors			
			Fed		Unfed	
			Number	Percent	Number	Percent
0	8/20/79	40	12	60	9	45
1	8/27/79	40	13	65	10	50
2	9/4/79	40	11	55	13	65
3	9/10/79	40	14	70	10	50
4	9/17/79	40	12	60	12	60
5	9/24/79	40	11	55	11	55
6	10/1/79	40	4	20	2	10

in the starved fish; swimming stamina was seriously impaired. The morphological hematology changed, i.e., volume of the red blood cells decreased and the white blood cell numbers were reduced. Albumin and globulin were markedly reduced. Separately any of these changes could impose serious limitations to the future survival of fish in a very active and competitive environment, not withstanding the rigors of the physiological changes induced by "smolting." Bams (1966) proposed that unless severe environmental conditions impose a serious constraint, the most important component of survival is stamina. Starvation seriously weakens fish and limits and impairs stamina.

#### Condition Factor

The coefficient of condition or condition factor is an expression of the relative well being of fish and fish populations (Hoar 1939; Everhart et al. 1976), and is based on the weight-length relationship of the fish and the cube law. The cube law simply states that masses of similarly shaped bodies vary as the cubes of the dimensions, provided that the densities of the masses are constant. A functional exponent  $b = 3$  (in the formula  $W = Kl^b$ ) indicates that isometric growth in weight increases as the cube of length as restated by Ricker (1975).

Apparent differences in condition factor in Phase I reinforced the assumption that the condition of an unfed presmolt fish would change markedly in 6 weeks. Significant differences were found in condition factors between fed and unfed fish in Phase II and Phase III. The differences in condition factors that were consistent throughout this study for fed and unfed fish could form the basis for initially categorizing presmolting coho salmon into the potentially chronically stressed condition

of starvation. Condition factors changed noticeably after 2 weeks of starvation: fed fish gained weight and unfed fish lost weight; small fed fish gained more weight than large fed fish and small unfed fish lost more weight than large unfed fish.

#### Hematology

In his classical review of depletion, Love (1970) stated that probably the most profound changes during starvation occur in the blood of fish. However, Blaxhall (1972) commented that in fish hematology, progress only seems to have been made in the diagnosis of anemia. Anemia is well known among fish culturists, and is easily detected by inspecting gill color (Kawatsu 1966). Two types of nutritional anemia of fish were postulated by Snieszko (1972), i.e., normochromic (acute or subacute) from a lack of elements permitting fish to produce blood, and macrocytic (chronic) from a lack of hematopoietic function. Kawatsu (1966) indicated that when erythrocytes became smaller in starving brook trout, Salvelinus fontinalis, the symptoms indicated microcytic anemia. Blaxhall and Daisley (1973) stated that erythrocyte counts when done visually are not precise because of inherent errors and so a greater reliance is placed on hematocrit and hemoglobin estimation as indicators of anemia. In this study, red blood cell count and hematocrit did not differ significantly between fed and unfed fish, but hemoglobin did. Using the relationship between red blood cell counts, hematocrit, and hemoglobin to compute red cell indices, a better understanding of the red blood cell can be obtained.

MCV is the average volume of the individual red blood cell; the MCV was significantly different between large and small fish and for fed and unfed fish. The volume was larger for the small fed fish than the large

fed fish, and in the unfed fish, the volume was reduced in both large and small fish.

The MCHC is the average hemoglobin concentration per 100 ml of packed red cells in percent. The MCHC demonstrated significant differences between holding times, water temperatures, fish sizes, and between fed and unfed fish. However, in general values were larger for Week 6, at higher temperatures, for large fish, and for the unfed fish.

The MCH is the amount of hemoglobin by weight in the average erythrocyte; MCH was not statistically significant for fish size or between fed and unfed fish.

Anemia can be defined as a reduction in the number of circulating red blood cells, or a condition in which the red cells of the blood are reduced in number or are deficient in hemoglobin (oxygen carrying capacity is reduced). Thus, in this study, anemia as defined above did not appear to occur in 6 weeks, but did result in anemia as observed in fish and defined by Kawatsu (1974) (the erythrocytes became smaller indicating microcytosis).

The leukocyte differential count expresses the relative number of blood cells present in the blood. Love (1970) indicated that immature blood cells were reduced and even failed to be produced after 4 weeks of depletion. Juvenile neutrophils were not numerous on the blood films examined, and were not associated exclusively with fed or unfed fish. Prolymphocytes differed significantly between fed and unfed fish (and time); lymphocytes were the most numerous white blood cells and were not statistically significant in this study. Macrophage cells were significantly different between fed and unfed fish, and were reduced in the unfed fish. The total white blood cell count was reduced in the unfed fish.

## Clinical Chemistry

The impact of starvation is measurable sooner in active than sluggish fish and seems to produce changes after several weeks (Love 1970).

Urea is the principal nitrogenous end product of protein metabolism and amino acid degradation; BUN is a laboratory expression of the urea nitrogen content of the blood. BUN ranged from 0.5 to 15.5 ml/dl and was variable throughout the study.

Love (1970) stated that liver synthesizes albumin from proteins, and albumin is the principal victim of starvation.

Albumin plays an important part in the distribution of body water because of its colloidal osmotic pressure. Albumin fractions are primarily responsible for maintenance of plasma volume, and because of its electrical characteristics, binds smaller organic molecules and salts, and is especially important in osmosis (Hoar 1975). Albumins were seriously reduced in the unfed fish in this study (see Table 5).

Plasma proteins, as a group, function in several different ways: buffer activity, oxygen transport, osmotic pressure, blood coagulation, and immune responses (Hoar 1975); they also function to weakly bind hormones and enzymes (Donaldson et al. 1979). A low level of total serum protein commonly occurs when both the albumin and globulins are depressed; this occurs commonly in humans with protein-calorie malnutrition.

Several investigators have suggested that protein fractions play an important part in antibody production for an immune response. Klontz et al. (1965) suggested that certain protein changes in serum macroglobins may be of immunological significance in sockeye salmon, O. nerka, following infection with infectious hematopoietic necrosis (IHN) virus. Amend and Smith (1974) found that in rainbow trout, Salmo gairdneri, infected with IHNV alpha fractions, the serum protein was altered.

Love (1970) indicated that different fractions of protein in the blood decrease during starvation with the sole exception of the slowest fraction, which shows a relative increase; in my study, increases in Beta II were evident for the smaller fish, but Beta II decreased for the larger unfed fish. The implication is that the larger fish may be more susceptible to a loss of Beta II during starvation and have less resistance to diseases. In general, the other globulin fractions (Alpha I, Alpha II, and Beta I) all decreased during starvation and were influenced in specific cases by holding time, water temperature, and fish size.

It was suggested that the examination of serum proteins in hatchery reared fish may be important in predicting outbreaks of disease in fish (Phillips et al. 1960); specifically, the A/G ratio. The A/G ratio in brown trout, Salmo trutta, affected with kidney disease was the reverse of the value obtained from fish not affected by this disease. Snieszko (1972) reported that the A/G ratio is very important in diagnosis; healthy carp, Cyprinus carpio, with an A/G ratio of 40% were compared to very sick carp with an A/G ratio of 12%. In the present study on presmolt coho salmon, unfed fish at Week 1 had a mean A/G ratio of 37% (as did fed fish); at Week 6 the mean A/G ratio of unfed fish was 21% (as opposed to an A/G ratio of fed fish at 31%).

#### Swimming Performance

Swimming performance is an important component of viability as it relates to a fish's capacity to maintain station against current, avoid predators, and acquire food (Beamish 1978). Bams (1966) proposed that unless severe environmental conditions impose a serious constraint, the most important component of survival is [swimming] stamina. Beamish (1978)

classified progress of fish in water as sustained, prolonged, and burst swimming; sustained is greater than 200 minutes without muscular fatigue, prolonged is 20 sec to 200 min and ends in fatigue, burst is a period of less than 20 sec. Lindsey (1978) pointed out that two different muscle systems were involved in fish locomotion, i.e., red and white muscle. Red muscle is usually slow, with low contractile power, and is used for prolonged activity sustained by aerobic metabolism; white muscle is faster, more powerful, and is capable of burst speed activity which may be anaerobic. Prolonged swimming speed was chosen to measure changes in stamina between fed and unfed coho salmon.

Griffiths and Alderdice (1972) tested swimming performance of juvenile coho salmon at various temperature levels and found that optimum (ultimate maximum) performance occurred at a combination of acclimation and test temperatures near 20°C. Glova and McInerney (1977) investigated swimming speeds of coho salmon from fry to smolt stages. They showed that coho salmon are capable swimmers on a size-related basis in freshwater and estuarine conditions; their apparent failure to survive premature seaward migration cannot be explained by their inability to perform important locomotor dependent behavior.

In this study, the swimming endurance of unfed fish measured by critical fatigue during prolonged swimming declined steadily until Week 4 and remained at a level of about 4% of the output of the fed fish. The unfed fish were simply unable to maintain their position in 0.45 m/sec velocity for over 8 minutes (fed fish maintained position for over 60 minutes) at and after Week 3. This reduced stamina could have many implications including the inability to forage for food, avoid obstructions, and escape from predators.

### Predator-Prey Study

Bams (1966) and Coutant (1973) have developed the design of a predator-prey study to determine the effect of a stressor on the prey fish. In this study, their design was adopted and adapted to determine the effects of starvation on presmolting coho salmon. This study design primarily investigates the effect of starvation on the burst speed muscles (white muscle). Starvation apparently did not place presmolting coho salmon at a disadvantage to predators; there was no significant difference between fed and unfed prey. Observation at the time of testings did reveal that the unfed fish were "pinheads," and were not active when placed in the predator tank--in fact, they were so still they resembled sticks. The more aggressive, fed presmolts usually darted and flashed producing a feeding frenzy in the predators. It was evident that more fish were eaten by the predators each week the experiment progressed, and that both fed and unfed fish were hardly a challenge to the wild cutthroat predators.

### Additional Factors

The rationale exists that starvation could be a potential threat to presmolt fish in the Columbia River, and this remains to be field tested. It is a fact that little is known about the effect of starvation on salmonids, although many field biologists have suspected this to be a problem in the lower Columbia River. Fish culturists and fish nutritionists know the importance of a proper diet for salmonids, and have recently reviewed the subject (Halver 1972). In that review, Ashley (1972) reflected that impoverished and unbalanced diets result in greater susceptibility to disease and inanition (exhaustion from lack or nonassimilation of food-emptiness), and consequently greater losses from

natural predation; he suggested more research regarding losses resulting from fish malnutrition. Inadequate diets often result in lowered resistance to infection and to degenerative diseases; this synergism may result in greater damage to the organism than would result from the sum of uncombined infections and degenerative diseases.

Snieszko (1972) defined nutritional fish diseases as those which can be attributed to deficiency, excess, or improper balance of components present in the food available. Snieszko stated that such diseases usually have a gradual onset, because symptoms do not appear until one or more of the components of a diet drop below the critical level of the body reserves. Further, he noted that very little is known about fish diseases caused solely by malnutrition.

Fish feed more intensely and food intake is increased following periods of starvation (Peter 1979). Thus, deficits in the body reserves of fish are made up as quickly as possible when provided the opportunity. However, when deprivation proceeds beyond a certain point, compensation by increased food intake is no longer possible. This was illustrated by the starvation and refeeding experiments done with young sockeye salmon fry by Bilton and Robins (1973). Fry starved 3 weeks, then fed for 8 weeks were at control levels. Fry starved for 4 weeks or longer were not able to overcome the deficits and to grow rapidly upon refeeding; mortalities were high in these latter fish, indicating that the more prolonged starvation had caused some irreversible effects.

Although starvation has not previously been associated with mortalities of hatchery reared salmon in the Columbia River, it is a serious cause of mortality in saltwater fish. In a colloquium on larval

fish mortality, Hunter (1976) pointed out that the major cause of larval fish mortality of saltwater species is starvation, predation, or both. Hunter suggested mechanisms of density dependent mortality caused by starvation were intra and interspecific competitions for food and expansion of a spawning population into areas where food was restricted to the developing larvae. He further suggested that the study of starvation should include laboratory and field studies, and most important, transitional studies that would help apply findings of laboratory studies to the field. Hunter stated that it was of major importance in any study of starvation to examine the possible interactions between starvation and predation for if slower growing larvae and larvae weakened by starvation are ingested much more frequently than healthy ones, the controlling mechanisms must be sought in food abundance rather than predation alone.

The ironic part of a hypothesis that excessive mortalities could occur as a result of starvation is that man most certainly could cause or has caused the problem. Man has stepped in to the natural life cycle of salmonids (prior to complete development of hydroelectric dams) and through constantly improving hatchery techniques has substantially improved survival from egg to smolt. Man then releases the product into an environment without food pellets, hoping the fish will respond innately to the limited natural food supply.

The Columbia River, however, has responded to environmental changes imposed (by man) by producing more zooplankton (food for smaller fish) and less benthic organisms and aquatic insects (food preferred by the larger fish - presmolt coho salmon).

Man has further acted to release more fish at the same time to the lower portion of the river than ever before in history. There are limited

records that show coho salmon were captured from April through November (Gauley et al. 1958) at Bonneville prior to major hatchery production. Recent results from collections at Jones Beach (Dawley et al. 1979) indicate a more narrow downstream passage window (May-July). It appears the presmolts used to move out of their natal streams in a more random fashion. Most coho salmon juvenile are released during late April and May; which means simply that all the fish are in the major rivers at the same approximate time--even though only 20% could be considered as "smolting" fish (see page 2). It may be that the necessity for raceway space for newly hatched fry is the present triggering mechanism for the release of the 1+ coho salmon at a coho hatchery, rather than the maturation of the fish into smolts.

An interesting factor to note in the effects of starvation on fish was provided by Love (1970) who stated hormones and enzymes are reduced during starvation. Thyroid hormone and enzyme metabolism appears to be influenced by starvation and feeding (quantity and composition of food). More specifically, starvation depresses thyroid function, plasma hormone levels, plasma  $T_4$  and  $T_3$  loss (Thyroxine), and peripheral metabolism of  $T_3$  and  $T_4$  (Donaldson et al. 1979). Levels of plasma thyroxine ( $T_3$  and  $T_4$ ) that are elevated have been associated with smoltification (Folmar and Dickhoff 1979), as have elevated  $Na^+-K^+$  ATPase levels (Zaugg and McLain 1970).

Thyroid hormones are bound to the plasma proteins; they buffer the plasma against excessive free  $T_3$  or  $T_4$  by binding them weakly. The hormones without this binding could be lost to the liver with subsequent elimination from the body (Donaldson et al. 1979). These factors pose an

interesting question for further research investigations, i.e., can starving presmolting fish in fresh water make the physiological transition to salt water?

Barnhart (1969) recognized that biologists needed to define "normality" in rainbow trout, and studied the effects of time (age), sex, diet, and strain on hematological characteristics of rainbow trout. He noted that average values for many hematological variables were small, and although significant statistically may be insignificant biologically. Wedemeyer and Chatterton (1971) recognized the value of the "normality" definition for juvenile coho salmon, and measured some associated blood chemistry values. Novotny and Zaugg (1979) have measured and are presently measuring some variables of blood chemistry in coho juveniles at various conservation hatcheries in the Columbia River Basin associated with investigations on "homing studies."

#### SUMMARY

This study was designed and conducted to characterize differences between fed and unfed presmolt coho salmon. A summary outline of the results follow:

1. Condition factor changed noticeably in unfed fish after 2 weeks; small fed fish gained more weight than large fed fish and small unfed fish lost more weight than large unfed fish.

2. In general, the unfed fish reflected a reduction in red blood cell volume (MCV), an increase in hemoglobin, and a reduction in numbers of white blood cells; large fish had higher hematological related values.

3. Total proteins (albumins, globulins, and the albumin/globulin ratio) were reduced in the unfed fish, and in general small fish had higher values.

4. Swimming stamina of unfed fish declined rapidly (to Week 3) and remained at 4% of the output of the fed fish to the termination of the study--verifying the poor condition of the fish reflected by the decreasing condition factor.

5. There was no apparent difference in susceptibility to predation between fed and unfed coho salmon presmolts, however both were readily dispatched by the predator fish (wild cutthroat trout).

#### CONCLUSIONS

The following conclusions resulted from this study:

1. Starvation reduces the swimming stamina of coho presmolts and could place them at a disadvantage in the environment.

2. The diagnosis of malnutrition needs to be characterized with a multiplicity of variables, e.g., condition factor, serum proteins, and red and white blood cell morphology and indices.

3. Malnutrition could cause mortalities in presmolt coho salmon from hatcheries.

#### RECOMMENDATIONS

As a result of the conduct of this study and of a review of the literature, the following recommendations are forwarded:

1. There is an urgent need to establish an adequate baseline of data to determine the normal physical, chemical, and physiological state of Columbia River salmonids in and after being released from hatcheries.

2. There is a need to include lipid analysis in future starvation studies.

3. The effect of malnutrition on smoltification of coho salmon needs to be investigated.

4. There is a need to investigate optimum release time from hatcheries and the rate and timing of movement after release from hatcheries.

5. There is a need to develop techniques to increase the standing crop of preferred food for presmolt salmonids in the lower Columbia River.

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