DEVELOPMENT OF TECHNIQUES FOR SALMON AND STEELHEAD TROUT HATCHERIES

Annual Report
July 1, 1976-September 30, 1977

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Technical Services
Fish Culture
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National Marine Fisheries Service
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Anadromous Fish Act
Project No. AFC 77-1
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CONTENTS

FIGURES

TABLES (cont'd)

Development of Techniques for Salmon
and Steelhead Trout Hatcheries

HATCHERY PRACTICES

Highlights

Experimental groups of coho salmon of transferrin phenotypes AA and AC were compared to determine relative growth and survival before release and total yield as 2- and 3-year olds.

In experiments with coho salmon no effect of inbreeding depression was associated with either inbreeding coefficients of F = 0.25 or F = 0.125 groups.

Production level experiments with Sandy Hatchery coho tested the interrelationship of water exchange rate and fish density.

Studies with Klaskanine and Fall Creek coho salmon indicate that time of release does not significantly influence marine distribution. Based on actual recoveries, a later release date produced significantly more 3-year-old fish at both hatcheries.

Cooperative Studies for Improvement of Fish Hatchery Stocks

Transferrin Studies

Two groups of 1973-brood coho were selectively bred according to their transferrin phenotype and were designated genotypically as groups AA and AC. The relative yield of each group was of primary interest in that preliminary data, obtained from 1968-brood 3-year-old returns, indicated AC fish produced greater yields than AA. We also noted a disparity in yield of 3-year-old males between the AA and AC groups and theorized that this may have been the result of an early maturation tendency of AA males (McIntyre and Johnson, 1973). Growth rates and survival before release, fishery recoveries, and hatchery returns for 2- and 3-year olds of the experimental 1973-brood groups are described here.

Blood samples for transferrin analysis were obtained from 266 adult coho (1970 brood) at Big Creek Hatchery on November 8, 1973; each fish was marked with a numbered disc tag and returned to the holding pond. On November 16, eggs from AA type females were fertilized with sperm from either AA or CC type males, yielding groups of AA and AC type offspring, respectively.

The AA and AC groups were held in separate rearing units until they reached an average fork-length size of 5.5 cm; each fish was then marked with a coded wire tag that identified its transferrin phenotype. Adipose fins were removed from 3% of each group and all fish were reared in a single pond. In late February 1975 fish with clipped adipose fins were removed from the rearing pond, and all other fish were marked adipose. From the 3% preliberation

sample of fish, coded wire tags were processed, and fork lengths were measured to the nearest millimeter. These fish were the source of data used to estimate mortality and growth for each group and to determine the percentage retention of coded wire tags. All fish were liberated on April 15, 1975.

Tags were recovered from 1976 Pacific Northwest marine and Columbia River gill-net landings, and from 1975 and 1976 Big Creek Hatchery returns. These data were used to calculate yield of the two transferrin groups.

Numbers of eggs resulting from the experimental matings were 39,000 AA type and 36,000 AC type. The proportion of juveniles surviving to time of release was significantly greater ($P < 0.01$) for AA than for AC fish (Table 1). Total yield of AA and AC type fish was equal; however, the yield of AC fish as 3-year olds was greater than that of AA fish. This disparity results from the greater tendency of AA males to mature in their second year as jacks, Table 1.

At time of liberation, the average fork length of 789 AA fish (130 mm) was significantly greater ($t = 6.25$) than that of 645 AC fish (125 mm). The experimental design eliminated the possibility that this difference was related to rearing environment. We therefore concluded that AA type fish grew faster than AC fish in the hatchery as a result of genetic influence.

Differences in survival to time at release and in yield values of the two groups were probably influenced by the growth rate of the juveniles. The faster growing AA fish may have maintained some competitive advantage over the slower growing AC fish in the hatchery, thereby reducing survival of AC fish. Because of their faster growth rate and/or larger size at release AA fish tended to produce a greater yield of jacks and a lower yield of 3-year-old males than did the AC types.

Suzumato, et al. (1971) showed that the transferrin AA phenotype in coho was more sensitive than the AC and CC phenotypes to the effects of bacterial kidney disease, a result suggesting that individuals with the C-allele have a selective advantage in the presence of this disease organism. Results of this study indicate that AA fish had a selective advantage in the hatchery because of their faster growth. Perhaps AC fish would have a selective advantage in years when environmental conditions were favorable for epizootics of bacterial kidney disease and be at a disadvantage in other years, relative to the faster growing AA fish.

These data and considerations indicate the futility of attempting to increase the yield of coho salmon by maximizing the frequency of biochemical phenotypes that display only a temporary advantage. Because of the existence of these complex gene-environment interactions, brood stock selection procedures should be developed that minimize losses of inherent variation to ensure the survival and maintenance of a stable population of propagated salmon.

Inbreeding Studies

The coefficient of inbreeding (F) is defined as the probability that two alleles at a locus are identical by descent. It also represents the average reduction in heterozygous loci per individual or per population, measured from some base generation before the inbreeding began.

In November 1975, 21 matings of coho salmon were made to evaluate the effects of inbreeding on the survival and growth of eggs, fry, and fingerlings. Adults used for these matings were 1972-brood genetic study returns of known lineage. Three crosses were full-sib matings (F = 0.25), and four were half-sib matings (F = 0.125). The remainder were control matings involving males and females used in the full- and half-sib matings and nonrelated males and females. Eyed eggs resulting from the matings were transferred from Big Creek Hatchery to Oregon State University in December 1976. After hatching fish were fed a constant ration (gram diet/gram body weight) until the study was terminated, July 6, 1976. Mortality and growth were determined for each group throughout the study period. No effect of inbreeding could be associated with either the F = 0.25 or F = 0.125 groups.

Results of this study are reported in a masters thesis entitled "Effects of Inbreeding Coho Salmon *(Oncorhynchus kisutch)*" by Mario Solazzi, March 9, 1977, Oregon State University, Corvallis, OR.

Pond Loading Studies

Ongoing studies at Sandy Hatchery have been designed to assess techniques for determining raceway loading levels. Previous pilot experiments tested the interrelationships of the weight of fish per cubic foot of rearing space as a function of fish size, i.e., Density Factor (DF) = $\frac{\text{lbs/ft}^3}{\text{fish length}}$, against the number of theoretical complete water interchanges per hour in a rearing unit, i.e., Turnover Rate (R), where R = $\frac{\text{lbs/ft}^3 \times 8}{\text{lbs/GPM}}$, (Westers, 1970). Table 2 expresses the relationship between fish weight per unit inflow (L) and the above variables.

Results from studies with 1974-brood coho suggested a relationship where a doubling of theoretical water exchange rates allowed a doubling of fish densities (FY 1977 Annual Report). Production level experiments designed to test this relationship (Table 2, Ex. #3) were initiated using 1975-brood Sandy coho.

Turnover rate values of 0.4, 0.8, and 1.6 were chosen for testing with replication in 20' x 80' flow-through raceways. The 0.4 R value approximates summer rearing conditions at Sandy and served as a control. Due to a limited water supply, raceway depth was reduced to an 18.5" average and flows of 122, 244, and 500 GPM were respectively supplied to achieve the desired R values. Flows were monitored throughout the duration of the experiment by implementation of 120° V-notch weirs calibrated to staff gauges mounted in the keyways of each raceway.

During the third week of May 1976, fish averaging 1.65 gms in weight were randomly stocked to produce DF values of 0.02, 0.04, and 0.08, respectively. These values were targeted to produce near maximum loadings at liberation the following year. The various combinations of R and DF values produced similar loadings defined as fish weight per unit inflow (Table 3). All treatments were fed OMP-2 according to the standard feeding schedule prepared for Sandy Hatchery. Regular management practices were observed and applied equally to all groups. During October, a random sample of approximately 23,000 fish

Table 2. Theoretical Interrelationships Between Interchanges Per Hour and Fish Weight Per Unit Volume and the Resulting Pond Loadings

R = rate of water interchanges per hour
D = weight of fish per unit volume (lbs/ft^3)
L = weight of fish per unit inflow (lbs/GPM)
C = constant unit

	R	x	D	=	L
Example #1:	C		1		1
	C		2		2
	C		4		4
Example #2:	1		C		1
	2		C		$\frac{1}{2}$
	4		C		$\frac{1}{4}$
Example #3:	1		1		C
	2		2		C
	4		4		C

Table 3. Comparison of Initial Pond Loading Production Data on 1975-Brood Coho at Sandy Hatchery

R (Flow)	Number of Fish	Grams per Fish	Total Pounds Fish	Pounds per Foot^3	Density Factor	Pounds per GPM
0.4 (122 GPM)	29,290	1.65	106	0.043	0.02	0.87
0.8 (244 GPM)	58,570	1.65	213	0.087	0.04	0.87
1.6 (500 GPM)	117,140	1.65	425	0.173	0.08	0.85

from each group were marked Ad+CWT. The experiment was continued through liberation on April 27, 1977.

Table 4 summarizes final production data. The apparent differences in fish size, lbs per GPM, feed conversion, and mortality rate were not significantly different when tested by analysis of variance ($P \geq 0.05$). Monthly water quality measurements were made of dissolved oxygen levels, ionized and unionized ammonia concentrations, pH, and conductivity. At no time were acceptable levels exceeded.

Final analysis will be based on yield of adults in 1978.

Table 4. Comparison of Final Pond Loading Production Data on 1975-Brood Coho at Sandy Hatchery

R (Flow)	Number of Fish	Grams per Fish	Total Pounds Fish	Pounds per Foot^3	Density Factor	Pounds per GPM	Feed Con-version	Percentage Mortality
0.4 1/ (122 GPM)	28,500	27.85	1,751	0.71	0.13	14.35	1.76	2.61
0.8 (244 GPM)	55,800	25.85	3,177	1.29	0.24	13.02	1.90	4.81
1.6 (500 GPM)	112,100	25.50	6,303	2.57	0.47	12.61	1.92	4.30

1/ *Values for R = 0.4 and R = 0.8 are means of replicates.*

Mullen & Crew could write report monitor returns 7.

Coho Time of Release Study

Studies are in progress at Fall Creek and Klaskanine hatcheries to determine the approximate release date which will provide the greatest yield of coho salmon. Also, we wish to determine if differences in release date influence marine distribution and harvest levels. At each hatchery, 1973-brood coho smolts marked adipose plus coded wire tag (Ad+CWT) were released on February 28 and April 30, 1975 (Table 5).

Table 5. Summary of Release Data, 1973- and 1974-Brood Coho Time of Release Study, Fall Creek and Klaskanine Hatcheries

Hatchery	Release Date	Number Released	Fish/Lb	Mark	Binary Code
1973-Brood					
Fall Creek	2/28/75	51,033	14.4	Ad+CWT	7-1/11
	4/30/75	49,382	12.5	Ad+CWT	7-1/12
Klaskanine	2/28/75	45,098	14.3	Ad+CWT	7-1/9
	4/30/75	44,053	14.6	Ad+CWT	7-1/10
1974-Brood		Jacks in 1977		Adult in 1978	
Fall Creek	3/1/76	28,690	15.3	Ad+CWT	9-3/8
	4/1/76	27,412	13.8	Ad+CWT	9-3/9
	5/1/76	29,690	13.7	Ad+CWT	9-3/10
Klaskanine	3/1/76	27,221	14.7	Ad+CWT	9-3/5
	4/1/76	26,927	13.8	Ad+CWT	9-3/6
	5/1/76	28,202	14.4	Ad+CWT	9-3/7

Based on actual fishery recoveries and hatchery returns, (Table 6), the April 30 release produced significantly more 3-year-old fish than did the February 28 release at both hatcheries ($X^2 = 36.76$ for Fall Creek; $X^2 = 24.58$ for Klaskanine). At Klaskanine Hatchery, the later release group contributed significantly more fish ($X^2 = 16.55$) to the Oregon fisheries. While a similar tendency appeared between Fall Creek releases, the difference was not significant.

Time of release did not appear to influence marine distribution (Figs. 1 and 2). However, a comparison of the combined recoveries from both releases at each hatchery indicated a greater number of Klaskanine coho were more northerly distributed. The difference was significant ($X^2 = 106.66$).

Fork length measurements were obtained from adult hatchery returns. At both hatcheries, the February release produced significantly ($t = 2.93$ for Fall Creek; $t = 2.65$ for Klaskanine) larger adults (Table 7). All data were statistically analyzed using the 5% level of significance.

Table 6. Summary of Recovery Data, 1973-Brood Coho Time of Release Study, Fall Creek and Klaskanine Hatcheries.

Hatchery	Release Date	Marine Recoveries				Col. R. Net	Total	Hatchery Recoveries		Combined Recoveries	
		Can	Wash	Ore	Calif			Adult	Jacks	Adults	Adults & Jacks
Fall Creek	2/28/75	5	69	443	159	--	676 1/ (1.32)	759 (1.49)	544 (1.06)	1,435 (2.81)	1,979 (3.88)
Fall Creek	4/30/75	9	81	484	218	--	792 (1.60)	927 (1.88)	237 (0.48)	1,719 (3.48)	1,956 (3.96)
Klaskanine	2/28/75	5	80	190	71	7	353 (0.78)	56 (0.12)	106 (0.24)	409 (0.91)	515 (1.14)
Klaskanine	4/30/75	22	103	273	85	7	490 (1.11)	61 (0.14)	154 (0.35)	551 (1.25)	705 (1.60)

1/ *Figures in parentheses indicate percentages of respective numbers released.*

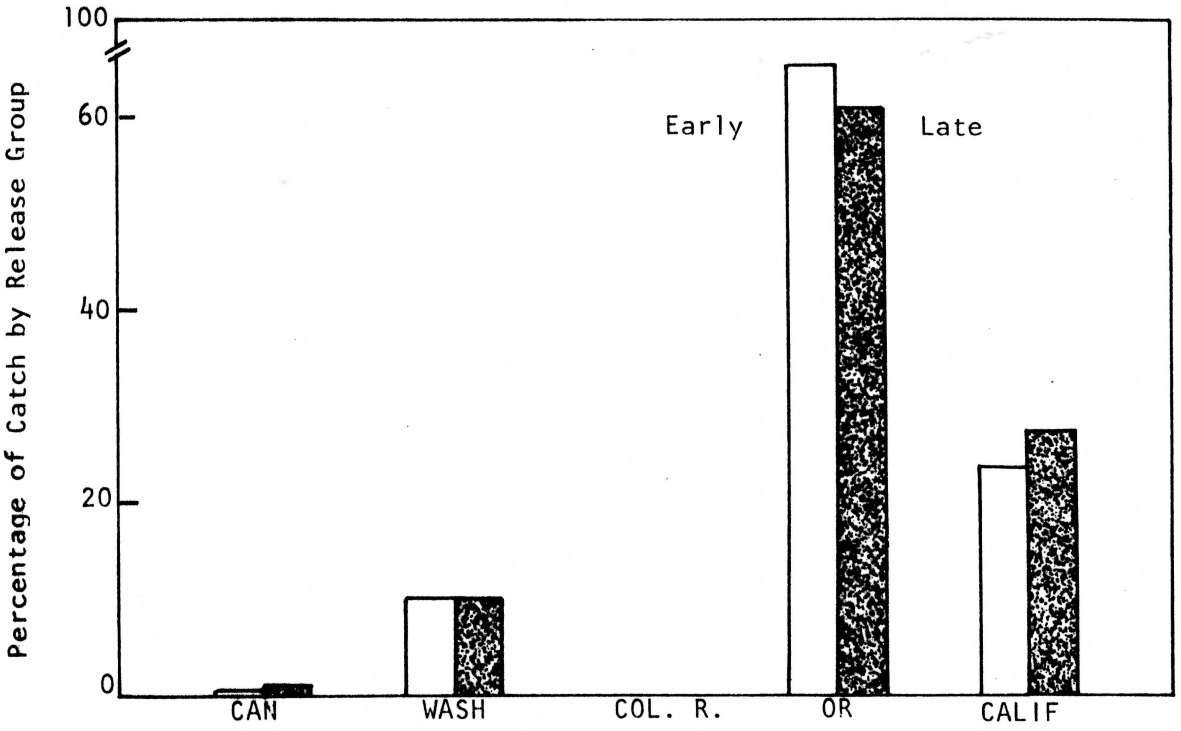

Fig. 1. Relative Distribution of 1973-Brood Coho by Release Group from Fall Creek Hatchery

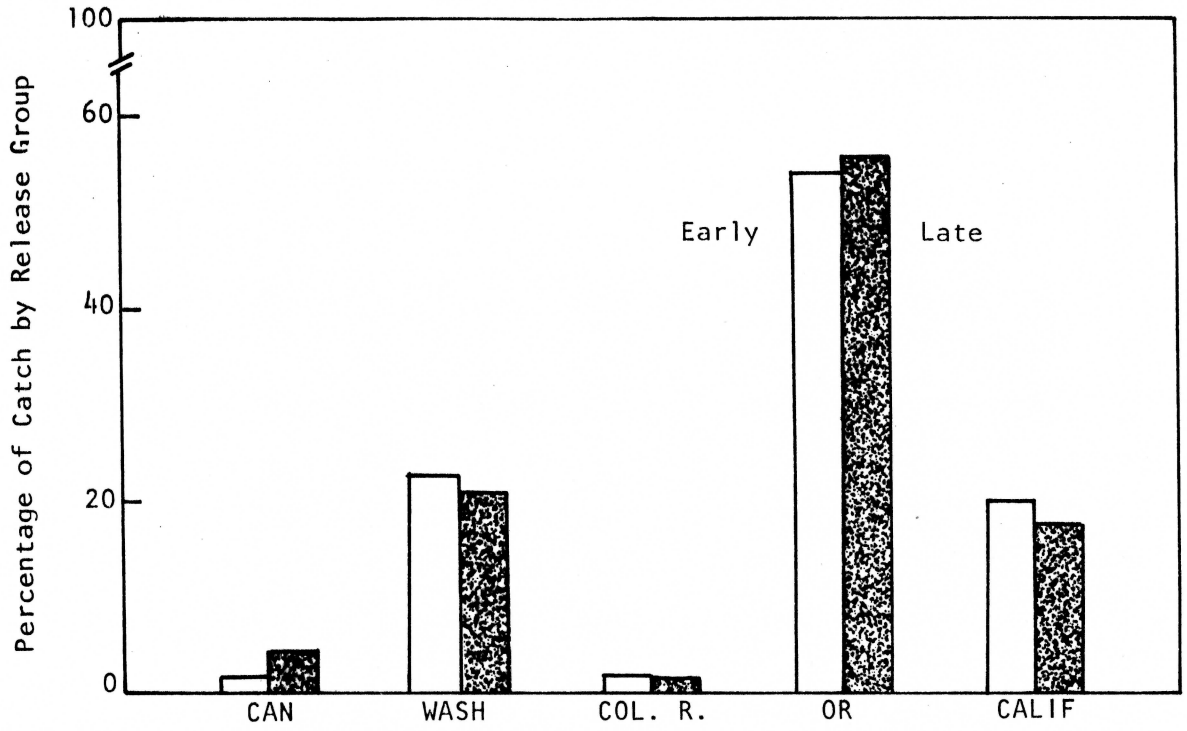

Fig. 2. Relative Distribution of 1973-Brood Coho by Release Group from Klaskanine Hatchery

Table 7. Comparison of Mean Adult Fork Lengths of 1973-Brood Coho Time of Release Study Hatchery Returns

Hatchery	Release Date	N	Mean Length (cm)	s	Length Range (cm)
Fall Creek	2/28/75	748	67.25	6.09	38-87
Fall Creek	4/30/75	920	66.37	5.99	41-85
Klaskanine	2/28/75	56	68.68	6.10	52-84
Klaskanine	4/30/75	61	65.39	7.36	41-80

Releases of Ad+CWT-marked 1974-brood coho smolts were made on March 1, April 1, and May 1, 1976 (Table 5). Jack returns to Fall Creek Hatchery in 1976 included 13 (0.05%), 31 (0.11%), and 31 (0.10%) of the releases made on the above dates, respectively. At Klaskanine Hatchery, the jack return in 1976 included 5 (0.02%), 12 (0.04%), and 8 (0.03%) of the releases made on the above dates, respectively.

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INFECTIOUS DISEASES

This report summarizes disease investigations conducted during fiscal year 1977 at 11 "coastal-stream" hatcheries. More detailed information is available in fish examination reports and special reports prepared during this period.

Hatchery Disease Examinations

Data presented in Table 8 show that furunculosis and bacterial gill disease were the most often diagnosed bacterial diseases; however, bacterial kidney disease (BKD), cold-water disease (CWD), and redmouth disease were also prevalent. *Costia* was the most commonly found parasite, though gill ameba, *Hexamita* and *Ichthyophthirius* were often present.

Infectious hematopoietic necrosis (IHN) virus was diagnosed in 1975-brood fall chinook being reared at Elk River Hatchery in 1976 and in "jack" fall chinook that returned to this hatchery in the fall of 1977.

Ceratomyxa shasta. Consistent poor returns of adult steelhead from hatchery plants in the Nehalem River coupled with the finding of *C. shasta* in adult spring chinook, steelhead, and coho from this stream promoted this study of the seasonal distribution of this myxosporidan parasite in the Nehalem.

Livebox experiments with steelhead trout were conducted during the spring and fall of 1976 to determine the seasonal occurrence of the infectious stage of *C. shasta*. During the spring groups of steelhead were exposed at weekly intervals from March 15 to May 18; in the fall each group was exposed for bi-weekly intervals from September 30 to January 7. After the exposure period in the Nehalem River each group of fish was transported to the Oregon State University Fish Disease Laboratory and held at 17.8 C for the duration of the experiment (30-60 days). Each group of fish was observed daily and intestinal scrapings from dead fish were examined microscopically (440X) for the presence of *C. shasta* spores.

Results from the spring exposures indicated the infectious stage was first present in the group of fish exposed from April 7 to 14 (Table 9). The level of infection in this group and the next exposure group April 14-21 was low (10%). In subsequent groups the level of infection was considerably higher reaching >90% in the two groups exposed during May.

The fall exposures indicated the infectious stage of *C. shasta* was present until December (Table 10). The level of infection remained extremely high (>85%) in the groups exposed during September, October, and the first part of November. With the November 24 to December 9 and the December 9 to 22 exposures the level of disease dropped to <20%. *Ceratomyxa shasta* was not found in the December 22 to January 7 group.

Table 8. Fish Disease Incidence and Treatment July 1, 1976 Through June 30, 1977, Coastal Anadromous Fisheries Hatcheries

Hatchery	Species	Brood Year	Disease or Other	Months J	A	S	O	N	D	J	F	M	A	M	J	Recommendations--Treatment, etc.
Alsea Salmon	Coho	1975	Furunculosis--*Epistylis*	X												3% TM-50 daily for 20 days
	F. Chinook	1975	Gill amoeba--*Costia*--Furunculosis		X											1:6,000 formalin 1 hr 3 times for amoeba 3% TM-50 daily for 15 days for furunculosis
	F. Chinook	1975	Gill amoeba--*Hexamita*		X											1:4,000 formalin 1 hr 2 times
	F. Chinook	1975	Gill amoeba--furunculosis				X									1:4,000 formalin 1 hr 2 treatments 3% TM-50 daily for 10 days for furunculosis
	Coho	1975	*Epistylis*--fungus							X						M.G. 1:2,000,000 3 days on then 3 days off then 1:1,500,000 for 3 days
	Coho	1975	Prelib exam--light *Epistylis*									X				Liberate as scheduled
	Coho	1976	CWD										X			3% TM-50 25 days
Alsea Steelhead	W. Sthd & Ct	1976	Bacterial gill disease	X												Hyamine 1622 2 ppm 3 times
	Cutthroat	1976	Bacterial gill disease			X										Hyamine 1622 2 ppm 4 times
	S. Steelhead	1976	"Redmouth"							X						3 gms sulfa--2 gms furoxone/100 lbs fish 10 days
	W. & S. Sthd	1976	Prelib exam--*"Gyro"--Trichodina--Epistylis*										X			M.G. 1 ppm 3 times then liberate
Bandon	Cutthroat	1976	Bac. gill disease--*Costia--"Gyro"* & Bac. hemorrhagic septicemia		X											Bac. gill disease--hyamine 1622 2 ppm 1 hr 4 treatments--3% TM-50 for 10 days
	S. Steelhead	1976	Bac. gill disease--*Costia*		X											Hyamine 1622 2 ppm 1 hr 4 times
	W. & S. Steelhead	1976	Bac. gill disease--*"Ich"*--light *Costia*			X										Formaline 1:8,000 1 hr 2 times
	Cutthroat	1974	Pretransfer exam--no pathogens							X						1 month on sulfa to prevent BKD
	W. Steelhead	1976	Pretransfer exam--no pathogens							X						Transfer as scheduled
	Brood cutthroat	3&4 yrs old	Prelib exam--no pathogens							X						Liberate as scheduled

Table 8. (cont'd)

Hatchery	Species	Brood Year	Disease or Other	Month J	A	S	O	N	D	J	F	M	A	M	J	Recommendations--Treatment, etc
Cedar Creek (Nestucca)	S. Steelhead	1976	Bacterial gill disease--CWD	X												Hyamine 1622 2 ppm 1 hr 4 times 3% TM-50 daily 14 days for CWD
	F. Chinook	1975	Prelib exam--light *Costia*	X												Liberate as scheduled
	F. Chinook	1975	Pretransfer exam--light *Costia*			X										Transfer as scheduled
	W.&S. Sthd	1976	Pretransfer exam--*Costia*			X										1:6,000 formalin 1 hr--then transfer
	F.&Sp. Chinook	1975	Prelib exam--light *Costia*				X									Liberate as scheduled
	W.&S. Sthd	1976	Light *Costia*				X									None
	Sp. Chinook	1976	Air bladder fungus							X						None: no treatment known
	Cutthroat	1976	*Epistylis* & *"Gyro"*							X						1:6,000 formalin 1 hr
	W.&S. Sthd	1976	*Epistylis*							X						None
	Cutthroat	1976	*Epistylis*									X				None
	W.&S. Sthd	1976	Prelib exam--*Epistylis*									X				Liberate as scheduled
	Sp. Chinook	1976	No pathogens									X				None
	Cutthroat	1977	CWD												X	3% TM-50 daily for 15 days
Elk River	F. Chinook	1975	IHN virus	X												
	F. Chinook	1975	Furunculosis	X												3% TM-50 for 20 days
	F. Chinook	1975	Gill amoeba		X											1:6,000 formalin 1 hr 2 times
	F. Chinook	1976	Prelib exam--marking stress mortality			X										3% TM-50 for 10 days
	Adult Chinook		BKD found in 4 of 86 sampled					X								None
	Adult Chinook		IHN virus					X								None
N. Nehalem	W. Steelhead	1976	*Costia* & furunculosis		X											3% TM-50 for 20 days for furunculosis 1:6,000 formalin 1 hr for *Costia*
	Coho	1975	Light *Costia*--furunculosis		X											3% TM-50 for 20 days
	F. Chinook	1975	Prelib exam--*Costia* & furunculosis					X								1:6,000 formalin 1 hr for *Costia* 3% TM-50 for 14 days for furunculosis
	W. Steelhead	1976	*Trichodina*							X						1 ppm M.G. 1 hr twice weekly for 4 weeks
	Coho	1975	*Trichodina* & CWD							X						1:6,000 formalin 1 hr.
	Coho	1975	Prelib exam--CWD--*Epistylis*--BKD									X				Liberate as scheduled
	W. Steelhead	1976	Prelib exam--BKD--light *Trichodina*									X				Liberate as scheduled

Table 8 (cont'd)

Hatchery	Species	Brood Year	Disease or Other	Months J	A	S	O	N	D	J	F	M	A	M	J	Recommendations--Treatment, etc.
N. Nehalem (cont'd)	Coho	1976	*Costia*									X				1:6,000 formalin 1 hr
	Coho	1976	CWD										X			3% TM-50 for 14 days
	Coho	1976	Light *Costia*													Split and thin population then 1:6,000 formalin 1 hr
	Coho	1976	Light fungus--BKD 1 of 30												X	None
	W. Steelhead	1977	Pinhead mortality												X	None
Rock Creek	Coho	1975	Fungus--*Epistylis*--sulfa toxicity	X												Reduce amt of sulfa being fed
	S. Steelhead	1976	*"Ich"*--light bacterial gill dis.			X										Drip formalin into ponds at 30 ppm for 8 hours
	Coho	1975	Prelib exam--light *Epistylis* & *Trichodina*								X					Liberate as scheduled
	Sp. Chinook	1975	Prelib exam--no pathogens								X					Liberate as scheduled
	S. Steelhead	1976	No pathogens								X					None
	Coho	1975	*Trichodina--Epistylis*--misc. bacteria									X				Treat daily M. green at 1 ppm
Salmon River	Adult Coho		*Myxosoma squamalis*--BKD 1 of 18 furunculosis--CWD						X							Salmon R. coho adults have their share of infectious diseases
Siletz	Coho	1975	*Trichodina--N. salmincola*					X								None
	Coho	1975	*Trichodina--Epistylis*--BKD							X						None
	Coho	1975	Prelib exam--*Trichodina--Epistylis N. salmincola*--BKD 10/12									X				Liberate as scheduled
Trask	F. Chinook	1975	Prelib exam--fluke metacercariae	X												Liberate as scheduled
	F. Chinook	1975	Mortality due to otters		X											None--a stress loss
	F. Chinook	1975	*"Ich"thyopthirius*		X											1:6,000 formalin 1 hr 4 consecutive days
	F. Chinook	1975	Prelib exam--furunculosis				X									3% TM-50 daily for 10 days then release
	Sp. Chinook	1975	Prelib exam--no pathogens				X									Liberate as scheduled
	Coho	1975	Prelib exam--BKD--CWD--*Epistylis*									X				Liberate as scheduled
	Sp. Chinook	1976	Prelib exam--no pathogens									X				Liberate as scheduled
	Coho	1976	CWD										X			3% TM-50 daily for 28 days
	Coho	1976	Pretransfer exam--*Costia*											X		1:6,000 formalin for 1 hr then transfer
	Coho	1976	Prelib exam--no pathogens												X	Transfer as scheduled

Table 9. Seasonal Occurrence of the Infectious Stage of *Ceratomyxa shasta* in the Nehalem River, Spring 1976 1/

Date of Exposure	Number Fish Exposed 1/	No. Fish Infected *C. shasta*	Percentage Infected *C. shasta*
March 15-24	16	0	0
March 24-31	10	0	0
March 31-April 7	18	0	0
April 7-14	21	2	10
April 14-21	19	1	5
April 21-28	18	10	56
April 28-May 5	17	10	59
May 5-11	25	23	92
May 11-18	15	14	93

1/ *Alsea winter steelhead (7-10/lb) from Cedar Creek Hatchery were used in these experiments.*

2/ *All fish were exposed in the main-stem Nehalem at river mile 8. After the exposure period these fish were brought to the Fish Disease Laboratory at Corvallis, Oregon, and held at 64 F (17.8 C) for the duration of the experiment (30-40 days).*

Table 10. Seasonal Occurrence of the Infectious Stage of *Ceratomyxa shasta* in the Nehalem River, Fall 1976 1/

Date of Exposure	Number Fish Exposed 2/	No. Fish Infected *C. shasta*	Percentage Infected *C. shasta*
September 30-October 14	17	15	88
October 14-29	16	14	88
October 29-November 10	20	18	90
November 10-24	18	18	100
November 24-December 9	20	4	20
December 9-22	19	2	11
December 22-January 7, 1977	20	0	0

1/ *Alsea winter steelhead (10-15/lb) from Cedar Creek Hatchery were used in these experiments.*

2/ *All fish were exposed in the main-stem Nehalem at river mile 8. After the exposure period these fish were brought to the Fish Disease Laboratory at Corvallis, Oregon, and held at 64 F (17.8 C) for the duration of the experiment (50-60 days).*

NUTRITION-PHYSIOLOGY

General

Portions of the work described in this report were performed using experimental fish and study sites outside of the coastal region. This was done with the knowledge and concurrence of the NMFS because: (1) the problems under investigation are of a broad nature; (2) results will be widely applicable to management of coastal anadromous fishes; and (3) the research facilities are either unique or they are the most practical and convenient sites available.

Highlights

A moist pellet ration containing 35% commercial soybean meal caused high losses in lots of spring chinook fingerlings after 5 weeks of feeding. Supplementation of the diet with methionine, lysine, valine, threonine, and dicalcium phosphate did not improve soybean meal performance.

In vitro tests demonstrated that soybean meal phytic acid has considerable binding capacity for nutritionally important divalent cations.

Short-term experiments indicated that anchovy, menhaden, and tuna oils produce acceptable results as lipid supplements to Oregon Pellets. Further in-hatchery testing is planned on a pilot scale.

Extensive investigations produced no hard evidence as to the etiology of a skin lesion/anemia condition observed in spring chinook fingerlings.

Laboratory studies suggested that, at times, Oregon Pellets may not provide vitamin C, folic acid, biotin, vitamin K, and iron in amounts sufficient to insure optimum fish health.

Fisheries recoveries of marked coho salmon from 1972-brood experiments continue to support previous data which indicated: (1) fish fed "high-fat" diets survive at increased rates; and (2) groups fed herring oil survived at significantly higher rates than fish fed soybean oil.

Results to date show no benefit associated with feeding an oral vaccine against *Vibrio anguillarum* to coho salmon raised in conventional freshwater hatcheries.

Field studies confirmed that feeding diets containing 8% NaCl to coho salmon for 8 weeks prior to liberation causes significant elevations in gill ATPase (Na, K-stimulated form).

Costs of production manufactured Oregon diets increased from an average cost of 23.8¢/lb to an average of 27.78¢/lb in a 6-month period, primarily due to precipitous increases in prices of fish meal and oil supplements.

Nutrition Studies

General

In FY 1977 we conducted laboratory and field work to: (1) explore ways for improving the performance of soybean meal as a component of fish feeds; (2) evaluate the nutritional characteristics of commercially available fish oils; (3) determine the etiology of a dermal "necrosis"/anemia condition afflicting spring chinook fingerlings; and (4) continue evaluations of Abernathy Dry Diet for Pacific salmon.

Cooperative projects conducted jointly with Oregon State University Department of Food Science and Technology included: (1) soybean meal utilization studies; and (2) testing of various fish oils and oil processing methods. University investigators provided the necessary biochemical and *in vitro* work while ODFW researchers were responsible for the biological aspects.

Utilization of Soybean Meal

General. Soybean meal is a potential source of protein for salmon feeds and its use could result in reduced dependence upon fish meal and lowered feed costs. Present cost per unit of soybean protein is about 40% less than protein from herring meal. All sources of protein are increasing in cost; however, the price of fish meal may increase at a faster rate than soybean because of decreased fish resources and growing interest in using some fishes, such as herring, for human consumption.

Supplementation with Amino Acids, Calcium, and Phosphorous. Soybean protein is generally inferior in quality to fish protein, but is much superior to other commercially available sources of concentrated plant proteins. Assuming equivalent bioavailability of amino acids, soybean meal is deficient in methionine, valine, lysine, and threonine compared to herring meal; however, theoretically only its methionine content fails to meet salmonid requirements. The work of Rumsey and Ketola (1975) suggested that supplementation of soybean meal with only methionine was not sufficient and that amino acid supplements simulating concentrations in trout eggs or isolated fish protein were needed to improve rainbow trout growth.

Soybean is also deficient in calcium and phosphorous compared with herring meal. Of these minerals, trout are thought to obtain phosphorous preferentially from their diet rather than the water environment. Ketola (1975) found that supplementing a diet containing 70.2% soybean meal with 0.75% inorganic phosphorous resulted in a 65% increase in growth of Atlantic salmon fingerlings in a short-term test.

This year we conducted a laboratory feeding trial with spring chinook salmon to see if dietary supplements of methionine, valine, lysine, threonine, and dicalcium phosphate would improve the performance of soybean meal rations. The basal diet contained 35% soybean meal and 45% fresh fish hydrolysate (3.5:1 herring:tuna viscera) yielding a soybean:fish protein ratio of about 2:1. We had found that fish ate this combination satisfactorily during

palatability tests last year. The test diets were fed to duplicate lots of 200 fish each for 7 weeks when the experiment was terminated due to excessive mortality.

Mortality began occurring after 5 weeks of feeding. The dying fish were large individuals that had definitely been eating the experimental diets. Many of the dying fish appeared to suffer a "tetany" of "rigor" when the deaths were precipitated by handling stress. Also many of the fish showed moderate scoliosis in the region between their heads and dorsal fins. This lateral flex seemed to be temporary and was not apparent after death. The fish fed soybean diets also had a high incidence of cataracts, about 5-fold higher than in the control lots. No fish pathogens could be isolated from moribund fish and histological examinations yielded no clues as to cause of death. The high losses seemed to be due to some factor(s) in soybean meal, and mortality rates were significantly aggravated when the fish were fed dicalcium phosphate.

The effects of amino acid and dicalcium phosphate supplementation are summarized in Tables 11 and 12, respectively. Weight gain was not affected by addition of methionine, lysine, or threonine; however, valine supplementation significantly depressed gain. Feed efficiency was not affected by methionine, lysine, or valine, but threonine supplements significantly depressed efficiency. Mortality was not significantly influenced by any of the amino acid supplements. Addition of calcium and phosphorous did not improve feed efficiency or weight gains.

The results of this short test suggested a possible mineral imbalance in diets containing soybean meal. Such a mineral imbalance may involve trace minerals rendered unavailable by binding factors.

Mineral Binding by Soybean Meal. Many vegetable feedstuffs, including soybean products, contain factors capable of binding or complexing with metal ions (Liener, 1969). One major binding constituent is phytic acid which forms insoluble complexes with divalent cations at pH values between four and seven. The pH values found in the upper small intestine (pH 6-7) of many animals are favorable for formation of stable, insoluble phytate complexes with essential minerals. For example, phytate in isolated soy protein has been demonstrated to reduce the availability of magnesium, copper, and zinc for poultry causing deficiency symptoms. It is conceivable that similar conditions in the salmon gastrointestinal tract could interfere with the availability of important cations such as Ca, Mg, Fe, Zn, Cu, Mn, and Co. Soybean meal contains a variable amount of phytic acid depending upon the variety of bean but the level could average in the vicinity of 1.0 to 1.5% of the diet, and the theoretical metal binding capacity could be considerable. For example, if the meal contained 1.4% phytic acid, the theoretical complexing capacity would be about .84% zinc, .52% calcium, and .31% magnesium (wt/wt). Because of these considerations, and the implications of symptoms displayed by fish fed soybean meal in the test reported above, we conducted several *in vitro* studies to investigate the metal binding capacity of soybean meal.

Table 11. Summary of Results, Amino Acid Supplementation of Soybean Meal, 1976 1/

Amino Acid Supplements (%) 2/				Weight Gain	Food Conversion	Mortality
Threonine	Lysine	Valine	Methionine	(%)	(As Fed)	(%)
-	-	-	-	48.4	2.83	16.25
-	-	-	0.267	54.6	2.50	10.00
-	-	0.389	-	52.8	2.64	12.00
-	-	0.389	0.267	48.5	2.92	18.25
-	0.218	-	-	53.7	2.63	16.50
-	0.218	-	0.267	54.4	2.61	12.00
-	0.218	0.389	-	49.2	2.79	17.25
-	0.218	0.389	0.267	48.9	2.71	11.25
0.048	-	-	-	55.4	2.63	15.00
0.048	-	-	0.267	47.8	2.88	12.25
0.048	-	0.389	-	50.2	2.79	13.00
0.048	-	0.389	0.267	50.2	2.90	17.50
0.048	0.218	-	-	48.0	2.96	16.25
0.048	0.218	-	0.267	51.1	2.81	11.50
0.048	0.218	0.389	-	49.9	2.80	15.25
0.048	0.218	0.389	0.267	50.0	2.77	12.50

1/ *Data are averages of duplicate lots at the end of 7 weeks feeding.*
2/ *L-amino acids were supplemented to levels in herring meal per unit of protein. All diets contained 2% anhydrous dicalcium phosphate ($CaHPO_4$)*

Table 12. Summary of Results, Dicalcium Phosphate Supplementation of Soybean Meal, 1976 1/

Diet Supplements		Weight Gain	Food Conversion	Mortality	Cataracts
$CaHPO_4$ (%) 2/	Amino Acids 3/	(%)	(As Fed)	(%)	(%)
0.0	No	54.3	2.63	7.50	70.8
2.0	No	48.4	2.83	20.00	68.8
0.0	Yes	56.3	2.54	5.75	60.4
0.5	Yes	52.8	2.63	15.25	Not Sampled
1.0	Yes	50.4	2.78	15.75	Not Sampled
1.5	Yes	46.8	2.96	25.00	Not Sampled
2.0	Yes	50.0	2.77	21.00	77.1
2.5	Yes	50.4	2.90	19.00	Not Sampled
0.0 Control 4/	No	76.2	1.86	0.25	12.5

1/ *Data are averages of duplicate lots at end of 7 weeks feeding, except that mortality is for an 8-week period.*
2/ *Anhydrous form.*
3/ *Amino acid supplement contained 0.048% threonine, 0.218% lysine, 0.389% valine, and 0.267 methionine.*
4/ *Protein in control diet supplied entirely from fish meal and fresh fish.*

We tested the binding of commercial soybean meal alone, in combination with herring meal, and in a pelletized ration. Two approaches were tried. One involved absorption of cations from an aqueous solution by soybean meal. The loss of cations from solution and the insolubility of phosphorous were used as measures of binding. The second technique involved pepsin digestion of the feed ingredients to simulate natural digestion followed by pH adjustment to yield insoluble phytate complexes which were isolated. The cation and phosphorous contents of these isolated complexes were used as measures of cation binding.

Results confirmed that phytate in soybean meal has considerable potential for complexing divalent cations. However, it was not possible to make precise estimates of the amount of dietary cation that would be required to compensate for phytate binding. Values based upon cation content found in insoluble, isolated complexes indicated that 4.39 mg Ca, 0.31 mg Mg, and 0.7 mg Zn would be required per gram of soybean meal to compensate for phytate in rations containing a mineral supplement such as the Bernhart-Tomerelli salt mix. The theoretical binding capacity of soybean meal based upon total soybean phophorous (index of phytate content) and a similar ration cation content would be equivalent to 5.7 mg Ca, 0.44 mg Mg, and 0.11 mg Zn.

If soybean meal phytate can induce a mineral deficiency in salmonids, the more chemically active micro constituents such as Cu, Zn, and Mn are most probably involved. Supplementation of soybean meal with Zn to form insoluble complexes with the phytate might improve the performance of soybean meal as a source of protein for salmonids.

Nutritional Quality of Fish Oils

We specify herring oil as the lipid supplement for Oregon Diets because it has usually produced better results than the plant oils we have tested (soybean and corn oils). Fish oils in general are thought to be superior to plant oils; however, we have had little experience with fish oils other than herring. Herring oil that meets our limit of 3% free fatty acids (FFA) is often difficult to obtain, and feed manufacturers must occasionally use soybean oil. We also require that the herring oil not be "reprocessed." Reprocessed oils have been treated in various ways, such as by addition of alkali, to reduce FFA levels. We do not know if reprocessing techniques have positive or detrimental effects on oil nutritional quality.

Since our sources of good fish oil need to be expanded we conducted two laboratory experiments this year to: (1) test samples of commercially available oils made from menhaden, tuna, anchovy, and herring; (2) determine the nutritional effects of alkaline reprocessing and "bleaching" fish oil of poor quality; and (3) investigate selected chemical assays as indices of oil quality.

Fifteen different samples of fish oils were incorporated in Oregon Pellets for comparison with a soybean oil control pellet in feeding trials with spring and fall races of chinook. Table 13 gives a description of the test rations.

Table 13. Treatment Descriptions, Fish Oil Evaluation Studies, FY 1977

Diet No.	Oil Description	FFA (%)	Peroxide Value	TBA 4/ Number	Total Nitrogen (mg/100 gm)
1	Tuna, black color, appeared quite polymerized	1/	-	8.7	128.8
2	Tuna, same as #1, except alkaline reprocessed 2/ and bleached with 1.5% earth 3/	1.1	10.0	31.4	44.9
3	Herring	14.3	6.8	4.7	40.0
4	Herring, same as #3, except alkaline reprocessed	0.6	16.2	15.7	28.6
5	Herring, same as #3, except bleached with 1.5% earth	14.2	24.5	27.0	39.7
6	Herring, same as #3, except bleached with 7.5% earth	14.0	34.0	22.7	37.8
7	Herring, Canada	8.0	4.5	4.0	47.5
8	Herring, Canada	5.6	7.2	5.4	34.1
9	Herring, Canada	2.6	7.5	7.4	35.5
10	Anchovy, California, may have been reprocessed at manufacturing plant	1.8	26.8	93.3	12.6
11	Tuna, California, may have been reprocessed at manufacturing plant	4.3	15.9	314.3	72.9
12	Menhaden, Gulf coast	2.3	17.3	34.2	28.3
13	Menhaden, Gulf coast	2.5	24.3	43.3	22.1
14	Menhaden, Atlantic coast	2.0	13.4	16.6	34.4
15	Menhaden, Atlantic coast	2.0	13.4	20.1	24.6
16	Soybean, food grade	0.01	1.9	0.2	22.9

1/ *Oil was too dark to titrate.*
2/ *Reprocessed by saponifying free fatty acids with NaOH.*
3/ *"Bleaching" accomplished by mixing oils with "bleaching earth" at 110-120 C for 30 minutes. Earth with absorbed materials removed from oil by centrifuging and vacuum filtration.*
4/ *2-Thiobarbituric Acid.*

The first feeding test (spring chinook) lasted only 12 weeks of a planned 16-week period because the fish developed a dermal necrosis/anemia condition that appeared unrelated to dietary treatments. There were some apparent differences in feed performance (Table 14) but those differences were not statistically significant ($P > 0.05$).

Table 14. Summary of Results, Fish Oil Evaluation Experiment with Spring Chinook, FY 1977 1/

Diet Number	Weight Gain %	Food Conversion (As Fed)	Mean Hematocrit (%)
1	71.8	2.00	25.4
2	82.8	1.84	24.2
3	93.9	1.70	26.2
4	82.2	1.94	25.0
5	77.6	1.96	26.7
6	87.0	1.83	26.6
7	88.1	1.77	25.6
8	85.4	1.74	25.9
9	82.0	1.88	25.9
10	85.2	1.78	25.4
11	83.4	1.82	27.0
12	84.2	1.80	28.2
13	89.2	1.80	26.9
14	90.0	1.74	24.9
15	77.4	1.92	24.7
16	68.1	1.91	24.3

1/ *Values represent means of duplicate lots.*

In the second trial we used the same oils in pellets fed to fall chinook fingerlings. We had no trouble with skin lesions or anemia in this experiment; however, the fish did not withstand the stress of weighday activities very well, and some mortality occurred. No fish pathogens were detectable as obvious cause(s) of the loss. Fish samples are presently being examined for virus diseases. Table 15 shows a summary of results at the end of 15 weeks of feeding. No significant differences could be detected ($P > 0.05$) between oil types or processing methods.

These experiments indicated that fish oils other than herring hold promise as components of fish diets; however, these data are short term, and further evaluations must be made before using the oils in production feeding. In the near future we plan to compare menhaden, anchovy, tuna, and herring oils in a year-long production scale feeding trial with spring chinook reared under cold-water conditions.

Table 15. Summary of Results, Fish Oil Evaluation Experiment with Fall Chinook, FY 1977 1/

Diet Number	Gain in Avg. Wt. (%)	Food Conversion (As Fed)	Mortality (%)	Mean Hematocrit (%)
1	508	1.21	1.50	37.9
2	519	1.20	1.50	38.6
3	508	1.19	2.50	36.4
4	552	1.12	1.50	37.2
5	518	1.20	0.50	38.4
6	507	1.20	1.33	36.4
7	534	1.17	1.50	36.1
8	520	1.18	1.33	39.0
9	521	1.18	1.00	38.0
10	534	1.16	1.33	36.0
11	539	1.15	1.67	37.4
12	484	1.26	1.17	36.6
13	525	1.18	1.00	34.9
14	552	1.12	1.00	39.0
15	543	1.10	1.00	37.8
16	544	1.10	3.00	37.2

1/ *Data are means for duplicate lots.*

Spring Chinook Skin Lesions and Anemia

General. During the last 3 years, spring chinook juveniles at several ODFW hatcheries and our nutrition laboratory have developed skin lesions and anemia which usually appear in late summer or fall and become progressively worse through the winter. Incidence of afflicted fish varies widely between lots in both the field and laboratory, ranging from less than 10% to over 40%. Fish death rates associated with the condition are generally elevated but not severe. Fish frequently "recover" as spring approaches but they may still be anemic even though skin lesions have healed.

The condition is characterized by scaleless, dark patches, or blotches on the fish's backs and sides. The affected areas may be reddish or purple, resembling a hematoma. The discolored patches are occasionally slightly swollen or "raised" and some fish have petechiae on their abdomens or sides. The lesions generally are not open sores.

Afflicted fish usually have abnormally low hematocrits, hemoglobin values, and red cell counts. Examination of blood smears suggests that the red cells are frequently macrocytic, and sometimes we see abnormal red cell sizes and shapes which resemble descriptions of folic acid deficiency symptoms. Serum chemistry profiles conducted on samples of diseased and "healthy" fish revealed no dramatic differences. Limited data suggested that fish with lesions may have somewhat elevated serum lactic dehydrogenase activities and albumin: globulin ratios.

Fish have been examined by two histopathologists. Observations were inconsistent and neither histologist could suggest plausible causes of the condition. Extensive tests for fish pathogens have been performed by both the ODFW Infectious Diseases Section and OSU Department of Microbiology. All examinations have yielded no evidence of a known infectious agent.

Since this condition has significant implications for the quality of our spring chinook and their ability to survive following release, we set up a program this year to monitor the course of the disorder in selected ponds of fish at hatcheries and to see if feed contaminants or other nutritional factors might be implicated in the disease.

Mycotoxin Assays. Because some aspects of the disorder were similar to symptoms reported for mycotoxin poisoning in domestic animals, we had samples of Oregon Pellets and cottonseed meal screened for Aflatoxins, T-2, F-2, and Ochratoxin (A and B) -- none were detected.

Hatchery Monitoring. Beginning in July 1976, we sampled fish monthly at Trask, Marion Forks, McKenzie, and Willamette hatcheries to follow blood condition (hematocrit) and watch for appearance of skin disorders. The fish at all study hatcheries, except Trask, developed skin lesions in varying degrees. The fish at Trask were liberated earlier (fall of 1976) all other fish (March 1977) which may have interfered with our ability to detect lesions. Hematocrit values appeared satisfactory in all pond populations originally set aside for monitoring.

In January 1977, we began more extensive sampling of additional fish at McKenzie Hatchery to evaluate the use of a special Oregon Pellet containing an increased vitamin package (2X normal) which we had recommended for use during the final 3-4 months of rearing for 1975-brood spring chinook at our hatcheries on the Willamette River system. Results of the examinations are shown in Table 16. Pond 2 was the population originally selected for monitoring in July 1976. Data obtained for other ponds during the winter of 1976-77 suggested that the Pond 2 group had not been representative of the conditions at this hatchery. Pond 23 was more typical of the overall situation and was selected as a control to be fed regular pellets. Pond 21 was used as the reference pond receiving the doubled vitamin supplement. All other fish at the hatchery also received increased vitamins. We could not conclusively demonstrate that the added vitamin supplements had an effect; however, hematocrit values for fish fed 2X vitamins appeared to improve substantially during the last month of feeding before the fish were liberated.

Oregon Test Diet vs Oregon Pellets. In our first search for clues as to a possible nutritional basis for the disease, we conducted a laboratory feeding experiment to see if the disorder would appear in fish fed diets which differ widely in composition, such as the Oregon Test Diet (OTD) and production formula Oregon Pellets. The OTD is made with purified ingredients (except for the lipid supplement) and is believed to be nutritionally adequate. The Oregon Pellet is manufactured with crude feedstuffs and contains many impurities. The OTD we used contained the Oregon Pellet vitamin premix which is only slightly different from the regular OTD vitamin pack. The OTD also did not contain any added vitamin A or D, although a vitamin A assay of the completed diet revealed a concentration of about 13,500 I.U./kg, presumably from herring oil lipid supplement.

Table 16. Effects of Doubling the Vitamin Package in Oregon Pellets Fed to Spring Chinook at McKenzie Hatchery, 1976-77

Vitamin Pack	Pond No.	Incidence of Skin Lesions (%) 12/28/76	2/3/77	3/9/77	Hematocrit (%) 12/28/76 Mean	12/28/76 <30 (%)	2/3/77 Mean	2/3/77 <30 (%)	3/9/77 Mean	3/9/77 <30 (%)
Regular	2	NS 1/	5	21	38.0	0	35.3	12	31.6	29
Regular	23	42	17	33	32.5	33	28.8	46	28.3	75
Double Rate	21	44	39	37	31.2	26	23.7	88	31.1	33

1/ *Not sampled.*

The two diets were fed for 25 weeks to single lots of 200 spring chinook fingerlings. No skin lesions or anemia were detected after 13 and 17 weeks, but at 25 weeks nearly 30% of the fish fed the OTD had developed the characteristic symptoms while fish fed Oregon Pellets appeared normal. Hemoglobin values, hematocrits, and red cell counts were significantly lower ($P < 0.05$) in fish fed the OTD, and mean corpuscular volume indices were significantly larger indicating a macrocytic anemia. The OTD was found to contain appreciably less vitamin B_{12} than Oregon Pellets (3.6 vs 14.1 ug/100 g), slightly less folic acid (.50 vs .61 mg/100 g), and less iron (7.4 vs 24.9 mg/100 g). However, it contained 240 mg of vitamin C/kg compared to no detectable amount in the Oregon Pellet. Fish maintained on the production pellet eventually developed typical skin lesions several months later.

Vitamin C Therapy. Some aspects of the disease resemble scurvy symptoms in other animals (skin hemorrhages and anemia). The condition in fish might involve a vitamin C deficiency because recent assays of Oregon Pellets had indicated the feed often contained low amounts of that vitamin. Therefore, we conducted an 8-week test using spring chinook with skin lesions to see if we could obtain remission by feeding mega-doses of vitamin C. Three Oregon Pellets were fed: (1) a control with no additional ascorbic acid beyond that in the original premix; (2) a test pellet with 2,880 mg/kg additional vitamin C; and (3) a ration containing 10,000 mg additional ascorbic acid per kg. Assays of the test feeds showed no vitamin C in the control, 1,370 mg/kg in the second diet, and 3,540 mg/kg in the third. The incidence of skin disorders increased in all lots regardless of treatment, and there was no significant ($P > 0.05$) effect of vitamin C on relative rates of increase. The fish were not anemic at the end of the test.

Vitamin K, Vitamin C, and Oxtetracycline Therapy. Vitamin K deficiency in other animals produces some symptoms similar to those noted in vitamin C deficiency states and the condition we observe in afflicted fish. In the past, the vitamin K supplement to Oregon Pellets has been considerably less than the amount recommended for salmon diets by the National Research Council. We thought it conceivable that combined deficiencies of vitamins K and C could be contributing factors in our problem with spring chinook.

Our discussions with other fisheries workers had revealed that a seemingly similar condition termed "strawberry disease" occurred in trout reared in Idaho. That disease reportedly is infectious and is "cured" by feeding oxytetracycline. We thought it reasonable to try such an approach with our fish.

We chose to test vitamin C supplementation (5,000 mg/kg feed), increased vitamin K (2,667 mg/kg), oxytetracycline therapy (TM-50 @ 3% of the diet), and all possible combinations thereof in Oregon Pellets. The vitamins C and K were added in addition to the regular vitamin premix. The diets were fed for 8 weeks to duplicate lots of spring chinook which had a skin lesion incidence of 75% and a mean hematocrit of 25.5% at the start. By the end of the experiment, the incidence of lesions had dropped to an average of about 6% and mean hematocrit had increased to 31.0%. Fish fed the control diet improved as well as any of the lots receiving vitamin or drug therapy.

Interrelations between Vitamins A and C. Hypervitaminosis A in other animals has been associated with symptoms very similar to those of scurvy. Oregon Pellets often contain high levels of vitamin A, averaging in excess of 85,000 I.U./kg of diet (six assays). We hypothesized that the spring chinook disorder might be due to chronic hypervitaminosis A or the synergistic effects of excess Vitamin A and deficient vitamin C. To examine this possibility we used the OTD to produce three dietary conditions: (1) excess Vitamin A (100,000 I.U. added per kg); (2) no vitamin A added with a normal amount of vitamin C; and (3) increased vitamin C (5,000 mg/kg) with no vitamin A added. These diets were fed for 8 weeks to spring chinook afflicted with the lesions. We could detect no effects due to diet. Fish condition improved in all lots regardless of treatment.

Liver Feeding. Historically, liver feeding has been used as a shotgun method for recovering fish suffering from "unknown" nutritional deficiencies. This year we tried feeding liver to afflicted fish for 6 weeks to see if their condition would improve. Two lots of fish were fed Oregon Pellets and two received 100% beef liver. Fish fed pellets started with 35.2% skin lesions and a mean hematocrit of 34.0% while those receiving liver began with 26.6% lesions and a hematocrit of 34.1%. Again, all lots improved regardless of diet used. The percentage skin lesions decreased to 7.7% and 4.7% for pellet-fed controls and liver diet, respectively. When expressed as a percentage of the initial lesion incidence the difference was not significant ($P>0.05$). Final hematocrits were 37.1% and 38.5% for pellet-fed fish and liver treatments, respectively. The differences were significant ($P<0.05$), suggesting that the pellet ration might be somewhat deficient in nutrients required for hematopoesis.

Increased Vitamins C, K, Biotin, and Folic Acid. It is well known that some vitamins are unstable during storage, particularly in the presence of moisture. Oregon Pellets contain about 28% water and may be held in frozen storage for 3 months, or longer, before being thawed for approximately 14 hours prior to feeding. This year we assayed pellets submitted to such a storage and handling regime and found that significant losses of certain vitamins occurred (Table 17). Vitamins C and K were largely destroyed and the remaining amounts failed to meet NRC recommendations. Biotin and folic acid were only marginally adequate. The level of vitamin E appeared to be too high in relation to NRC recommendations; however, increased quantities of vitamin E could be needed in the presence of the large amounts of unsaturated fats present in the Oregon Pellet.

If these preliminary data are indicative of changes occurring in the field, it is conceivable that simultaneous deficiencies of these four vitamins could be contributing to the development of the skin lesion/anemia condition. To test this hypothesis, we started a feeding trial with spring chinook fingerlings in May 1977 to see if we could prevent the disease by feeding and Oregon Pellet diet containing increased supplements of the four vitamins (vitamin C = 3,000 mg/kg, vitamin K - 60 mg/kg, biotin - 2 mg/kg, and folic acid = 30 mg/kg). These supplements are expected to deliver modest excesses of vitamins to the fish. The test ration and a control pellet are being fed to duplicate lots of fish (no skin lesions at the start) and the trial will last until the spring of 1978.

Table 17. Comparison of Oregon Pellet Vitamin Supplements, NRC Recommended Rates, and Amounts Found in Feed Following Storage and Thawing

Vitamin	Amount (mg/kg of feed as fed)		
	Supplement	Assayed 1/	NRC Recos. 2/
Biotin	0.595	0.757	0.72
B_6	17.7	19.9	7.2
B_{12}	0.060	0.281	0.014
C	893	<10.0	72
Choline	3,040	4,750	2,160
E	503	432	21.6 3/
Folic acid	7.11	5.26	3.6
Myo-inositol	562	1,540 4/	288
K_3	18.6 5/	2.03 6/	57.6
Niacin	188	227	108
Pantothenic acid	106	99.5	28.8
Riboflavin	52.9	52.7	14.4
Thiamine	23.6	22.9	7.2

1/ *Includes natural levels in feed ingredients.*
2/ *Adjusted for 28% moisture in Oregon Pellets.*
3/ *Oregon Pellets probably require higher level because of fish oil content.*
4/ *May include phytic acid, which is inactive.*
5/ *Assuming 1 mg MSBC has 1 mg of activity.*
6/ *Assuming K_1 has 1/3 the activity of K_3.*

Injection Studies. Some characteristics of both the skin disorders and poor blood condition noted in afflicted spring chinook suggest deficiencies of one or more vitamins (e.g., folic acid, vitamin B_{12}, biotin, vitamin C). Feed assays (Table 17 above) also lend circumstantial support to such a possibility because some of the suspected nutrients appear to be in short supply or at least borderline at times. If this is indeed true, then fish should respond to increased amounts of those nutrients if supplied directly by injection. We pursued this idea by conducting a trial in the spring of 1977 to see if injections of certain materials known to be involved in either hematopoesis or maintenance of epidermal tissue would affect fish hematology and/or skin lesions.

Experimental animals were obtained from pond 23 (see Table 16) at McKenzie Hatchery in early March. Our sampling had indicated this particular population had a skin lesion incidence of about 33% and the fish showed packed red cell volumes averaging less than 30%. The test fish held in 10-11 C spring water at the Sandy Laboratory for about 4 weeks before use in any experiments. During that time they were fed production grade Oregon Pellets ad libitum.

Upon examining the fish before their use in our tests we found they were recovering from the disorder. The incidence of skin lesions had dropped to less than 10%, and hematocrit values averaged about 35%. Even though the fish appeared to be healing, we proceeded with our test injections to see if selected materials would influence blood condition or prevent recurrence of skin disorders.

The tests were performed in two phases. During the first phase, duplicate lots of about 30-35 fish (50-60 grams average weight) were held at 10-11 C and injected interperitoneally, on a weekly basis, with folic acid, vitamin C, vitamin B_{12}, niacin, and iron dextran (once monthly). Control was provided by lots injected with buffered saline (0.9%) and lots not submitted to handling. The second phase employed 4-6 C water for 8 weeks. Similar types of treatments were used except that a biotin containing treatment was added in place of the nonhandled controls, and iron dextran was injected biweekly. Details on experimental treatments are given in Table 18. The fish were fed production grade Oregon Pellets ad libitum 5 days per week at 10-11 C and every-other-day during rearing at 4-6 C.

The incidence of skin disruptions and discolored blotches had virtually disappeared from all lots by the end of the experiment and there was no difference between treatments. Toward the end of the trial we noted a variance in the condition of the caudal fins between lots. In some groups the caudal fins were considerably more split, frayed, and eroded. Scoring of fin quality 2 weeks before the end of the trial and at termination revealed that the differences between treatments were significant ($P < 0.05$). The data (Table 19) suggest that injection of vitamin C and folacin was associated with better quality fins. Folic acid by itself did not produce the beneficial effect.

Hematology data also showed differences between treatments. Injection of both folacin and iron caused significant ($P < 0.05$) increases in hemoglobin concentrations. Iron injection was also associated with higher hematocrits. Data for red cell counts seemed to parallel the hemoglobin measurements but the differences were not significant at the 5% level. Biotin injection was associated with a significant decrease in both red cell counts and hemoglobin values. A concomitant reduction in hematocrit was not significant.

These results indicate that the feed used in this experiment was not supplying folic acid, vitamin C, and iron in amounts sufficient to insure maximum health. Analyses of the feed revealed the following concentrations (mg/kg of feed): folic acid = 5.2, vitamin C - 130, iron = 100, biotin - .22, B_{12} = .28, and niacin = 246. The folacin level appears to be adequate compared to NRC guidelines. Perhaps the bioavailability is poor in Oregon Pellets. The iron assay was low. Normal concentrations in past assays exceeded 200 mg/kg. Vitamin C appeared to be present in adequate amounts for normal conditions. The biotin level was less than 50% of NRC recommendations. We have no satisfactory explanation for the lack of positive response to biotin injection.

Dry Diets for Coho Salmon

Last year we began a third trial with 1974-brood fish at Big Creek Hatchery to evaluate the Abernathy Dry Diet for coho salmon. Results of the hatchery rearing phase were reported in our FY 1976 annual report. The fish were identified with fin marks at release, and this year we began collecting data on fish returning from the sea. Only a very few 2-year-old fish returned to the hatchery in the fall of 1976, and as a result we recovered only small numbers of marked fish from our study groups. Lots fed Oregon Pellets produced only six jacks while only two fish were recovered from the group fed Abernathy Diet.

Table 18. Treatments Used in Vitamin and Mineral Injection Experiment with Spring Chinook Yearlings, 1977

Phase		Nominal Dose (ug/kg of fish)						
		Folacin	C	B_{12}	Niacin	Biotin	Fe	Frequency
4 weeks @ 10-11 C	Control (Nonhandled)	-	-	-	-	-	-	-
	Control (Saline)	-	-	-	-	-	-	Weekly
	Folacin	1,400	-	-	-	-	-	"
	Folacin, C	1,400	42,000	-	-	-	-	"
	Folacin, C, B_{12}	1,400	42,000	2	-	-	-	"
	Folacin, C, B_{12}, Niacin	1,400	42,000	2	35,000	-	-	"
	Folacin, C, B_{12}, Fe 1/	1,400	42,000	2	-	-	10,000	2/
8 weeks @ 4-6 C	Control (Saline)	-	-	-	-	-	-	Weekly
	Folacin	2,800	-	-	-	-	-	"
	Folacin, C	2,800	70,000	-	-	-	-	"
	Folacin, C, B_{12}	2,800	70,000	2	-	-	-	"
	Folacin, C, B_{12}, Niacin	2,800	70,000	2	49,000	-	"	"
	Folacin, C, B_{12}, Fe	2,800	70,000	2	-	-	10,000	3/
	Folacin, C, Biotin	2,800	70,000	-	-	500	-	"

1/ *In form of iron dextran.*

2/ *Iron injected once monthly. Folacin, C and B_{12} injected weekly.*

3/ *Iron injected biweekly. Folacin, C, and B_{12} injected weekly.*

Table 19. Results of Blood Tests and Caudal Fin Examinations 1/ Vitamin and Mineral Injection Experiment, Spring Chinook Yearlings, 1977

Treatment	End of 10-11C Phase			End of 4-6C Phase			
	Hemoglobin (g/100 ml)	Hematocrit (%)	RBC 2/	Hemoglobin (g/100 ml)	Hematocrit (%)	RBC 2/	Caudal Fin Quality 3/
Control (Nonhandled)	11.54	35.1	1.432	-	-	-	-
Control (Saline)	11.86	35.4	1.489	10.70	32.4	1.327	1.43
Folacin	12.16	38.0	1.520	11.50	34.2	1.398	1.22
Folacin, C	11.47	36.0	1.430	11.44	34.4	1.406	1.74
Folacin, C, B_{12}	11.62	37.3	1.474	11.52	33.8	1.468	1.39
Folacin, C, B_{12}, Niacin	11.40	36.2	1.437	11.34	31.8	1.392	1.70
Folacin, C, B_{12}, Fe	12.48	37.4	1.519	12.23	38.4	1.526	1.75
Folacin, C, Biotin	--	--	--	10.22	32.4	1.282	1.88

1/ *Data are means of duplicate lots.*

2/ *Millions of cells per cubic millimeter.*

3/ *Averages for examinations conducted on 6/21/77 and 7/6/77. Fin condition scored: 3 = good, 2 = fair, 1 = poor, and 0 = bad or missing.*

Fish Quality Studies

General

This year we continued field research to: (1) study the effects of lipid nutrition on survival rates of fish following liberation from hatcheries; and (2) determine if feeding diets containing added sodium chloride will influence the marine survival of coho salmon. In addition, we collected data concerning marked fish recovered from experiments designed to see if feeding Oregon Pellets containing a *Vibrio anguillarum* bacterin affects the post-release survival of coho salmon.

Lipid Nutrition and Survival

During the past several years, we have been investigating the effects of lipid nutrition upon survival of hatchery-reared fish. This year we continued to compile information concerning fishery recoveries of marked fish we released from our experiments with 1972-brood coho. Details of that experiment were presented in our FY 1974 annual report. The results of studies to date (Table 20) indicate that: (1) fish fed "high-fat" diets survived at higher rates; and (2) the type of lipid supplement used (vegetable vs fish oil) had a marked effect on total survival. Groups fed herring oil in the 1972-brood test survived at a rate approximately 60% higher than lots fed soybean oil ($P < 0.05$).

Because the availability of herring oil is limited and variable, this year we began an experiment with coho salmon at Sandy Hatchery to determine if the survival benefit associated with feeding herring oil could be attained by using less than the normal 6% supplementation rate. Five Oregon Pellet formulations containing different ratios of herring:soybean oils were fed to five groups of approximately 60,000 1975-brood fish from June 21, 1976 to May 4, 1977. In order to evaluate survival to adulthood, all fish in each treatment were identified with distinctive coded wire tags and marked with an adipose clip (Table 21).

Disease Immunity and Survival

In the previous 2 years, we have carried out studies with 1973- and 1974-brood coho at Fall Creek and Sandy hatcheries to determine if any benefit would result from feeding a *Vibrio anguillarum* vaccine to fish raised in conventional freshwater hatcheries. Details of those experiments were discussed in our FY 1975 and FY 1976 annual reports. In FY 1976 we continued to recover experimental fish returning to hatcheries from those experiments.

The results to date (Table 22) suggest no consistent effects associated with feeding the oral vaccine. In two of the experiments, the controls have been recovered at higher rates ($P < 0.05$). In the other two tests, there is no significant difference in recovery rates. Final data will become available in the fall of 1977 with the return of adults from the 1974-brood.

Table 20. Lipid Nutrition vs Survival, Spring Chinook and Coho Salmon, Summary of Liberation Data, and Marked Fish Recoveries Through 1976

Species	Brood Year	Diet Treatment 1/	Liberation Data: Avg. Wt. (g)	Liberation Data: Number Released	Liberation Data: Fin Mark	Recoveries of Marked Fish: Fisheries (No.) 2/	Recoveries of Marked Fish: Hatcheries (No.) 3/	Recoveries of Marked Fish: Total (No.)	Recoveries of Marked Fish: Total (%)
Spring Chinook	1968	Soybean Oil							
		Low-fat	22.9	84,623	Ad-OTC 4/	--	103	103	0.122
		High-fat	23.7	85,285	Ad	--	161	161	0.189
	1969	Soybean Oil							
		Low-fat	27.2	79,827	D-RP	100	217	317	0.397
		High-fat	27.6	88,937	D-LP	124	204	328	0.369
Coho	1970	Herring Oil							
		Low-fat	31.0	80,681	LV-LM	45	93	138	0.171
		High-fat	32.0	80,380	RV-RM	86	194	280	0.348
	1972	Soybean Oil							
		Low-fat	23.4	172,112	RV-LM	91	523	614	0.357
		High-fat	23.4	181,584	LV-LM	193	1,009	1,202	0.662
		Herring Oil							
		Low-fat	23.0	183,944	RV-RM	181	1,012	1,193	0.649
		High-fat	23.2	176,983	LV-RM	270	1,475	1,745	0.986

1/ *Test diets in all cases were OP-2.*
2/ *Includes both sport and commercial fisheries. Numbers represent only the actual observed marks.*
3/ *Spring chinook adults recovered at Marion Forks Hatchery. Coho recovered at Bonneville, Cascade, and Oxbow Salmon hatcheries.*
4/ *OTC = oxytetracycline mark in bone tissue.*

Table 21. Summary of Data from Preliberation Samples, Lipid Nutrition vs Survival Experiment, 1975-Brood Coho, Sandy Hatchery

Diet Lipid Supplement				Average Size		
Herring (%)	Soybean (%)	Identification	Number Released	Weight (g)	Fork Length (mm)	Release Date
0	8	Ad+CWT 1/	59,983	29.7	140.7	5/6/77
2	6	"	60,195	29.6	141.2	"
4	4	"	57,212	29.9	141.4	"
6	2	"	58,753	30.9	141.6	"
8	0	"	60,607	30.3	141.1	"

1/ *Adipose fin clip plus coded wire tag.*

Salt Feeding and Survival

Last year we ran a field study at Sandy Hatchery to see if feeding 8% NaCl to coho yearlings for 8 weeks prior to liberation would affect their ability to migrate and survive the rigors of adaptation to the marine environment (see FY 1976 annual report). In the fall of 1976, we recovered jacks returning to the hatchery from this experiment. Very low numbers of fish returned from both the experimental and control groups (Table 23).

In FY 1977, we also began two field studies at Sandy and Big Creek hatcheries with 1975-brood coho to replicate the above experiment. Two lots of fish at each station were fed Oregon Pellets containing 8% NaCl for 8 weeks just prior to liberation. Two comparable lots were fed regular pellets without added salt. The feeds containing salt were fed at slightly higher rates to compensate for the displacement of nutrients by the added salt. Just before the fish were released, we collected samples of gill tissue from 40 fish per lot and analyzed them for ATPase activity. At both hatcheries, the fish fed salt showed significantly ($P < 0.05$) elevated Na, K-stimulated ATPase and significantly lower Mg ATPase than did the controls (Table 24). All fish in each treatment were marked with single ventral fin clips and the replicates were identified with oxytetracycline marks in their bone tissue. The first returns of marked fish from these tests are expected in the fall of 1977.

Technical Assistance

Feed Specifications and Contracts

Feed specifications were revised for the July to December 1977 period. Important changes included: (1) eliminating the need to calculate the protein level supplied by fish meal, and substituting a requirement that 30% fish

Table 22. Immunization of Coho Salmon Against Vibriosis, Marine Survival Studies, Summary of Liberation Data, and Marked Fish Recoveries Through 1976, Fall Creek and Sandy Hatcheries

Hatchery	Brood Year	Treatment	Liberation Data: Avg.Wt. (g)	Liberation Data: Number Released	Liberation Data: Fin Mark	Marked Fish Recoveries at Hatcheries: 2-Year Old (No.)	2-Year Old (%)	3-Year Old (No.)	3-Year Old (%)	Total (No.)	Total (%)
Sandy	1973	Vaccine	30.9	118,637	LV	238	0.201	1,101	0.928	1,339	1.129
		Control	31.2	123,009	RV	323	0.263	1,404	1.141	1,727	1.404
	1974	Vaccine	30.1	64,936	LV	74	0.114	1/	1/	74	0.114
		Control	30.3	65,170	RV	61	0.094	1/	1/	61	0.094
Fall Creek	1973	Vaccine	31.1	52,464	RV	292	0.557	575	1.096	867	1.653
		Control	31.5	52,490	LV	294	0.560	520	0.991	814	1.551
	1974	Vaccine	30.4	56,314	LV	36	0.064	1/	1/	36	0.064
		Control	31.1	50,235	RV	53	0.106	1/	1/	53	0.106

1/ *Data not yet complete. Adults to return in the fall of 1977.*

Table 23. Salt Feeding Experiment, Post-Release Survival Study, Summary of Liberation Data and Marked Fish Recoveries Through 1976, Sandy Hatchery, 1974-Brood Coho

Treatment	Fin Mark	Number Released	Average Weight (g)	Recoveries of Marked Fish 2-Year Olds	
				Number	%
Salt Diet	LV-LM	61,750	30.6	29	0.047
Control	RV-LM	61,217	30.6	27	0.044

Table 24. Salt Feeding Experiments, Post-Release Survival Studies, Summary of Data from Preliberation Samples, 1975-Brood Coho, Sandy and Big Creek Hatcheries

Hatchery	Treatment	Fin Mark	Number Released 1/	Average Size		ATPase Activity 2/	
				Weight (g)	Fork Length (mm)	Na,K	Mg
Sandy	Salt Diet	RV	60,600	29.1	139.0	5.3	6.9
	Salt Diet	RV+OTC 3/	61,105	30.2	141.5	5.6	6.3
	Control	LV+OTC	60,705	29.8	140.3	3.7	8.4
	Control	LV	61,257	30.1	141.7	2.9	8.1
Big Creek	Salt Diet	RV	84,436	30.4	139.7	4.7	6.0
	Salt Diet	RV+OTC	84,135	29.8	138.8	4.7	6.2
	Control	LV+OTC	82,904	30.7	140.3	2.9	6.7
	Control	LV	81,799	29.7	138.9	3.2	6.6

1/ *Sandy Hatchery fish released 4/29/77. Big Creek fish released 4/20/77.*
2/ *Micromoles inorganic phosphorous released per hour per mg protein.*
3/ *Oxytetracycline mark in bone tissue.*

meal must be used in the case of anchovy and hake and 31% fish meal if menhaden is used; (2) increased level of vitamin K in vitamin package to 870 mg/lb; and (3) required use of a cereal carrier for the vitamin premix.

Bioproducts, Inc., Warrenton, Oregon, held the feed manufacturing contract from July through December 1976, with an average price of 20.5¢/lb. Moore/Clark Company, LaConner, Washington, was awarded the contract from January through June 1977, at an average price of 23.8¢/lb. Recently, the contract for July through December 1977 was awarded to Bioproducts, Inc., at 27.78¢/lb. The abrupt jump in cost was attributed to skyrocketing prices of fish meal and oil supplements.

Quality Control of Production Feeds

We conducted eight unannounced spot checks at the manufacturing plants to see if specifications were being met. Only one significant problem was detected. Insufficient fish meal and supplemental oil were being used when employing optional fish meals and wet fish ingredients. The problems were corrected.

Feed Programming

This year we only prepared feeding schedules for those hatcheries where a change in liberation date or size was required. Programs were computed for use the first time with coho salmon at Salmon River Hatchery. All other coastal hatcheries are using programs prepared in past years.

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