DEVELOPMENT OF TECHNIQUES FOR SALMON AND STEELHEAD TROUT HATCHERIES

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OREGON DEPARTMENT OF FISH AND WILDLIFE
TECHNICAL SERVICES
FISH CULTURE
FISH DIVISION

NATIONAL MARINE FISHERIES SERVICE
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
DEPARTMENT OF COMMERCE
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PROJECT NO. AFC 77–2
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and Steelhead Trout Hatcheries

HATCHERY PRACTICES

Highlights

An apparent increase in the survival rate of experimental groups of coho salmon was demonstrated after two generations of selective breeding.

Recoveries of 2-year-old coho were obtained from experimental groups of interstock and intrastock parent crosses.

Intergeneration matings of coho salmon indicated that more 2-year-old offspring were produced from 2-year-old male parents than from 3-year-old male parents.

Continuing studies with Klaskanine and Fall Creek Coho salmon supported previous data that time of release did not appear to influence marine distribution. However, a significant increase in the survival rate of 3-year olds from a later release date was not verified.

When water exchange rates and coho densities were increased proportionally, return rates of 2-year-old fish did not differ significantly.

Improvement of Hatchery Stocks

Selection Experiments

A study of the role of genetic factors in the characteristics of coho salmon was initiated at Big Creek Hatchery in 1967. An experiment designed to test the hypothesis that the yield ([recoveries/smolts released] x 100) of coho can be enhanced by selective breeding has been ongoing at Big Creek since 1971. This report summarizes progress since inception of the selective breeding experiment in addition to describing work accomplished in FY '78.

After arrival to the hatchery of 3-year-old coho from the 1968-brood, adipose marked fish were separated from production fish. Those that were overripe or green prior to a predetermined spawning time were sacrificed; microtags were removed and decoded. During the spawning operation, gametes obtained from each adult were placed in an individual container and identified by a number corresponding to a numbered disc tag applied to the parent carcass. Gametes were stored at 4.4 C. Microtags from all carcasses were removed and decoded; gamete containers were assigned their respective sib group via the disc-tag number. Numbers of adults returning to the hatchery from each sib group were then added to the numbers recovered in that year's Pacific marine and Columbia River fisheries and the yield for each group was determined. This procedure became standard for each successive return of 3-year-old fish.

Criteria for selection of the F_1 generation were yield values for the 60 families from the 1968 brood (Table 1, page 3, FY '75 Annual Report). Ten families had a yield greater than 1.0 (mean = 1.49). Individuals from these select families were mated one male to one female to produce 30 select families for the 1971 brood. Full-sib matings were avoided. A control group was produced by mating 15 pairs of adults obtained from unmarked production fish.

The 45 distinct 1971-brood families thus produced were incubated separately. After hatching, they were reared in separate fiberglass tanks until injection with distinctively coded microtags in May 1972, after which they were pooled in a common raceway environment. An adipose clip was applied to all fish prior to liberation in 1973. These incubating, rearing, and marking procedures also became standard for successive broods.

Tagged-fish returns from the 1971 brood were obtained from 2-year olds at the hatchery in fall 1973, and from 3-year olds in summer-fall fisheries, and the hatchery in 1974 (Table 1).

Table 1. Smolts Released and Microtagged Fish Recovered from Select and Control Families of 1971-Brood Big Creek Coho Salmon

	Select	Control
Smolts released	40,520	15,488
3-year olds recovered:		
Marine fishery	409	156
Columbia River gill net	50	12
Sport fishery	1	1
Big Creek Hatchery	94	27
2-year olds recovered	170	80
Totals	724	276

Average yield values of 3-year olds for the select and control groups were 1.37 and 1.27, respectively. While the select group displayed an advantage in yield, chi-square analysis showed no significant difference ($P \geq 0.05$) between groups. Total yield values (2- and 3-year olds combined) were 1.79 and 1.78, respectively. Males and females from families producing the highest yield values were selected as parents of 22 select F_2 generation 1974-brood families. Mature, unmarked production fish were obtained to produce 12 unselected control families. In all matings, one male was used with two females; each family consisted of all the progeny from a single female.

Tagged-fish returns from the 1974-brood were obtained from 2-year olds at the hatchery in fall 1976, and from 3-year olds in summer-fall fisheries, and the hatchery in 1977 (Table 2).

Average yield values of 3-year olds for the select and control groups were 0.32 and 0.06, respectively. Yield for the combined select families was greater than the yield for combined control families ($X^2 = 38.8$) indicating a significant ($P \geq 0.05$) effect due to selection. Total yield values for the select and control groups were 0.45 and 0.09, respectively.

It appears that the effect of selection has been to increase the yield of 3-year olds in the select group (Table 3). Lower values for the 1974 brood reflect the generally low coho harvest and escapement levels on the entire Pacific Coast in 1977.

Table 2. Smolts Released and Microtagged Fish Recovered from Select and Control Families of 1974-Brood Big Creek Coho Salmon

	Select	Control
Smolts released	37,355	20,617
3-year olds recovered:		
Marine fishery	55	3
Columbia River gill net	3	2
Sport fishery		
Big Creek Hatchery	61	7
2-year olds recovered	49	7
Totals	168	19

Table 3. Yield Values of 3-Year Olds in Select and Control Groups of Big Creek Coho Salmon

Brood Year	Yield		
	Select	Control	Gain
1971	1.37	1.27	0.10
1974	0.32	0.06	0.26

The difference (R) between select and control groups is the product of the heritability (h^2) of the trait under selection, the standard deviation of the trait (σp), and the selection intensity (i) (Falconer, 1967). In this report, i is the difference between the average yield of all families available for selection and the average yield of families retained for breeding; σp is the standard deviation of yield for all 1968-brood families. By rearranging:

$$h^2 = \frac{R}{i \ \sigma p};$$

and with the results obtained here:

$$h^2 \text{ (1971 brood)} = \frac{0.10}{1.679 \times 3.93} = 0.015$$

$$h^2 \text{ (1974 brood)} = \frac{0.26}{1.094 \times 3.93} = 0.060$$

Obviously, the results were not consistent for the two generations of selection, precluding any accurate prediction of gains to be expected with continued selection.

Since the trend from F_1 to F_2 generations was to increase the gain in yield between select and control groups, an F_3 generation was composed in FY '78. From a total of 53 available 1974-brood select adults, gametes from five males and 10 females were mated producing 10 families to continue the high survival lines. Parent family yield values are given in Table 4. Six males and 12 females were randomly chosen from Big Creek production stock to provide 12 control families for the 1977 brood. Again, each family consisted of all the progeny from one female.

Table 4. Parent Family Yield Values for Composition of Select Families of Big Creek Coho Salmon

Combined Parent Families	Yield Mean	Yield Range
1974-Brood Offspring	1.75	1.00-2.84
1977-Brood Offspring	0.44	0.21-0.70

In May 1978, a total of 52,000 1977-brood coho were implanted with microtags distinctive for each family. All fish were then placed in a common raceway environment for rearing until liberation in spring 1979. The first returns from these groups are expected in fall 1979.

Interstock Experiment

An alternative to selective breeding within a given stock is to compare the performance of crosses between stocks. To evaluate this approach for Big Creek coho, gametes from adults returning in 1975 to the Umpqua and Sol Duc rivers were collected and transported to Big Creek Hatchery for mating with gametes from Big Creek stock in the following manner:

1) Big Creek males x Sol Duc females
2) Big Creek females x Sol Duc males
3) Big Creek males x Umpqua females
4) Big Creek females x Umpqua males
5) Big Creek males x Big Creek females

The five groups were incubated and reared separately until injection with distinctively coded microtags in spring 1976, after which they were pooled in a common raceway environment until liberation in April 1977. During FY '78, 2-year-old marked fish were recovered. Preliberation and recovery data through 1977 are summarized in Table 5. Recoveries of 3-year-old coho will be conducted in summer-fall 1978.

Intergeneration Experiment

Yield of 3-year-old coho of the 1969 and 1972 broods has been low at Big Creek. Sufficient numbers of spawning adults have not been available to conduct selection experiments similar to the 1968-71-74-77-brood series. Therefore, an intergeneration experiment was designed to overcome any possible genetic basis for the poor performance of the former series.

Table 5. Summary of Preliberation Data and Marked Coho Salmon Recoveries Through 1977 of the 1975-Brood Genetic Study Interstock Experiment at Big Creek Hatchery

Parent Cross	Preliberation Size 1/ Mm Length Mean	S.D.	Gms Weight Mean	S.D.	Tagged Fish Released 2/	Recoveries of 2-Year-Old Fish Number	% of Release
BC ♂ x SD ♀	126	10	24.4	5.4	22,140	44	0.199
BC ♀ x SD ♂	122	10	22.4	5.2	12,275	6	0.049
BC ♂ x Ump ♀	129	10	26.5	5.5	15,564	35	0.225
BC ♀ x Ump ♂	126	10	24.6	5.9	11,036	10 3/	0.091
BC ♂ x BC ♀	127	10	25.9	5.8	17,279	48	0.278

1/ *Figures are based on a 3% sample.*
2/ *Fish were released 4/18/77.*
3/ *Includes one recovery from the Washington troll fishery. All other recoveries shown are hatchery returns.*

In 1975, 2-year-old males were mated with 3-year-old females. A total of 14 females were randomly divided into two groups for replication. One-half the eggs from each female were fertilized with sperm from 3-year-old males to provide controls. All progeny in each of the four resultant groups were injected with distinctively coded microtags and pooled in a common raceway environment in spring 1976 for rearing until liberation.

During FY '78, 2-year-old marked fish were recovered. Preliberation and recovery data are summarized in Table 6. Recoveries of 3-year-old coho will be conducted in summer-fall 1978.

COHO TIME OF RELEASE STUDY

Studies have been in progress at Fall Creek and Klaskanine hatcheries to determine the approximate release date which will provide the greatest yield of coho salmon. We also wish to determine if differences in release date influence marine distribution and harvest levels. At each hatchery, groups of micro-tagged coho smolts of the 1973 and 1974 broods were released at variable times (Table 7). Returns of 3-year-old fish from March 1, April 1, and May 1 releases of the 1974 brood were monitored in FY '78.

Based on actual fishery recoveries and hatchery returns (Table 8), time of release from Klaskanine Hatchery had no significant effect on 3-year-old survival. At Fall Creek Hatchery, no significant difference in survival was observed between the March and April release groups, but the latter produced significantly (X^2 = 14.92) more 3-year olds than did the May release. No signficant advantage in contribution to the Oregon marine fisheries was observed from any of the three release dates at Fall Creek. Differences in contribution to the Oregon marine and Columbia River net fisheries from variable release times at Klaskanine also were not significant.

Table 6. Summary of Preliberation Data and Marked Coho Salmon Recoveries Through 1977 of the 1975-Brood Genetic Study Intergeneration Experiment at Big Creek Hatchery

Parent Cross	Preliberation Size 1/ Mm Length Mean	Mm Length S.D.	Gms Weight Mean	Gms Weight S.D.	Tagged Fish Released 2/	Recoveries of 2-Year Fish No.	% of Release
Female group 1:							
x 2-yr. male	142	10	33.4	6.4	4,806	31 3/	0.645
x 3-yr. male	143	11	34.5	7.3	5,970	17	0.285
Female group 2:							
x 2-yr. male	138	9	32.0	6.1	8,313	28 4/	0.337
x 3-yr. male	135	9	30.1	5.9	7,039	10	0.142

1/ *Figures are based on a 1.5% sample.*
2/ *Fish were released 4/18/77.*
3/ *Includes two recoveries from the Washington marine fisheries.*
4/ *Includes one recovery each from the Canadian and Oregon marine fisheries. All other recoveries shown are hatchery returns.*

Table 7. 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					
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

Table 8. Summary of Recovery Data, 1974-Brood Coho Time of Release Study, Fall Creek and Klaskanine Hatcheries

	Release	Marine Recoveries				Col.R.		Hatchery Recoveries		Combined Recoveries	
Hatchery	Date	CAN	WA	OR	CA	Net	Total	3-Yr Olds	2-Yr Olds	3-Yr Olds	2+3-Yr Olds
Fall Cr.	3/1/76	3	4	24	4	0	35 (0.12) 1/	76 (0.26)	13 (0.05)	111 (0.39)	124 (0.43)
"	4/1/76	0	3	22	0	0	25 (0.09)	61 (0.22)	31 (0.11)	86 (0.31)	117 (0.43)
"	5/1/76	0	4	16	0	0	20 (0.07)	27 (0.09)	31 (0.10)	47 (0.16)	78 (0.26)
Klaskanine	3/1/76	0	5	22	1	2	30 (0.11)	6 (0.02)	5 (0.02)	36 (0.13)	41 (0.15)
"	4/1/76	0	4	14	0	5	23 (0.09)	9 (0.03)	12 (0.04)	32 (0.12)	44 (0.16)
"	5/1/76	1	15	21	1	2	40 (0.14)	9 (0.03)	8 (0.03)	49 (0.17)	57 (0.20)

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

These data deviate from results obtained with the 1973 brood (FY 1977 Annual Report), where a significant 3-year-old survival advantage was observed from an April 30 release compared to a February 28 release at both hatcheries. The later release group from Klaskanine had also contributed more fish to the Oregon fisheries.

One or all of several possible factors for the inconsistencies may exist. There was an extremely low number of marked 1974-brood coho recovered. Seasonally fluctuating environmental conditions may have had an impact. Relative to the 1973 brood at Fall Creek, survival of 1974-brood 3-year olds from the early and late releases displayed a reversal. While results with the 1974 brood at Klaskanine were not significantly different, the combined recoveries of 3-year olds from the May release (49) vs those from the March release (36) tended to follow the pattern established with the 1973 brood. Perhaps different optimal times of release exist for coho produced at Columbia River and coastal hatcheries. It appears that further investigation of the subject is needed.

Consistent with data from the 1973 brood was the observation that, within each hatchery, time of release did not appear to influence marine distribution (Figures 1 and 2). All analyses were made at the 5% level of significance.

POND LOADING STUDIES

In spring 1976, a production level experiment was initiated with 1975-brood coho at Sandy Hatchery to test the relationship where a doubling of raceway water turnover rates (R), i.e., the number of theoretical complete water interchanges per hour, allows a doubling of fish density. R values of 0.4, 0.8, and 1.6 in 20' x 80' flow-through raceways held at a depth of 18.5" were obtained by respectively supplying water inflows of 122, 244, and 500 gpm. Density factors, $\left(\frac{lbs/ft^3}{fish\ length}\right)$, of 0.02, 0.04, and 0.08 were initially stocked to produce fish densities of the same proportion as the chosen R values. These density factors were targeted to produce near maximum loadings at liberation. The various combinations of R values and density factors produced similar loadings defined as fish weight per unit inflow. Replication of each combination resulted in six lots of fish.

Approximately 23,000 fish from each lot were randomly selected for injection with distinctively coded microtags in fall 1976. All groups were liberated on April 27, 1977. A more detailed description of the experimental methods and a summary of production data can be found in the FY 1977 Annual Report.

A 1:2:4 ratio of R values was tested. While initial fish densities were of the same ratio, increasing density produced a growth suppression that resulted in a density ratio at liberation slightly less than 1:2:4. However, loading levels as defined above were sufficiently comparable that we hypothesized there would be no significant differences in survival.

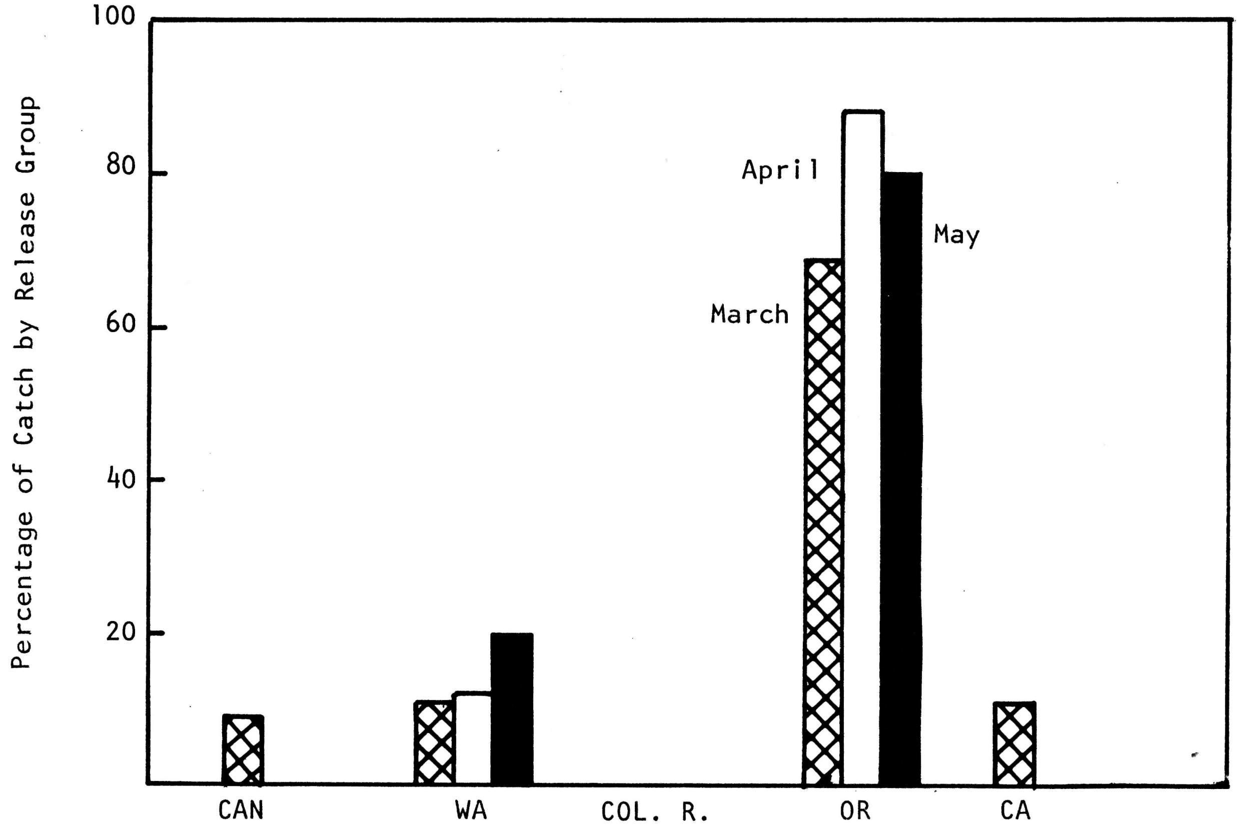

Figure 1. Relative Distribution of 3-Year-Old 1974-Brood Coho by Release Group from Fall Creek Hatchery

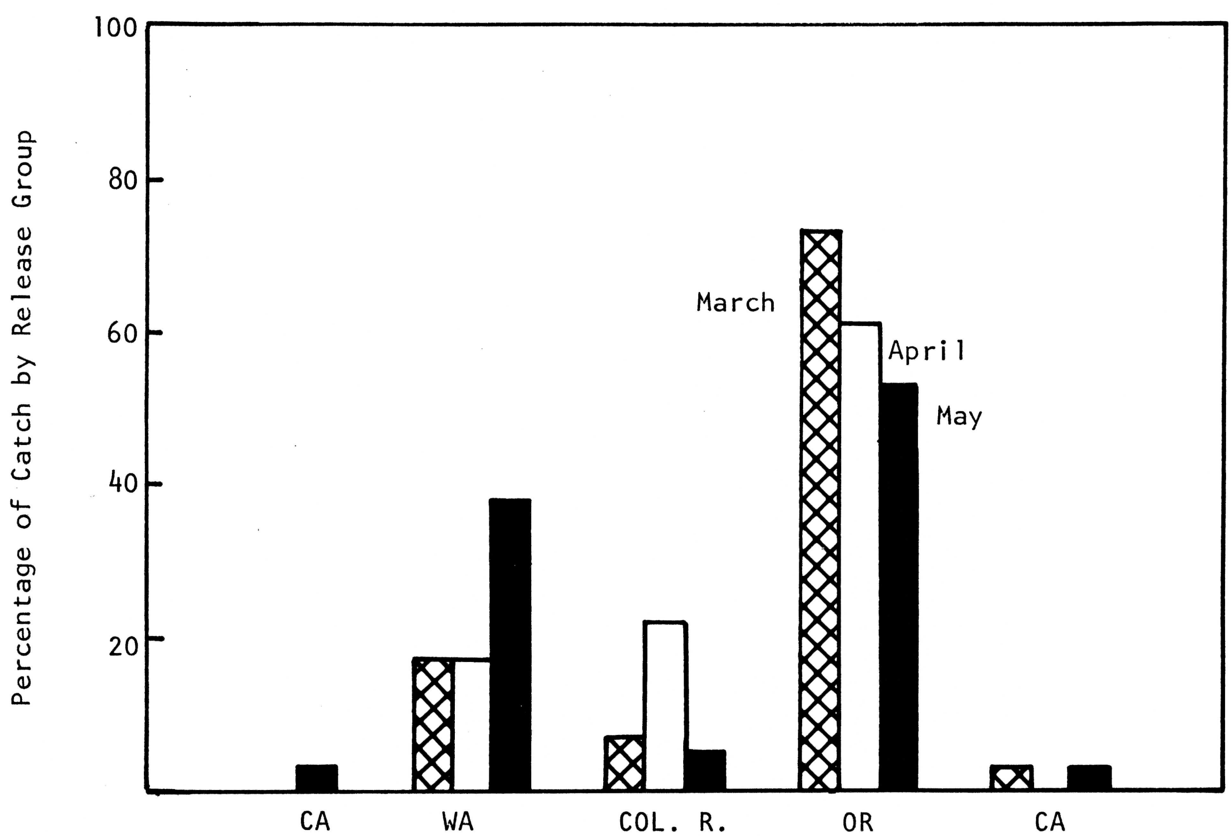

Figure 2. Relative Distribution of 3-Year-Old 1974-Brood Coho by Release Group from Klaskanine Hatchery

During FY '78, 2-year-old marked fish returning to Sandy Hatchery were recovered. Preliberation and recovery data are summarized in Table 9. Recoveries of 3-year-old coho will be made in summer-fall 1978.

LITERATURE CITED

Falconer, D. S. 1967. Introduction to quantitative genetics. Ronald Press, New York, 365 pages.

Table 9. Summary of Preliberation Data and Marked Coho Salmon Recoveries Through 1977 of the 1975-Brood Pond Loading Study at Sandy Hatchery

R (flow)	Density Factor	Lbs per Foot^3	Total Lbs Fish	Grams[1/] per Fish	Tagged Fish Released	Recoveries of 2-Yr-Old Fish No.	Recoveries of 2-Yr-Old Fish % of Release
0.4 (122)	0.13	0.71	1,752	27.82	24,893	24	0.096
"	0.13	0.71	1,751	27.82	25,813	17	0.066
mean		0.71	1,752				0.081
0.8 (244)	0.24	1.32	3,254	26.59	22,854	20	0.088
"	0.23	1.26	3,100	25.21	24,477	16	0.065
mean		1.29	3,177				0.076
1.6 (500)	0.48	2.62	6,436	25.67	23,416	19	0.081
"	0.46	2.51	6,170	25.09	20,139	16	0.079
mean		2.57	6,303				0.080

1/ *Values are for tagged fish only in their respective populations.*

INFECTIOUS DISEASES

This report summarizes disease investigations conducted during Fiscal Year '78 at 10 coastal hatcheries. More detailed information is available in fish examination reports and special reports prepared during this period.

Hatchery Disease Examinations

Data presented in Table 10 indicate that furunculosis and bacterial kidney disease were the most frequently diagnosed bacterial diseases. However, bacterial gill disease, cold water disease, and red-mouth disease were also prevalent. *Costia* and *Trichodina* were the most commonly found ectoparasites, though *Ichthyophthirius*, *Gyrodactylus*, *Epistylis*, gill ameba were also encountered.

Infectious hematopoietic necrosis (IHN) virus was detected in fingerling fall chinook (1977 brood) at Elk River Hatchery.

Immunization Studies

Intraperitoneal injection of cutthroat brood stock at Alsea steelhead hatchery with furunculosis and bacterial kidney disease antigens in Freund's complete adjuvant resulted in increased antibody titer against each. This procedure may afford a means to reduce loss caused by these diseases.

Ozone Treatment of Water

Incubation of approximately 100,000 coho eggs and resultant fry in ozonated water at Alsea Salmon Hatchery apparently resulted in enhanced fish condition, fungus inhibition, and reduced loss. Bacterial content (evidenced by plate count of cold water disease like colonies) was much less in ozonated water.

Table 10. Fish Disease Incidence and Treatment, July 1, 1977 Through June 30, 1978

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	1976	Furunculosis	x												3% TM-50 for 14 days
	F.Chinook	1976	Gill amoeba & furunc.		x											1:5,000 formalin 1 hr for gill amoeba 3% TM-50 for 14 days for furunculosis
	F.Chinook	1976	Bacterial gill disease			x										4 consecutive day treatments hyamine 1622 at 1,2,2,2 ppm for 1 hr
	F.Chinook	1976	Prelib exam grey tail				x									Liberate as soon as possible
	Coho	1976	Prelib:no pathogens								x					Liberate as scheduled
	Coho	1977	Cwd								x					3% TM-50 for 14 days
	Coho	1977	Prelib:cwd										x			Liberate as scheduled
	F.Chinook	1977	Prelib:no pathogens										x			Liberate as scheduled
	Coho	1977	Prelib:no pathogens												x	Liberate as scheduled
	F.Chinook	1977	Redmouth												x	3% TM-50 for 10 days
	Coho	1977	Redmouth & furunc.												x	3% TM-50 for 10 days
Alsea Trout (steelhead)	Cutthroat	1977	"*Ich*," *Costia*, *Epistylis*, furunc.	x												3% TM-50 for 15 days for furunc. 30 ppm formalin for 7 hrs & flush for "*Ich*"
	Cutthroat& Steelhead	1977	"*Ich*" & furunc.		x											3% TM-50 for 15 days for furunc. 30 ppm formalin for 7 hrs & flush for "*Ich*"
	Cutthroat	1977	Prelib:no pathogens									x				Liberate as scheduled
	Steelhead	1977	Prelib:"*Gyro*," *Epistylis*, & *Trichodina*									x				Liberate as scheduled
	W.Steelhead	1976 1977	Prelib:"*Gyro*," *Epistylis*, & *Trichodina*									x				Liberate as scheduled
	Cutthroat	1978	Bacterial gill dis.												x	Hyamine 1622 1 ppm then 2,2,2 for 4 days
			Bacterial gill dis. Redmouth												x	Hyamine 1622 4 x 1 hour--3% TM-50 for RM

Table 10 (cont'd)

Hatchery	Species	Brood Year	Disease or Other	J	A	S	O	N	D	J	F	M	A	M	J	Recommendations--Treatment, etc.
Bandon	Cutthroat	1978	*Costia* & furunc.										x		x	1:6,000 formalin for *Costia* & 3% TM-50 for furunculosis
	Cutthroat	1978	No pathogens												x	Prophylactic formalin--M.G. for *"Ich"*
	W.Steelhead S.Steelhead	1978	Some *Costia*												x	0.1 ppm M.G. & 25 ppm formalin 3 days week for *Costia*
Cedar Creek (Nestucca)	Cutthroat	1977	Prelib:no pathogens		x											Liberate as scheduled
	W.Steelhead	1977	Pretransfer:no pathogens		x											Transfer as scheduled
	Sp.Chinook	1976														
	F.Chinook	1977	Prelib:no pathogens				x									Liberate as scheduled
	Cutthroat	1977	Pretransfer:*Epistylis*								x					Transfer as scheduled
	S.Steelhead	1977														
	W.Steelhead	1977	*Epistylis*								x					None
	Cutthroat		Prelib:no pathogens									x				Liberate as scheduled
	S.Steelhead	1977														
	W.Steelhead	1977	Prelib:no pathogens									x				Liberate as scheduled
	F.Chinook	1977	Coagulated yolk in fry									x				None
	Steelhead	1978	No pathogens											x		None
Elk River	F.Chinook	1977	IHN virus,*Costia*, & CWD									x				1:6,000 formalin 1 hr for *Costia*
	W.Steelhead	1977	*Gyrodactylis*									x				Liberate as scheduled
	F.Chinook	1977	Gill amoeba, red mouth												x	1:4,000 formalin for 2 days for 1 hr for amoeba 2 g furox--50/3 g sulfa/100 lbs fish/day for RM

Table 10. (cont'd)

Hatchery	Species	Brood Year	Disease or Other	J	A	S	O	N	D	J	F	M	A	M	J	Recommendations--Treatment, etc.
				Month												
N. Nehalem	Coho	1976	*Costia* & furunc.	x												3% TM-50 for 20 days
	Sp.Chinook	1976	Furunculosis		x											3% TM-50 for 14 days
	W.Steelhead	1977	"*ICH*" & *Trichodina* furunculosis		x											1:6,000 formalin--0.5 ppm M.G. over 4 days for ectoparasites. 3% TM-50 for 14 days for furunc.
	Coho	1976	Furunculosis		x											3% TM-50 for 14 days
	Sp.Chinook	1976	Opportunistic *Aeromonas-pseudomonas* bacteria		x											None
	Cutthroat	--	Ceratomyxa exam--negative		x											None
	Sp.Chinook	1976	Prelib:no pathogens			x										Liberate as scheduled
	W.Steelhead	1977	BKD								x					None
	Coho	1976	Prelib-BKD								x					Liberate as scheduled
	Coho	1977	CWD								x					3% TM-50 for 14 days
	Coho	1976	Prelib.BKD & CWD								x					Liberate as scheduled
	Coho	1977	No pathogens									x				None
	W.Steelhead	1977	BKD--furunculosis									x				Liberate as scheduled
	Coho	1977	CWD										x			3% TM-50 for 14 days
	Coho	1977	Light CWD											x		None
	W.Steelhead	1978	*Trichodina*											x		2 treatments 1 ppm M.G. 1 hr 3 days apart
	Coho	1977	Fungus												x	M.G. treatment
Salmon River	Sp.Chinook	1976	Bacterial gill disease	x												Increase water flow Hyamine 1622 4x for B.G.D.
	F.Chinook	1976	*Trichodina*		x											Liberate as scheduled
	Sp.Chinook	1976	*Trichodina* & furunc.			x										1 ppm M.G. 1 hr for 3 days for "*Trich*" 3% TM-50 for furunc.
	Coho	1976	Light BKD			x										None
	Sp.Chinook	1976	Light furunculosis			x										Liberate as scheduled
	F.Chinook	1976	Light furunculosis			x										Liberate as scheduled
	Coho fry	1977	Gas bubble disease CWD								x					N_2 supersaturation not a cause of G.B.D. TM-50 for CWD
	Coho	1976	CWD & BKD								x					Liberate as scheduled
	F.Chinook	1977	*Costia*											x		1:6,000 formalin 1 hr.
	Coho	1976	BKD & opport.bact.											x		Liberate as scheduled
	Coho	1977	BKD--*Costia*--CWD													1:6,000 formalin 1 hr for *Costia*

Table 10 (cont'd)

Hatchery	Species	Brood Year	Disease or Other	J	A	S	O	N	D	J	F	M	A	M	J	Recommendations--Treatment, etc.
Siletz	Coho	1976	Furunculosis	x												3% TM-50 for 15 days
	Coho	1976	Light B.K.D.								x					Liberate as scheduled
	Coho	1977	*N. salmonicola* & *Costia*												x	1:6,000 formalin 1 hr for *Costia*
Trask	Sp.Chinook	1976	Furunculosis		x											4% TM-50 for 14 days
	Sp.Chinook	1976	Prelib:furunculosis			x										3% TM-50 for 14 days then liberate
	F.Chinook	1976	Prelib													
	Sp.Chinook	1976	Furunculosis				x									3% TM-50 for 14 days then liberate
	Coho	1976	Light BKD								x					None
	Coho	1977	CWD								x					3% TM-50 for 14 days
	Coho	1977	Pretransfer, no pathogens													Transfer as scheduled
	Coho	1976	*Costia*											x		1:6,000 formalin for 1 hour
	Coho	1977	Furunculosis												x	4% TM-50 for 14 days

NUTRITION STUDIES

Highlights

Zinc supplementation of a moist diet containing 40% soybean meal reduced mortality and improved feed conversion.

An acidified combination of hake and tuna viscera stored at ambient temperatures up to 8 months appeared satisfactory as the wet component of OMP in a laboratory study.

Fish suffering from a skin lesion/anemia malady had altered serum proteins and mineral composition. Blood smears suggested an inflamatory response.

Vitamin C losses in OMP during manufacturing, storage, and thawing varied widely, but occasionally left deficient amounts. A fat encapsulated vitamin C product was quite stable in OMP.

Coho fingerlings were tagged with coded wires in experiments designed to determine the needed level of fish oil and effectiveness of various fish oils.

An oral vibrio vaccine did not increase post-release survival of hatchery coho salmon.

The antioxidants tertiary butylhydroquinone (TBHQ) and ethoxyquin were not toxic to coho salmon.

The Abernathy Diet produced comparable coho survival rates to OMP.

Cooperative Projects with OSU

Cooperative projects conducted jointly with Oregon State University, Seafoods Laboratory, included fish meal replacements, acidified wet fish, vitamin C losses, and antioxidant toxicity. OSU personnel provided the necessary biochemical and *in vitro* work, while ODFW investigators were responsible for feeding trials and other biological aspects.

Technical Services

Feed Specifications and Contracts

Oregon Moist Pellet (OMP) and mash specifications were prepared for the January-June and July-December 1978 contract periods. Changes in vitamin supplementation included increasing the level of folacin and decreasing levels of inositol and vitamin K. Beginning in July we allowed use of reprocessed herring oil and required an increase in fish meal when steam injection is used to pasteurize the wet fish. Current formulations are given in Tables 11 to 13.

Both contracts were awarded to Bioproducts, Inc., Warrenton, Oregon. The average price in January was 26.71¢/lb, and in July it was 29.8¢/lb.

Table 11. Formula and Ingredient Specifications, Oregon Pellet (OP-2), July 1978

Ingredient	Percentage of Diet
MEAL MIX	
Fish meal--herring meal (min. 70% protein, max. 3% NaCl) must be used as 100% of the fish meal in each batch of 1/16-inch and smaller pellets, and at no less than 50% of the fish meal in each batch of larger pellets. Anchovy, domestic or Peruvian (min. 65% protein); hake (min. 68% protein); or menhaden (min. 60% protein) may be used as the remaining portion for larger pellets provided the total fish meal is increased to 30% of the diet (31% if menhaden meal is used).	28.0-32.0
Cottonseed meal--prepressed, solvent extracted, min. 48.5% protein, max. 0.055% free gossypol.	15.0
Dried whey product--partially delactosed, min. 15% protein	5.0
Wheat germ meal--min. 23% protein and 7% fat	Remainder
Shrimp or crab meal--max. 3% NaCl, min. 25% protein	4.0
Corn distillers dried solubles--may contain up to 30% "grains" in place of "solubles." Level depends on fish meal and oil levels	4.0
VITAMIN PREMIX	1.5
WET MIX	
Wet fish--limited to tuna viscera, salmon viscera, "bottom fish" (whole or fillet scrap), herring, hake 1/, and dogfish, with the following provisions: (1) all must be pasteurized; (2) two or more must be used in combination with no one exceeding 15% of the diet; (3) 1/32- and 3/64-inch pellets must contain at least 7.5% tuna viscera but no fillet scrap; and (4) visceral products must contain livers but no heads or gills. If steam injection is used to pasteurize, the fish meal must be increased by 1.0% of the diet.	30.0
Herring oil--stabilized with 0.3% BHA-BHT (1:1), free fatty acids (FFA) not more than 3%. The BHA-BHT must be added at time of reprocessing if reprocessed oil is used.	6.0-6.75 1/
Choline Chloride--liquid, 70% product	0.5
	100.0

1/ *Special condition when using hake--add 0.5% oil for every 10% hake in total diet.*

Table 12. Formula and Ingredient Specifications, Oregon Mash (OM-3), July 1978

Ingredient	Percentage of Diet
MEAL MIX	
Herring meal--min. 70% protein, max. 3% NaCl	48.0
Wheat germ meal--min. 23% protein and 7% fat	10.0
Dried whey product--partially delactosed, min. 15% protein	10.0
VITAMIN PREMIX	1.5
WET MIX	
Tuna viscera--no heads or gills, with livers, pasteurized	10.0
Pasteurized salmon viscera (no heads or gills), herring, and/or turbot	10.0
Herring oil--stabilized with 0.3% BHA-BHT (1:1), free fatty acids (FFA) not more than 3%. The BHA-BHT must be added at time of reprocessing if reprocessed oil is used	10.0
Choline chloride--liquid, 70% product	0.5
	100.0

Table 13. Oregon Vitamin Premix Specifications, July 1978 1/

Vitamin	Guaranteed Minimum Analysis (mg/lb Premix)	Source Limitation
d-Biotin	18.0	
B_6	535.0	Pyridoxine HCL (650 mg)
B_{12}	1.8	
C	27,000.0	Ascorbic acid
E	15,200.0	Water dispersible alpha tocopheryl acetate
Folacin	385.0	Folic acid
Myo-inositol	8,000.0	Not phytate
K	180.0	Menadione sodium bisulfite complex (545 mg)
Niacin	5,700.0	
d-Pantothenic acid	3,200.0	d-Calcium pantothenate (3,478 mg), or d L-Calcium Pantothenate (6,957 mg)
Riboflavin	1,600.0	
Thiamin	715.0	Thiamine mono-nitrate (778 mg)

1/ *Diluent must be a cereal product.*

Quality Control

OMP production at the manufacturer's plant was inspected periodically, and samples of ingredients and pellets assayed to determine compliance with specifications. Much of the herring meal used appeared to contain less protein and more salt than specified. Assays of wet fish revealed higher moisture than expected, due to use of steam injection to pasteurize the wet fish, and this prompted a change in feed specifications to compensate for dilution of protein.

Feed Programming

Feeding schedules were prepared for 12 coho and three spring chinook hatcheries. The coho were programmed for a May 1, 1979, release at 18 to 20 fish/lb, and most spring chinook were scheduled for March 15, 1979, at 8 or 10 fish/lb.

Fish Meal Replacements

Zinc Supplementation of Soybean Meal

We conducted a laboratory feeding trial to determine effects of adding zinc to a moist ration containing 40% soybean meal. Soybean meal contains phytic acid and other mineral binding agents, and zinc supplementation has given other workers good results with rats.

We began our experiment with six levels of zinc sulfate in the soybean diets, supplying from 0 to 4,000 ppm zinc, and compared results with a positive control diet with protein supplied entirely from fish sources. The diets were fed to duplicate lots of spring chinook fingerlings averaging 9.5 grams each at the start, and reared at 10-11 C. The fish refused to eat the soybean diets containing more than 500 ppm zinc, and they were terminated early; the others were fed for 79 days. We fed all lots the same weight of food each day if it was at all possible.

Results at the end of the trial are summarized in Tables 14 and 15. Weight gain was significantly reduced when soybean protein was used, and the addition of zinc had no effect on weight gain. The diets containing soybean meal were converted to fish weight less efficiently than the all fish protein control, but zinc significantly improved efficiency of these diets. Mortality was greatly increased by soybean meal without added zinc, but there was no signficant difference in mortality between the all fish protein control and the zinc supplemented soybean diets. All lots were afflicted with skin lesions and anemia, and there was no effect on this condition due to diet.

In Table 15 serum profiles revealed low albumin/globulin ratios in all groups, including the all fish protein control, suggesting an infectious disorder. The fish fed soybean meal without supplemental zinc also had elevated copper, reduced zinc, and reduced alkaline phosphatase levels in their blood serum. Liver assays showed reduced zinc and copper in fish fed soybeans without added zinc. Supplementation of zinc in the soybean diets resulted in "recovery" of serum zinc and alkaline phosphatase levels, and also increased zinc and copper stores in the liver.

The poor growth suggested there were more problems with soybean meal, as a protein source for fish, than just zinc deficiency.

Table 14. Summary of Results, Soybean-Zinc Feeding Trial, 1977 1/

Diet Description							
Source of Protein	Zn Added (ppm)	Weight Gain (%)	Feed Conversion (As fed)	Total Mortality (%)	Skin Lesions (%)	Hematocrit (%)	Feed Acceptance Index 2/
Fish	0	47.3a	2.13a	4.8a	54a	23.9a	2.4a
Soybean	0	20.4b	6.14c	26.8b	60a	21.3a	6.4c
Soybean	100	26.4b	3.98b	6.3a	64a	22.4a	4.0ab
Soybean	500	21.4b	4.92b	9.0a	60a	22.5a	5.6bc

1/ *Mean values in a column with the same letter did not vary significantly from each other (P = >0.05).*

2/ *Average number of feedings required to consume daily ration.*

Table 15. Results of Serum and Liver Analyses, Soybean-Zn Feeding Experiment

Parameter	Units	Fish Protein Control	Soybean Meal Diets (0 ppm Zn)	(100 ppm Zn)	(500 ppm Zn)
Serum Assays					
Proteins					
Alpha globulins	g/100cc	2.4	2.1	2.4	2.1
Beta globulins	"	0.7	0.6	0.5	0.8
Gamma globulins	"	0.4	0.2	0.4	0.3
Total globulins	"	3.5	2.9	3.3	3.2
Albumin	"	0.2	0.1	0.3	0.1
Total Protein	"	3.7	3.0	3.6	3.3
A/G Ratio		0.06	0.03	0.09	0.03
Metals & Ions					
Zn	mcg/100cc	1,379	750	1,271	1,262
Cu	"	98	151	109	97
Ca	Meq/l	4.8	4.5	4.7	4.6
Mg	"	1.7	1.5	1.7	1.7
P	"	6.0	5.9	7.2	6.9
Na	"	131	135	130	134
K	"	4.7	4.3	5.5	5.4
Cl	"	106	107	101	110
Enzymes					
Alkaline Phosphatase	IU/l	40	13	24	24
Lactic Dehydrogenase	"	3,421	3,890	4,124	3,462
Other					
Glucose	mg/100cc	154	107	114	118
LIVER ASSAYS					
Zinc	mg/kg (wet)	20	15	19	21
Copper	"	59	4.6	42	26
Iron	"	85	79	72	71

Robert Smith's Soybean Diet and Meal

Dr. Robert Smith, Tunison Laboratory of Fish Nutrition, reported promising results with both trout and salmon fed his dry diet containing 80% full fat cooked soybeans. This year we tested both his meal, as an isonitrogenous replacement for 50% of the fish meal in OMP and his dry diet. The diets were fed in the laboratory to duplicate lots of spring chinook salmon and compared with OMP. Our fish wouldn't eat the dry soybean diet and it was terminated after 6 weeks. The OMP containing his full fat cooked soybean meal was eaten fairly well, but after 12 weeks, weight gain was significantly less (174% vs 280%) and feed conversion significantly higher (1.80 vs 1.18) than regular OMP. Mortality was also significantly higher from the OMP containing soybean meal, but these were mostly emaciated fish and not the good appearing fish suffering from tetany that we noted from soybean meal last year.

Acidified Wet Fish

Energy costs to freeze and store wet fish for OMP are becoming more expensive. Eliminating the need for frozen storage by stabilizing liquified fish against microbial growth with various acids has been used effectively by other industries. This procedure might enable OMP manufacturers to process and cheaply store large quantities of fishery products in bulk without refrigeration as they become available in season from the fishery or processing plants. This should reduce feed costs and ease wet fish supply problems for OMP.

Storage Trial

The effects of storage up to 8 months at ambient temperatures on various chemical and biological qualities of autolysed fish processing wastes and whole fish was investigated. The autolysates were treated with phosphoric acid to pH 3.25 and potassium sorbate to control microbial spoilage. No antioxidant was used. Table 16 summarizes results.

The acidified fish products were stable to microbial growth at all storage treatments. Hydrolytic rancidity (FFA) did not progress during storage. Oxidative rancidity (TBA) was not controlled during storage, and was especially bad in the fish processing wastes. Proteolysis (glycine equivalents) did not occur during storage; levels of available lysine and tryptophan remained constant. Protein efficiency ratio for rats (PER) was not affected by storage of whole hake, English sole waste, or rockfish wastes; but storage had a significant overall effect and this occurred during the first 4 months. PER's from processing wastes were inferior to whole fish.

Feeding Trial

We tested whole hake combined with tuna viscera, and various combinations of fish processing wastes (fillet scrap), acidified to pH 3.25 with phosphoric acid, and stored up to 8 months at ambient temperatures in a factorial experiment. These were compared with relatively fresh samples of hake/tuna viscera and rockfish processing waste, which were not acidified. The stocks of stored fish were those used in the previously described storage trial. We fed these products in OMP to duplicate lots of coho salmon for 24 weeks in the laboratory. Initial fish size averaged 6.3 grams.

Table 16. Results of Acidified Wet Fish Storage Trial

Fish Product	Storage Time (Months)	Microbes (N/g x 10^3)	FFA (%)	TBA (mg/kg)	Glycine Equiv. (mg/1g g N)	Avail.Lysine (g/16 g N)	Tryptophan (g/16g N)	PER (% ANRC Casein)
Whole Hake	0	5.0	7.6	2.3	9.5	6.4	1.8	104
	4	4.8	6.3	4.8	9.4	6.9	1.7	99
	8	4.6	6.4	5.5	9.4	6.5	2.2	100
Whole Dogfish	0	5.5	3.1	3.5	3.7	4.6	0.9	101
	4	6.7	6.2	8.3	3.0	4.5	0.8	87
	8	7.3	6.1	10.4	3.4	4.4	1.0	92
Eng.sole Waste	0	52.0	7.1	5.2	6.3	6.1	3.1	81
	4	17.0	6.9	14.7	6.0	6.7	3.0	76
	8	18.0	7.4	13.9	6.7	5.6	3.4	80
Dover Sole Waste	0	3.0	11.8	4.9	6.4	5.3	2.9	-
	4	5.4	11.2	11.7	6.3	5.7	2.6	-
	8	15.2	10.5	16.2	6.5	5.4	2.7	-
Rockfish Waste	0	46.0	6.2	6.8	7.3	5.8	3.1	86
	4	36.0	7.7	16.4	7.0	5.6	3.0	82
	8	21.3	8.1	16.0	6.9	5.4	3.3	78
True Cod Waste	0	30.0	19.4	5.5	7.0	5.6	2.6	-
	4	34.0	23.5	12.5	8.0	5.9	3.0	-
	8	21.4	22.8	6.4	7.6	5.4	3.4	-
Whole hake: tuna visc.	0	TFC1/	22.3	7.0	11.7	5.9	2.2	-
	4	4.6	24.9	16.7	12.0	6.4	2.0	-
	8	3.6	21.5	11.9	11.8	6.2	2.3	-

1/ *Too few to count.*

Results are summarized in Table 17. We found that weight gain did not vary significantly with storage time, but the difference between stored samples of processing wastes and whole hake/tuna viscera was significant. Feed conversions varied significantly by both storage time and fish type, and there was a significant interaction, indicating that both processing waste diets were inferior to whole hake/tuna viscera and they were especially poor at 4-months storage. "Streaked" kidneys, resembling nephrocalcinosis, were confined to the processing waste diets, and this was significant at 4- and 8-months storage.

These results, similar to those from the storage trial, suggest an acidified whole fish/viscera combination should be tested on a hatchery scale, while acidified carcass wastes need further investigation in the laboratory.

Skin Lesions/Anemia

Our FY '77 progress report described this problem that affects most of the ODFW spring chinook hatcheries.

Increased Vitamin Supplementation

We conducted a long term (45 week) laboratory feeding trial to determine if the skin lesion/anemia problem was in any way related to vitamin deficiency. A vitamin supplement was formulated to boost all levels in the OMP beyond National Research Council recommendations, taking into account expected losses during processing and storage. This supplement was compared with the regular OMP supplement, which may have been deficient in several vitamins. The test fish were spring chinook from South Santiam Hatchery, a station where the skin lesion/anemia condition has been a problem during the last several years.

Hematocrits were taken periodically during the course of the trial, and all were satisfactory. At the end of the trial, differences in weight gain, feed conversion, and hematocrit were not significant. No skin lesions were found in either treatment; all fish were examined.

Serum and Tissue Tests

This year we collected samples of blood and tissue from spring chinook at selected sites to see if we could find clues to the etiology of the skin lesion/anemia problem. Samples were collected from afflicted fish and compared with samples from apparently healthy fish. We selected spring chinook with skin lesions at South Santiam Hatchery, Oakridge Hatchery, and Sandy Field Laboratory for examples of "sick" fish, and Cole Rivers Hatchery, Eagle Creek NFH, and Little White Salmon NFH were used for samples of "healthy" fish. The fish at Eagle Creek NFH appeared normal at time of sampling, but skin lesions were subsequently found at that hatchery.

Blood from 300-600 fish at each station was pooled to provide enough material for serum chemistry profiles. Tissue assays for metals were conducted using pooled liver samples. Results are summarized in Tables 18 and 19.

Table 17. Results of Acidified Wet Fish Feeding Trial 1/

Fish Product	Storage Time (mos.)	Weight Gain (%)	Feed Conversion	Mortality 2/ (No.)	Hematocrit 2/ (%)	Streaked Kidney (%)
Whole Hake & Tuna Visc.	Fresh, not acidified	298bc	1.60b	2	32.5	0
Rockfish Waste		324ab	1.50a	2	32.8	0
English Sole &	0	305abc	1.61bc	0	32.2	2.8a
Dover Sole	4	260d	1.78e	7	29.5	22.2b
Wastes	8	282cd	1.68cd	3	29.9	27.8bc
Rockfish &	0	288cd	1.68cd	2	31.0	2.8a
True cod	4	260d	1.72de	3	28.9	38.0c
Wastes	8	295bc	1.58ab	2	31.2	22.2b
Whole Hake &	0	313abc	1.61bc	3	31.0	0
Tuna	4	335a	1.56ab	2	32.2	0
Viscera	8	328ab	1.56ab	1	34.6	0

1/ *Mean values in a column with the same letter did not vary significantly from each other ($P = >0.05$).*

2/ *Differences not significant ($P = >0.05$).*

Table 18. Summary of Blood Chemistries and Tissue Assays

"Healthy" Fish vs Fish with "Dermal Necrosis Disorder"
Spring Chinook Fingerlings (1976 Brood)

		Healthy Appearing		"Healthy"?? 2/	Afflicted with "Skin Lesions" 3/		
Parameter	Units	Cole Rivers	Little White 1/	Eagle Cr. NFH	S.Santiam	Sandy Lab	Oakridge 4/
Sampling Data							
Date		11/3/77	3/2/78	12/21/77	10/25/77	12/15/77	1/13/78
App. Fish Size	No/lb	10.5	24	15-20	12	31	12-13
Water Temp.	F	49.5	40	39.5	49	50-51	44
Sampling Time		AM	AM	AM	AM	AM	PM
Date Last Fed		11/2/77	3/1/78 AM	12/20/77	10/24/77	12/12/77	1/12/78 AM
Serum Proteins							
Alpha glob.	g/100cc	1.8	2.7	3.4	2.8	2.4	3.6
Beta glob.	"	0.4	0.2	0.4	0.9	0.7	0.7
Gamma glob.	"	0.2	0.0	0.2	0.4	0.4	0.6
Total glob.	"	2.4(74.9) 5/	2.9(87.9)	4.0(88.9)	4.1(93.2)	3.5(94.6)	4.9(90.8)
Albumin	"	0.8(25.1)	0.4(12.1)	0.5(11.1)	0.3(6.8)	0.2(5.4)	0.5(9.2)
Total Protein	"	3.2	3.3	4.5	4.4	3.7	5.4
A/G Ratio	"	0.33	0.14	0.12	0.07	0.06	0.10
Enzymes							
LDH	IU/l	1,207	1,310	1,690	2,193	3,421	2,874
Alkaline Phos.	"	--	18	33	--	40	11
SGO-T	"	-- 6/	115	184	--	--	171
SGP-T	"	--	23	13	--	--	17
Minerals & Ions							
Fe	mcg/100cc	121	136	155	109	--	64
Cu	"	34	54	49	55	98	73
Zn	"	1,627	2,020	1,623	1,818	1,379	1,275
Ca	Meq/l	--	5.5	5.6	--	4.8	6.3
Mg	"	--	1.6	--	--	1.7	2.0
Na	"	--	156	152	--	131	152
K	"	--	2.0	1.7	--	4.7	1.5
P	"	--	6.7	6.2	--	6.0	8.1
Cl	"	--	125	130	--	106	126
Fe Binding Cap.	mcg/100cc	387	333	359	352	--	411
Transferin Satur.	%	31.3	40.8	43.2	31.0	--	15.6

Table 18. (cont'd)

Parameter	Units	Healthy Appearing		"Healthy"?? 2/	Afflicted with "Skin Lesions" 3/		
		Cole Rivers	Little White 1/	Eagle Cr. NFH	S.Santiam	Sandy Lab.	Oakridge 4/
Vitamins							
Folic acid	ng/ml	32.9	50.7	81.2	104.4	--	95.9
Vitamin C	mg/100cc	0.6	0.6	0.9	1.1	--	1.3
Other							
Glucose	mg/100cc	--	109	92	--	154	106
Uric acid	"	--	0.2	1.5	--	--	0.8
Creatinine	"	--	0.6	0.2	--	--	0.4
BUN	"	--	6	3	--	--	2
Bilirubin (Total)	"	--	0.1	0.1	--	--	0.2
Cholesterol	"	--	267	394	--	--	290
Liver Tissue Assays							
Fe	mg/kg wet	--	209.4	105.3	--	85	78.5
Cu	"	--	8.7	9.3	--	59	22.6
Zn	"	--	20.7	31.6	--	20	26.7
Mg	"	--	225.0	176.8	--	--	169.5
Pb	"	--	71.3	16.8	--	--	14.1
Cd	"	--	31.2	4.8	--	0.1	3.8

1/ *Fish at this station had low level of kidney disease.*
2/ *Appeared healthy at sampling. Fish exhibiting skin lesions were subsequently discovered at this hatchery.*
3/ *Only fish with typical skin lesions were collected for blood sampling.*
4/ *South Santiam stock held at Oakridge (Pond 21).*
5/ *Values in parentheses are percentages.*
6/ *-- means no data.*

Table 19. Summary of Hematological Tests
"Healthy" Fish vs Fish with "Dermal Necrosis Disorder"
Spring Chinook Fingerlings (1976 Brood)

		Healthy Appearing		Healthy ?? 2/	Afflicted with "Skin Lesions" 3/		
Parameter	Units	Cole Rivers	Little White NFH 1/	Eagle Cr. NFH	S.Santiam	Sandy Lab 4/	Oakridge 5/
Sampling Date		--	3/1/78	12/20/77	--	12/2/77	1/12/78
No. of Fish		--	9	10		8	10
Hematocrit							
Mean	%	--	31.7	34.5	--	17.6	25.8
Range			28-33	31-40		14-22	21-32
Red Cell Count	Mill/mm^3						
Mean		--	1.249	1.718	--	0.770	0.996
Range			1.090-1.532	1.532-2.255		0.600-1.088	0.885-1.190
MCV	Microns^3						
Mean		--	255	203	--	230	259
Range			209-294	146-241		202-254	229-297
Lymphocytes & Thombocytes	No./mm^3						
Mean		--	33,371	31,137	--	19,840	44,548
Range			24,279-46,129	17,428-56,194		7,954-35,178	22,638-79,968
Granulocytes	No./mm^3						
Mean		--	607	751	--	697	1,228
Range			246-1,023	306-1,077		56-1,926	483-2,098
Macrophages? 6/	No./mm^3						
Mean		--	50	62		546	2,757
Range			0-157	0-161		151-1,255	1,015-6,507

1/ *These fish reportedly had a low incidence of BKD.*
2/ *Appeared to be normal when examined during blood sampling. Fish with high incidence of skin lesions were subsequently discovered in other ponds at this hatchery in January 1978.*
3/ *Only fish with typical skin lesions were sampled.*
4/ *These fish were very "sick!" They were off feed, lethargic, and did not survive handling well.*
5/ *South Santiam stock held in ponds 21, 23, and 28.*
6/ *Cells that resemble those that would be called monocytes or macrophages in other animals.*

The data in Table 18 suggest fish with skin lesions had the following differences from normal appearing fish: (1) relative decreases in albumin and increases in globulins, (2) elevated lactate dehydrogenase activity (LDH), (3) increased serum copper, (4) reduced serum iron and zinc, (5) increased liver copper, and (6) reduced liver iron.

Hematological examinations (Table 19) again demonstrated that spring chinook with these skin lesions are commonly very anemic, with reduced red cell numbers and hematocrits. Mean corpuscular volumes (MCV) did not appear to differ between afflicted and healthy fish, but so far we have no good indication of the type of anemia. The most striking characteristics of blood from afflicted fish, an increase in numbers of macrophages, indicated an inflamatory response. Also, there is an apparent shift in white cell age toward more immature forms, suggesting the fish are trying to combat some agent.

Vitamin C Losses in OMP

The OMP is supplemented with a vitamin premix that theoretically supplies 89.3 mg vitamin C per 100 g of ration as fed. Previous analyses of the vitamin C content of OMP suggested that amounts reaching the fish are frequently below the detectable level. This year we conducted *in vitro* investigations to determine: (1) the characteristics of vitamin C destruction in OMP, (2) the relative level of decomposition that occurs through the feed system, and (3) means of protecting vitamin C in OMP.

Vitamin C Losses in OMP During Laboratory Preparation and Thawing

A test sample of OMP was prepared by mixing the wet and dry components. The vitamin C content of the dough with respect to time was determined. A sample at the end of 5 minutes mixing was pelletized and frozen at - 30 C.

Vitamin C was shown to decompose rapidly during initial mixing of wet and dry components. Analysis showed the dry mix (including the vitamin premix) contained 146.7 mg vitamin C/100 g which would yield a theoretical 93.1 mg/100 g in the completed ration. After mixing for 5 minutes the vitamin C content of the dough was 79.7 mg/100 g, a 14.4% loss from theoretical in a short period.

The loss of vitamin C in samples of OMP thawed at 2 C and 25 C showed the marked effect of temperature on the rate of decomposition. Using regression methods, the rate of decomposition computed between 2 and 8 hours was 1.78 and 5.23 mg vitamin C/100 g/hr at 2 C and 25 C, respectively.

This laboratory evaluation suggested that the majority of the vitamin C loss probably occurs after the diet is fabricated.

Relationship of Initial Vitamin C Concentration to Rate of Decomposition

One means of increasing the vitamin C available to the fish might be to increase the OMP supplementation rate. That could yield a greater than proportional increase if the oxidation potential is finite. This could also support use of biological inactive erythrobate, which is cheaper and possesses identical chemical characteristics to spare dietary vitamin C.

OMP rations were prepared with 50, 100, 200, and 400 mg vitamin C/100 g. The percentage vitamin C retention after 24 hours increased in a linear manner ($r = 0.9351$) up to 200 mg/100 g. The 400 mg level yielded a disproportionate decrease in level of retention.

Loss of Vitamin C During Frozen Storage of OMP

Laboratory evaluation of the decomposition of vitamin C in OMP indicated that a maximum 30% of the supplemented level might be lost during formulation, pelletizing, and freezing. Investigations were initiated to estimate the level that might be lost during frozen storage.

Frozen storage resulted in a surprisingly rapid loss of vitamin C, a rate of 0.644 mg/100 g/day at -17.8 C (0 F) ($r = 0.9654$). One-half of the initial level was lost after 54 days. Complete destruction would occur in 108 days if the linearity of the regression was maintained.

Vitamin C Loss in OMP Made with Acidified Wet Fish

The stability of vitamin C is markedly affected by the pH of its environment, it being most stable under acid conditions. This year we investigated the effect of using acidified wet fish on the stability of vitamin C in OMP. Coupled with this was an evaluation of two vitamin premixes, one with a calcite extender and the other utilizing a wheat flour extender. The basicity of calcite was suspected of contributing to the instability of vitamin C.

Test OMP rations were prepared with the wet fish acidified with four levels of phosphoric acid (up to 4%). Replicates at each level were prepared with the vitamin premix extended with calcite and wheat flour. Decomposition rates were determined at 2 C and 25 C.

Acidification of the wet fish reduced the rate of vitamin C decomposition in a linear manner. Phosphoric acid at 4% of the wet fish reduced decomposition from 17.5% to 35.2%, depending on temperature but not extender. Calcite did not appreciably affect the stability of vitamin C nor clearly affect the protection afforded by acidification.

Vitamin C Loss in Commercially Produced OMP Under Laboratory Conditions

Investigations were conducted to estimate losses that might occur under normal hatchery practices. Frozen OMP is usually removed from the freezer in late afternoon and allowed to thaw overnight at "room" or "cooler" temperatures. Under these conditions, OMP could be out of the freezer between 14 and 24 hours before feeding.

The effect of thawing practices was evaluated using three 50 lb sacks of OMP obtained fresh frozen from one commercial production batch and subjected to the following thawing regimes: (1) room temperature (55 F) for 22 hours; (2) room temperature for 16 hours plus 6 hours at 34 F, and (3) cooler (34 F) for 22 hours. Each bag was sampled at zero time (frozen) and at 6, 16, and 22 hours.

Results are given in Table 20. The vitamin C content of OMP thawed in 50 lb sacks declined rapidly at room temperature; only 46% of the initial level remained after 22 hours. An overnight (16 hrs) thaw at room temperature followed by 6 hours at 34 F increased retention to only 48.7%. Under these temperature regimes, retention of the original supplementation level was only 24.9% and 27.8%, respectively. Thawing at refrigerated temperature (34 F) reduced the rate of vitamin C loss; after 22 hours 67.1% of that found in the frozen pellets and 37.2% of the supplementation level remained.

The loss of vitamin C through commercial processing, frozen storage, and simulated hatchery handling was evaluated. Samples of the vitamin premix, complete dry mix, dough, and frozen pellets were obtained from one production batch at the manufacturing plant. Results are given in Table 21. They suggest considerable loss can occur during frozen storage and only a small portion of the original supplement reaches the fish.

Levels of Vitamin C in OMP at the Hatchery

The vitamin C content of frozen and thawed OMP at various hatcheries was determined to estimate levels reaching the fish. Results are given in Table 12. The requirement of trout and salmon for vitamin C may range from 10 to 50 mg/100 grams dry diet, depending on stress and criteria used to measure need. Dr. Halver in "Fish Nutrition" suggested 20 mg/100 g as a "compromise value." The data in Table 22 suggest considerable loss of vitamin C due to thawing and that deficient amounts reaching the fish are rather common.

Protected Sources of Vitamin C

Use of commercially available protected sources of vitamin C was investigated. The protective mechanism of these sources relies on exclusion of oxygen and/or moisture. The stability of two products in OMP was evaluated at 2 C and 25 C. One, a fat encapsulated product, offered considerable protection (76.4% retention when thawed at 2 C for 16 hours). The other product, ethylcellulose coated, offered little protection. Protection afforded by the fat coated product during frozen storage of OMP is presently under investigation. The cost difference between the fat encapsulated product and crystalline vitamin C, and the protection afforded, suggest a lower level of the encapsulated product might be used and costs would be lower despite higher initial costs per unit of the protected product.

Particle size of the fat encapsulated product will not meet present OMP specifications. This may present a real problem for the smaller pellet sizes, but a revision of specifications may be in order for the larger sizes.

Lipid Nutrition

Previous studies demonstrated a significant increase in survival of hatchery coho by feeding OMP containing herring oil instead of soybean oil. Herring oil is now specified for OMP. Herring oil that meets our specifications for free fatty acids (maximum 3%) is in short supply, and OMP manufacturers must occasionally use soybean oil. Present studies are designed to determine how much herring oil is needed in OMP, and whether other fish oils can take its place and maintain the survival benefit.

Table 20. Decomposition of Ascorbic Acid in Commercial 50 Lb Sacks During Thawing

Temperature Regime	Time (hrs)	Ascorbic acid (mg/100 gm)	Percentage of Zero Time	Percentage of Theoretical 1/	Regression of Ascorbic Acid Content on Time
Ambient	0	48.2 $\pm$ 4.4	100.0	54.0	r = -.9174 2/
(non-heated)	6	42.4 $\pm$ 2.5	88.0	47.5	m =-1.1597
room temp.	16	30.5 $\pm$ 6.0	63.3	34.1	
(55 F)	22	22.2 $\pm$ 6.5	46.0	24.9	
Ambient	0	50.9 $\pm$ 4.4	100.0	57.0	r = -.9149 2/
unheated	6	45.0 $\pm$ 1.3	88.4	50.4	m =-1.1598
room temp.	16	34.5 $\pm$ 7.5	67.8	38.6	
(55 F) plus	22	24.8 $\pm$ 4.0	48.7	27.8	
6 hrs at 34 F					
Refrigerated	0	49.5 $\pm$ 2.5	100.0	55.4	r = -.9247 2/
temperature	6	44.7 $\pm$ 2.1	90.3	50.1	m = -.6928
(34 F)	16	40.1 $\pm$ 2.2	81.0	44.9	
	22	33.2 $\pm$ 3.2	67.1	37.2	

1/ *Based upon 89.3 mg ascorbic acid/100 gm.*

2/ $P \geq .005$.

Table 21. Loss of Vitamin C in OMP During Manufacturing, Storage, and Simulated Thawing at the Hatchery

Sample Description	Mg Vitamin C Per 100 Grams	% of Theoretical Supplement
Oregon vitamin premix	6,671.7	112
Complete dry mix	147.0	105
OMP Dough (moist)	81.9	92
Frozen OMP (zero time)	62.4	70
Frozen OMP (2 mos @ 0 F)	10.4	12
Thawed OMP (16 hrs @ 34 F)	8.9	10

Table 22. Levels of Ascorbic Acid in Oregon Pellet Rations Sampled at Various Hatcheries

Source/Sample Description	Production Date	Sampling Date	Sample Condition	Ascorbic Acid (mg/100 gm)
Cole Rivers	8/15/77	9/20/77	Frozen	49.8 ± 0.0
1/8 OP2 2-11 PBFSVTV			Auto-feeders	19.0 ± 0.4
South Santiam	7/6/77	9/22/77	Freezer	20.2 ± 0.4
OP2 PBFSVTV DB 1-11			Thawed	14.9 ± 0.9
McKenzie	9/6/77	9/21/77	Freezer	52.3 ± 0.0
OP2 PBFSVTV DB 1-111			Thawed (cart)	62.9 ± 0.9
Marion Forks	8/2/77	9/22/77	Freezer	40.4 ± 0.0
OP2 PBFSVTV DB 1-111			Thawed	11.5 ± 0.4
Oakridge	9/1/77	9/21/77	Freezer	44.5 ± 0.4
OP2 PBFSVTV SF Joe 2-1			Thawed (cooler cart)	6.2 ± 0.0

Needed Level of Herring Oil

Last year at Sandy Hatchery we fed five Oregon Pellet formulations containing different ratios of herring to soybean oil to marked 1975-brood coho for about 10 months prior to liberation from the hatchery. Details of that experiment were discussed in our FY '77 annual report. This year we recovered jacks returning to the hatchery from that experiment (Table 23). Survival of these fish with coded wire tags was low compared to survival of fin marked jacks from another experiment at the same hatchery (Table 24). Adults will be recovered at the hatchery in the fall of 1978.

Also this year we began a study at Sandy Hatchery with 1976-brood coho to replicate the herring oil level experiment. Five Oregon Pellet formulations, modified by replacing 2% wet fish with 2% additional supplemental lipid and

Table 23. Summary of Liberation Data and Marked Fish Recoveries Through 1977, Fish Oil Levels, 1975-Brood Coho

Supplemental Lipid (Percentage of Diet)		Liberation Data				Hatchery Recoveries of 2-Year-Old Fish	
				Mean Fish Size			
Herring (%)	Soybean (%)	Identification	Number Released	Weight (g)	Fork Length (mm)	(No.)	(%)
0	8	Ad+CWT 1/	59,983	29.7	140.7	46	0.077
2	6	"	60,195	29.6	141.2	48	0.080
4	4	"	57,212	29.9	141.4	67	0.117
6	2	"	58,753	30.9	141.6	67	0.114
8	0	"	60,607	30.3	141.1	47	0.078

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

Table 24. Summary of Liberation Data and Marked Fish Recoveries, Immunization of Coho Salmon Against Vibriosis, 1973- and 1974-Brood Coho

			Liberation Data				Hatchery Recoveries					
				Mean Fish Size			2-Yr Old		3-Yr Old		Total	
Hatchery	Brood Year	Treatment	Fin Mark	Weight (g)	Length (mm)	Number Released	(No.)	(%)	No.	(%)	No.	(%)
Sandy	1973	Vaccine	LV	31.3	143.4	59,224	103	0.174	476	0.804	579	0.978
			LV+OTC1/	30.4	142.2	59,413	135	0.227	625	1.052	760	1.279
		Mean		30.9	142.8			0.201		0.928		1.129
		Control	RV+OTC	31.4	143.1	61,790	182	0.295	717	1.160	899	1.455
			RV	30.9	143.5	61,219	141	0.230	687	1.122	828	1.353
		Mean		31.2	143.3			0.263		1.141		1.404
	1974	Vaccine	LV	30.1	142.3	64,936	74	0.114	232	0.357	306	0.471
		Control	RV	30.3	143.0	65,170	61	0.094	202	0.310	263	0.404
Fall Creek	1973	Vaccine	RV	31.1	137.6	52,464	292	0.557	575	1.096	867	1.653
		Control	LV	31.5	139.4	52,490	294	0.560	520	0.991	814	1.551
	1974	Vaccine	LV	30.4	137.8	56,314	36	0.064	75	0.133	111	0.197
		Control	RV	31.1	138.6	50,235	53	0.106	85	0.169	138	0.275

1/ *Oxytetracycline mark in bone tissue.*

containing different ratios of herring to soybean oil, were fed to five groups of about 33,000 coho from June 10, 1977, until release on May 2, 1978. All diets were fed according to rates prescribed in the 1977-78 feeding schedule for Sandy Hatchery except for minor adjustments to insure comparable fish sizes between treatments. To evaluate dietary influence on post-release survival, all fish were marked with distinctive coded wire tags prior to liberation.

Fish Oil Types

This year we began a study to evaluate the effect of using menhaden or anchovy oil as the supplemental lipid in OMP on the post-release survival of coho. Four OMP formulations containing menhaden, anchovy, herring, and soybean oils were fed to four groups of about 33,000 1976-brood coho from July 1977 to May 1978 at Sandy Hatchery. All fish in each of the four treatments were marked with coded wire tags before release. The first returns to the hatchery of marked fish are expected in the fall of 1978.

Oral Vibrio Vaccine

Beginning in FY '75, we carried out studies with 1973- and 1974-brood coho at Fall Creek and Sandy hatcheries to see if immunization with an oral vaccine against *Vibrio anguillarum* would improve estuarian and marine survival of hatchery-reared coho. Details of the experiments have been outlined in our FY '75 and FY '76 annual reports. This year we completed the recovery of marked fish returning to the hatcheries.

Final results (Table 24) indicate that exposure to the oral vaccine did not increase marine survival of coho salmon. In two of the experiments, control fish were recovered at significantly higher rates than the immunized group. In the other two experiments, recovery rates were not significantly different.

Salt Feeding and Survival

In the previous 2 years, we have carried out field studies with the 1974- and 1975-brood coho at Sandy Hatchery and with 1975-brood coho at Big Creek Hatchery to determine the effect on survival of feeding OMP containing 8% added sodium chloride to coho yearlings for 8 weeks just prior to release. Details of those experiments were presented in our FY '76 and '77 annual reports. This year we recovered marked fish returning to the hatcheries from those experiments.

Results to date (Table 25) are inconclusive. Recoveries of control jacks at Big Creek Hatchery were significantly greater than recoveries of salt-fed jacks. In the other two experiments, recovery rates were not significantly different. Data from the final returns of marked fish will be available in the fall of 1978.

Table 25. Summary of Liberation Data and Marked Fish Recoveries Through 1977, Salt Feeding Experiments, 1974- and 1975-Brood Coho

Hatchery	Brood Year	Treatment	Fin Mark	Liberation Data: Mean Fish Size Weight (g)	Length (mm)	Number Released	Hatchery Recoveries: 2-Yr Old (No.)	2-Yr Old (%)	3-Yr Old (No.)	3-Yr Old (%)	Total (No.)	Total (%)
Sandy	1974	Salt Diet	LV-LM	30.6	142.6	61,750	29	0.047	182	0.295	211	0.342
		Control	RV-LM	30.6	143.4	61,217	27	0.044	148	0.242	175	0.286
	1975	Salt Diet	RV	29.1	139.0	60,600	108	0.178	1/	1/	108	0.178
			RV+OTC2/	30.2	141.5	61,105	149	0.244	1/	1/	149	0.244
		Mean		29.7	140.3			0.211				0.211
		Control	LV+OTC	29.8	140.3	60.705	110	0.181	1/	1/	110	0.181
			LV	30.1	141.7	61,257	131	0.214	1/	1/	131	0.214
		Mean		30.0	141.0			0.198				0.198
Big Creek	1975	Salt Diet	RV	30.4	139.7	84,436	260	0.308	1/	1/	260	0.308
			RV+OTC	29.8	138.8	84,135	242	0.288	1/	1/	242	0.288
		Mean		30.1	139.3			0.298				0.298
		Control	LV+OTC	30.7	140.3	82,904	354	0.427	1/	1/	354	0.427
			LV	29.7	138.9	81,799	302	0.369	1/	1/	302	0.369
		Mean		30.2	139.6			0.393				0.393

1/ *Data not yet complete. Adults to return in the fall of 1978.*

2/ *Oxytetracycline mark in bone tissue.*

Antioxidant Toxicity Test

TBHQ and Ethoxyquin

The Food and Drug Administration may remove butylated hydroxytoluene (BHT) from the GRAS ("generally recognized as safe") list. We presently specify a combination of BHT and BHA (butylated hydroxyanisole) as antioxidants in OMP. The manufacturer of BHT and BHA suggested we might want to replace them with tertiary butylhydroquinone (TBHQ), which they claim offers an outstanding stabilization effect on unsaturated fats, such as the fish or soybean oils used in OMP. Ethoxyquin is also reputed to be very effective in meeting our needs.

This year we tested TBHQ and ethoxyquin for toxicity to coho salmon in a 24-week laboratory feeding trial. The antioxidants were fed in OMP at 10 times their recommended levels, 0.2% of the total dietary oil for TBHQ and 0.15% of the diet for ethoxyquin, and compared with BHA/BHT (1:1) at 0.018% of a control diet. We detected no significant differences in weight gain, mortality, or hematocrit at the end of the trial. The diet containing TBHQ was associated with a feed conversion significantly better than the control.

We plan to follow this toxicity test with tests of oil stability before changing the antioxidant system in OMP.

Abernathy Diet vs OMP

Abernathy Diet for Coho Salmon

Our study of the Abernathy Diet for coho salmon was completed this year, with return to Big Creek Hatchery of the 1974 brood. Liberation and recovery data are presented in Table 26. Overall returns from 3 brood years of fin clipped fish indicate no significant difference in survival between coho fed the Abernathy Diet and those fed OMP. Feed costs to rear the fish at the hatchery favored the Abernathy Diet each year. The 1974-brood smolts were afflicted with bacterial kidney disease, which appeared to be worse in the Abernathy-fed fish.

Table 26. Liberation and Recovery Data, Abernathy vs OMP Diets for Coho Salmon

Brood Year	Diet Fed	Fin Mark	Liberation Data		Hatchery Recoveries					
					2-Yr Olds		3-Yr Olds		Total	
			Fish/Lb	No.Released	No.	%	No.	%	No.	%
1971	OMP	LV	14.5	73,037	101	0.138	39	0.053	140	0.192
	Abernathy	RV	14.4	68,574	97	0.141	32	0.047	129	0.188
1972	OMP	LV	14.9	76,176	207	0.272	120	0.158	327	0.429
	Abernathy	RV	13.8	68,730	313	0.455	100	0.145	413	0.601
1974	OMP	LV	15.4	166,546	6	0.004	24	0.014	30	0.018
	Abernathy	RV	15.9	153,079	2	0.001	10	0.007	12	0.008