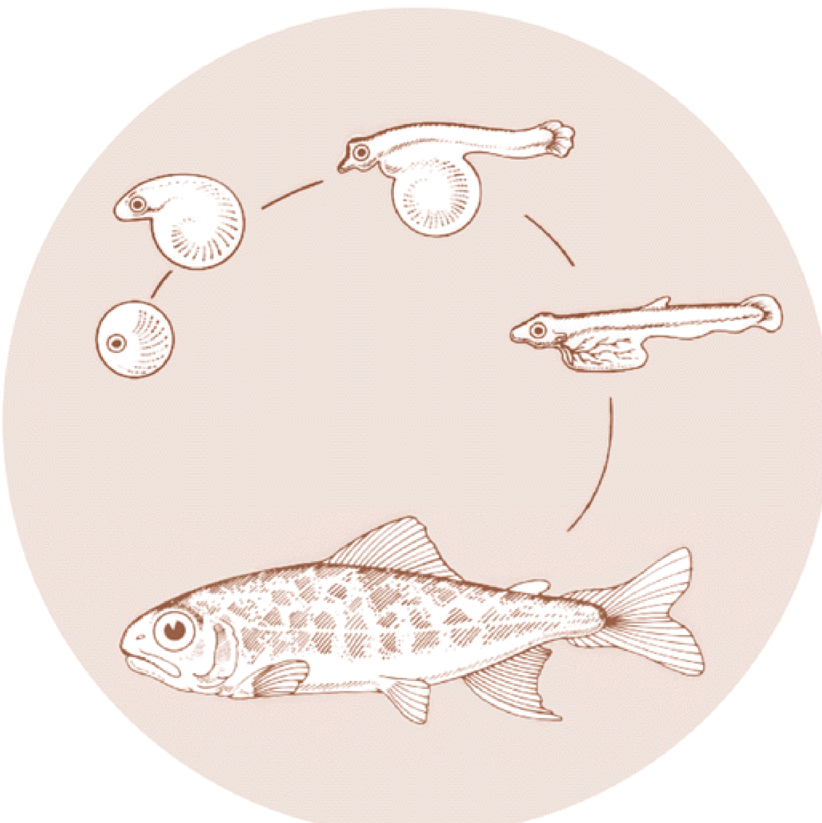
March 1990

DEVELOPMENT OF RATIONS FOR THE ENHANCED SURVIVAL OF SALMON

Annual Report 1998



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DEVELOPMENT OF RATIONS FOR THE ENHANCED SURVIVAL OF SALMON

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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, may be reduced in quality from poor drying techniques during manufacture. Dietary stress in the hatchery may result. This investigation tests the hypothesis that protein quality of fish rations can influence the survival of smolts and the ultimate return of adults. The test involves a comparison between performances of coho (Oncorhvnchus kisutch) and chinook salmon (0. tshawvtscha) reared on rations containing very high quality protein derived from vacuum dried meals and those of fish reared on commercial rations, with commercial fish meal as a source of protein. Survival and return of several brood years of test and control fish are used to measure the influence of ration on survival.

Rearing and release of tagged fish to date include 1982, 1983, 1984 and 1985 broods of coho salmon (Sandy stock); the 1983 and 1984 broods of fall chinook (tule stock) salmon; and the 1985 and 1986 broods of fall chinook (upriver bright stock) salmon. This report includes recovery data from these marked fish collected through September 1989.

Recovery data of coho salmon suggested an improved survival for fish supplied test rations. Recovery rates varied significantly ($P \leq 0.05$) by brood year and ration treatment. The interaction of ration and brood year was not significant. Recovery data (as of September 1989) of fall chinook salmon did not suggest a significantly greater survival rate for fish supplied test rations. Brood year and the interaction of ration and brood year were significant. Also, the control ration produced better survival for the 1984

brood fall chinook salmon. This latter result was due to the use of an unpalatable test ration for three months prior to release, The fish oil supplement used was highly susceptible to autooxidation, could not be stabilized with antioxidants, and quickly became rancid.

INTRODUCTION

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

Time of release, natural abundance of food, fish size, and health or "fitness" play important roles in determining survival of hatchery-reared smol ts and their ultimate return as adult fish. It is believed that nutritional quality determines the health of smolts. Ration regimes containing high quality components in uniform and fine-free pellets produce good fish growth and minimize loss of nutrients, resulting in fish that are less susceptible to disease and of more uniform size at release. Smolts produced by these high quality feeds are thought to migrate rapidly to the sea and successfully adapt to salt water.

Quality in fish feed is determined in large part by its protein complement. Protein is the major food component in fish rations. The most successful fish rations rely on large quantities of fish protein in the form of fish meal. Plant sources of protein, such as soybean and cottonseed meal, are tolerated to a certain extent, but an excessive replacement of fish protein with plant protein results in a reduction in feed consumption, conversion, and/or weight gain. This reduction creates a dietary stress that affects smolt 'fitness."

Commercial fish meal supplies used to formulate successful rations are declining in availability and quality. Industrial round (whole) fish that, in the past, formed the raw material base for high quality meal is no longer available because of costs and regulations dicating its use for human food. Carcass waste is replacing round fish as a raw material. The resulting meals have a lower protein content and an elevated mineral level because of the removal of muscle tissue for human food. The majority of fish meals are produced by high-temperature direct-flame dryers. Excessive heating damages the proteins and initiates lipid-protein interactions. Both of these effects reduce the biological value of fish proteins.

Meals and fish protein concentrates produced from round fish and/or fish-processing waste using processes with low temperatures and reduced pressures yield protein of optimum quality. These gentle drying and concentration procedures coupled with the use of fat antioxidants limit heat damage to proteins and lipids, and markedly reduce lipid-protein interactions. Ration regimes that incorporate these sources of protein are more costly, but additional feed costs may be offset by the greater survival of smolts and increased return of adult fish. Efficiency of hatchery production would thus be improved.

The basic hypothesis of this investigation is that protein quality of the rations can influence the survival of smolts and the return of adult salmon to the Columbia River basin. The general approach to test this hypothesis involves the rearing of coho (Oncorhvnchus kisutch) and chinook (0. tshawytscha) salmon on rations containing high quality fish protein. Fish reared on hatchery rations with commercial fish meals as a source of protein were used as controls. Coded-wire tagging experiments were 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 (upriver bright stock) salmon were assessed for physiological changes associated with smoltification and correlated with ration type and smolt "fitness." These physiological changes are discussed in other reports.

Project rearing and release of tagged fish to date include 1982-, 1983-, 1984-, and 1985-brood coho salmon, the 1983-, and 1984-brood fall chinook salmon (tule stock) and the 1985- and 1986-brood fall chinook salmon (upriver bright stock). This report includes preliminary recovery data on these release groups collected through September 1989.

METHODS AND MATERIALS

General Project Ooeration

This project combines the facilities and expertise of the Seafoods Laboratory of the Department of Food Science and Technology, Oregon State University, and the Oregon Department of Fisheries and Wildlife (ODFW). ODFW carried out fish husbandry tasks involved in feeding trials at their Sandy and Bonneville hatcheries and conducted coded-wire tagging of experimental and control groups. Acquisition and production of ration components and manufacture of test rations were carried out at the Seafoods Laboratory. The Department of Fisheries and Wildlife, Oregon State University, and ODFW examined physiological changes due to diet during the course of Parr-smolt transformation in the fall chinook.

Formulation and Production of Test Feeds

The usual hatchery supply of Oregon Moist Pellets (OMP) 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 OMP. Coho salmon were supplied with two test rations deriving their major protein complement from vacuum-dried carcasses of salmon collected from hatcheries and from vacuum-dried round Pacific hake. A test ration containing vacuum-dried salmon meal as the major protein source was supplied to fall chinook. Protein complements in the test diets were supplemented by hydrolyzed and vacuum-dried salmon carcasses.

Ration Component Production and Acquisition

Advanced Hydrolyzing Systems, Inc. of Astoria, OR, in cooperation with the Seafoods Laboratory, produced high-quality vacuum-dried meal, using the facilities, power, and steam of the Seafoods Laboratory. Concentrated hydrolysates were produced-in the company's own facilities. Salmon carcasses were provided by ODFW. Hake and groundfish carcasses were purchased on the open market.

Fish meals were prepared by placing coarse ground fish into a steam-jacketed chamber equipped with a stirring-scraping device and subjecting the meal to a vacuum of 25-27 inches of Hg. Product temperature was maintained at 101-105 °F except for pasteurization, when the temperature of the moist feed was raised to 180 °F for 5.0 minutes. All vacuum-dried meals, if not used immediately for ration preparation, were sacked and held frozen at ≤0 °F.

Concentrated fish hydrolysates were prepared by exposing coarse ground fish to a temperature of approximately 140 °F with mechanical agitation until sufficient liquefaction was achieved to allow screen removal of bones. The temperature of the liquefied material was raised to 180 °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 frozen before storage at ≤0 °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 OMP.

Test Ration Formulation and Production Protocol

Test rations were formulated to contain 28 lbs of protein derived from meal and 7.7 lbs of protein from concentrated hydrolyzed salmon for each 100 lb of ration. Water and wheat germ meal were added to yield rations with 76% solids (24% moisture). Herring oil was added in amounts needed to provide a fat:protein caloric ration of 0.95 (protein = 4.0 kcal/g, fat = 9.0 kcal/g). A computer controlled the percentage of vacuum dried meal and concentrated hydrolyzed salmon or hake used for each batch of ration. 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 placed in 50 lb sacks and held frozen at 0 to -30 °F if not immediately used to prepare rations.

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 pellets of the desired length and diameter, screened to remove fines, placed into 40 lb (1/32-inch pellets only) or 50 lb sacks, 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 consumption, protein consumption, and conversion. Random samples from all pellet sizes and production dates (if possible) were taken from the control rations. -Test rations were sampled during production. At least two samples were selected for analysis from each 150-250 lb batch. The composition of a particular lot of pelletized feed was estimated from the mean of all samples from that lot. The mean composition of each pellet size of control ration was used to compute dry weight and protein consumption and conversion.

Husbandry Protocol

Coho Salmon (Sandy Hatchery): Coho salmon (Sandy stock) were reared in 20 x 80 x 4 ft (variable depth) raceways with a 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 that varied from 38 to 59OF (four 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.

Groups of 600,000 to 650,000 unfed fry were placed in one pond during late March or early April at about 1,100 fish/lb (0.4 g/fish). Fish were supplied starter ration and progressed through the pellet size guide for salmon recommended by the ODFW for moist pelletized feeds:

Pellet size (in.)	Fish size	
	fish/lb	g/fish
Starter	1,000-700	0.4- 0.6
1/32	700-500	0.6- 0.9
3/64	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 at 195 to 212 fish/lb (2.1 to 2.3 g/fish) were randomly distributed in 10-lb lots into six ponds on April 30 to June 11. Final numbers were 54,000 to 60,000 fish per pond. Control rations and two test rations were randomly assigned to provide duplicate ponds for each ration type.

Control rations and two test rations were supplied to fish for about 10 months from June to release on April 30. 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)
1,200-800	8-10
	6
500-250	4
250-150	3
150- 15	1-2

Control fish were supplied feed according to a feeding guide which schedu'led 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 (Bonneville Hatchery): At Bonneville Hatchery, fall chinook salmon (upriver bright and tule stocks) reared in well water (49-51'F) in 17.5 x 75 x 3 ft. raceways (3,948 cu ft.; 29,456 gal.). Flow rate was gradually increased from 300 gpm/pond for swim-up fry to 550 gpm/pond for fingerlings. Maximum loading occurred at 6 lbs of fish/gpm at liberation.

Approximately 600,000 unfed fry of the tule stock were stocked in each pond in late December at an average size of 750 to 1,100 fish/lb (0.4 to 0.6 g/fish). About 200,000 to 400,000 unfed fry of the upriver bright stock were stocked in each pond in February or March at an average size of 980 to 1,060 fish/lb (0.4 to 0.5 g/fish). Tule stock were fed on a demand basis until release in early May. Upriver bright stock were fed at a rate designed to achieve a target release size of 13 fish/lb in mid-October. The 1986-brood fish were liberated early due to an emergency low water supply and did not reach the target size. Control and test fish were initially supplied starter rations and then progressed through the pelllet size guide recommended by the ODFW for moist pelletized feeds listed above.

Pathological Assessment

ODFW 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.

Physiological Assessment

Methodology and results are reported in past reports. No further analyses were provided during the past year.

Growth Response Parameters

Fish weight, feed consumption, feed conversion and mortality information were 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 were collected for the determination of body composition.

Mean fish weight and length were 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 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. Determinations of body composition were based upon the means of duplicate analyses of three randomly selected samples of ten fish from each replicate.

The emergency release of 1986-brood fall chinook salmon (upriver bright stock) from Bonneville hatchery precluded the above sampling schedule. One sample from each replicate pond (293-329 fish each) was obtained at release and immediately frozen. Fish were thawed, weighed, and measured. Pooled samples were used to determine body composition. Blood hematocrit levels were not determined in these groups.

Coded-Wire Tagging Experiments

Groups of 25,000 to 31,000 coho salmon in each experimental and control replicate were tagged and marked with an adipose fin clip during September or October. 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. Tule stock fall chinook salmon were similarly tagged and marked in April. Groups of 75,000 to 80,000 fish were marked in each replicate of control and test fish. Upriver bright stock of fall chinook salmon were tagged and marked in August. Groups of about 32,000 to 47,000 fish were marked in each replicate of control and test fish. Fish were randomly selected using a procedure similar to that used for coho salmon. Tag retention in fish from each replicate was determined prior to release, except for the emergency release of 1986-brood fall chinook salmon. In these groups, tag retention numbers were determined from frozen samples used to determine weight and length measurements.

Analysis of Recovery Data

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. Data were converted from percent to arcsin derivatives for analysis. All statistical comparisons were made at the significance level of $P<0.05$.

RESULTS AND DISCUSSION

Releases of coded-wire tagged fish to date include the 1982, 1983, 1984 and 1985 broods of coho salmon (Sandy River stock, Sandy Hatchery), the 1983 and 1984 broods of fall chinook salmon (tule stock, Bonneville Hatchery) and the 1985 and 1986 broods of fall chinook salmon (upriver bright stock, Bonneville Hatchery). Preliminary recoveries of coded-wire tags from the 1982, 1983, 1984, and 1985 broods of coho salmon and 1983, 1984, 1985, and 1986 broods of fall chinook salmon through September of 1989 have been described in earlier reports. Recoveries include those from the hatchery and from the fishery. The recoveries from the fishery may or may not have been expanded depending upon the fishery and the brood year.

Test rations, those containing vacuum-dried salmon and hake meal, appear to alter the survival of coho salmon, but not in a uniform manner (Table 1). Analysis of variance of the percent of the tags recovered to date (September 89) from the 1982-, 1983-, 1984-, and 1985-brood releases in a 3 x 4 factorial design showed that the recovery rates varied significantly by brood year and ration treatment. The interaction of ration and brood year was significant.

Recovery rate did not vary significantly between groups for the 1982-brood coho salmon, but recovery rate of the 1983-brood fish fed salmon meal and hake meal were significantly greater than those of the control groups fed

OMP. Recovery rate of the 1984-brood for coho salmon fed salmon meal was statistically greater than the other two treatments. However, the recovery rate of the 1985-brood coho salmon supplied with hake meal was significantly lower than those from coho salmon fed OMP or salmon meal diets. Furthermore, means of all individual recoveries differed by brood year (1982, 1983, 1984 and 1985).

Results from the recovery of coded-wire tags from the 1983 and 1984 broods (tule stock) and the 1985 and 1986 broods (upriver bright stock) of fall chinook salmon are preliminary and represent data available as of September 1989 (Table 2). Due to incompleteness, the recovery data from the 1986 brood were not included in the statistical analysis. Analysis of variance (2 x 3 factorial design) of tag recovery data to date shows a significant variation with respect to brood year. The interaction between ration and brood year varied significantly, but there was no significant variation with ration treatmentts.

For the 1984 brood, the recovery rate of the control treatment was significantly greater than the test ration. This is not a surprising result. Growth of fish between mid-February, 1985, and release in May, 1985, was compromised in an intermittent manner by poor palatability of the test rations that resulted in reduced feed consumption and conversion. Poor palatability was traced to one of two lots of herring oil used to prepare rations. The lot of herring oil that produced problems was not oxidized when the ration was made (based upon chemical analysis), but contained only traces of antioxidant. Although antioxidant protection was increased to four times that normally incorporated into the ration, the ration became rancid and unpalatable to the fish.

Table 1. **Summary of preliminary tag recoveries from coho salmon reared at Sandy Hatchery.**

Brood year, diet	Tag code	Tagged fish release	Number recovered at age- 2	3	Total	Percent of release[2]
1982						
OMP						
	7-29-13	25,763	4	492	496	1.93
	7-29-06	26,983	3	475	478	1.77
	Mean					1.85[a]
Salmon meal						
	7-29-12	25,250	4	444	448	1.77
	7-29-09	26,573	8	513	521	1.95
	Mean					1.86[a]
Hake meal						
	7-29-10	26,654	7	515	522	1.96
	7-29-07	26,095	3	447	450	1.72
	Mean					1.84[a]
1983						
OMP						
	7-30-45	25,683	20	1,929	1,949	7.59
	7-31-05	26,459	53	2,046	2,099	7.93
	Mean					7.76[b]
Salmon meal						
	7-30-48	26,673	66	2,396	2,462	9.23
	7-31-06	25,743	68	2,229	2,297	8.92
	Mean					9.08[c]
Hake meal						
	7-30-47	25,493	48	2,212	2,260	8.86
	7-31-07	25,827	55	2,299	2,354	9.11
	Mean					8.99[c]
1984						
OMP						
	7-37-46	27,623	11	907	918	3.32
	7-36-20	27,974	6	845	851	3.04
	Mean					3.18[d]
Salmon meal						
	7-37-45	28,079	9	1,104	1,113	3.96
	7-36-19	27,115	10	1,029	1,039	3.83
	Mean					3.90[e]

Table 1. Continued

Brood year, diet[2]	Tag code	Tagged fish release	Number recovered at age[1] 2	3	Total	Percent of release[3]
1984 (continued)						
Hake meal						
	7-36-18	27,489	6	866	872	3.17
	7-36-23	27,542	7	889	896	3.25
	Mean					3.21[d]
1985						
OMP						
	7-44-47 R2	32,011	44	1602	1646	5.14
	7-41-19 R2	31,475	51	1536	1587	5.04
	Mean					5.09[f]
Salmon meal						
	7-44-42 R2	30,839	52	1343	1395	4.52
	7-41-21 R2	30,927	62	1680	1742	5.63
	Mean					5.08[f]
Hake Meal						
	7-44-41 R2	29,410	16	509	525	1.79
	7-41-11 R2	28,560	31	709	740	2.59
	Mean					2.19[g]

1 *Includes catch and escapement data available through September 1998.*
2 *Mean values with same exponent letters* **are** *not significantly different (P>0.05).*

Table 2. Summary of preliminary tag recoveries of fall chinook salmon (tule and upriver bright stock) reared at Bonneville Hatchery.

Brood year, diet	Tag code	Tagged fish release	Number recovered at age[1]				Percent of release[2]
			2	3	4	Total	
1983							
OMP							
	7-31-20	80,348	5	9	4	18	0.02
	7-31-21	80,048	6	39	1	46	0.06
	Mean						0.04[a]
Salmon meal							
	7-31-22	80,138		53	3	65	0.08
	7-31-23	81,282	9	28	5	40	0.05
	Mean						0.07[a]
1984							
OMP							
	7-33-22	78,367	314	1,867	340	2,521	3.22
	7-33-23	78,962	304	1,701	294	2,299	2.91
	Mean						3.07[b]
Salmon meal							
	7-33-24	80,242	147	1,334	389	1,870	2.33
	7-33-25	79,750	109	1,226	305	1,640	2.06
	Mean						2.12[c]
1985							
OMP							
	7-37-52	46,579	21	198	1	220	0.47
	7-37-53	47,268	36	172	1	209	0.44
	Mean						0.46[d]
Salmon meal							
	7-36-35	46,852	19		2	222	0.47
	7-36-36	47,250	26	163	2	191	0.40
	Mean						0.44[d]

Table 2. Continued

Brood year, diet[2]	Tag code	Tagged fish release	Number recovered at age[1] 2	3	4	Total	Percent of release[3]
1986							
OMP				1			
	7-47-19 R2	31,944	24	2	-	25	0.08
	7-47-21 R2	32,196	11		-	13	0.04
	Mean						0.06
Salmon Meal			18			18	
	7-47-22 R2	32,283	30	-		32	0.06
	7-47-25 R2	31,823		2	-		0.10
	Mean						0.08

1 *Includes catch and escapement data avai7ab7e through September 1989.*
2 *Mean values with same exponent 7etters are not significant7y different (P>0.05).*

APPENDIX I. Monthly Water Temperatures (^{o}F) at Sandy Hatchery.

Month	1983			1984			1985		
	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.
January	44	42.5	43.3		40	41			38
February	45	44	44.5	45	42.5	44	39	37	38
March	47	45	46	47	44	45.5	48	40	41.5
April	49.5	45	47	47.5	44	45.8	52	44	4946
May	54.5	49.8	52.1	51	47	49		47	
June	56	52	54	61	54	52	56	51	53.5
July	56.6	53.4	55	62	56	57.5	66	58	62
August	61	56	58.5			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

Month	1986			1987		
	Max.	Min.	Av.	Max.	Min.	Av.
January	42.5	41.0	41.8	41.0	39.8	40.4
February	42.1	40.9	41.5	44.6	42.6	43.6
March	47.9	44.4	46.0	46.9	44.2	45.5
April	47.9	44.3	46.1	51.2	46.5	48.8
May	52.2	47.7	49.9			
June	60.2	54.7	57.4			
July	60.0	54.8	57.4			
August	64.7	58.8	61.8			
September	64.0	49.0	54.3			
October	54.0	46.0	49.8			
November	44.3	42.7	43.5			
December	41.6	39.9	40.8			

APPENDIX II. Ration Formulations. Components are designated as percentages of total wet weight of ration.

Component	Control Ration	Test Rations: Hake Meal	Test Rations: Salmon Meal
Fish meal	28.0 (min)[11]	40.0-48.4[13]	37.7-41.4[13]
Cottonseed meal[1]	15.0	0.0	0.0
Dried whey product[2]	5.0	2.0	2.0
Wheat germ meal[3]	Remainder	Remainder	
Corn distillers solubles[4]	4.0	0.0	
Trace mineral premix[5]	0.1	0.1	0.1
Vitamin premix[6]	1.5	1.5	1.5
Spray dried blood meal[7]	0.0	2.0	2.0
Sodium bentonite	0.0	19.7-22.3	19.7-22.0
Concentrate hydqlyzed fish[8]	0.0		
Choline chloride	0.5	0.5	0.5
Pasteurized wet fish[10]	30.0	0.0	0.0
Fish oil	6.0-6.75[12]	1.8-7.5[14]	7.7-10.6[14]
Water	0.0	8.1-12.6	8.1-10.5
Total	100.0	100.0	100.0

1 Preprocessed, solvent extracted, min. 48% protein, max. 0.055% free gossypol.

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

3 Min. 23% protein and 7% fat

4 May contain up to 30% "grains" in place of solubles

5 Gm/lb: Zn ($ZnSO_4$), 34.0; Mn, 34.00 ($MnSO_4$) 9.10; Fe, Cu ($CuSO_4$) 0.70; I (ethy7enediamine dihydroiodide), 4.54; diluted to 1.00 lb with cereal product

6 Mg/lb: d-biotin, 18.0; vitamin B_6, 535.0 (pyridoxine HCl, 650 mg); B_{12}, 1.8; vitamin C (ascorbic acid), 27,000; vitamin E (water-dispersab7e a7pha tocopheryl acetate), 15,200; fo'lacin (folic acid), 385; Myo-inosito7 (not as phytate sa7t), 4000; vitamin K, 180 (menadione sodium bisulfite comp7ex, 545 mg); niacin, 5700; d-pantothenic acid, 3200 (d-ca7cium pantothenate, 3478 mg or d,l-ca7cium pantothenate, 6957 mg); riboflavin, 1600; thiamine, 715 (thiamine mononitrate, 778 mg); di7ute to 1.0 lb with cereal product.

7 Spray dried who7e blood

8 Concentrated bone-free hydro7ysate of salmon carcasses, groundfish carcass waste and whole Pacific hake

9 Liquid, 70%

APPENDIX II (Continued)

10 Two or more of the following, with none exceeding 50% of the combination; (1) Salmon of tuna viscera (no heads or gills, with livers); (2) who7e herring; (3) bottom fish (whole **or** fillet scrap); (4) dogfish; (5) whole hake; and (6) who7e salmon. Approved enzymes used to aid liquefaction.

11 Herring meal (min. 67.5% protein) used at no 7ess than 50% of the fish mea7 in each batch. Anchovy (min. 65% protein), cape7in (min. 67% protein), **or** hake (min. 67% protein) mea7s may be used as the remainder. Level to supply not 7ess than 21.5% fish mea7 protein; max. 5% NaCl; 8-12% fat; max 17% ash.

12 Herring, sa7mon, menhaden, dogfish (not more than 3%), or refined tuna oi7-stabilized with 0.4% BHA-BHT **(1:l);** free fatty acids not more than 3%; 8HAr BHT must be added at the time of reprocessing if reprocessed oi7 is used. Special condition when using hake as a wet fish: add 0.5% oi7 for every **10%** hake in tota ration.

13 Vacuum dried

14 Herring oi7; stabilized with 0.02% BHA-BHT **(1:l);** free fatty acids not more than 3%.