DEVELOPMENT OF RATIONS FOK THE ENHANCED SURVIVAL OF SALMON

Annual Report

BY

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Abstract

The nutritional quality of feed plays an important role in determining the health and "fitness" of smolts. commercial fish meal, the major source of protein in salmon rations, is subject to heat damage during drying and chemical interaction of fat oxidation products with proteins. Protein bioavailability is reduced and dietary stress may be introduced into hatchery feeds. This investigation tests the hypothesis that ration protein quality can influence the survival of smolts and the ultimate return of adults. improved survival production wouid be better able to reestablish natural runs of saimon in the Columbia River system and maintain and improve the genetic integrity of specific stocks.

The general approach being used involves a comparison of coho and chinook salmon reared on rations containing very high quaiity protein derived from vacuum dried meals and commercial rations relying on commercial fish meal as a source of protein. Survival and return of replicate brood-years of coded wire tagged test and control fish are being used to determine the influence of ration on survivai.

Project rearing and release of tagged fish to date include 1982, 1983 and 1984-brood replicates of coho salmon; the 1983 and 1984-brood replicates of fall chinook (tule stock) salmon; and the 1985-brood of fall chinook (up-river-bright stock) saimon. Ihe 1985-brood year replicate of coho salmon is presently being reared and has been tagged for release in April 1987. The rearing of the MB--brood replicate of fall chinook (up-river bright stock) salmon has been initiated. This report covers the rearing and release of the 1984-brood coho and the 1985-brood fall chinook (up-river-bright stock) salmon.

Duplicate lots of coho salmon were reared on two test rations containing vacuum dried salmon or hake meais and a control ration composed of the hatchery supply of Oregon pellet ration from 11 June 1985 to release on 30 April 1986. Fall chinook (up-river-bright stock) salmon were reared on a test ration containing vacuum dried salmon meal and a control ration composed of the hatchery supply of Oregon pellet ration from 12 April 1986 to 17 October 1986. The salmon meal ration produced more rapid growth for both coho and fall chinook salmon with less feed through superior conversion. Coho, supplied the hake meal ration, grew at a slower rate on less feed that was converted at an equal rate to the control ration.

Plasma cortisol and thyroxine (T_4) level, gill Na'/K+-ATPase, osmoregulatory performance, immunocompetency and total hepatic/gill microsomal lipid content were monitored from early June to mid-October 1.986 to assess the physiological condition of fall chjnook saimon. Results indicated that on several sampling dates early in the 1986 rearing period fish supplied the control ration were physiologically different than fish receiving the salmon meal ration. The probable presence of a VEN (viral erythrocytic necrosis)-like blood cel l virus infection in test and control fish may have complicated observed physiological parameters.

Incomplete recovery of coded wire tags from 1982 and 1983-broods of coho salmon (Sandy stock) revealed an improved ($P<.05$ survival for fish supplied test rations. Improved survival was only observed for the 1983-brood which enjoyed a superior ($P\geq.001$) survival over the 1982-brood; a significant ($P\geq.025$) interaction of ration x brood year was observed. Recovery of coded wire tags from the 1983 and 1984-brood years of fall chinook (tule stock) are still too preliminary for valid and statisically based conclusions.

Introduction

The natural habitat for the spawning and rearing of salmon in the Columbia River system has been reduced by hydroelectric development and other encroachments. Artificial production of salmon in hatcheries has become a critical link in the restoration of natural stocks.

Time of release, natural abundance of food, fish size and the health or “fitness” of smolts play important roles in determining survivai and the ultimate return of adult fish. it is believed that nutrition quality plays a major role in determining the effectiveness of hatchery production and the health and/or “fitness” of smolts. Ration regimes containing high quality components in uniform and fine-free pellet forms produce efficient growth response and minimize loss of nutrients. Quality feeds produce fish less susceptible to disease and of a more uniform and desirabie size at release. High quality smolts would help to optimize out-migration and successful adaptation to salt water.

The success of a ration in rearing high quality salmon smolts is dependent upon the quaiity and quantity of their protein complement. Although adequate levels of quality energy, essential fatty acids, vitamins and minerals are needed for optimum growth and “fitness” , protein is the major food component in rations. The most successful fish rations rely. on large quantities of fish protein in the form of fish meal. Plant sources of protein (soybean and cottonseed meal) are tolerated to a certain extent based upon growth response, but an excessive replacement of fish protein results in a reduction in feed consumption and growth response parameters (conversion and/or weight gain). Their presence in rations represent a dietary stress factor affecting smolt “fitness”.

Commercial fish meal supplies needed for formulating successful rations are declining in availabilty and quality. Industrial round (whole) fish that in the past formed the raw material base for high quality meal production is disappearing because of cost and/or regulation dictating its use for human food. Carcass waste is replacing round fish as a raw material. Resulting meals have a lower protein content and quality and contain an elevated minerai level because of the removal of muscle tissue for human food. The majority of meals are produced by high-temperature efficient direct flame dryers to meet the specifications of the poultry industry. Variability in raw materials and the need to meet protein content requirements for marketability have encouraged excessive heating during drying. Excessive heating damages proteins directly and initiates lipid-protein interactions. Both of these effects reduce the biological value of fish proteins.

The basic hypothesis of this investigation is that ration protein quality can also influence the survival of smolts and ultimate return of adult salmon to the Columbia River system. It is believed poor quality fish meals based upon composition dictated by raw materiais or processing damage introduces a dietary stress into fish ration formulations that can affect survival of smolts and the ultimate return of adult fish.

Meais and fish protein concentrates produced from round fish and/or upgraded fish processing waste using processes employing iow temperature and reduced pressure yields protein of optimum quality. These gentle drying and concentration procedures coupled with the use of fat antioxidants limits heat damage to proteins and markedly reduce lipid-protein interactions. Ration regimes incorporating these sources of protein would be more costly, but additional feed costs could be offset by more favorable survival of smolts and return of adult hatchery fish. Hatchery production efficiency would be improved and more hardy smolts would be less susceptible to disease and mortality.

The general approach being used to test this hypothesis involves the rearing of coho and chinook salmon on nutrient dense rations containing a high quality fish protein complement. Fish reared on the hatchery supply of commercial ration relying on commercial fish meals as a source of protein serve as a control. Coded wire tagging experiments are being conducted on replicate brood years of test and control fish to determine the influence of ration protein on survival. Beginning with the 1985-brood, fall chinook (up-river-bright stock) salmon are being assessed for physiological changes associated with smoltification and correlated with ration type and smolt “fitness”.

Project rearing and release of tagged fish to date include 1982, 1983 and 1984-brood replicates of coho salmon, the 1983 and 1984-brood replicates of fall chinook (tule stock) salmon and the the 1985-brood of fall chinook (up-river-bright stock) salmon. The 1985-brood year replicate of coho salmon are presently being reared and have been tagged. Rearing of the 1986-brood of fall chinook (up-river-bright stock) salmon has just been initiated. This report covers the rearing and release of the 1984-brood coho and the 1985~-brood fall chinook (up-river-bright stock) salmon replicates.

Methods and Materials

General Project Operation

This project combines the facilities and expertise of the Oregon Department of Fish and Wildlife and Oregon State University through their Seafoods Laboratory of the Department of Food Science and Technology and the Department of Fisheries and Wildlife. The Oregon Department of Fish and Wildlife carried out required fish husbandry tasks invoived in survival feeding trials at their Sandy and Bonneville Hatcheries and conducted coded wire tagging survivai experiments. The task of ration component acquisition and/or production and test ration production and characterization were carried out at the Seafoods Laboratory. The Department of Fisheries and Wildlife at Oregon State University carried out a determination of the physiological changes associated with smoltification of fall chinook salmon to assess smolt "fitness" and its relationship to the type of ration supplied fish.

Husbandry Protocol

Coho Salmon; Oregon Department of Fish and Wiidlife Sandy Hatchery: Coho salmon (*Oncorhynchus kisutch*) (Sandy stock) were reared in 20 x 80 x 4 ft. (variable depth) raceways with an actual volume of 4,290 cu. ft. (32,089 gal.) at a maximum water depth of 3.5 ft. Raceways were supplied with 228 to 396 gpm/pond of Cedar creek water at 38 to 59 °F (three year monthly mean range) (Appendix I). The lowest flow rates occurred during the summer, and the highest during the spring before release of smolts. The hatchery had north and south facing banks of ten ponds each with a separate head box for each bank. The north head box was constructed so that only a single pass of water will go into each pond. The south head box was equipped with a pipe and pump system that was used to recirculate water into the head box (along with the normal creek water). This system was used only during the summer and early fall when the water flow in the creek was too low to meet the needs of the hatchery. Under normal circumstances, the pump is used only three months during the year.

Unfed fry were ponded on April 4-5, 1985 at 1,150 fish/lb (0.394 g/fish) at a rate of 636,351 (pond 7) and 654,250 (pond 17) fish/pond. Fish were supplied starter ration and progressed through the pellet size guide for salmon recommended by the Oregon Department of Fish and Wildlife for moist pelletized feeds:

Pellet size (in.)	Fish size fish/lb	g/fish
Starter	1000-700	0.4-0.6
1/32	700-500	0.6-0.9
	500-250	0.9-1.8
1/16	250-150	1.8-3.0
3/32	150-50	3.0-9.1
1/8	50-13	9.1-34.9

Fish (197.816 fish/lb; 2.29 g/fish) were randomly distributed (in 10 lb lots) into six ponds at a rate of 58,512 to 58,670 fish/pond on May 28, 1985. Control and two test rations were randomly assigned to duplicate ponds/ration; one pond located in the south bank and the other in the north bank of raceways.

Control and two test rations were supplied to fish from June 11, 1985 to release on April 30, 1986. Each ration in recommended pellet sizes was fed by hand to replicate ponds of fish at the feeding frequencies listed as follows:

Fish size (fish/lb)	Feeding frequency (times/day)
1200-800	8-10
800-500	6
500-250	4
250-150	3
150-15	1-2

Control fish were supplied feed according to a feeding guide which schedules fish to be 15 fish/lb (30.24 g) at liberation. Fish supplied test rations were fed at a rate less than the feeding rate guide to achieve equal size at liberation.

Fall Chinook Salmon; Oregon Department of Fish and Wildlife Bonneville Hatchery: Fall chinook salmon (up-river-bright stock) (Oncorhynchus tshawytscha) were reared in 17.5 x 75 x 3 ft. raceways (3,938 cu ft.; 29,456 gal.). Ponds were supplied with well water (49-51 °F) at a rate of 300 to 550 gpm/pond. Water flow rate was gradually increased from 300 gpm/pond for swim-up fry to 550 gpm/pond and/or to a maximum of 6 lbs of fish/gpm at liberation.

Unfed fry were ponded on March 18, 1986 into six ponds at a rate of 200,172 (ponds C-3 and C-4), 202,745 (pond C-5) and 200,673

(pond C-6) fish/pond at 982 fish/lb (0.46 g/fish). Duplicate ponds were supplied the control and test rations from April 12, 1986 until October 17, 1986. Fish were supplied rations composing the Oregon pellet feed system between March 18 and April 12, 1986. Experimental lots of control and test ration fish were split during this time period to meet projected pond water flow/fish weight requirements.

Control and test fish were initially supplied starter ration and then progressed through the pellet size guide recommended by the Oregon Department of Fish and Wildlife for moist pelletized feeds listed above. Test ration feeding began with the 3/64-inch pellet size. Rations were supplied with Garon automatic feeders at a rate designed to achieve a target release size of 13 fish/lb.

Pathological Assessment

Oregon Department of Fish and Wildlife pathologists responded to any increase in mortality rates that occurred. At the pathologists discretion, appropriate diagnostic tools were employed to determine the causative agent and remedial treatments were prescribed. Examinations were summarized and reports became a permanent record of the lot of fish involved. During their experimental rearing period coho saimon at Sandy Hatchery were inspected four times (Appendix V), while fall chinook (up-river-bright stock) at Bonnevilie Hatchery were examined twice (Appendix VI). Both included a preliberation examination.

Physiological Assessment

Physiological changes associated with smoltification were determined and correlated with ration type and smolt "fitness". Growth, plasma cortisol, thyroxine (T_4) levels, gill Na^+/K^+-ATPase activity, osmoregulatory performance, immunocompetency, and total hepatic/gill microsomal lipid content were monitored from early June to mid-October 1986.

Growth was monitored by measuring fork length and wet weights. Plasma cortisol and thyroxine levels were measured by radioimmunoassay; gill Na+/K+-ATPase activity was measured spectophotometrically. Osmoregulatory performance was assessed using the plasma sodium regulatory approach with fish placed for 24 hours into 20 ppt artifical seawater. Plasma sodium and potassium concentrations were determined using ion-specific electrodes. Total hepatic and gill microsomal lipid content was determined spectrophotometrically following chloroform/methanol extraction.

Fish samples were obtained with a dip net in a manner that provided as representative sampling of the fish in each raceway as possible. Sampling was performed on June 1, July 1, August 5 and

18, September 4 and 18, and October 1 and 15, 1986. Fish were released on October 17, 1986. Although an attempt was made to work with fish before June, they were too small for obtaining adequate samples. Fifteen fish were collected from each pond, in duplicate (30 fish/ration) for each treatment. Fish were weighed, measured, and then blood, gill, liver and head kidneys were collected and prepared for later assay.

Growth Response Parameters

Fish weight, feed consumption, feed conversion and mortality information was determined at monthly intervals and reported at two to three month intervals for coho and fall chinook salmon. At liberation, fork length, weight and blood hematocrits were measured and samples of fish from each pond collected for the determination of body composition.

Mean fish weight and length was based on the measurement of three to six randomly selected samples (varying in weight depending on fish size) of the pond populations. Feed consumption and mortality were recorded daily. Feed conversion (feed/gain) was computed wet weight on a cumulative and period basis for interim reporting purposes and on both a wet and dry weight basis for the entire rearing period at liberation. The blood hematocrit level for each pond replicate was the mean of twelve to fourteen fish. Body composition determinations were based upon the mean of duplicate analysis of three randomly selected samples of ten fish/pond replicate.

Coded Wire Tagging Experiments

Coho salmon were injected with a distinctive coded wire tag on 11-18 September 85 at a rate approximating 28,000 fish/pond replicate of control and test fish and marked with an adipose fin clip. Coho were randomly selected for tagging by passing the entire pond of fish over a sampling table which was adjusted to select the desired percentage of fish. Fall chinook salmon were similarly tagged and marked on 19-25 August 86 at a rate approximating 47,000 fish/pond replicate of control and test fish. Fish were randomly selected using a procedure similar to that used for coho salmon. Tag retention numbers from each pond replicate were determined prior to the release of both coho and fall chinook salmon.

Protein Evaluation Design

The hatchery supply of rations composing the Oregon pellet feed system served as a control ration for both coho and fall chinook salmon. This included, when applicable, Biomoist Starter Ration and the OP-4 and OP-2 formulations of the Oregon pellet feed. Coho salmon were supplied with two test rations deriving their

major protein complement from vacuum dried salmon hatchery carcasses and round Pacific hake. A single test ration containing vacuum dried salmon meal as the major protein source was supplied fall chinook. The major protein complements provided by both vacuum dried saimon and hake were supplemented by hydrolyzed and vacuum concentrated bone-free fish derived from round hatchery salmon carcasses.

Ration Component Production and Acquisition

Advanced Hydrolyzing Systems, Inc. of Astoria, OR, in direct cooperation with the Seafoods Laboratory, produced high quality vacuum dried meal with their equipment using Seafood Laboratory facilities, power and steam. Concentrated hydrolysates were produced in company facilities. Hatchery carcasses were provided by the Oregon Department of Fish and Wildlife. Hake and groundfish carcass waste were purchased on the open market.

Fish meals were prepared by subjecting coarse ground fish in a steam jacketed chamber equipped with stirring-scraping device to a vacuum equivalent to 25-27 inches of Hg. Product temperature was maintained at 101-105 ^{o}F except for a time period of approximately 5.0 minutes while the product was still moist when the product temperature was allowed to rise to 180 ^{o}F to achieve pasteurization. Product temperatures upon completion of drying were <110 ^{o}F. All vacuum dried meals, if not used immediately for ration preparation, were sacked and held frozen <0 ^{o}F.

Concentrated fish hydrolysates were prepared by exposing coarse ground fish to a temperature approximating 140 ^{o}F with mechanical agitation until sufficent liquefaction was achieved to allow screen removal of bones. The temperature of the liquefied material was raised to 180 ^{o}F to achieve pasteurization and then concentrated in vacuum with scraped surface heat transfer equipment to approximately 50% solids. Concentrates were sacked or boxed, cooled and then frozen and held at <0 ^{o}F.

Remaining components required for ration preparation were purchased from commercial firms that either produce moist pelletized fish rations or provide components to the fish feed industry. All purchased components met specifications for the Oregon pellet feed.

Test Ration Formulation and Production Protocol

Test rations were formulated to contain 28 lb of protein derived from meal and 7.7 lb from concentrated hydrolyzed fish/100 lb of ration. Water and wheat germ meal were balanced to yield rations with 76% solids (24% moisture). Herring oil was added in amounts needed to yield a total ration fat content that provided a ration fat:protein caloric ration of 0.95 (protein = 4.0, fat = 9.0

kcal/g). Computer controlled formulation using the above criteria relied upon the determined compositions of vacuum dried meal and concentrated hydrolyzed fish used for each batch of ration and the general and accepted composition of remaining components. The formulation of test and control rations is listed in Appendix II. Ration dry components (vacuum dried fish meal, wheat germ meal, dried whey product, spray dried blood, mineral and vitamin premixes and sodium bentonite) were mixed in 600-1000 lb batches and hammer milled to achieve a fine particle size. Milled dry mix was sacked in 50 lb units and held frozen at 0 to -30 "F if not immediately used to prepare ration.

Milled dry mix was mechanically mixed with remaining "moist" components (antioxidant stabilized herring oil, choline chloride, concentrated hydrolyzed fish and water) in 150-250 lb batches. The thoroughly mixed components were then mechanically extruded into desired length-diameter pellet forms, screened to remove fines, sacked into 40 lb (l/32-inch pellets only) or 50 lb units and immediately frozen at -30 °F.

Ration Composition Control

The proximate analysis (moisture, ash, protein and fat content) of test and control rations was determined to assure composition and for computation of dry weight and protein consumption and conversion. The entire hatchery supply of control ration was sampled by pellet size and if possible, by production date. Test rations were sampled during production at a rate of at least two samples from each 150-250 lb mixer batch. The mean for all samples derived from each production day lot which was prepared from the same dry mix formulation was used as the composition of a particular lot of pelletized ration. The composition of control rations was related to feed consumption at the hatchery only by pellet size. The mean composition of each pellet size derived by sampling was used to compute dry weight and protein consumption and conversion. The composition of test rations was directly related to the actual feed consumed.

Analysis of Growth Response Data

Growth response data were analyzed using analysis of variance (one-way classification) procedures. Tag recovery information was analyzed using a factorial design for analysis of variance. The significance of differences between treatment means was determined using "least significant difference" (LSD) procedures.

Results and Discussion

Rearing Results: 1984-Brood Coho Salmon

Duplicate lots of coho salmon (2.29 g initial average weight) were reared on two test rations containing vacuum dried salmon and hake meals and a control ration composed of the Sandy Hatchery supply of Oregon peilet feed system rations from 11 June 1985 to release on 30 April 1986. The number of fish initially supplied test rations, the number reieased and the numbers released with recognizable coded wire tags are listed below. The raw growth response data for control and test rations are detailed in Appendix III.

Ration	Pond No.	Initial No. fish	No. fish released	No. recognizable tags released
Salmon	4	58,489	57,767	28,079
	12	58,458	57,430	27,115
Hake	6	58,646	57,899	27,489
	16	58,486	57,860	27,542
OMP	5	58,490	57,865	27,623
	13	58,492	57,893	27,974

Coho saimon, from which experimental lots were derived, often experience a cold water disease epizootic prior to the ponding at Sandy Hatchery. Fish were supplied feed medicated with 3% TM-50 from April 14 to June 6, 1985 as a prophylaxes against cold water disease. A cold water disease epizootic occurred in early May and Furox NF-180 was supplied from May 3 to May 27 at a rate of 36 g/100 lbs fish/day. At final splitting and initiation of feeding experimental rations, cold water disease was not evident.

in late July of 1985 increased mortality was observed for some ponds of fish. An examination on 7/30/85 identified an epizootic of Columnaris (Appendix V). Ponds experiencing mortality involved fish supplied salmon and hake rations. Ration medicated with 5% TM-50 were prepared and supplied to ponds requiring treatment from August 2 to 25, 1985. Losses were observed to be normal by August 10. No symptoms of disease were observed on 14 April prior to release on 30 April, 1986.

The quantity of feed consumed by test and control fish varied (P>.025) on a wet and dry weight basis, but protein consumed did not vary (P>.05) (Table 1). The lower feed consumption by test

fish (P=.O5) was a function of the programmed feed schedule based upon ration composition (Table 2) and conversion which was designed to achieve an equal size to control fish at release. Test fish were supplied with more (P=.O5) ration containing hake than salmon meal as major sources of protein.

Conversion of test and control rations varied on a wet weight basis (P>.005) on the basis of dry weight (P>.01); and based upon the ratio of feed protein to body protein gain (P>.025) (Table 1). Test rations containing salmon meal were converted more efficiently (P=.05) than either the test ration containing hake meal or the control ration. Conversion of the test ration containing hake meal was equal (P=.O5) to that of the control ration on a the basis of feed wet and dry weights and on the basis of the quantity of feed protein deposited as body protein.

The gain produced by fish supplied test and control rations during the rearing peroid varied significantly (P>.005) and resulted in the release of fish varying in size (P>.005) (Table 3). Test fish supplied salmon meal ration produced the greatest (P=.O5) gain and were the largest fish released (P=.O5). The control ration produced superior (P=.O5) weight gains to the test ration containing hake meal and were larger at release (P=.O5).

Salmon meal rations produced rations superior (P=.O5) gain with less feed (P=.05) through a more efficient conversion of feed and feed protein (P=.O5) than either the control or test ration containing hake meal (Table 3). The superior gain (P=.O5) of the control ration over the hake meal test ration was a function of the consumption of less feed (P=.O5). The slightly better feed conversions (NS P=.O5) observed for the hake meal ration was not sufficient enough to produce a weight gain equal to the control ration.

Fish supplied control and test rations did not vary (P<.05) in length, condition factor, blood hematocrit, mortality (Table 3) upon release. Small variations (NS P=.05) in condition factors for fish receiving the different rations reflected significant variations in fish weight and differences in length that were not significant (PL.05).

Fish supplied control and test rations did not vary (P<.05) in body composition (Table 4) at release on a wet or dry weight basis. The relatively large differences between control fish pond lots complicated the ability of statistical relationships to reflect the higher fat content of test rations (Table 2).

Table 1. Feed consumption and conversion. 1984-Brood coho salmon; Sandy Hatchery.

Ration	Pond No.	Feed consumption (kg) Wet wt.	Dry wt.	Protein	Feed/gain Wet wt	Dry wt.	Feed protein/protein gain
Salmon	4	2159.1	1597.3	880.2	1.227	.908	2.925
	12	2070.6	1532.4	844.2	1.170	.866	2.841
Hake	6	2336.4	1722.3	919.5	1.395	1.029	3.270
	16	2269.3	1673.9	893.6	1.364	1.006	3.170
OMP	5	2480.2	1816.4	905.6	1.444	1.058	3.067
	13	2454.8	1797.8	896.3	1.436	1.051	3.085
Salmon	Mean	2114.9[a]	1564.9[a]	862.2	1.199[a]	.887[a]	2.883[a]
Hake	Mean	2302.9[b]	1698.1[b]	906.6	1.380[b]	1.017[b]	3.220[b]
OMP	Mean	2467.5[c]	1807.1[c]	900.9	1.440[b]	1.054[b]	3.076[b]

Significant relationships: feed (wet wt.), $P \geq .025$; feed (dry wt.), $P \geq .025$; feed (wet wt.)/gain, $P \geq .005$; feed (dry wt.)/gain, $P \geq .01$; feed protein/protein gain, $P \geq .025$.

Mean values for rations in a column with same exponent letter did not vary significantly (P=.05).

Table 2. Ration composition. 1984-Brood coho salmon; Sandy Hatchery

Ration		Composition (% wet wt.) Moisture	Ash	Fat	Protein
Salmon	Mean	25.82	7.05	16.47	40.97
n = 11	S.D.	.705	.281	1.070	1.162
Hake	Mean	26.42	9.69	16.66	39.25
n = 18	S.D.	2.151	1.364	.900	1.665
OMP	Mean	26.77	7.62	13.96	36.63
n = 9	S.D.	.705	.318	.401	1.514

n = Number of lots of experimental ration; number of samples of hatchery feed supply taken through rearing period for OMP.

Table 3. Fish release size, gain, length, condition factor, hematocrit and mortality. 1984-Brood coho; Sandy Hatchery.

Ration	Pond No.	Fish Release wt. (g)	Weight gain (g/fish)	Length (mm)	Condition factor[1]	Hematocrit (%)	Mortality (%)
Salmon	4	32.79	30.49	142.5	1.133	34.7	1.2
	12	33.14	30.85	143.5	1.121	40.1	1.8
Hake	6	31.24	28.95	141.3	1.108	37.5	1.3
	16	31.07	28.78	141.1	1.106	36.3	1.1
OMP	5	32.00	29.70	142.9	1.096	33.7	1.1
	13	31.85	29.56	142.3	1.106	34.5	1.0
Salmon	Mean	32.97[a]	30.67[a]	143.0	1.127	37.4	1.5
Hake	Mean	31.16[b]	28.86[b]	141.2	1.107	36.9	1.2
OMP	Mean	31.92[c]	29.63[c]	142.6	1.127	34.1	1.0

[1] [100000 x wt. (g)]/[length (mm)^3].

Significant relationships: fish release wt. (g), $P \geq .005$; weight gain (g/fish), $P \geq .005$.

Mean values for rations in a column with same exponent letter did not vary significantly (P=.05).

Table 4. Body composition. 1984-Brood coho; Sandy Hatchery.

Pond No. /Ration	Composition (% wet wt.)				Composition (% dry wt.)		
	Moisture	Ash	Fat	Protein	Ash	Fat	Protein
4/ Salmon	73.28	2.30	8.17	17.10	8.62	30.57	64.00
	.272	.013	.335	.071	.116	.937	.720
12/ Salmon	73.37	2.21	8.25	16.79	8.32	30.98	63.09
	.327	.029	.510	.126	.128	1.554	1.149
6/ Hake	73.45	2.37	7.85	16.79	8.95	29.56	63.25
	.224	.005	.065	.185	.088	.245	1.218
16/ Hake	73.70	2.34	7.72	16.95	8.92	29.34	64.45
	.113	.058	.034	.130	.255	.189	.729
5/ OMP	73.72	2.34	7.55	17.19	8.91	28.72	65.42
	.142	.016	.198	.150	.096	.607	.643
13/ OMP	73.95	2.33	6.92	16.99	8.93	26.55	65.21
	.158	.045	.131	.127	.125	.461	.291
Salmon Mean/S.D.	73.33	2.26	8.21	16.94	8.47	30.77	63.54
	.046	.044	.042	.152	.150	.206	.455
Hake Mean/S.D.	73.58	2.36	7.78	16.87	8.93	29.45	63.85
	.128	.015	.067	.055	.015	.109	.598
OMP Mean/S.D.	73.83	2.33	7.23	17.09	8.92	27.64	65.32
	.115	.008	.316	.102	.009	1.084	.104

n = 3 replicate samples/pond.

Mean values for rations in columns did not vary significantly ($P \leq .05$).

Rearing Results: 1985-Brood Fall Chinook Salmon

Fall chinook salmon (up-river-bright stock) were reared on a test ration containing vacuum dried salmon meal and a control ration composed of the Bonneville Hatchery supply of Oregon feed system rations from 12 April to 17 October 1986. Experimental lots of control and test ration fish were split during this time period to meet projected pond water flow/fish weight requirements according to the following schedule:

Split date	Ration:	OMP		Salmon meal	
	Pond:	5	6	3	4
4/12/86	No. fish:	201,935	200,118	199,474	199,585
	G/fish:	.933	.867	.963	.909
6/9/86	No. fish:	103,130	103,260	100,441	102,000
	G/fish:	3.965	3.917	4.637	4.447
8/12/86	No. fish:	47,472	49,085	48,261	47,897
	G/fish:	13.700	13.197	14.013	14.168

Fish were coded wire tagged during the period 19 to 25 August, 1986; the numbers released and released numbers with recognizable coded wire tags are listed below. The raw growth response data for control and test fish are listed in Appendix IV.

Release date	Ration:	OMP		Salmon meal	
	Pond:	5	6	3	4
10/17/86	Released	47,042	47,795	47,980	47,630
	Tagged	46,579	47,265	46,852	47,250

Pathological evaluations at Bonneville Hatchery were carried out on 3 October 1986 in response to increased mortality in one pond lot that was not a part of this experiment (Appendix VI). Mortalities were diagnosed to be caused by cold water disease and Furox medicated food a 50 g/ 100 lbs fish/day for ten days was supplied this specific pond. Ponds with diagnosed cold water disease continued to exhibit elevated mortality until liberation. Mortality for ponds without diagnosed cold water disease remained low until liberation. Preliberation evaluation of fish diagnosed cold water disease in four ponds at Bonneville Hatchery that were not a part of this experiment. VEN (viral erythrocytic necrosis)-like blood cell virus was detected in seven ponds; two

of which involved one replicate pond from the experimental control ration and the salmon meal ration.

The salmon meal ration supplied to fall chinook during the rearing period contained a higher fat and protein content than the control ration (Table 5). Fish were supplied significantly smaller quantities of saimon meal ration during the rearing period (wet wt., PL.025; dry wt., P>.025; protein, P>.05) than control ration (Table 6). The conversion of the salmon ration on a wet (P>.01) and dry (P>.005) weight basis was superior to that produced by the control ration (Table 6). Salmon meal ration protein was converted to body protein (feed protein/protein gain) more efficiently (P>.05) than the protein content of the control ration.

Fish supplied the salmon meal ration gained more (P>.025) weight during the rearing period. The fish released weighed more (P>.025) and were longer (P>.05) than fish supplied the control ration (Table 7). The condition factor, blood heaatocrit level and mortality of control and test fish were statistically equal (P<.05) at release. Blood hematocrit level from individual fish for all pond replicates, regardless of the ration supplied, were highly variable. It is believed that this observation was a direct reflection of a VEN (viral erythrocytic necrosis)-like blood cell virus infection diagnosed at Jiberation.

The salmon meal ration produced more growth through superior conversion of less feed. Despite the feed restrictions applied, fish were released at a size significantly greater than fish supplied the control ration. Husbandry goals were designed to produce fish of equal size at release. Our ability to achieve reiease size goal was partially hampered by an early release time (approximately two weeks) dictated by construction at Bonneville Hatchery.

Dispite a higher fat content in salmon meal rations (Table 5), the body composition of fish supplied the salmon meal and control ration did not vary (P<.05) on both a wet and dry weight basis (Table 8). The higher ration fat content was most probably utilized in sparing the use of protein for energy and enhancing the conversion of less (P=.O5) consumed protein into body protein.

Table 5. Ration composition. 1985-Brood fall chinook; Bonneville Hatchery.

Ration		Composition (% wet wt.)			
		Moisture	Ash	Fat	Protein
Salmon	Mean	27.99	6.65	16.91	40.59
n = 14	S.D.	.77	.30	.61	1.02
OMP	Mean	27.70	7.08	14.12	38.00
n = 7	S.D.	.47	.46	.46	1.30

n = Number of lots of experimental ration; number of samples of hatchery feed supply taken through rearing period for OMP.

Table 6. Feed consumption and conversion. 1985-Brood fall chinook; Bonneville Hatchery.

Ration	Pond No.	Feed consumption (kg)			Feed/gain		Feed protein/protein gain
		Wet wt.	Dry wt.	Protein	Wet wt.	Dry wt.	
OMP	5	2846.3	2055.3	1075.5	1.18	.85	2.77
	6	2932.5	2117.4	1107.8	1.21	.87	2.87
Salmon	3	2571.9	1851.8	1040.3	.99	.71	2.53
	4	2594.5	1867.9	1049.1	.98	.70	2.47
OMP	Mean	2889.4[a]	2086.3[a]	1091.6[a]	1.20[a]	.86[a]	2.82[a]
Salmon	Mean	2583.2[b]	1859.9[b]	1044.7[b]	.98[b]	.71[b]	2.50[b]

Significant relationships: feed (wet wt.), $P \geq .025$; feed (dry wt.), $P \geq .025$; feed protein, $P \geq .05$; feed (wet wt.)/gain, $P \geq .01$; feed (dry wt.)/gain, $P \geq .005$; feed protein/protein gain, $P \geq .05$.

Mean values for rations in a column with same exponent letter did not vary significantly (P=.O5).

Table 7. Fish size, gain, length, condition factor, hematocrit, mortality. 1985-Brood fall chinook; Bonneville Hatchery.

Ration	Pond No.	Fish release wt. (g)	Weight gain (g/fish)	Length (mm)	Condition factor[1]	Hematocrit (%)	Mortality (%/day)
OMP	5	30.73	29.80	136.6	1.204	27.0	.379
	6	30.77	29.90	137.2	1.192	31.6	.268
Salmon	3	33.67	32.71	140.9	1.203	25.6	.314
	4	34.48	33.58	142.6	1.189	28.2	.287
OMP	Mean	30.75[a]	29.85[a]	136.9[a]	1.198	29.3	.324
Salmon	Mean	34.08[b]	33.14[b]	141.8[b]	1.196	26.9	.301

[1] [100000 x wt. (g)]/[length (mm)^3].

Significant relationships: fish release wt. (g), P>.025; weight gain/fish (g), P>.025; length (mm), P>.05.

Mean values for rations in a column with same exponent letter did not vary significantly (P=.O5).

Table 8. Body composition. 1985-Brood fall chinook; Bonneville Hatchery.

Pond No. /Ration	Composition (% wet wt)				Composition (% dry wt.)		
	Moisture	Ash	Fat	Protein	Ash	Fat	Protein
5/ OMP	75.21	2.03	7.35	16.16	8.17	29.64	65.17
	.025	.042	.154	.078	.163	.644	.334
6/ OMP	76.36	1.98	6.58	15.98	8.37	27.79	67.60
	.450	.040	.493	.172	.304	1.581	1.627
3/ Salmon	75.41	1.98	7.70	15.74	8.07	31.32	64.01
	.182	.012	.144	.051	.103	.530	.629
4/ Salmon	74.48	2.00	8.19	16.02	7.85	32.06	62.77
	.343	.009	.630	.076	.070	2.062	1.148
OMP Mean/S.D.	75.78	2.00	6.96	16.07	8.27	28.71	66.38
	.575	.025	.386	.090	.096	.926	1.218
Salmon Mean/S.D.	74.94	1.99	7.95	15.88	7.96	31.69	63.39
	.465	.010	.244	.138	.108	.374	.619

n = 3 replicate samples/pond

Mean values for rations in a column did not vary significantly ($P \leq .05$).

Assessment of Physiological Condition: 1985-Brood Fall Chinook Salmon

Plasma cortisol and thyroxine (T4) levels did not differ in any consistent fashion between the two rations (Oregon pellet control and salmon meal ration) (Fig.1). In general, cortisol levels decreased between July and the middle of September and then increased during late September prior to the time of release. This is the same general trend our laboratory has documented for coho salmon (although the timing of the pattern differs on a seasonal basis). On the last sampling date, fish fed the salmon meal ration had a higher cortisol level than those fed Oregon pellets. It is possible that the fish fed control ration were physioiogically less advanced than the salmon meal group at the time of release. The apparent peak in plasma cortisol observed on 1 October was accompanied by great variability and is probably indicative of rising levels at this time rather than a peak followed by a precipitous drop. Such a trend is apparent when one examines the raw data. The majority of fish on 1 October had low cortisol levels, while a few animals had relatively high values.

Plasma T4 levels in the fish supplied the control ration followed the same general pattern as cortisol levels, i.e., a gradual decline between July and September followed by an increase prior to release. In contrast, T_4 levels for fish receiving the salmon meal ration were more variable during the course of this study. However, T_4 levels did not differ according to ration regime at the time of release.

The considerable movement of fish during the particular rearing cycle may have confounded recognition of differences between the two groups and the replication in subsequent years are necessary. Because of the small volumes of plasma in some of the early samples, it was decided to run assays on complete series for the above two hormones. The remainder of the plasma samples will be assayed for triiodothyronine (T_3) at the beginning of the next phase of this study.

Gill Na+/K+-ATPase activity in fish from both control and salmon meal groups followed the same general pattern as cortisol levels, aithough the timing of the changes observed was relatively more advanced (Fig. 2). Activity appeared to differ between the two ration groups early in the rearing period, with fish supplied the control ration having higher activities. By early August and thereafter, however, the ATPase activities between the two ration groups did not differ. These results suggest either differential effect of ration on development rate or some other influence. The former is supported by our experience with coho salmon where we found effects of hatchery practices on developmental physiology of parr. Results also may have been confounded by sampling or the

numerous times the fish were moved to different ponds at the hatchery during the course of sampling.

Surprisingly, all fish used in the saltwater challenge tests were able to regulate their plasma sodium levels (Fig. 3). This is contrary to prior experience and other reports for this species. We suspect that the lack of a seasonal trend showing enhanced sodium regulatory ability through time was attributable to the relatively low level of sodium (20 ppt) use for the test. This level was selected based on earlier, preliminary tests designed to establish a salinity level that all test groups could tolerate for 24 hours, but not necessarily adapt to with an extended exposure time. Obviously, this level of salt was within the capacity of even the youngest parr to adapt. Consequently, a more severe seawater challenge will have to be employed in subsequent tests.

Initially,. it was proposed to assess immunocompetence of fish at each sampling date using an in vivo approach in which fish were to be injected intraperitoneally with Vibrio anguillarum antigen and held in tanks for 17 days. After this time period, the ability of anterior kidney lymphocytes to generate antibody-producing cells was to be quantified. A test of this nature was initiated on 3 June, but proved unsatisfactory as fish held in tanks would not eat, and consequently were in a poor state of health prior to day 17 (a significant number of fish died).

A redesigned protocol for assessing immunocompetence was developed. An in vito assay approach was chosen in which the head kidney was removed from the fish using aseptic techniques and cells were cultured in a sterile medium containing a lymphocyte antigen for 9 days. After culturing, the number of antibody-producing cells were quantified. Using this method solved the problems incurred with the in vivo approach, however, it proved to be more labor intensive and expensive. It was therefore decided to assess immunocompetence once an month and at the time of release rather than at each sampling date.

Assessment of immunocompetence did not show any consistent trends during the limited period of observation available (Table 9). Data for June and July samples are not available because of problems with the assay. The apparent greater number of antibody-producing cells (and greater variability) observed during October in fish receiving the control ration was a reflection of the fact that approximately one-half of these fish completely failed to demonstrate an immune response in vitro while the other half demonstrated a very high degree of response. It is believed that these results do not accurately reflect a greater degree of immunocompetence in these fish as compared to those supplied the test ration. It is very likely that these fish were sick, having viral erythrocytic necrosis at the time of release. Both groups

were roughly equally unhealthy, thereby masking any differences detected by the assay.

No consistent differences between control and salmon rations were observed in either total hepatic or gill microsomal lipid content (Fig. 4). Total hepatic lipid levels were relatively constant during the course of the investigation. In contrast, gill microsomal lipid content gradually declined. The physiological significance of this trend is presently unclear.

A high performance liquid chromatography (HPLC) technique was worked out during this reporting period that can be used to identify the nature of specific membrane fatty acids. Briefly, this method consists of converting endogenous fatty acids to pentafluorobenzyl esters, which can be identified using ultraviolet detection following reversed-phase HPLC. Specific hepatic membrane lipids will be determined using this technique early in the next phase of this project.

In summary, we believe that the physiological parameters monitored in the present study are sensitive and reliable indicators of early saimonid development and fitness. Our results indicate that on several sampling dates early in the 1986 rearing period, the fish fed control and test rations were physiologically different. It is conceivable that these differences would have been even more pronounced had the fish at the hatchery not been handled (moved from one raceway to another) quite so often. Further, the fact that both groups of fish were unhealthy may have further obfuscated differences due to ration type. It is very important that we repeat this investigation in subsequent years in order to clarify these issues.

Table 9. Mean[1] number of antibody producing cells per head kidney (A.P.C./organ). 1985-Brood fall chinook; Bonneville Hatchery.

Date	Ration	A.P.C./organ	n
5 Aug 86	Salmon	166 + 30	24
	OMP	174 + 58	23
4 Sept 86	Salmon	154 + 32	24
	OMP	112 + 29	23
1 Oct 86	Salmon	74 + 24	24
	OMP	360 + 120	23
15 Oct 86	Salmon	113 + 30	22
	OMP	353 + 23	23

[1] Mean with standard error

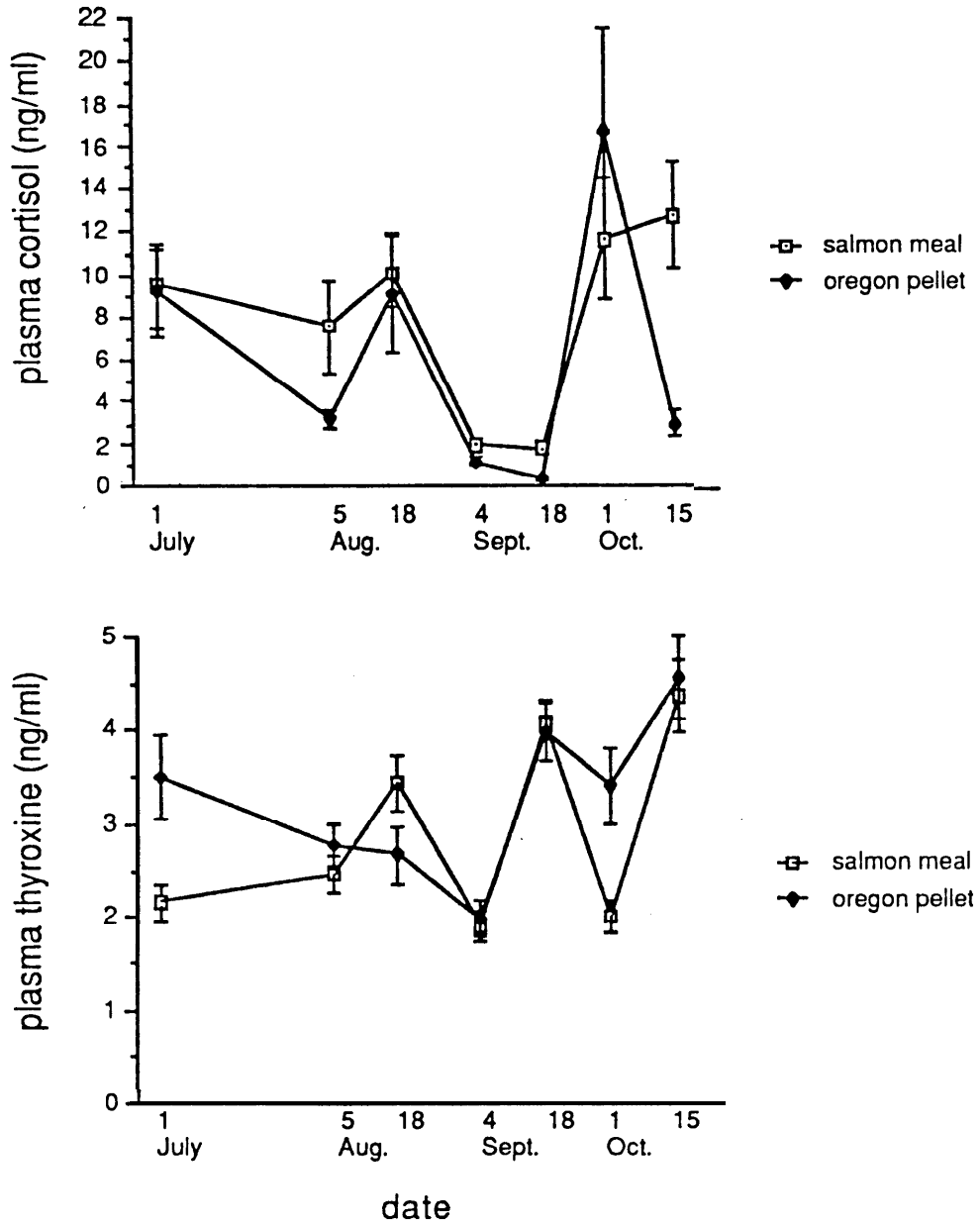

Figure 1. Mean plasma cortisol and thyroxine concentrations. 1985-Brood fall chinook; Bonneville Hatchery. Vertical line indicates S.E.; n = 20.

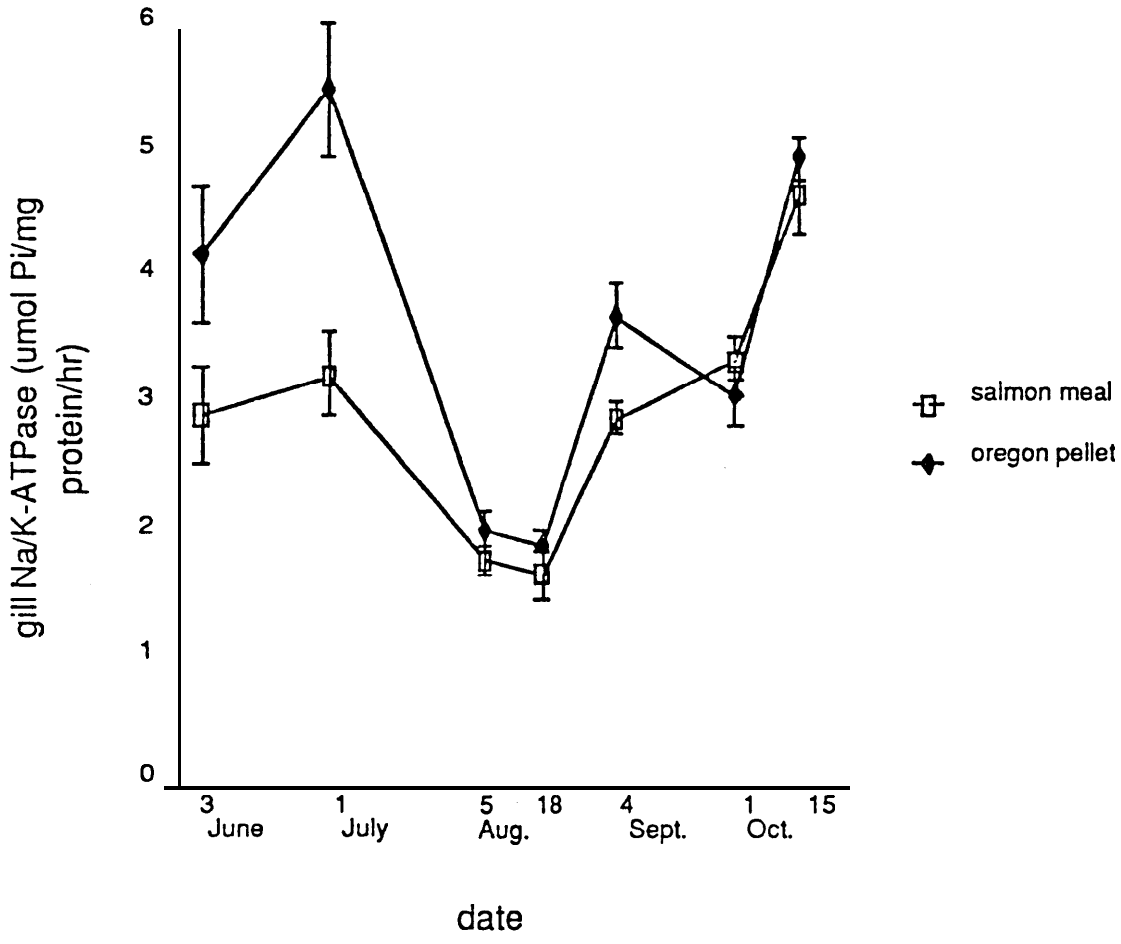

Figure 2. Mean gill Na/K-ATPase levels. 1985-Brood fall chinook; Bonneville Hatchery. Vertical line indicates S.E.; n = 30.

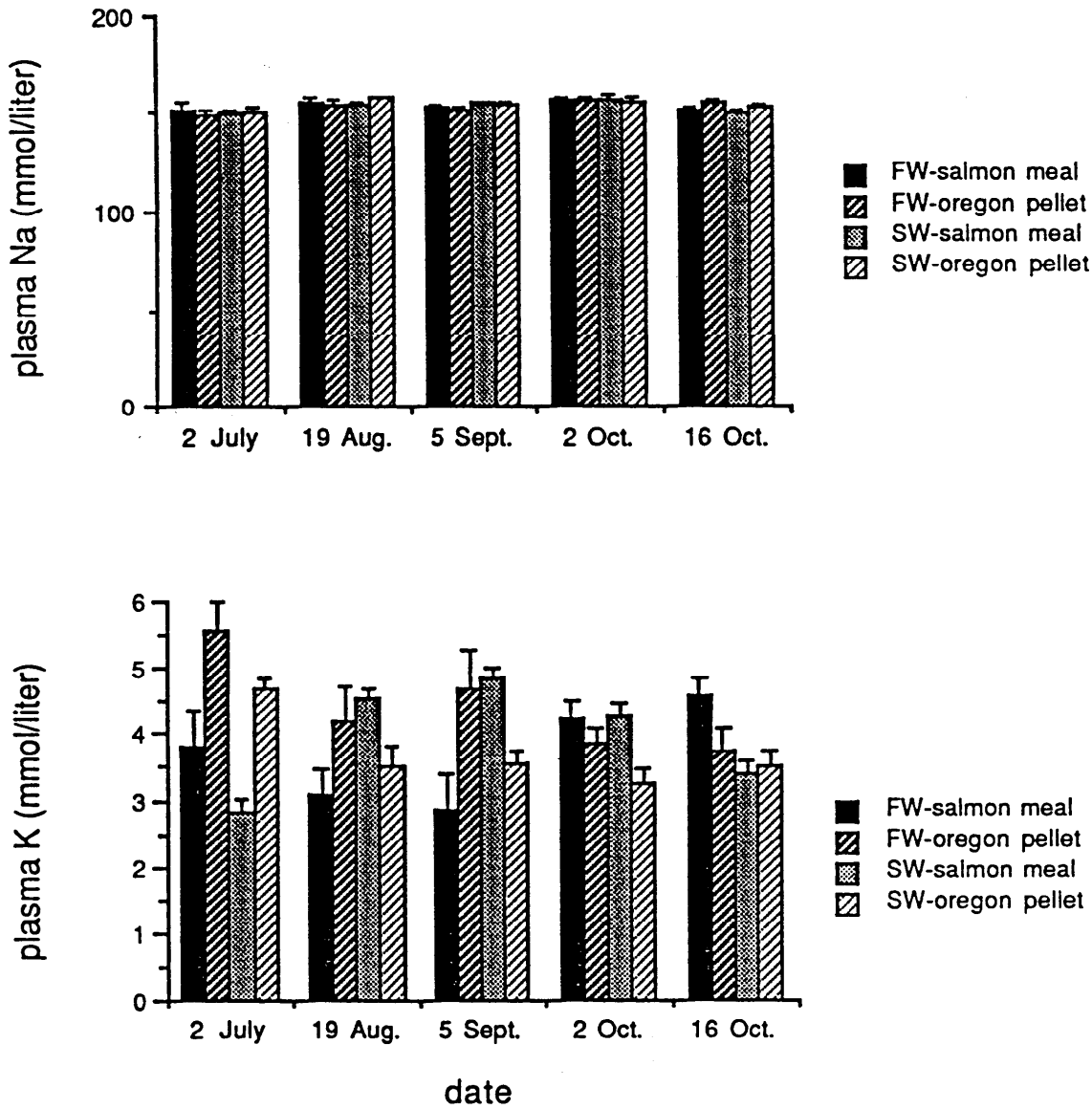

Figure 3. Mean plasma Na and K concentrations in salmon reared in freshwater (FW) and 24 hours after transfer to 20 ppt artificial seawater (SW). 1985-Brood fall chinook; Bonneville Hatchery. Vertical line indicates S.E.; n = 10 - 20.

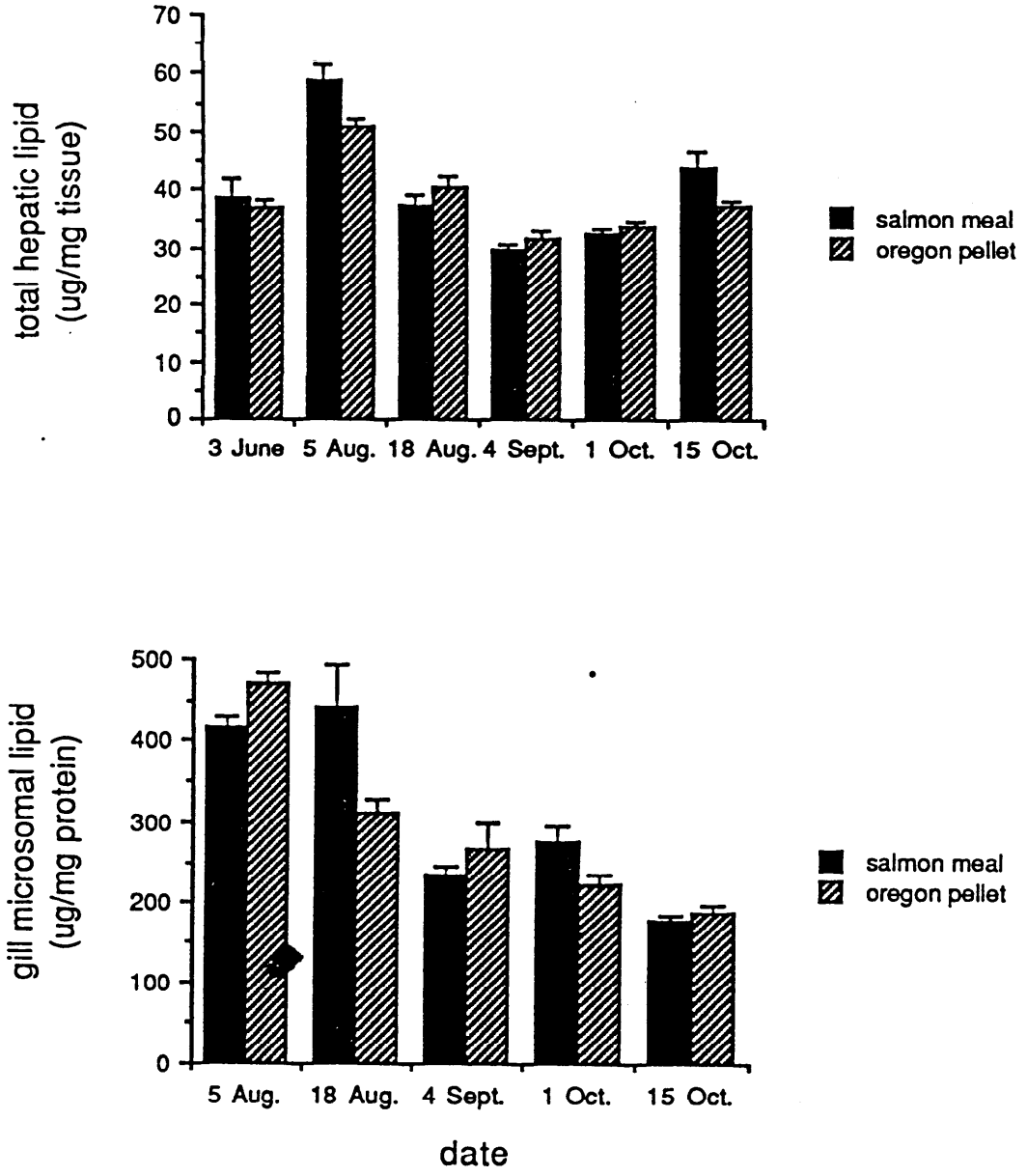

Figure 4. Mean liver and gill lipid content. 1985 Brood fall chinook; Bonneville Hatchery. Vertical line indicates S.E.; n = 10 (hepatic lipid), n = 20 (gill microsomal lipid).

Preliminary Coded Wire Tag Recovery for Coho Salmon (Sandy Stock), Sandy Hatchery; Fall Chinook Salmon (Tule Stock), Bonneville Hatchery

Project releases of coded wire tagged fish to date include the 1982, 1983, and 1984-broods of Sandy stock coho salmon, the 1983 and 1984-broods of tule stock fall chinook salmon and the 1985-brood of up-river-bright stock fall chinook salmon. Preliminary recovery coded wire tags from the 1982 and 1983--broods of coho salmon (Sandy stock) and 1983 and 1984-broods of fall chinook (tule stock) have become available in sufficient numbers for evaluation. Coho recoveries include those from the hatchery (not expanded) and expanded recoveries from the fishery. Recoveries of fall chinook from both the hatchery and fishery were not expanded.

Test ratians, those containing vacuum dried salmon and hake meal, favorably altered the survival of coho salmon, but not in a uniform manner (Table 10). Analysis of variance of the percent of recovered tags released from the 1982 and 1983-broods in a 2 x 3 factorial design revealed that fish supplied either the salmon or hake ration produced superior (P>.05) survival to fish receiving the control OMP ration. The hake ration produced better survival than the salmon ration, but the magnitude of the difference was not significant (P<.05). However, analysis revealed the recovery of tags to be greater (P>.001) for the 1983 than the 1982-brood and a significant (PL.025) ration x brood year interaction was observed. Inspection of individual recovery means revealed the significant interaction to reflect the fact that recovery of tags from test ration fish did not vary from the control for the 1982-brood, but did for the 1983-brood. All individual recovery means for 1983-brood fish were significantly (P=.O5) better than any of the means for the 1982-brood. Although recovery of coded wire tags to date from the 1983-broods of coho indicated that test ration did significantly improve survival, the effectiveness of superior quality rations may be dependent upon the general survival success of a particular brood-year.

Results from the recovery of coded wire tags from the 1983 and 1984-broods of fall chinook (tule stock) are very preliminary at this time (Table 11). The recovery of tagged fish from the 1983-brood appears to show an improved survival by fish supplied the salmon meal ration. However, the low number of recoveries obtained to date for both test and control fish and lack of expanded numbers does not allow a definitive and statisically based conclusion.

The overall survival of the 1984-brood fish appears to be far superior to that of the 1983-brood if the preliminary numbers of recovered two year old fish are compared. Preliminary recovery of 1984-brood two year olds indicates a more favorable recovery of

fish supplied the control over the test ration. This is not a surprising result. The growth response of fish between mid February of 1985 to release in May was compromised in an intermittent manner by poor test ration palatability resulting in reduced feed consumption and conversion. Poor ration palatibility was traced to the herring oil component of the ration derived from one lot of two lots of oil being used to prepare rations. The lot of herring oil producing problems was not oxidized based upon chemical analysis, but contained only traces of antioxidant. It possessed an unusual potential for oxidation when incorporated into the ration. This was the case even when antioxidant protection against autooxidation was strengthened to four times that normally incorporated into the ration.

Table 10. Summary of preliminary tag recovery. Coho salmon; Sandy Hatchery

Brood Year	Ration	Tag Code	Tagged Fish Release	Number Recovered 2-Year	3-Year	4-Year	Total	Percent of Release
1982	OMP	7-29-13	25,763	4	488		492	1.910
		7-29-06	26,983	3	471		474	1.757
		Mean						1.833[a]
	Salmon meal	7-29-12	25,250	4	436		440	1.743
		7-29-09	26,573	7	509		516	1.942
		Mean						1.843[a]
	Hake meal	7-29-10	26,654	5	511		516	1.936
		7-29-07	26,095	3	435		438	1.678
		Mean						1.807[a]
1983	OMP	7-30-45	25,683	19	1,327		1,346	5.241
		7-31-05	26,459	52	1,400		1,452	5.488
		Mean						5.364[b]
	Salmon meal	7-30-48	26,673	66	1,588		1,654	6.201
		7-31-06	25,743	67	1,423		1,490	5.788
		Mean						5.995[c]
	Hake meal	7-30-47	25,493	46	1,538		1,584	6.213
		7-31-07	25,827	53	1,601		1,654	6.404
		Mean						6.309[c]

Significant relationships, 2 x 3 factorial design: percent of release; brood year, $P \geq .001$; ration, $P \geq .05$; brood year x ration interaction, P.025.

Ranking of level means (means with same underline did not vary significantly; P=.05):

Brood year 1982 < Brood Year 1983

OMP < Salmon < Hake

Mean values in a column with same exponent letters did not vary significantly (P=.05).

Table 11. Summary of preliminary tag recovery. Fall chinook salmon (tule stock); Bonneville Hatchery

Brood Year	Ration	Tag Code	Tagged Fish Released	Number Recovered 2-Year	3-Year	4-Year	Total	Percent of Release
1983	OMP	7-31-20	80,348	3	8		11	.014
		7-31-21	80,048	3	16		19	.024
		Mean						.019
	Salmon meal	7-31-22	80,138	4	29		33	.041
		7-31-23	81,282	5	21		26	.032
		Mean						.037
1984	OMP	7-33-22	78,367	125			125	.160
		7-33-23	78,962	150			150	.190
		Mean						.175
	Salmon meal	7-33-24	80,242	54			54	.067
		7-33-25	79,750	48			48	.060
		Mean						.064

APPENDIX I.

Water Temperatures (^{o}F) by the Month at Sandy Hatchery

	1983			1984			1985		
Month	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.
January	44	42.5	43.3	42	40	41	39	37	38
February	45	44	44.5	45	42.5	44	39	37	38
March	47	45	46	47	44	45.5	43	40	41.5
April	49.5	45	47	47.5	44	45.8	48	44	46
May	54.5	49.8	52.1	51	47	49	52	47	49.5
June	56	52	54	54	50	52	56	51	53.5
July	56.6	53.4	55	61	54	57.5	66	58	62
August	61	56	58.5	62	56	59	61.4	55.2	58.3
September	60	45	54	60	48	54	59	48	53.2
October	51	47	49	49	47	48	49	46	47.5
November	47	46	46.5	45	44	44.5	41	40	46.5
December	41	39	40	41	40	40.5	37	36	36.5

APPENDIX II.
Ration Formulation

Component	Control ration	Test Rations	
		Hake meal	Salmon meal
Fish meal	28.0 (min)[1]	40.0-48.4[14]	37.7-41.4[14]
Cottonseed meal	15.0[2]	0.0	0.0
Dried whey product[3]	5.0	2.0	2.0
Wheat germ meal[4]	Remainder	Remainder	Remainder
Corn distillers solubles[5]	4.0	0.0	0.0
Trace mineral premix[6]	0.1	0.1	0.1
Vitamin premix[7]	1.5	1.5	1.5
Spray dried blood meal[8]	0.0	2.0	2.0
Sodium bentonite	0.0	2.0	2.0
Concentrated hydrolyzed fish[9]	0.0	19.7-22.3	19.7-22.3
Choline chloride[10]	0.5	0.5	0.5
Pasteurized wet fish[11]	30.0	0.0	0.0
Fish oil	6.0-6.75[12]	1.8-7.5[13]	7.7-10.6[13]
Water	0.0	8.1-12.6	8.1-10.5
Total	100.0	100.0	100.0

[1]Herring meal (min. 67.5% protein) used at no less than 50% of the fish meal in each batch. Anchovy (min. 65% protein), capelin (min. 67% protein), or hake (min. 67% protein) meals may be used as the remainder. Level to supply not less than 21.5% fish meal protein; max. 5% NaCl; 8-12% fat; max 17% ash.

[2]Preprocessed, solvent extracted, min. 48% protein, max 0.055% free gossypol.

[3]Min. 12% protein, max. 6% moisture, max. 10% ash, max. 3% salt

[4]Min. 23% protein and 7% fat

[5]May contain up to 30% "grains" in place of solubles

[6] Gm/lb: Zn,34.00 ($ZnSO_4$, 84.g/lb); Mn, 34.00 ($MnSO_4$, 94 g/lb); Fe, 4.50($FeSO_4.7H_2O$, 22.5 g/lb); Cu, 0.70 ($CuSO_4$, 1.75 g/lb); I, 0.23 (KIO_3 0.38 g/lb); diluted to 1.00 lb with cereal product.

[7] Mg/lb: d-biotin, 18.0; vitamin B_6 535.0 (pyridoxine.HCl, 650 mg); B_{12}, 1.8; vitamin C, 27,000.0 (ascorbic acid); vitamin E, 15,200.0 (water dispersible alpha tocopheryl acetate): folacin, 385.0 (folic acid); Myo-inositol, 4000.0 (not phytate); vitamin K, 180.0 (menadione sodium bisulfite complex, 545 mg); niacin, 5700.0; d-pantothenic acid, 3200.0 (d-calcium pantothenate, 3478 mg or d,l-calcium pantothenate, 6957 mg; riboflavin, 1600.0; thiamine, 715.0 (thiamine mononitrate, 778 mg); dilute to 1.0 lb with cereal product

[8] Spray dried whole blood

[9] Concentrated bone-free hydrolysate of salmon carcasses, groundfish carcass waste and whole Pacific hake

[10] Liquid, 70%

[11] Two or more of the fol lowing , with none exceed@ 50% of the combination; (1) Salmon or tuna viscera (no heads or gills, with livers) ; (2) whole herring; (3) bottom fish (whole or fillet scrap); (4) dogfish: (5) whole hake; and (6) whole salmon. Approved enzymes used to aid liquefaction.

[12] Herring, salmon, menhaden, dogfish (not more than 3%) or refined tuna oil; stabilized with 0.4% BHA-BHT (1:l); free fatty acids not more than 3%; BHA-BBT must be added at the time of reprocessing if reprocessed oil is used. Special condition when using hake as a wet fish: add 0.5% oil for every 10% hake in total ration.

[13] Herring oii; stabilized with 0.02% BHA-BHT (1:l); free fatty acids not more than 3%.

[14] Vacuum dried

Ethoxyquine antioxidant protection: control ratio, 0.009% in the ration; test rations, 0.015% of the ration excluding concentrated hydroiysed fish, choline chloride, fish oil, and water components.

APPENDIX III.
Growth Response: 1984-Brood Coho

Ration:	OMP		Salmon meal		Hake meal	
Pond:	5	13	4	12	6	16
Date feeding initiated	6/11/85	6/11/85	6/11/85	6/11/85	6/11/85	6/11/85
Initial av. fish wt. (g)	2.293	2.293	2.293	2.293	2.293	2.293
Initial av. fish wt. (fish/lb)	197.816	197.816	197.816	197.816	197.816	197.816
Release av. fish wt. (g): Mean	31.997	31.851	32.786	33.145	31.240	31.074
S.D.	.156	.054	.238	.289	.370	.217
Release av. fish wt. (fish/lb)	14.176	14.241	13.835	13.685	14.520	14.597
Fish release length (mm): Mean	142.916	142.257	142.479	143.538	141.285	141.096
S.D.	7.077	7.172	7.076	6.900	7.180	7.575
Var.	50.086	51.436	50.068	47.610	51.552	57.376
n	691	673	729	645	627	658
Inital No. fish	58490	58492	58489	58458	58646	58486
Date tagged	10/18/85	10/16/85	10/18/85	10/16/85	10/17/86	10/11/86
Binary code	7-37/46	7-36/20	7-37/45	7-36/19	7-36/18	7-36/23
Release date	4/30/86	4/30/86	4/30/86	4/30/86	4/30/86	4/30/86
No. fish released	57865	57893	57767	57430	57899	57860
Recognizable tags released	27623	27974	28079	27115	27489	27542
Feed consumed (kg): Wet wt.	2480.243	2454.842	2159.100	2070.649	2336.455	2269.323
Dry wt.	1816.370	1797.768	1597.340	1532.409	1722.341	1673.890
Protein	905.620	896.275	880.170	844.223	919.504	893.759
Body composition (%): Moisture	73.716	73.947	73.282	73.375	73.449	73.705
Ash	2.341	2.326	2.304	2.216	2.375	2.344
Fat	7.550	6.918	8.169	8.253	7.849	7.716
Protein	17.195	16.990	17.097	16.793	16.792	16.947
Condition factor[1]	1.0961	1.1064	1.1335	1.1208	1.1077	1.1062
Blood hematocrit (%): Mean	33.75	34.50	34.75	40.08	37.50	36.33
S.D.	2.24	2.36	2.52	2.25	2.96	2.17
n	12	12	12	12	12	12
Total No. fish lost	625	599	722	1028	747	626
Mortality (%)	1.069	1.024	1.234	1.759	1.274	1.070

[1] $[100{,}000 \times \text{fish wt. (g)}]/[\text{length (mm)}^3]$

APPENDIX IV.
Growth Response: 1985-Brood Fall Chinook

Ration	Salmon meal		OMP	
Pond No. (4/12-6/9/86)	C-3	C-4	C-5	C-6
Pond No. (6/9-8/12/86)	A-8	A-9	A-12	A-13
Pond No. (8/12-10/17/86)	C-3	C-4	C-5	C-6
Initial No. fish (4/12/86)	199474	199585	201935	200118
No. fish before first split (6/9/86)	198979	199159	201327	199707
No. fish after first split (6/9/87)	100441	102000	103130	103260
No. fish before second split (8/12/86)	100124	101688	102832	103015
No. fish after second split (8/12/86)	48261	47897	47472	49085
No. fish released (10/17/87)	47980	47630	47042	48795
No. fish released w/recognizable tag	46852	47250	46579	47265
Date tagged	8/19/86	8/20/86	8/21/86	8/25/86
Tag code	7-36-35	7-36-36	7-37-52	7-37-53
Initial fish size (g) (4/12/86)	.963	.909	.933	.867
Fish wt. before first split (g) (6/9/86)	4.637	4.447	3.965	3.917
Fish wt. after first split (g) (6/9/86)	4.637	4.447	3.965	3.917
Fish wt. before second split (g) (8/12/86)	14.013	14.168	13.700	13.197
Fish wt. after second split (g) (8/12/86)	14.013	14.168	13.700	13.197
Fish release wt. (g/fish): Mean	33.671	34.485	30.731	30.768
S.D.	.533	.955	.274	.519
Fish release wt. (fish/lb): Mean	13.475	13.164	14.761	14.746
S.D.	.213	.369	.131	.250
Body composition (%): Moisture	75.41%	74.48%	75.21%	76.36%
Ash	1.98%	2.00%	2.03%	1.98%
Fat	7.70%	8.19%	7.35%	6.58%
Protein	15.74%	16.02%	16.16%	15.98%
Feed (kg wet wt.) (4/12-6/9/86)	610.082	605.546	687.193	691.728
Feed (kg wet wt.) (6/9-8/12/86)	986.564	1000.171	1052.334	1133.981
Feed (kg wet wt.) (8/12-10/17/86)	975.224	988.832	1106.766	1106.766
Feed (kg wet wt.) (4/12-10/17/86)	2571.869	2594.549	2846.293	2932.475
Feed (kg dry wt.) (4/12-6/9/86)	442.190	438.786	500.659	503.900
Feed (kg dry wt.) (6/9-8/12/86)	709.314	719.030	758.742	817.583
Feed (kg dry wt.) (8/12-10/17/86)	700.323	710.085	795.875	795.875
Feed (kg dry wt.) (4/12-10/17/86)	1851.827	1867.901	2055.275	2117.359
Feed protein (kg) (4/12-6/9/86)	249.202	247.233	268.949	270.601
Feed protein (kg) (6/9-8/12/86)	400.492	405.899	395.487	426.110
Feed protein (kg) (8/12-10/17/86)	390.580	395.942	411.053	411.053
Feed protein (kg) (4/12-10/17/86)	1040.274	1049.074	1075.489	1107.763
Hematocrit (%): Mean	25.583	28.250	27.000	31.583
S.D.	3.475	3.854	2.041	6.958
Fish length (mm): No. measured	658.000	645.000	655.000	688.000
Mean	140.913	142.614	136.655	137.188
S.D.	9.593	8.783	11.241	11.493
Condition factor[1]:	1.203	1.189	1.204	1.192
Mortality:				
No fish lost 4/12-6/9/86	495	426	608	411
No. fish lost 6/9-8/12/86	317	312	298	245
No. fish lost (8/12-10/17/86)	281	267	430	290
Weighted mortality (%)	.313922	.287568	.378967	.268397

[1] $[100{,}000 \times \text{fish wt. (g)}]/[\text{length (mm)}^3]$

OREGON DEPARTMENT OF FISH AND WILDLIFE

FISH EXAMINATION

SOURCE Sandy

EXAM DATE(S) 5/30/85

ADDRESS

COMPLETION DATE 5/30/85

SAMPLE ~~11.84 Co~~ ~~250/lb~~

Lot No. - Species - Fish Size

REASON FOR EXAM: ☐ Certification ☐ Increased Loss ☐ Preliberation ☐ Routine

☒ Other prelib p 17 & belated pretransfer to S. Santiam

SIGNS OF DISEASE:

None; loss very low at 5/day/pond and no evidence of any CWD epizootic.
Fish all final split and thin into all ponds.

RESULTS:

No disease

RECOMMENDATIONS:

Liberate 17. So far excellent looking fish.

See Reverse

c: Source
Pathology - Clackamas
Pathology - Corvallis
Fish Culture - Portland
Regional Office
Other S. Santiam

6/4/85
Date

Terry Kreps
Pathologist(s)

Appendix V (Continued)

Previous Diseases, Treatments, Stress Conditions of This Fish Stock:

Slight and controlled via furox CWD epizootic.

Diet _____ Water Temperature 52 F Loss 5/day/pond

Microscopic Exam:

Gill structure good; no bugs

Culture Exam:

ND--no loss

Tissues ☐ Kidney ☐ Gill ☐ Other _____

Media ☐ TSA ☐ BHI ☐ Cytophaga ☐ Other _____

Additional Comments:

Appendix V (Continued)

OREGON DEPARTMENT OF FISH AND WILDLIFE

FISH EXAMINATION

SOURCE Sandy

EXAM DATE(S) 7/30/85

ADDRESS

COMPLETION DATE 8/6/85

SAMPLE 11.84 Co 70/lb

Lot No. - Species - Fish Size

REASON FOR EXAM: ☐ Certification ☒ Increased Loss ☐ Preliberation ☐ Routine

☐ Other

SIGNS OF DISEASE:

Columnaris gill lesions and body "scruffy" lesions. Loss apparently confined to black and blue diets only (salmon meal and hake meal) loss normal at 1-5/day in the other diet groups. 80% of the loss show columnaris symptoms.

RESULTS:

Columnaris on gills and scruffy body areas. Currently confined to the 4 ponds on the salmon and hake meal.

RECOMMENDATIONS:

TM-50 at 45 grams/100 lbs fish/day for 21 days. Treat only those ponds with increased loss. Ponds with normal low loss do not treat unless loss starts increasing. As of 8/6 loss declining in the 4 ponds and no increase in loss in other ponds. Due to unusual wet pellets and poor binding consistency in the black and blue diets, diet remade and TM-50 incorporated at 5% level rather than the "roll your own" approach. Loss normal as of 8/10.

See Reverse

c: Source
Pathology - Clackamas
Pathology - Corvallis
Fish Culture - Portland
Regional Office
Other

8/9/85
Date

Terry Kreps
Pathologist(s)

Previous Diseases, Treatments, Stress Conditions of This Fish Stock:

None since CWD in April-May

Diet various Water Temperature 60-70 F Loss black & blue diets 20-40/day other diets 1-5/day

Microscopic Exam:

Gill structure good; no "bugs" except for haystacks of columnaris in the gill lesion areas.

Culture Exam:

Tissues [x] Kidney [ ] Gill [ ] Other _____

Media [x] TSA [ ] BHI [x] Cytophaga [ ] Other _____

Cultures show columnaris, no other pathogens. This time columnaris grew out strongly on TSA which is a bit unusual. This has also been noted with CWD on occasion.

Additional Comments:

Appendix V (Continued)

OREGON DEPARTMENT OF FISH AND WILDLIFE

FISH EXAMINATION

SOURCE Sandy

EXAM DATE(S) 9/18/85

ADDRESS

COMPLETION DATE

SAMPLE 11.84 Co 38/1b

Lot No. - Species - Fish Size

REASON FOR EXAM: ☐ Certification ☒ Increased Loss ☐ Preliberation ☐ Routine

☒ Other analy is of recirculated water

SIGNS OF DISEASE:

A few crinkle back dropout runts by the screens of ponds 8, 9, & 10. These 3 ponds probably get most of the recirculated water. Ponds 1 through 7 get fresh water and some recirc. They and the other bank which is straight freshwater do not show these crinkleback fish.

RESULTS:

CWD & Costia

RECOMMENDATIONS:

Number of crinklebacks is low and is probably an aftermath of the spring cwd epizootic. Recommend dipping these out once a morning to eliminate the Costia "Reservoir."

See Reverse

c: Source
Pathology - Clackamas
Pathology - Corvallis
Fish Culture - Portland
Regional Office
Other

10/1/85
Date

Terry Kreps
Pathologist(s)

Appendix V (Continued)

Previous Diseases, Treatments, Stress Conditions of This Fish Stock:

Diet _____ Water Temperature 48-55 F Loss normal

Microscopic Exam:

Good fish good gill structure--no bugs
crinkleback fish, fair gills moderate to heavy Costia

Culture Exam:

Tissues [X] Kidney [ ] Gill [ ] Other _____

Media [x] TSA [ ] BHI [x] Cytophaga [ ] Other _____

cwd grew out as expected

Additional Comments:

Based on gill structure, conversion food factor of 1.4, low loss etc.; there is no definite direct adverse effect of recirculation. The hatchery crew, however, did note the physical activity (feeding, etc.) of the recirc. pond fish was less than that of the corresponding nonrecirc. straight pass ponds.

Appendix V (Continued)

OREGON DEPARTMENT OF FISH AND WILDLIFE

FISH EXAMINATION

SOURCE Sandy

EXAM DATE(S) 4/18/86

ADDRESS

COMPLETION DATE 4/18/86

(5/1/86)

SAMPLE 11.84 Co 15/1b

Lot No. - Species - Fish Size

REASON FOR EXAM: ☐ Certification ☐ Increased Loss ☒ Preliberation ☐ Routine

☐ Other

SIGNS OF DISEASE:

Fish look very good. Loss at 0-1/day last 3 months to current lib on 5/1 on 86. If BKD is present in the Sandy system, it has not created any acute epizootic.

RESULTS:

No disease in evidence. VEN exam pending on future report.

RECOMMENDATIONS:

Liberate

See Reverse

c: Source
Pathology - Clackamas
Pathology - Corvallis
Fish Culture - Portland
Regional Office
Other

5/22/86
Date

Terry Kreps
Pathologist(s)

Previous Diseases, Treatments, Stress Conditions of This Fish Stock:

No disease problems with this brood from initial ponding to current lib except for minor cwd at ponding in April-May 1985.

Diet _____ Water Temperature 50 F Loss 0-1/day

Microscopic Exam:

Gill structure very good; no "bugs!'
Body scrapings negative.

Culture Exam: ND No loss

Tissues q Kidney ☐ Gill ☐ Other _____

Medi a ☐ TSA ☐ BHI ☐ Cytophaga q Other _____

Additional Comments:

Coho transferred from Sandy to S. Santiam did apparently have a problem with chronic cwd and also VEN EIB's found in blood smears.

OREGON DEPARTMENT OF FISH AND WILDLIFE

FISH EXAMINATION

SOURCE Bonneville Hatchery

EXAM DATE(S) 10/3/86

ADDRESS

COMPLETION DATE 10/8/86

SAMPLE 95.85 ChF 14f/lb.
Lot No. - Species - Fish Size

REASON FOR EXAM: Certification Increased Loss Preliberation ☐ Routine

☐ Other

SIGNS OF DISEASE:

Increased loss in C-l, URB-ChF smolts. Dying fish have pale gills, and some have fungused tails or damaged eyes. Few with CWD lateral body wall lesion.

RESULTS:

Blood cell virus (VEN) and CWD epizootic. Multiple and large single inclusions in 16 of 19 fish blood smears. Extremely heavy levels of inclusions, i.e., inclusions/field in 12 of 19 fish. Active as well as anemic fish had much inclusions in their blood cells.

RECOMMENDATIONS:

T. Kreps recommended Furox medicated food at 50g/100lbs fish/day for 10 days for the CWD infish in raceway C-l.

See Reverse

cc: Source
Pathology - Clackamas
Pathology - Corvallis
Fish Culture - Portland
Regional Office Columbia
Other H. Hansen, J. Lagasse - Sandy

11/4/86
Date

Richard Holt
Holt and Kreps
Pathologist(s)

NOV 0 7 1986

Previous Diseases, Treatments, Stress Conditions of This Fish Stock:

Diet Biomoist **Water Temperature** 49-50°F **Loss** up to 300/day in pond C-1

Microscopic Exam:

Pinacyanol chloride stain of blood smears - (9 apparently healthy fish and others anemic) 19 smears were examined for inclusions: 12 were extremely heavy for inclusions (1-10 inclusions/field), 3 fish were moderate (every 3rd-6th field had 1 inclusion) and 4 blood samples were negative. Th ose smears with a lot of inclusions had both multiple basophilic types and large rose colored inclusions.

Culture Exam:

Tissues [X] Kidney [ ] Gill q Other Brain

Media q TSA [ ] BHI [X] Cytophaga [ ] Other

17 dead fish cultured:

Fish #l-7: Cultures from brain: all positive for C. psychrophila (on C.A.).
#8-11: Cultures from kidney: all positive for?. psychrophila on C.A.
812-17: Cultures from kidney: all positive for?. psychrophila on TSA.
These isolates grew very well on TSA.

Additional Comments:

Appendix VI (Continued)

OREGON DEPARTMENT OF FISH AND WILDLIFE

FISH EXAMINATION

SOURCE Bonneville 'Hatchery

EXAM DATE(S) 10/10/86

ADDRESS

COMPLETION DATE 10/17/86

SAMPLE 95.85 ChF 14f/lb. C - -
Lot No. - Species - Fish Size

REASON FOR EXAM: c l Certification c X l Increased Loss [X] Preliberation c l Routine

[X] **Other Check for prevalence of blood cell virus.**

SIGNS OF DISEASE:
CWD and VEN diagnosed in C-l on 10/3/86; since then slight increased loss in C-2, B-27 and B-30 (30 fish/day). Dying fish have signs of CWD, i.e., anemic gills, or no external signs, 10% of dead fish have fungused tails, also some damaged eye. In general, active fish look okay, most raceways show no loss.

RESULTS:
CWD and VEN in C-l,C-2, B-27, B-30.
Blood cell virus in fish in C-1, C-2, C-4, C-S, C-13, B-27, B-30
B-28 no VEN detected.

RECOMMENDATINS:
Because of construction, fish must be liberated. VEN apparently quite prevalent throughout Batterys B & C in URB ChF. Losses remain very low in all groups except C-l, 6-2, B-27 and B-30, all of which have CWD. So as per Kreps' recommendation feed Furox medicated food to those groups where loss occurring and liberate as scheduled. ChF in Battery A intended to be held until spring - should be checked for VEN. If at all possible effluent from ChF (95.85's) ponds should not be passed over yearling coho.

See Reverse

cc: Source
Pathology - Clackamas
Pathology - Corvallis
Fish Culture - Portland
Regional Office Columbia
Other H. Hansen, J. Lagasse, J. Westgate

11/4/86
Date

Holt and Amandi
Pathologist(s)

NOV 07 1986

Appendix VI (Continued)

Previous Diseases, Treatments, Stress Conditions of This Fish Stock:
C-l receiving Furox medicated food for CWD.

c-1,2 = Biomoist
c-3,4 = Salmon meal
Diet C-5,6 = OP2 Water Temperature Batt B - 47°C, Batt C - 49-50°F loss C-l 100-200/day, B-30 30/day
Most OP2

Microscopic Exam:
Pinacyanol chloride stain of blood smears - healthy fish collected from several raceways. See below.

Culture Exam:
Tissues q Kidney ☐ Gill ☐ Other _____

Media [X] **TSA** ☐ **BHI** [X] **Cytophaga** ☐ **Other** _____

Raceway	No. fish (morts)	Results
c-2	8	4 positive for C. psychrophila on Cyto agar and TSA both.
B-27	6	5 positive for C. psychrophila on Cyto agar and TSA both.
B-30	6	6 positive for C. psychrophila on Cyto agar and TSA both. –

Blood Cell Virus check

Diet	Raceway	No. Fish	Results
Biomoist	C-l	10	9 fish had 1-10 affected rbc per field. 1 had one inclusion in 3-5 fields.
Biomoist	c-2	8	4 fish - l-10 inclusions/field 2 fish moderate levels (1 inclusion every 3-5 fields). 2 fish negative
Salmon meal	C-4	7	3 fish - l-5 inclusions/field. 3 fish few to moderate inclusions (1 inclusion/5 fields). 1 fish negative
OP2	c-5	10	8 fish - l-5 inclusions/field 1 fish - 1 inclusion/2 fields 1 fish - 3 inclusions total
OP2	c-13	10	2 fish few inclusions 8 fish negative
OP2	B-27	10	1 fish - 2-3 inclusions/field 2 fish moderate levels (1 inclusion/2-5 fields). 7 fish negative.
OP2	B-28	10	All negative.
OP2	B-30	7	6 fish (l-10 inclusions/field) 1 fish negative.