NORTHWEST FISH CULTURE CONFERENCE

DECEMBER 1960

★ DIETS & FISH CULTURE TECHNIQUES
★ FISH DISEASES
★ FISH PLANTING
★ RETURNS OF PLANTED FISH—MISCELLANEOUS
★ FISH NUTRITION STUDIES

Olympia, Washington

1960

ELEVENTH ANNUAL

NORTHWEST FISH CULTURAL CONFERENCE

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Following are the abstracts of the papers presented at the meeting held in the Jade Room - Olympian Hotel - Olympia, Washington on December 6th and 7th, 1960.

The abstracts are presented as received without editing except typographical errors.

Appreciation is given to those who expended interest and effort in preparation of the papers. Participation of those who asked questions on the subject matter of the individual papers also are thanked for their contribution. I also wish to thank one and all who, in informal groups both large and small, discussed the various problems and activities in fish culture. These informal discussions mean much to our working knowledge of present day fish culture.

Mr. Chris Jensen of the Oregon Game Commission, Portland, Oregon, was selected as chairman for the 1961 Conference.

John M. Johansen

1960 Chairman

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DIET TESTS ON SILVER SALMON
EAGLE CREEK NATIONAL FISH HATCHERY

John Parvin
U. S. Fish and Wildlife Service

A decision was made to test four brands of dry feed during the calendar year 1960 on silver salmon. During the previous years, tests have been made of various brands of dry feed using silver salmon as the test animal. Mixed success had been experienced which encouraged further work.

We decided to test the following brands of feed: Dinafish, manufactured by Willis H. Small Feed Company of Eugene, Oregon, Clark's New Age Complete Trout Feed, manufactured by J. R. Clark Company of Salt Lake City, Utah, Rainbrook Silver Salmon Feed, manufactured by Stockton Hay and Grain Company, Ltd of Stockton, California.

In addition to the above closed formula brands of feed, we were able to obtain sufficient feed for testing from Glenco Mills of Glenco, Minnesota. This was an open formula feed developed by Mr. Harvey Willoughby, Hatchery Manager of the McNenny National Fish Hatchery, Spearfish, South Dakota. This feed was designated as McNenny #13. The control group was fed the regular production wet diet which contained no raw salmon products.

Every precaution was taken to set up the tests so that the least number of variable would be present. Sufficient number of eggs were selected from one day's egg take to supply the number needed for the entire test. The eggs, when eyed, were placed in a vat and thoroughly mixed, then placed on trays to hatch. Fifty pounds of fry, ready to feed, were placed in four troughs for each of the diets tested, including the control. Twenty troughs were used for the feeding test. The number of fish in each group amounted to 66,850 fish. The fish started to feed on April 15, 1960.

The five groups of fish appeared to grow in a satisfactory manner during April and most of May. The wet diet produced the best growth, while the McNenny #13 was a close second. During the latter part of May the fish fed the Dinafish diet began to show an alarming mortality. The mortality developed so rapidly that we suspected a toxicity. However, we were unable to determine if this were so, or if the mortality was due to dietary deficiency.

On June 1, 1960 three of the troughs being fed Dinafish, were placed on the regular production diet, while the fourth trough was continued on Dinafish. The fish in this trough continued to die although those placed on the wet diet recovered. The Dinafish portion of the test was discontinued on July 1, 1960. The one trough which had been retained on Dinafish, was transferred to production diet and left in the hatchery to determine total mortality.

On June 1st, the fish being fed the test diets were transferred to ponds. Each group of fish being fed one diet was placed in one 8' x 80' raceway. During the feeding tests Mr. Earnest Hesser of the Willard Laboratory, made monthly hemopoietic surveys of the fish on the test diets. The attached chart shows the progress of this picture as well as the total growth and mortality picture. The hematocrit readings were listed as indicative of the blood condition.

During the latter part of July, an epizootic was experienced which was diagnosed by Mr. Joe Uxman of the Western Fish Disease Laboratory as parasitic amoeba. The attack of this pathegen had responsibility for biasing the mortality picture. The fish fed the McNenny #13 diet suffered the greatest mortality from this source.

On September 12, 1960, the test of McNenny #13 diet was discontinued and the fish placed on Stockton diet. At this time, the hemopoietic picture of this group of fish had worsened until the hematocrit reading was down to a figure of 29.6%. This was much lower than the other groups. Subsequently, some loss was experienced in the pond from anemia.

The data for this report was obtained as of November 15, 1960. The diet tests will continue for some additional time to view the performance of the diets during cold water conditions.

Some interesting observations can be drawn from the attached charts. The wet control diet performed best during the early portion of the tests, when the water temperatures were relatively cold. Certain of the dry diets were rejected because of their inability to perform satisfactorily with silver salmon. The dry diets which proved successful for the entire period of the test did not perform as well as the control during the early cold water periods. However, later during warm water periods, they overtook the control and in one instance exceeded it, considering growth potential alone.

The mortality picture is not as favorable to the dry diets. One dry diet mortality was over twice that of the control. The other diet was slightly higher in loss. Most of the mortality of the fish being fed the dry diets, occurred as a result of the poor performance of these during the early cold water periods. As mentioned earlier, an epizootic of parasitic amoeba tended to bias the mortality figures.

The cost of pounds of fish produced was quite favorable for the dry diets. The cost per pound of fish produced was approximately equal for one of the dry diets and control. One of the dry diets was less costly. Conversions of 2.0 and 2.1, respectively, were slightly better than those experienced with dry feed in former years.

1960 DRY DIET TESTS - EAGLE CREEK NATIONAL FISH HATCHERY
SUMMARY OF DATA BY WEIGHT PERIODS.

DIET	PERIOD ENDING	CONVERSION	% GAIN IN WEIGHT	% MORTALITY	FISH SIZE # PER LB.	HEMATOCRIT READING
CONTROL	4/30/60	2.7	20.6%	1.6%	1095	
CLARK'S	"	2.2	16.1%	1.6%	1137	
STOCKTON	"	1.9	18.2%	1.8%	1111	
MCNENNY	"	1.7	20.2%	1.4%	1101	
DINAFISH	"	1.9	18.8%	1.9%	1104	
CONTROL	5/15/60	3.5	36.0%	1.4%	790	37.1
CLARK'S	"	2.7	26.2%	.4%	893	38.12
STOCKTON	"	2.7	27.1%	.4%	871	32.36
MCNENNY	"	2.2	30.6%	.4%	840	38.6
DINAFISH	"	2.1	33.0%	.2%	828	34.05
CONTROL	5/31/60	3.8	33.3%	1.2%	585	
CLARK'S	"	2.6	28.5%	.8%	690	
STOCKTON	"	2.1	34.6%	8%	642	
MCNENNY	"	2.2	37.8%	.7%	605	
DINAFISH	"	29.4	2.3%	11.2%	726	
CONTROL	6/14/60	2.2	67.4%	.4%	259	33.6
CLARK'S	"	1.6	51.8%	2.3%	447	28.8
STOCKTON	"	1.4	59.2%	1.4%	398	32.2
MCNENNY	"	1.4	58.0%	.5%	383	26.8
CONTROL	6/30/60	4.2	32.8%	.3%	259	
CLARK'S	"	2.1	35.9%	1.7%	324	
STOCKTON	"	1.4	60.9%	.6%	246	
MCNENNY	"	2.1	40.4%	1.2%	268	
CONTROL	7/14/60	3.3	49.4%	.2%	176	36 06
CLARK'S	"	2.0	37.3%	1.6%	236	34.51
STOCKTON	"	2.4	33.6%	.5%	184	36.01
MCNENNY	"	2.2	35.4%	.8%	199	32.44
CONTROL	7/31/60	5.0	43.0%	.5%	121	
CLARK'S	"	4.4	21.9%	3.3%	187	
STOCKTON	"	3.1	28.6%	1.9%	140	
MCNENNY	"	19.2	4.0%	12.5%	167	
CONTROL	8/15/60	4.5	34.1%	.9%	90	38.3
CLARK'S	"	2.2	37.2%	.4%	134	35.5
STOCKTON	"	2.3	33.0%	.5%	107	38.7
MCNENNY	"	2.3	32.5%	2.2%	124	30.1
CONTROL	8/31/60	3.6	32.8%	.1%	68	
CLARK'S	"	2.5	37.5%	.2%	97	
STOCKTON	"	2.3	40.4%	.1%	76	
MCNENNY	"	2.5	41.5%	.3%	87	
CONTROL	9/15/60	4.3	22.0%	.1%	56	36.7
CLARK'S	"	2.0	37.1%	.1%	71	38.7
STOCKTON	"	1.8	37.6%	.01%	55	39.9
MCNENNY	"	1.8	39.0%	.1%	62	29.6

DIET	PERIOD ENDING	CONVERSION	% GAIN IN WEIGHT	% MORTALITY	FISH SIZE # PER LB.	HEMATOCRIT READING
CONTROL	9/30/60	2.9	29.3%	.1%	43	
CLARK'S	"	1.4	40.6%	.1%	50	
STOCKTON	"	1.5	41.7%	.2%	39	
*MCNENNY	"	1.4	44.0%	1.0%	43	
CONTROL	10/14/60	3.0	17.1%	.1%	37	44.1
CLARK'S	"	2.1	20.1%	.7%	41	38.9
STOCKTON	"	2.5	14.4%	.2%	34	43.2
CONTROL	10/28/60	3.0	9.9%	.05%	33	
CLARK'S	"	1.9	13.8%	.4%	36	
STOCKTON	"	1.5	14.2%	.2%	29	
CONTROL	11/15/60	4.2	5.5%	.02%	32	37.1
CLARK'S	"	3.0	5.3%	.2%	32	34.2
STOCKTON	"	3.1	4.3%	.1%	28	35.2

SUMMARY AS OF 11/15/60

DIET	DIET TEST ENDED	CONVERSION	CUMULATIVE MORTALITY IN %	FISH SIZE # PER LB.	COST PER LB. OF FISH PRODUCED
CONTROL	11/15/60	3.6	6.3%	32	.292
CLARK'S	"	2.1	14.4%	32	.296
STOCKTON	"	2.0	8.4%	28	.245
MCNENNY	9/12/60	2.1	18.4%	43	.315
DINAFISH	5/31/60	3.62	12.2%*	726	.502

* Loss until May 31, 1960, and does not reflect later loss in trough maintained on Dinafish.
* McNenny changed to Stockton 9/30/60.

FREQUENCY OF FEEDING DRY FEEDS TO CERTAIN SIZE SOCKEYE SALMON FINGERLING

Alfred C. Gastineau
Leavenworth National Fish Hatchery

U. S. Fish Wildlife Service

In our first successful attempt in feeding Sockeye Salmon Fingerling certain dry trout feeds, which was reported to this group in 1959, we fed at least once every hour.

This year, we tried to determine how frequent Sockeye Salmon fingerling should be fed. We started with fish that were 552 per pound in size, terminating the project at the end of our rearing season at which time the fish were approximately 44 per pound in size. The length of the period was 142 days. No change in frequency of feeding was made due to increase in size of fish.

We selected 278,760 fish, thoroughly mixed them and divided into four equal groups. One group was fed four times daily, one six times, one eight times and one group sixteen times daily. Clark's dry feeds were used, amounts fed as per their feeding charts. Pilot troughs were used to determine the progress; these being weighed every two weeks to determine the progress and the amounts to be fed adjusted accordingly.

DATA

Sockeye Salmon Fingerling	4 Feeds Per Day	6 Feeds Per Day	8 Feeds Per Day	16 Feeds Per Day
Number of fish at start	69,690	69,690	69,690	69,690
Number per lb. at Start 5/3/60	552	552	552	552
Number per lb. at End 9/26/60	43.8	44.7	43.8	45.6
Percent mortality	6.23	5.68	6.56	5.83
Percent gain	1,082	1,065	1,079	1,041
Conversion	1.99	2.01	2.00	2.07

In summary, it appears that Sockeye Salmon Fingerling growing from size 552 per pound to 44 per pound need not be fed more frequently than four times per day, perhaps less.

RECONSTITUTED DRY MEAL DIETS FOR CHINOOK SALMON

Salmon-Cultural Laboratory
Entiat, Washington

U. S. Fish and Wildlife Service

The feeding trials conducted during the 1961 season utilized a raw products control diet and several dry meal mixes partially reconstituted with water. The water served to control the protein intake and to provide a mush-type feed, similar in consistency to a raw products diet. The composition of the control diet and the meal mixtures are shown in the table.

All of the dry meal mixtures were fed at the 27.5 and 20 percent protein levels, at the 7 percent fat level, and with a vitamin supplement at double the amounts required (as determined for trout). In addition the E-2 mixture was fed at the 3, 4, 7 and 10 percent fat level and with the vitamin supplement at single double, and triple the requirements. Corn oil was used as a fat supplement.

The feeding trials were concluded after 16 weeks because of the marked debilitation and high mortality in the fish fed the reconstituted dry meal diets. Although high levels of vitamins were fed, hematocrits varied from 12.3 percent to 19.4 percent in contrast to 37.3 percent for the control group. The livers from fish fed the dry meal diets were yellow in color and granular in appearance.

Since we were unable to develop an adequate reconstituted diet from commercially available meals, we intend to formulate diets which contain dry meals supplemented with fresh meats in an effort to maintain the fish under conditions of near normal growth and condition.

COMPOSITION OF BASIC DIETS OF THE 1960 FEEDING TRIALS

Entiat Production Diet 1/	
Hog liver	12.5%
Beef lung	12.5%
Arrowtoothed halibut	25.0%
Salmon viscera	40.0%
Seal meal	5.0%
Distiller's solubles	5.0%
E-2. Meal Mixture 1/ 2/	
Whitefish meal	70.0%
Dried skim milk	7.0%
Dried brewer's yeast	10.0%
Distiller's solubles	10.0%
Cod liver oil	3.0%
E-3. Meal Mixture 1/ 2/	
Seal meal	35 0%
Whitefish meal	35.0%
Dried skim milk	7.0%
Dried brewer's yeast	10.0%
Distiller's solubles	10.0%
Cod liver oil	3.0%
E-4. Meal Mixture 1/ 2/	
Salmon meal	35.0%
Whitefish meal	35.0%
Dried skim milk	7.0%
Distiller's solubles	10.0%
Dried brewer's yeast	10.0%
Cod liver oil	3.0%
E-5. Meal Mixture 1/ 2/	
Seal meal	35 0%
Salmon meal	35.0%
Dried skim milk	7.0%
Dried brewer's yeast	10.0%
Distiller's solubles	10.0%
Cod liver oil	3.0%
E-6. Meal Mixture 1/ 2/	
Salmon meal	23.3%
Seal meal	23.3%
Whitefish meal	23.4%
Dried skim milk	7.0%
Dried brewer's yeast	10.0%
Distiller's solubles	10.0%
Cod liver oil	3.0%

1/ Salt added at the rate of 2 grams per 100 grams of diet.
2/ CMC added at the rate of 2 grams per 100 grams of diet. Corn oil was used to regulate the fat content of the reconstituted dry meal diets.

STAMINA TUNNEL TESTS OF CHINOOK SALMON FINGERLING

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U. S. Fish and Wildlife Service
Entiat, Washington

The ability of fish to withstand current has been selected as one criterion for determining quality differences of salmon fingerlings. A stamina tunnel has been designed and constructed which utilizes a 1,500 gpm axial flow pump circulating water through a 12-inch diameter plastic tunnel, 6 feet long. The speed of the pump can be regulated to create a velocity through the tunnel of from less than .2 feet per second to 5 feet per second. An electrical field at the tunnel outlet induces the fish to remain in the tunnel until they are partially exhausted.

The test of a group of fish consists of an orientation period and two changes in water velocity. The lower velocity eliminates the poorest performing fish from the group; the higher velocity eliminates all except the best. The test including the 5-minute orientation period, requires 30 minutes. As the fish leave the tunnel they are counted and the cumulative totals are recorded each minute.

In order to compare the performance of various groups of fish, the time when the first 25 percent have left the tunnel is added to the time when 75 percent are gone. This index measures both the poorest and best performance and eliminates minor variation. When the first and fourth quartiles of a group were segregated and rerun, only 5 percent of either quartile occurred in the other.

Certain factors must be controlled in order to make valid comparisons between groups of fish. These include water temperature, method of sampling, sample size, and the interval between feeding and testing. As little as a three degree difference in temperature has a measurable effect on performance. The higher the temperature, within the limits of normal environment, the better the performance. The method of sampling large groups of fish presents a problem. In order to assure an adequate sample, the entire group must be handled and systematically sampled with a vertical sampler. Dip net samples are not reliable for large groups. Samples varying from 13 to 264 fish have been tested in the tunnel with a trend toward better performance in the smaller samples. Samples of the same numbers from one group of fish, however, perform similarly. Fish which were tested immediately after feeding performed poorly, but fish starved from 16 to over 40 hours showed no difference in performance. When these variables are controlled, the performance of various groups of fish may be compared.

There is an extreme variability in performance within a group of hatchery-reared chinook fingerlings. Repeated tests have shown that the poorest performing 25 percent of a sample are significantly smaller than the best performing 25 percent. Low hematocrit has an adverse effect on performance but at 30 percent or above, it has no effect. Other differences undoubtedly exist which have not yet been detected.

Environment has a definite effect on performance. Early in September samples were drawn from each of four raceway-type ponds which had originally been stocked with equal poundages of fish. Two of the raceways were the conventional type and two the recirculating type, one of each composing a pair. One pair had 125 gpm water inflow and the other pair a 62.5 gpm inflow. Using the performance index and analysis of variance for paired samples, the performance of the fish in the low-flow recirculating raceway was found to be significantly lower than that of the other three ponds. The impaired performance was believed due to the higher ammonia concentration in the low-flow ponds. Although ammonia concentration was similar in both of the low-flow ponds, the high gradient existing in the conventional raceway subjected only those fish in the lower half of the pond to a truly unfavorable environment. Only a slight gradient exists in the recirculating raceway and all of the fish are subjected to essentially the same environment. Immediately after the stamina tunnel tests, the water inflow was increased in the low-flow ponds and four weeks later they were again sampled. The fish in the low-flow recirculating raceway had recovered markedly and their performance was comparable to that of the other raceways. This experiment indicates that perhaps hatchery-reared fish could be conditioned for better survival after release if, during the last month of holding, they were subjected to a faster water interchange by either increasing the inflow or lowering the pond depth. Water temperatures and pond loadings would have to be favorable for such a practice. Performance of the fish from the rectangular-recirculating pond was superior to that of fish reared in the raceways. The greater water velocity in the rectangular pond is thought to account for the better performance.

Tests were conducted on wild chinook fingerlings trapped on their downstream migration. When compared with hatchery fish in the stamina tunnel tests, no wild fish left the tunnel at the time when 25 percent of the hatchery fish were eliminated. The performance of the wild fish in the fourth quartile was slightly inferior to that of the hatchery fish, due probably to the fact that the wild fish were but half the size of the hatchery fish.

The indications are that the stamina tunnel will prove to be a very useful tool for measurement of performance differences.

1960 DIET TRIALS WITH PASTEURIZED AND AUTOLYZED SALMON PRODUCTS

Richard G. Bigej
Bureau of Sport Fisheries and Wildlife
Cook, Washington

In an effort to eliminate the feeding of infected flesh and viscera of adult salmon to fingerling salmon in our hatcheries, an investigation into the possibilities of sterilization of salmon products has been started.

Diet trials comparing two types of processed salmon products with a raw diet and with the Carson production diet were conducted at the Carson National Fish Hatchery, using fall chinook and silver salmon of the 1959 brood year to measure any differences in growth or mortality. In a joint effort, the Western Fish Disease Laboratory began testing the effectiveness of the processing methods in killing the disease organisms, primarily acid fast bacteria.

The two processing methods used were: (1) pasteurization; and (2) autolyzation. Pasteurization was done by the R. V. Moore Company in accordance with the standards set by the Oregon Fish Commission. This method consisted of heating the ground salmon products to 140° F. for a minimum of 30 minutes during which time the temperature was raised to 180° F. and held for 5 minutes then rapidly cooled down to 140° F. and held for the remainder of the original 30 minute period. The mixture was then cooled to about 60° F., packed and frozen. Autolyzation was done by the Wilbur-Ellis Company of Seattle, Washington under the supervision of Dr. Diptiman Chakravarti of the University of Washington College of Fisheries. This method consisted of reducing the pH of the ground salmon products to approximately 4 by the addition of acid. The mixture was then heated to about 130° F. for a period of 1 hour and then was allowed to start cooling. During the cooling period, the pH was brought back to approximately 7 by the addition of calcium hydroxide. Starch and gums were then added to stabilize or "bind" the mixture which was packaged and frozen. Both processing methods combined 1/3 salmon eggs, 1/3 salmon viscera, and 1/3 salmon flesh. Both end products were a thick slurry before freezing.

The fall chinook diet trials were set up with 50,000 fish, approximately 254 to the pound, in each of four 8 x 80 raceway ponds. The silver salmon diet trials were set up two weeks later with 50,000 fish approximately 180 to the pound in an additional four 8 x 20 raceway ponds.

The following diets were fed to fall chinook for 15 weeks and to silvers for 13 weeks:

Production Diet	Raw Control Diet	Pasteurized Diet	Autolyzed Diet
10% Beef Liver	25% Beef Liver	25% Beef Liver	25% Beef Liver
10% Hog Liver	25% Salmon Eggs	25% Past.Sal.Eggs	25% A.Sal.Eggs
15% Beef Spleen	25% Salmon Visc.	25% Past.Sal.Visc.	25% A.Sal.Visc.
13% Salmon Eggs	25% Salmon Flesh	25% Past.Sal.Flesh	25% A.Sal.Flesh
14% Salmon Viscera			
30% Salmon Flesh			
5% Distillers Solubles			
1% Fleischman's Type B-50 Yeast			
2% Salt			

All diets except the production diet were changed to include B.L. 23%, B.S. 15%, salmon products 60%, and salt 2% in order to get a better "bind" on the diet. These diets were fed an additional 6 weeks making the total feeding period 21 weeks for the chinook and 19 weeks for the silvers.

The following is a summary of the results of the fall chinook diet trials:

	Prod. Diet	Raw Control Diet	Past. Diet	Auto. Diet
% Gain Total Wt.	550.3	457.4	352.8	331.5
% Gain Avg. Wt.	567.0	476.0	369.3	344.7
% Mortality	1.96	2.43	3.40	3.39
Fish Per Lb. End	38	44	54	57
Avg. Wt. Per Fish End	11.94	10.31	8.40	7.96
Conversion	2.75	3.16	3.78	3.92
Cost Per Lb of Fish	0.185	0.226	0.346	0.359

These results indicate that one or more of the growth essentials are made less available to the fish by both processing methods, either by actual destruction or by the liquid form into which the feed is rendered permitting increased leaching.

The original four diets, plus two additional diets were fed to silver salmon. The two additional diets were fed during the last 10 weeks of the feeding trials and were set up with 46,000 fingerlings at 73 to the pound, in two additional 8 x 80 raceway ponds. These diets were designed to supply additional vitamins and minerals when it became apparent that the pasteurized and autolyzed diets were lacking in one or more of the growth essentials.

The supplemented diets were composed of the following:

Past-Supl. Diet	Auto-Supl. Diet
5% Beef Liver	5% Beef Liver
15% Hog Liver	15% Hog Liver
15% Hog Slpeen	15% Hog Spleen
19% Past. Salmon Eggs	19% Auto. Salmon Eggs
19% Past. Salmon Viscera	19% Auto. Salmon Viscera
19% Past. Salmon Flesh	19% Auto. Salmon Flesh
5% Distillers Solubles	5% Distillers Solubles
1% Yeast	1% Yeast
2% Salt	2% Salt

The following is a summary of the results of the silver salmon diet trials:

	Prod. Diet	Raw Control Diet	Past. Diet	Auto Diet	Past-Supl. Diet	Auto Supl. Diet
% Gain Total Wt.	372.7	299.3	229.9	206.8	82.1	81.1
% Gain Avg. Wt.	386.5	309.1	246.0	215.9	82.6	82.3
% Mortality	2.06	2.58	3.98	2.09	1.23	1.01
Fish Per # End	37	44	52	57	39	40
Avg. Wt. Per Fish End	12.26	10.31	8.72	7.96	11.63	11.34
Conversion	2.93	3.43	4.16	4.44	3.74	3.78
Cost Per # of Fish	0.197	0.245	0.380	0.406	0.352	0.357

The results of these trials also indicate that the processing methods used, destroyed or made less available one or more of the growth essentials. The supplemented-processed diets did not show an increase in growth over the non-supplemented diets after 10 weeks of feeding.

A diet free of infective organisms is a "must" if we are to raise better fish in our hatcheries. The results of these feeding trials show that more intensive studies under closely controlled conditions will be necessary to determine the cause of reduced growth and increased mortality brought about by processing salmon products by these two methods.

SUMMARY

First Phase Pilot Plant-A Progress Report

By

Warren E. Shanks

The intermediary between research and production is the pilot plant. It is normally a research function engaged in developmental research as distinguished from basic research. Both programs should be coordinated with each group cognizant of the problems and objectives of the other. In this way each may derive the benefits of contemporary criticism so vital to any form of research - or management as well.

In the initial stages of the program, emphasis must, of necessity, be given to the development of methods for evaluating biological availability and utilization of nutrients. Since protein is probably the most important single dietary ingredient, methods for measuring protein quality will be studied first. The procedures to be evaluated are:

1. Protein efficienty ratio. (P.E.R.). This is a feeding trial in which weight gained per weight protein fed is the index of quality. All groups of fish will receive a basal ration containing similar levels of vitamins, minerals, carbohydrates and fats. Protein will be the only variable.

2. Net protein retention (N.P.R.) N.P.R. is a modification of P.E.R. and includes a control group of fish which receive the basal ration (no protein) only. The final weight of this group is subtracted from the final weight of the test group to give net gain. Net gain divided by protein intake gives N.P.R. The advantages of this procedure include the measurement of proteins which do not produce gain in weight and eliminates the effect of intake on protein efficiency.

3. Protein repletion. The ability of a protein to restore lost weight in protein depleted animals is a measure of protein quality. This is an example of a so-called "short cut" method.

4. Nitrogen balance (N.B.). The N.B. of an animal is the ratio between the nitrogen as protein, ingested and nitrogen eliminated in the urine, feces and gills, in the case of fish. Under carefully controlled conditions this provides a quantitative measure of protein metabolism and shows specifically if the body is gaining or losing protein. An accurate N.B. experiment can provide a precise measure of the actual protein requirement in terms of a specific ration by determining the minimum intake which will provide maximum retention, or growth.

5. Biological value (B.V.). B.V. is a widely mis-used term. It denotes a measure of protein quality obtained in an animal experiment in which the percentage of the intake which is actually utilized is determined. The simplest calculation of B.V. is made as follows:

$$\frac{\text{N Intake} - (\text{Fecal N} + \text{Urinary N})}{\text{N Intake} - \text{Fecal N}} \times 100 = \text{B.V.}$$

THE EFFECTS OF SELECTED ANTIOXIDANTS AND SELENIUM ON FISH FEED

Duncan K. Law, Oregon State College Seafoods Laboratory, Astoria, Oregon

INTRODUCTION:

At our meeting with this group last year, we presented what we felt was strong evidence that oxidative rancidity was correlated with anemia, growth inhibition and high mortality. Evidence was also presented that alpha-tocopherol was either an essential vitamin, possessed vitamin-like action, or was acting as an antidote for the toxicity of oxidative rancidity. These results raised a number of questions of which we felt the following to be the most important. (1) Are the alphatocopherols the best biological antidote against oxidative rancidity? What are some of the in vivo effects of some of the common antioxidants? Would selenium be effective in place of vitamin E? (2) What are the dangers of toxicity from these antioxidants at levels higher than the level already allowable for Santoquin by the Food and Drug Administration? (3) Which of the antioxidants are most suitable in feeds?

It is well known that the pro-oxidant effects of fat increase with the degree of unsaturation. Since the presence of various types of fish oils are common in many fish feeds and pellets, the answers to these questions are very important. Perhaps the simplest way to solve the problem would be to minimize the presence and use of unsaturated fats and substitute saturated fats in their place. However, most all of our diet work with fish indicates that non-rancid, unsaturated oils supplemented with vitamin E produce the best results. On this basis, a more effective solution would seem to be to protect the unsaturated oils and the fish with the most suitable antioxidant available.

We have divided our 1960-61 diet series into three phases as mentioned earlier. John Westgate will give the biological, or in vivo phase. Cecil Whitmore will present the phase concerning toxicity and I am reporting to you on the in vitro effect of various antioxidants and selenium in feed.

EXPERIMENTAL PROCEDURE:

The antioxidants we elected to test for this diet series were chosen on the basis of reported effectiveness and on the results of a standard A.O.M. test on some of these antioxidants in lard. The antioxidants used were as follows:

1. Quercetin: (3,3',4',5,7,Pentahydroxy Flavone) commonly distributed in plants and particularly concentrated in rinds and barks. The test sample was obtained from the Central Development Department of the Weyerhaeuser Timber Company.

2. THBP: (2,4,5,-Trihydroxy butyrophenone). This is a relatively new antioxidant being developed by Eastman Chemical Company.

3. NDGA: (Nordihydroguaiaretic acid). The test sample was obtained from the Nordigard Corporation.

4. Propyl Gallate

5. BHA-BHT: (Butylated Hydroxyanisole-Butylated Hydroxytoluene).

6. Tenox VI: An Eastman developed antioxidant whose active ingredients are BHA-BHT, and Propyl Gallate.

7. Santoquin: (1,2,-Dihydro 6-ethoxy-2-2-4, trimethylquinoline). This is a Monsanto Chemical Company antioxidant.

8. Selenium: (Dosium selenite).

These antioxidants were mixed into a regular Oregon Diet formulation from which Tenox VI had been deleted. The antioxidants were added at the .015% level with the exception of selenium. Selenium was added at 0.1 part per million. The resultant mix was extruded into pellets, sharp frozen and held at -10° F.

DISCUSSION:

With the exception of Quercetin all the selected additives inhibited rancidity development in relation to the control (TableI). Quercetin appears to accelerate rancidity development in the Oregon pellet. The manner in which selenium inhibits rancidity development was not determined. At the end of the six-week period the pellets with Propyl Gallate and BHA-BHT gave lower TBA numbers than the initial value of the control. It is thought that the hydroperoxides initially present in the pellets combined with these antioxidants in such a manner as to prevent the formation of malonaldehyde. This would account for the lowered value.

CONCLUSION:

1. The control pellet compounded with vitamin E increases in rancid value after six-weeks storage. Vitamin E as alpha-tocopherol is not an effective feed antioxidant for Oregon pellets.

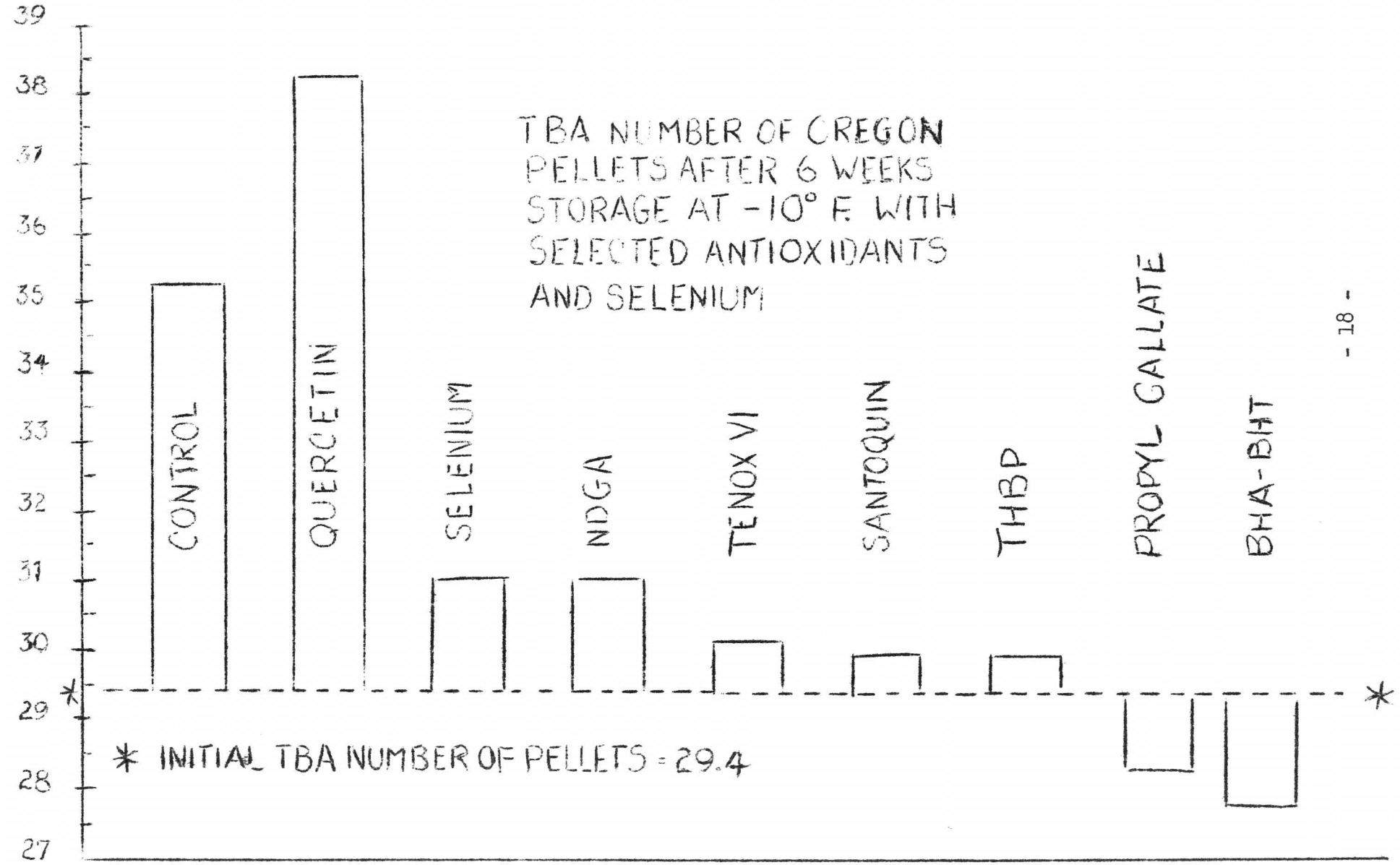
2. Quercetin appears to increase the rate of fat oxidation after six weeks storage. Quercetin is not an effective feed antioxidant for Oregon pellets.

3. Selenium and NDGA appear to have similar slight inhibitory effects to rancidity.

4. THBP, Tenox VI, and Santoquin inhibit rancidity development in Oregon pellets markedly. They can be considered effective antioxidants for Oregon pellets.

5. Propyl Gallate, and BHA-BHT show the strongest inhibitory action to rancidity. These two can be considered effective antioxidants for Oregon pellets.

TABLE 1


TBA NO.
39
38
37
36
35
34
33
32
31
30
29
28
27
TBA NUMBER OF OREGON PELLETS AFTER 6 WEEKS STORAGE AT -10° F. WITH SELECTED ANTIOXIDANTS AND SELENIUM
CONTROL
QUERCETIN
SELENIUM
NDGA
TENOX VI
SANTOQUIN
THBP
PROPYL GALLATE
BHA-BHT
* INITIAL TBA NUMBER OF PELLETS = 29.4

EFFECTS OF HIGH LEVELS OF FIVE ANTIOXIDANTS, SELENIUM AND ALGIT FED TO SPRING CHINOOK SALMON IN THE OREGON TEST DIET.

Cecil M. Whitmore - Oregon Fish Commission

INTRODUCTION

Dry or moist pelleted fish foods require the addition of antioxidants or allied compounds to prevent degradation of fats, vitamins, and other essential nutrients. Other agents, such as Algit, are needed to bind pellets into economical, readily reproducible, and palatable products. In order to eliminate potential antioxidants or binders which may be toxic or detrimental, a study was conducted at Willamette Hatchery in which young spring chinook salmon were fed high levels of five antioxidants, selenium, and Algit in the Oregon Test Diet for a period of 16 consecutive weeks.

PROCEDURE

Four hundred thirty-five chinook fingerlings were counted into each of sixteen 6-foot, circular, wooden tanks. Duplicate lots for each diet were chosen from a random numbers table. Fish were counted three times during the progress of the experiment to account for any hidden loss, and were weighed at the beginning, at 4 weeks, and at the termination of the experiment. Small numbers of fish were sacrificed periodically for blood tests or extensive examinations. For the first 11 weeks of the experiment the fish were treated weekly with 1:1,000,000 solution of Lignasan to control myxobacterial and algal problems endemic to the water supply.

All diets contained the same meal mixture, vitamin supplements and corn oil. They differed only in the antioxidant or binder added. Quercetin, an equal mixture of BHA and BHT, Propyl Gallate, THBP, and NDGA were added at the rate of 1.5 per cent of the dry weight of the diets. Selenium was added at the rate of 1 part per million of the complete diet. Algit was included in one mixture as five per cent of the dry weight. Antioxidants in foodstuffs for human consumption are eliminated if test animals show symptoms of toxicity when fed at 100 times the level normally needed for food protection. As revealed by the high rates of additives in the diets, ten times the normal level for animal consumption was considered adequate in these diets.

The diets were compounded weekly and immediately placed in the freezer (0° F. or lower). Food was fed daily closely approaching maximum consumption, six days a week. Mortalities were picked up daily and pertinent data recorded.

RESULTS

The effects of feeding high levels of antioxidants, selenium, and Algit are show in Tables 1 and 2. The maximum readings obtained from rancidity tests following 8 days freezer storage are recorded in column 2 (Table 1). The TBA numbers suggest that rancidity was not a problem in this experiment. Growth was relatively good in most diets, but an actual loss in weight occurred in groups of fish fed the diet containing NDGA. The losses of 68.5 and 69.5 per cent of the fish from the NDGA lots far exceeded losses in other diet lots. Lethargy, pinheadiness, slow spiralling and loss of equilibrium was noticeable in some fingerlings within the first two weeks of the experiment. Fifty per cent mortality occurred on the 55th day (last day of the 7th week) of the experiment in both NDGA lots.

Periodic blood tests showed nearly equal blood quality for all diets and replications within the limits of the tests used. The average hemoglobin and hematocrit values of 12 fish sacrificed at the end of the experiment from each lot are given in colums 5 and 6 (Table 1). A few low hemoglobin and hematocrit readings were obtained during July and August, but these were not generally restricted to a specific diet. A number of the sacrificed fish of Selenium diet lots, appeared anemic at the 4 week blood test, but this result did not recur. Contrary to expectations fish fed the NDGA diet showed satisfactory blood condition throughout the experiment. Counter to that found in the other experimental diets, immature red blood cells were seldom observed in blood smears from fish fed this diet. Since many of the moribund fish fed the NDGA diet were pinheaded it was expected that Hexamita would be prevalent in the intestine, but such was not the case. Hexamita was found in the intestine of fish fed some of the other diets.

Table 2 gives much the same information as Table 1, but emphasizes additional results. The mortality recorded in this table shows that in the NDGA lots, the large number of fish lost were small in size; whereas the losses in the other diets were few but generally larger in size. The individual weight gains show that the fish which survived in the lots fed the NDGA diet increased in weight but at a somewhat slower rate. Also concerning the NDGA diet -- during the first four weeks of the experiment the individual weight of one group dropped 0.2 gram while the other remained unchanged. There seems to be some basis, as suggested by these data and subsequent observations, that the main growth of these fish occurred after water temperatures became lower and fish metabolic demands lessened.

The addition of the various antioxidants, selenium, and algit produced pellets varying in color, consistency, and acceptability as fish food. Weight gain differences between some diets may be due principally to physical features of the diets rather than specific preferences by the fish. However, it should be noted that the selenium diet had an unexplained, superior taste appeal for chinook fingerlings.

With the exception of those fed the NDGA diet, all lots took between 1.3 and 1.7 grams of dry diet to produce a gram of fish, a very acceptable figure for test diets fed at maximum rates.

SUMMARY

Eight variations of the Oregon Diet with high levels of Quercetin, Selenium, BHA-BHT, Propyl Gallate, THBP, Algit and a control were fed to juvenile spring chinook salmon for 16 weeks. All diets produced satisfactory growth and fish condition with the exception of the diet containing NDGA, which produced severe mortalities, lethargic fish and retarded growth.

Table 1.
Effects of High Levels of Five Antioxidants, Selenium and Algit - Fed Spring Chinook Salmon-Oakridge, 1960

Diet	TBA No.	%Lot Gain A	%Lot Gain B	% Mort. A	% Mort. B	Hb. A	Hb. B	RBC Vol. % A	RBC Vol. % B
Control	3.8	264	309	1.3	0.7	9.7	10.9	35	38
Quercetin	5.7	271	297	1.5	0.5	10.7	11.4	37	35
Selenium	3.1	309	293	0.5	1.0	9.7	11.7	33	34
BHA-BHT	2.4	292	259	0.2	0.7	10.3	10.9	33	32
Prop. G.	5.4	237	246	0.2	0.0	10.2	11.3	39	38
THBP	6.0	245	231	1.4	0.2	10.6	10.9	36	32
NDGA	4.7	-35	-21	69.5	68.5	9.5	11.2	31	33
Algit	3.6	225	246	1.7	1.0	9.7	10.6	36	34

Table 2.
Data Summary of Eight Diets Fed Spring Chinook Oakridge, 1960

Diet	Ind. Wt. Gain-GMS A	Ind. Wt. Gain-GMS B	Mortality Nos. A	Mortality Nos. B	Mortality Ave. Wt. A	Mortality Ave. Wt. B	Taste Appeal
Control	9.6	11.6	5	3	7.6	2.7	Good
Quercetin	10.4	11.1	6	2	6.1	7.3	Good
Selenium	11.6	11.1	2	4	1.9	2.6	Excels
BHA-BHT	11.1	9.8	1	3	8.3	9.2	Good
Prop. G	8.9	9.3	1	0	3.5	.0	Fair
THBP	9.5	8.4	7	1	3.5	2.7	Fair
NDGA	3.9	4.9	287	283	1.9	2.0	Poor
Algit	8.8	9.4	7	4	8.3	6.6	Fair

IN VIVO EFFECTS OF SELECTED ANTIOXIDANTS AND SELENIUM IN A STRESS OREGON TEST DIET

John Westgate, Aquatic Biologist, Oregon
Fish Commission-Research Laboratory

The ability of antioxidants to retard rancidification of food and their toxicity to salmon, has been discussed. Probably less known and understood are the biological actions of certain antioxidants and other substances producing in vivo effects. These actions take place, not on the food, but inside an animal that has eaten rancid food. Theoretically, they would allow that animal to eat rancid food without suffering adverse effects.

The biological actions of these substances in fish fed rancid diets may be similar to those of vitamin E. Our experiments last year produced severe anemia and mortality when juvenile chinook salmon were fed moderately rancid diets deficient in vitamin E. Moderately rancid diets supplemented with vitamin E did not produce anemia, and mortality was not excessive unless the diet was extremely rancid. Last year's experiment also showed that the more rancid the diet the less weight the fish would gain. This inverse relationship between diet rancidity and fish weight existed both with and without the vitamin E supplement; however, the rate of decrease was less with vitamin E.

It is not yet understood if the anemia and mortality were caused by the toxicity of rancid fats, vitamin E deficiency, or a combination of both. Vitamin E has not been considered essential for trout. At any rate, our fish suffered no ill effects from moderately rancid diets supplemented with vitamin E, other than growing slower than the controls. Therefore, we concluded that vitamin E may act as an antidote to the toxicity of rancid fats.

The inclusion of an antioxidant in feed may not be adequate insurance against rancidity. For instance, the feed may still become rancid during high temperatures or prolonged storage; rancid ingredients may have been used during diet preparation; or the vitamin E content of the rancid feed may have been destroyed. It would be desirable, therefore, to have a substance in the feed, with properties similar to vitamin E, to combat anemia, mortality, and growth inhibition, the penalties for feeding rancid diets. A substance that would not be destroyed as easily as vitamin E would be especially valuable.

The purpose of our experiment at Clackamas this year, was to determine the biological activity, or in vivo effects, of selected antioxidants and selenium on juvenile spring-run chinook salmon. The antioxidants and selenium were added to an Oregon test diet containing an extremely rancid fat component and no vitamin E supplement. This was to simulate a production diet that had become rancid and its vitamin E destroyed. Selenium was selected because it is known to prevent a number of vitamin E deficiency symptoms in chickens. The antioxidants Quercetin and THBP were reputed to have in vivo actions. The antioxidants were added at 0.015 per cent of the diets and selenium at 0.1 p.p.m. The level of vitamin E used was 50 mg.

per 100 grams dry diet. Two lots of two-hundred fish each received each diet for 16 weeks. The fish averaged 103 per pound at start of the experiment.

Diets were prepared three days a week and stored at sub-zero temperatures until the day they were fed. All were fed within two days after preparation. This procedure was followed to minimize the in vitro effects of the antioxidants on diet rancidity.

Table 1 presents the results of the experiment. Duplicate lots are indicated by "A" and "B".

Despite the precautions taken to minimize antioxidant in vitro action, we were not successful in maintaining comparable rancidities when the diets were fed. TBA numbers that would be expected on the complete diets at time of preparation are 2 for Diet 1, and 35 for all the others. Values obtained after two days storage, the maximum rancidity fed, are shown in Table 1.

Average weight gains, clouded by unwanted differences in diet rancidity, allow us to make few conclusions concerning them. Diet 1, with low rancidity and vitamin E, did produce significantly more weight than the very rancid Diet 2. No differences were clearly demonstrated between Diets 2 or 3 and the ramaining diets. The antioxidants produced no definite improvement over the control, even though some were much less rancid. Selenium did show significant improvement, however, the selenium diet was slightly less rancid than the control.

Mortality and blood pictures are clearly shown, and do not appear to be clouded by the unwanted differences in rancidity. The influence of vitamin E in a rancid diet can be seen by comparing Diets 2 and 3. Excessive mortality and anemia occurred when the rancid diet was not supplemented with vitamin E. The addition of vitamin E prevented this mortality and anemia, even though rancidity was nearly equal. The addition of antioxidants BHA-BHT, Propyl Gallate, THBP and Quercetin, brought no improvement. Selenium did, however, lessen mortality and improve the blood picture. The fish fed selenium were not anemic, although their blood was not as good as that obtained from the vitamin E fed fish.

We can conclude then that selenium acts similarly to vitamin E in preventing anemia and mortality in juvenile chinook salmon fed a rancid diet. The antioxidants tested did not act in this manner.

Table 1. EFFECTS OF ANTIOXIDANTS AND SELENIUM, FED IN A STRESS DIET ON JUVENILE CHINOOK SALMON, CLACKAMAS, 1960.

Diet No.	Description	TBA No.	Average Wt. Gain(GMS) A	B	Mortality A	B	Mean Hb A	B	Mean Hematocrit A	B
1	Vitamin E	8	10.6	10.1	1	0	11.6	13.0	44.5	39.9
2	Vitamin E	85	8.9	8.2	1	1	11.7	12.2	36.7	42.7
3	Control	80	8.2	7.8	23	41	6.4	7.4	19.8	24.4
4	BHA-BHT	25	8.8	8.6	26	24	7.2	6.7	22.5	22.5
5.	Propyl Gallate	50	8.8	7.9	23	26	7.6	6.4	21.4	24.3
6	THBP	35	8.8	8.7	29	34	5.3	8.0	17.2	27.6
7	Quercetin	70	8.6	7.6	43	43	6.9	6.1	26.4	19.6
8	Selenium	75	8.8	9.3	3	2	9.2	9.7	31.3	28.7

FURTHER INFORMATION CONCERNING OREGON PELLETS IN PRODUCTION

Wallace F. Hublou
Oregon Fish Commission

The Oregon Fish Commission is now feeding a custom product called Oregon Pellets to all fish after they have reached a size of 300 per pound. Production use of Oregon Pellets was preceeded by several years of experimental diet studies in conjunction with the Food Tech. Dept. of Oregon State College. A detailed account of these studies may be found in the Oregon Fish Commission Research Briefs, Vol. 7, No. 1, July, 1959, in an article entitled "Development of the Oregon Pellet Diet". Oregon pellets are composed of a mixture of dry meals and wet fish products which results in a pellet of soft consistency. The pellets are frozen immediately after being manufactured and are kept in cold storage until used.

In 1958, upon summarizing the results of 9 production feeding experiments conducted from 1954-1958, it was estimated that if Oregon Pellets were used in place of the usual wet meat and fish diets the food cost to produce a pound of fish could be reduced from 46 to 30 cents, providing a savings of 34 per cent in food costs alone.

As a result of the diet studies, Oregon Pellets were put into production use in 1959, being fed to all silver and bluback salmon, and some of the spring chinook salmon and steelhead trout; after the fish reached a size of 300 per pound. At the end of the rearing period, it was found that some 167,000 pounds of fish were produced with approximately 313,500 pounds of pellets for an overall food conversion of 1.9. Pellet cost was 15.1 cents per pound, including a 1 cent charge for transportation costs. The resulting food cost to produce a pound of fish was 28.3 cents. Mortality was very low, the total for the entire period of pellet feeding ranged from as little as 0.2 per cent to a high of 6.0 per cent. A few cases of suspected anemia were encountered which resulted in short periods of meat feeding followed by further pellet feeding.

For 1960, pellet feeding was broadened and at the present time all fish larger than 300 per pound are being fed Oregon pellets. The results thus far, using figures based on fish samples, show that over 239,000 pounds of fish have been produced with 415,000 pounds of pellets for a food conversion of 1.7. Pellet cost, including 1 cent per pound for transportation charges, is presently 15.7 cents per pound, somewhat higher than last year. The food cost to produce a pound of fish is currently 27.1 cents. Mortality is very low; ranging from a total of 0.3 per cent to a high of 3.2 per cent. General fish condition is very good; no fish have been removed from the pellet diet because of anemia or other nutritional disorders.

The results of almost two years of production use indicates Oregon Pellets are living up to our expectations in providing economical fish production. The current cost to produce a pound of fish is 41 per cent less than in 1958 before the advent of pellet feeding. Furthermore, fish health is thought to be very good at the present time.

Realizing that the efficiency of hatchery production is only part of the problem, the fish produced in 6 of the 9 experimental diet studies were marked upon release to determine how well fish fed Oregon pellets would survive as compared to those fed the wet diets in use at that time. To date, returns are complete for 3 groups of silver salmon, and almost complete for the 4th. In summarizing, the information gathered from the mark recoveries of the 4 groups as follows, was found to be evident in every case:

1. Fish fed Oregon Pellets returned in numbers equal to or greater than those fed the wet diet. Actual recoveries ranged from 0.4 - 2.3 per cent for those fed pellets and from 0.2 - 1.0 percent for those fed the comparison wet diets.

2. Regardless of which diet was fed, more marks were recovered from the fish which were largest at the time of release. The pellet fed fish ranged from 18 - 24 fish per pound while those fed the wet diets ranged from 22 - 32 fish per pound.

3. In 2 of 3 experiments for which the data has been analysed the incidence of acid fast bacteria in liver samples has shown a significantly lower infection among fish fed Oregon Pellets.

4. The sex ratio of recoveries fed Oregon Pellets was always greater in favor of the females as compared to recoveries which had been fed the wet diet. What this may imply, in our opinion, is that a lower incidence of disease in pellet fed fish may have resulted in better survival of the females.

5. The females, which had been fed Oregon Pellets, returned to the hatchery in sufficient numbers to provide an egg-take which was always equal to or much greater than the number of parent stock marked. This was not always true of the fish fed the wet diets.

In summary, it appears that Oregon Pellets are doing a better job than the wet meat and fish diets it replaced in the feeding program of the Oregon Fish Commission.

EXPERIMENTAL HEATING OF POND WATER TO START RAINBOW FRY ON A DRY DIET

K. E. Norton
Wizard Falls Trout Hatchery
Oregon State Game Commission
Camp Sherman, Oregon

While many factors are involved in starting rainbow fry on dry diets, the most important problems at this station are reducing the loss of fry, elimination of pinheads and the reduction in number of man hours required to train fry to eat dry foods.

Because water temperature seems to be one of the most important factors in the success attained in starting fry on dry diets, the idea occurred to this writer the above problems might be materially reduced if heated water could be used during the critical spring months, when limited man hours are spread thin over a multitude of peak production chores.

Through the cooperation of the Central Electric Co-op. (REA), Redmond, and the Coates Electric Manufacturing Co. of Seattle Washington, the writer was able to make arrangements (at no cost to this department) for the necessary power and special water heater to conduct a pond heating test. The objective being to explore the feasibility and cost of heating pond water and compare with the result achieved.

The test was set up to cover a two months period; however, it was our feeling that more thansatisfactory results had been obtained at the end of forty-two days, and the heating was discontinued at that time.

This lot of approximately one million fry was the first, full-scale production lot ever to be started in an Oregon Game Commission hatchery on one hundred per cent dry diet.

As we do not have sufficient trough space to start such large lots, it is necessary to start them in circular nursery ponds.

Previous experience on smaller lots indicated that density was very important in starting fry in troughs on dry diets. The data indicates it is also very important in starting fry in circular ponds.

The attached charts show in detail the results achieved in the various methods used for the mass starting of fry on dry diets.

The data were not carried beyond the first of July because by that time all mortality had practically ceased. Only two-tenths of one per cent of the entire lot was lost during July. Liberations and grading operations required complete mixing of all groups and time did not permit a close follow-up of each in-

dividual group. It is regretted this was not possible as there was a very noticeable difference in the uniformity of the unheated ponds and, in particular, the unheated ponds started at the least density. The fish from the heated pond were by far the most uniform.

The water temperature in the heated pond was raised from the constant spring supply temperature of 50° to 56° by means of a special Coates Booster water heater, 3 phase, 230 volts, 32 KW. The heater was connected directly to the two inch inlet pipe and a by-pass valve arrangement permitted the mixing of unheated water with heated water to maintain a constant flow of thirty-five gallons per minute at 56° temperature, or six degrees above normal.

On a few afternoons toward the end of the test when the water temperature climbed to nearly 60°, as a result of the sun's heat, the heater was shut off for several hours and restarted at sunset. Since the temperature was noted to be 54° on only one morning and most all thermometer readings were exactly 56°, it is not believed these few fluctuations seriously affected the end result.

The circular ponds at this station are all 25 feet in diameter, 2 feet deep, and operated with 18 inches of water, or 735 cubic feet of water. Each pond has an individual inlet and no water is re-used.

All unheated ponds were hand fed every half-hour twelve hours per day.

A special twin set of Clark fry feeders, made by the Pam Company of Portland, and designed for outside use, were suspended over the heated pond. These were set to feed every ten minutes sixteen hours per day. The heated pond also received supplemental hand feeding once per hour ten hours per day. It is estimated that only one-half the man hours were required for feeding the heated pond compared with the unheated ponds. It is also felt these feeders played an important part in the results attained in the heated pond.

The cost of electricity, as computed by the power company, would have been $289.80 for the 42 days.* This would be a cost of 96.3 cents per thousand fish.

A heating period of 32 days would have probably been satisfactory to give the fry a good initial start; this would have cost 73.3 cents per thousand fish. It is possible this cost could be further reduced by covering the ponds or enclosing them with plastic tarps.

It is difficult to pin down the exact savings in dollars and cents. While this test was of a very preliminary nature, the results indicate that heating the water substantially reduced the

*The heater unit equipment and installation costs would have been approximately $500.

fry mortality. The value of the fish (at $10 per thousand) and man hours saved in the heated circular pond offset the cost of the power.

Conclusions:

Advantages of increasing the water temperature six degrees were the following:

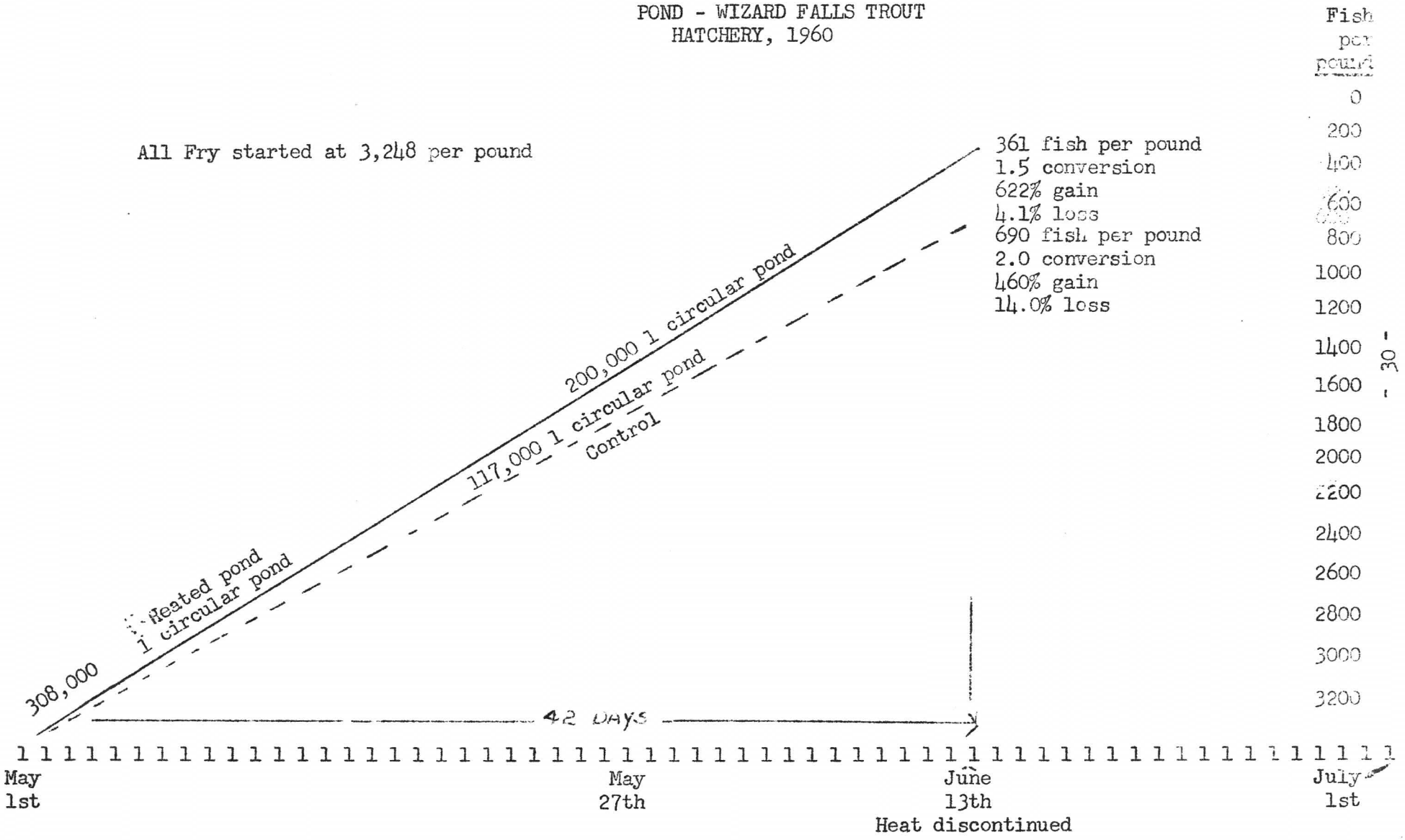
1. The fry which were reared in the heated pond were easier to start on dry food, had a lower mortality than other groups, and were much more uniform in size.

2. The cost of electricity for heating the experimental pond was calculated at approximately $0.96 per thousand fish for 42 days. For 32 days, the cost would have been approximately $0.73 per thousand fish.

3. Less manpower was required to rear fish in the heated pond because of faster rate of growth.

4. Density in numbers of fry per pond was found to be an important factor in rate of growth and mortality.

COMPARATIVE GROWTH RATES BETWEEN HEATED POND AND UNHEATED CONTROL POND - WIZARD FALLS TROUT HATCHERY, 1960



COMPARATIVE DATA ON POND HEATING TEST
WIZARD FALLS TROUT HATCHERY, 1960

Group No.		April 14th X	May 1st X	June 1st X	July 1st X	Per cent loss	Conversion	Fish per pound
1.	55,216	1 trough	2 troughs	1 circular pond Hand fed		22.3	1.85	172
		100% auto. fed						
2.	84,448	1 circular All hand fed				15.5	1.3	148
3.	402,800	1 circular All hand fed	2 circular ponds	4 circular ponds		8.8	1.34	172
4.	117,083	Control 50°	1 circular pond All hand fed			14.6	1.55	240
5.	308,618	Heated pond 56°	1 circular	1 circular pond hand fed*		2.81	1.22	199
			Auto fed plus hand feeding			4.1	1.5	361
				1 circular*		0.6	1.28	211
				Hand fed* 1 circular		0.4	1.7	233
				Combined data for heated pond		5.3	1.4	216
	968,165	Combined data for all ponds				9.7	1.4	193

All fry started at 3,248 per pound
All ponds divided equally at time of division. ^ Time mortality peaked
*100,000 each approximately.

PROGRESS REPORT ON AUTOMATION AT OREGON STATE GAME COMMISSION HATCHERIES

C. C. Jensen, Oregon State Game Commission

Fish hatcheries, although somewhat behind industry in development of labor saving devices, have nevertheless made rapid progress toward this objective in the last few years.

The Oregon Game Commission has, for instance, tried to incorporate in its hatchery program automatic devices which would tend to decrease the costs of production. The most current developments which have been adopted and/or are undergoing experimentation are discussed below.

Automatic pellet feeders have been undergoing development for approximately three (3) years, and during the past year, fifty feeders were constructed and delivered to thirteen hatcheries for experimental feeding. Double feeders, consisting of two individual hoppers were usually installed on pond walls between two ponds so that one feeder could feed both ponds. Single feeders were used on ponds which were isolated or in a position where a double feeder was not applicable. The pattern or spread of pellets from each feeder covers an area of approximately 20 X 40 feet. In a pond 20 X 100 feet, two single feeders can be used to feed one pond. Where ponds are located adjacent to each other, two double feeders will feed both ponds. Electric timers are connected to each battery of machines to regulate the interval of feeding.

Automatic fry feeders, manufactured by The Pam Company of Portland, Oregon, have been used successfully at many stations to start trout, steelhead and salmon fry. The feeders are used in troughs, live pens, concrete rearing tanks and in outside ponds. At cold water stations, fry have been fed successfully only when the feeders have been set to feed at intervals of not more than fifteen minutes for periods up to eighteen hours per day.

One of the more recent undertakings in the field of automation is the "Loader-Grader", also being developed by The Pam Company of Portland, Oregon, in cooperation with the Game Commission. It is anticipated that the "Loader-Grader" will automatically move fish from one pond to another pond, grade, weigh, record and hold fish for liberation or later distribution. Earlier experimentation with a scale model has proven successful to the point that a large size prototype is now under construction at Wizard Falls Hatchery. Preliminary testing has been successful. The present Loader-Grader is being built as a stationary piece of equipment, but it is believed that later models may be mobile.

The Morton fish grader has been modified by installing aluminum extrusions in place of the plastic space bars which were formerly used. The aluminum bars permit a finer adjustment, thus

enabling the fish culturist to grade fish at an earlier age. The plastic bars used in construction of the former models had a tendency to warp.

At the new Gnat Creek Hatchery on the lower Columbia River, an overhead trolley has been devised which enables the hatcheryman to move large numbers of fish from one pond to another or to a waiting liberation truck with a minimum expenditure of effort. The trolley operates on an overhead track approximately 90 feet in length and is equipped with an electric Zip hoist.

Another fish moving device, adaptable for use in ponds located adjacent to each other, utilizes a 20 foot length of 10 inch diameter aluminum pipe. The pipe with a built-in hopper at one end and a counterbalance at the other end operates on the pond wall like a teeter-totter in moving fish from one pond to the other. Approximately 20 to 30 pounds of fish are transported through the tube at one time.

Liberation equipment includes a 1,000 gallon refrigerated truck which maintains the water at a constant low temperature, a planting boat capable of transporting up to 1,000 pounds of fish and an automatic fish trolley which transports fish from the liberation truck to the stream bank by means of an overhead cable.

PROGRESS REPORT ON FISH DISEASE RESEARCH

James W. Wood
Washington State Dept. of Fisheries*

Ceratomyxa

A myxosporidian parasite belonging to the genus Ceratomyxa has been found in adult spring chinook in the Middle Fork of the Willamette River each year since 1955. The numbers of fish dying from the infection is believed to have been small until this year. During the 1960 holding season, losses due to columnaris disease were held to a minimum with the malachite green treatment method described at last years meeting. Losses, mainly due to Ceratomyxa amounted to 54% of the females entering the Dexter holding ponds. Fish holding under natural conditions in the Middle Fork, as well as those in the holding ponds, were infected.

During 1957, 1958 and 1959, substantial numbers of fall Chinook that had spawned successfully in the Snake River below Swan Falls Dam, were examined. The diagnosable incidence of infection varied from 40 to 100%.

In 1960, a total of 46 adult female silver salmon were transported from Roza Dam on the Yakima River to a pond at the Yakima trout hatchery for holding. A loss of 30 females (65%) was sustained primarily due to Ceratomyxa, previous to spawning. The finding of infected silvers in the Yakima at a later date indicates the fish contracted the infection before transfer to the hatchery holding pond.

The kidney, liver, and G.I. tract appear to be the most heavily infected organs. Perforated lesions in the intestine are a common manisfestation in terminal stages of infection.

The source of infection is unknown. However, observations incidental to other disease studies indicate that the adults pick up the parasite in the Columbia River on their upstream migration, since it has not been observed in salmon taken by the commercial fishery near the mouth of the river. All observations of the parasite in adult salmon to date have been from fall Chinook, spring Chinook and Silvers that have spent at least two months in fresh water.

Furunculosis

Relatively high losses due to a bacterial infection occurred among young silvers and spring Chinook at Dungeness Hatchery in late October 1960 at which time the weekly average water temperature was approximately 47°F. The bacterium was classified as Aeromonas salmonicida, the etiological agent of furunculosis disease, on the basis of biochemical and cultural tests. Furunculosis, usually considered a warm-water disease, is notable in this instance for the relatively low temperature at which fulminating losses occurred. The disease responded well to therapy with nf180 at a level of 5 gm./100 lbs. of fish/day for 10 days administered in the feed.

* Oregon Fish Commission previous to August 1960.

THE HEXAMITA PROBLEM - REAL OR IMAGINED?

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ABSTRACT

The intestinal flagellate, Hexamita salmonis (Moore), was described in 1922 from trout in hatcheries throughout New York State. At first associated with the so-called "whirling disease" of brook trout, now known to be a virus disease, Hexamita was subsequently held responsible for practically any otherwise unexplainable mortality in underyearling salmonids.

Moore's findings and assertions regarding the pathogenic nature of Hexamita provoked a series of intensive investigations which continued for many years. H. S. Davis, pioneer in fish disease research in the United States, published a detailed account in 1926 -"Octomitus salmonis, a parasitic flagellate of trout" - which sought to define and describe the life cycle and histopathology of hexamitiasis. This paper, however excellent in general, apparently stifled all further objective thinking on the role of Hexamita in fish culture. Subsequent researches were directed entirely toward suppression or elimination of the "parasite" from affected fishes. McKay and Tunison (in Davis, H.S., 1937) devised the calomel treatment which was later supplanted by the carbarsone treatment (Fish and McKernan, 1940; Nelson, 1941). Carbarsone, the drug of choice until recently, has been used regularly as a routine prophylactic or therapeutic in hatcheries throughout the country.

During all this time it is signifacant that no clear-cut experimental work has been performed to prove or disprove the alleged pathogenicity of Hexamita. Despite the excellent morphological, distributional, and life history information provided by Moore, Davis and others, we have no direct evidence that Hexamita is a primary pathogen. Nevertheless, we can now cure the "disease" more or less readily by appropriate addition to the diet of calomel, carbarsone, Furoxone, Fumagillin, and even epsom salts! Ignoring the known off-feeding tendencies of fish on medicated diets, the practice of Hexamita control has continued, and even flourished, with the additional help of the dry feed manufactureres who can make available (at slight additionsl cost) drug fortified feeds to help eliminate the ubiquitous (and probably harmless) Hexamita.

We wish to report briefly the results of a preliminary experiment which was conducted at the Quilcene National Fish Hatchery

during the summer of 1960. The objective of the experiment was to determine the effects of rigid Hexamita control on the growth response and mortality rate of juvenile silver salmon.

Fifteen standard hatchery deep troughs were stocked with ten pounds each of Hexamita-infected fingerling silver salmon from an outside production pond. Five treatments were imposed in triplicate. Control lots received Clark's Regular Crumbles daily throughout the experiment at the manufacturers' recommended levels. The experimental groups were treated and fed similarly excepting that medicated crumbles as described below were fed on 6/13, 6/14, 6/15, 6/16, 6/27, 7/11 and 7/25. All lots received prophylactic treatment for external parasites (Costia, Trichodina, and bacterial gill disease) using P.M.A. 1:500,000 on 6/22, 7/13, 7/20, 8/3, 8/17 and 8/31. All lots were inventoried and examined by-weekly to determine rate of gain, and incidence and intensity of Hexamita infection.

Medicated feeds were custom mixed by the J. R. Clark Co., using the basic formulation of "regular crumbles" plus medicants at the following levels:

1. 6% NF-180 (Hess & Clark).
2. 3% NF-180 " "
3. .1% Fumagillin (Abbor Laboratories).
4. 3% Magnesium sulfate (J. R. Clark Conditioner).

Essential details of the experimental results are shown in Table 1. It will be noted that complete Hexamita suppression was attained only in the Fumagillin and Conditioner treated groups. It is significant that the unmedicated controls showed superior growth response and food conversion despite prevailing high levels of Hexamita infection. It should be noted also that mortality rates in all lots were comparable and below expectation. We consider this low mortality rate directly attributable to elimination of suppression during the experimental period of the ectoparasites cited above.

Under the experimental conditions outlined here, it must be concluded that hexamitiasis is less harmful than any of the treatments invoked against it. The lack of significant mortality in the control groups suggests further that Hexamita is harmless to silver salmon of this age composition. It may be argues that the results of this experiment are confounded by the "drug effect" and that a less noxious or toxic drug therapy might produce Hexamita-free fish which would show a growth response superior to that reported here for the "diseased" fish. While the argument is hypothetical, it is possible valid and we propose, accordingly, to investigate this facet of the problem.

In the intermin, however, we hold to the theory that the benefits of Hexamita control are more imagined than real.

TABLE 1.

CUMULATIVE SUMMARY OF QUILCENE EXPERIMENT (HEXAMITA)[1]

Period 6/9/60 - 8/31/60

	Controls	NF-180 (6%)	NF-180(3%)	Fumagillin	Conditioner
No. per lb., start	152	152	152	152	152
No. per lb., end	42	58	55	47	48
No. fish, start	4,560	4,560	4,560	4,560	4,560
No. fish, end	4,548	4,529	4,540	4,540	4,548
Weight, end [2]	107.4	78.1	82.8	95.7	94.9
Weight, start	30.0	30.0	30.0	30.0	30.0
Gain in weight	77.4	48.1	52.8	65.7	64.9
Lbs. food fed	153.7	121.8	124.6	150.1	146.0
Conversion	1.99	2.53	2.36	2.28	2.25
Per cent gain	258.0	160.3	176.0	219.0	216.3
Mortality	12	31	20	20	12
Per Cent mortality	0.26	0.68	0.43	0.43	0.26
Hexamita incidence, start	90.0%	90.0%	90.0%	90.0%	90.0%
Hexamita incidence, end	86.7%	13.3%	20.0%	0.0%	0.0%
Hexamita intensity, start [3]	1.4	1.4	1.4	1.4	1.4
Hexamita intensity, end	1.73	0.13	0.17	0.0	0.0

1. Column entries are composites of triplicate treatments
2. Weight at end adjusted to account for fish sacrificed for disease inventory.
3. Intensity scale range 0 - 3.0

CRYPTOBIS SALMOSITICA, A HEMOFLAGELLATE PARASITE OF COHO SALMON [1,2]

By

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During a previous study of the normal hematology of the Coho salmon (Katz, 1949), an unidentified hemoflagellate was observed in stained smears of the blood of mature salmon. These protozoan parasites were first observed during the early part of the spawning season, and only a few fish were found infected. As the spawning season progressed, larger numbers of fish were infected until by November 21, all of the salmon examined contained the hemoflagellate. This high degree of infection persisted until the end of the spawning season.

Coincident with the arrival of the salmon was the appearance of leeches (Piscicola salmositica)(Meyer, 1946), adhering to the rocks on the bottom of the holding ponds and on the salmon. As the number of salmon increased, so did the number of leeches. As the peak of the run passed and the numbers of salmon declined, so did the number of leeches until by the end of the spawning run, all of the leeches had disappeared. Examination of the salmon-holding area at the Soos Creek Hatchery of the Washington State Department of Fisheries near Auburn, Washington, where these observations had been made, during the summer months failed to disclose any leeches.

Examination of the crop contents of the leeches found on the adult salmon revealed the presence of large numbers of hemoflagellates which were presumably the same organisms as were found in the blood of the salmon.

Not only were the adult salmon infected, but young salmon fingerlings have been found to contain the parasite. Young of the year coho salmon taken from Swamp Creek near Kenmore, Washington during November were found to have the parasite. The infection has not been observed in young salmon from the same stream during the spring and summer months.

A literature search indicated that this flagellate represented a new species, and it was described in detail and named Cryptobia salmositica (Katz, 1951). In 1954, some young coho salmon collected in Beaver Creek a small coastal stream flowing directly into the Pacific Ocean near Newport, Oregon, were found to contain this hemoflagellate in their blood (Davison, Breese and Katz, 1954.). Cryptobia are not restricted to this immediate area or to American salmonids. As long ago as 1906, cryptobids of other species were reported from the blood of brown trout, Salmo trutta, in France by Brumpt (1906a) and Gauthier (1920) later repeated this observation. The transmission of the flagellates to fish by the

1. Contribution No. 120, College of Fisheries, University of Washington-Seattle, Washington
2. This study is supported by National Institutes of Health Research Grant, E3664.

leech has been demonstrated by Brumpt (1906b) and by Robertson (1911). The only other published report of Cryptobia in North American salmonids is by Wales and Wolf (1955). They found Cryptobia in domesticated rainbow trout and yearling chinook salmon at the Mr. Shasta Hatchery, California. During 1950-1952, the same investigators found Cryptobia in several species of wild fish of the Klamath and Sacramento drainages of northern California: steelhead, brown trout, chinook salmon, coho salmon, Klamath large-scale suckers, and cottids. Wales and Wolf expressed the belief that many past spawning losses in rainbow brood fish have been due to the combined effects of Cryptobia infestations and secondary fungus invaders. Chinook salmon held for a year in hatcheries were found by these workers to be exceptionally vulnerable to Cryptobia and suffered high mortalities.

Because of the basic scientific interest in these organisms, the Division of Research Grants, National Institutes of Health, U. S. Public Health Service, awarded the Fisheries Research Institute College of Fisheries, University of Washington, a two-year grant to study the life history of the flagellate, the leech vector and their influence on the coho salmon host.

In this study, we will attempt to correlate the spawning migration of the coho salmon and the incidence and degree of infection of the mature fish with the blood parasite. At the same time, we will collect data on the size distribution, numbers, and life history stages of the leech vector. We will also study in detail the life history of the Cryptobia. The developmental forms of the flagellate will be described. In addition, we will also try to elucidate in detail the life history of the leech, Piscicola salmositica Meyer, measure its rate of growth and determine its abundance throughout the year, especially prior to, during, and after the salmon run. We are particularly interested in the reproductive cycle of the leech, where they go after the salmon spawning season and where and when they deposit their eggs.

Additional studies will be carried on to determine the pathological effects of the infection of the hemoflagellates upon the salmon hosts. At the present we feel that there is little to be concerned with in regard to the pathological effect of the leeches and the flagellates upon the adult salmon, because the fish die after spawning. It is of interest, however, to determine their effect upon the small fish of the year which are wintering in the fresh-water stream prior to their movement to the ocean. It is important to determine whether these parasites cause important pathological changes and whether the Cryptobia contribute to the important fresh-water mortalities of the young coho salmon.

At the present, most of the preliminary studies will be carried on at the Soos Creek Hatchery. Steve Fallert, Superintendent of the hatchery and his staff are most cooperative; and we also want to acknowledge the cooperation of Bud Ellis, Smock Brittain and Dick Noble of the Hatchery Division of the Washington State Department of Fisheries.

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FACILITIES USED IN VIRUS DISEASE STUDY AT THE COLEMAN NATIONAL FISH HATCHERY

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The study of the virus disease of the Chinook salmon fingerling at the Coleman National Fish Hatchery required three different water supplies - well water, which is assumed to be virus free, Battle Creek water, which is the regular station production water, and a mixture of Battle Creek and well water.

Various plumbing, pumping and trough arrangements were necessary to have available the water supplies and hatching trough space. A method of cooling the well water was devised by means of refrigeration and heat exchanger manifolds.

Through use of color slides the several water and trough arrangements were shown. These facilities consisted of source of well water cooled through a heat exchanger manifold, the water then delivered into a storage tank, in which a refrigerated cooling unit is immersed, the cooling effect obtained by use of a 1 ½ H. P. Freon refrigeration unit. Two G.P.M. of water were thus cooled from 62 degrees to 48 - 50 degrees. Burrows - Johnson type incubator trays are used to incubate the Chinnok eggs. This unit was used at the initial phase of the program, to conserve well water and until the other cooling system could be placed into operation.

The main well water facility was setup and this water cooled through a water cooling heat exchanging system of manifolds immersed in creek water. Creek water temperature is 46 degrees, and through this system about 50 G.P.M. are cooled from 62 to 50 degrees. Twenty four compartments are supplied from this source, and produce 24 separately controlled groups of fish stock for further study in the program. An emergency supply, involving a gravel filter was assembled to be used in the event of power failure and well water not available. The filter consists of a tank 16 feet by 30 feet by 48 feet, filled with washed and sterilizedgravel. Battle Creek water is supplied at one end and pumped out by means of a gasoline pump at the other, this supply then delivered into the well water system. While not entirely satisfactory, it is better than nothing.

Another portion of the study involves regular Battle Creek water, and a section of the hatchery is used for this purpose. The mixed water portion involves a like setup except that here well water and Battle Creek water are mixed at a ratio of 1 well to 8 Battle Creek water parts. Temperature was raised from 46 F. for Battle Creek Water to 50 F. mixed.

There are about 48 individual lots of eggs involved in the study, totaling some 4 to 5 million eggs when finally arranged.

These consist largely of Battle Creek and Keswick eggs, with about 400,000 from Spring Creek, 50,000 from the Klamath River, 50,000 from the American River.

Representative samples from these groups will be held in individual troughs for observation and progress of the virus. These studies are being conducted by Tom Parisot of the Western Fish Disease Laboratory.

An additional arrangement, whereby water will be re-circulated with a 20% or 20 G. P. M. waste is a part of the station program, looking for means of getting around the virus.

The final portion of the station program is also directed toward circumventing the Virus, by means of sterilization of eggs and equipment.

Problems arising are that of maintaining temperatures of the well water, since as Battle Creek temperature rises, our cooling of well water is not satisfactory and above 52 degrees the Virus is known to disappear. It is necessary that we maintain 52 degrees and below in the well water to be sure the Virus could devolop if it is on the fish or in the water.

A TEST OF INJECTED OXYGEN IN FISH DISTRIBUTION

Adrien F. Bernier
Fish and Wildlife Service
Hagerman, Idaho

To test the physical and economic advantages of adding injected oxygen to our fish distribution units.

On one standard 1,200 gallon tank we loaded 1,000 pounds of legal rainbow and ran the truck around the station grounds for 8 hours.

On another identical 1,200 gallon tank, equipped with a K cylinder of oxygen, flow meter set at 4 liters per hour, we loaded 1,500 pounds of legal rainbow and ran this truck around the grounds for 8 hours. On this tank, the oxygen was introduced into the water line on the intake side of the pump.

On each tank we took temperatures, oxygens and ammonias every 2 hours to learn what was happening and when.

At the end of the test period the fish were dumped into separate rearing ponds for observation. At the end of 24 hours the 1,500 pound load had a mortality of 12 fish. The 1,000 pound load had no loss. The fish were held for an additional 24 days and there was no more loss in either group.

The details of the experiment are shown in the two following tables:

	1,000 lbs Rainbow 5 to pound Control			1,500 lbs Rainbow 5 to pound Oxygen added		
Hours in tank	PPM Oxygen	PPM Ammonia	Temp. F.	PPM Oxygen	PPM Ammonia	Temp.F.
0	9.5 9.3	0	59	12.1 12.2	0	59 1/
2	7.7 6.8	0.5	50	12.1 12.0	0.5	50
4	5.5 5.4	1.1	58	9.6 9.4	1.6	58 2/
6	5.9 6.5	1.6	54	9.9 10.0	2.2	54
8	5.1 5.4	2.0	60	8.9 8.9	2.5	60
Mortality after 24 hours - 0				Mortality after 24 hours - 12		
Mortality after 14 days - 0				Mortality after 14 days - 0		

1/ 800 pounds ice added to each tank

2/ 500 pounds ice added to each tank

Averages -	Numbers of Fish	Pounds of Fish	Hours in Tank	Miles Traveled	Ice Used	Cost Per lb.
10 trips with standard load and no oxygen	8,376	841.7	5.25	274	730	$0.055
Averages - 10 same trips standard load oxygen added.	14,084	1,324	5.25	268	995	$0.0365

TRANSPORTATION OF FISH - PLASTIC BAGS WITH OXYGEN

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Winthrop National Fish Hatchery
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As Fish-culture knows, one of the biggest problems around a trout or warm-water hatchery is the transportation of fish of various sizes. To accomplish this as cheaply as possible, to utilize ones time to the utmost and have the end product delivered at its destination in the best of health are all items to be considered in the transportation of fish by any means that fits the situation.

I would like to quote from an article in the October 1958 issue of "The Progressive Fish-culturist" entitled "Transporting Small Live Trout in Sealed Polyethylene Bottles", by Mr. G. Norman Wilkenson, Jr., Bureau of Sport Fisheries and Wildlife, Creede, Colorado - 'Considerable work needs to be done to set up tolerance tables for ready reference for various species, sizes and number of fish in relation to water temperature, oxygen-water charge ratios, sedative drugs, metabolic-waste neutralizing agents and time factors' end quote. This I believe still must be done to accomplish the overall picture of fish transportation.

During the Spring of 1956 we attempted to anesthetize Rainbow trout running 6 per pound by using Chlorabutanol-anhydrous; place them in a moss filled wooden box with added ice and rig the box with a parachute and sir drop them into a remote creek in our area. In one box there were placed 50 fish which had been anesthetised only until they showed signs of breathing distress; the other box (same number of fish) were anesthetised until they turned on their sides; still another box was used as a control; this was handled as the other two boxes except that it was placed in the pickup and only hauled to the airport and returned to the hatchery water supply in the same length of time as those that were airlifted, dropped and placed in the stream by personnel who were already in the area. This method was not satisfactory as only 50 percent of the fish survived; of the 25 fish that were mortality it seems, even though the fish had been off feed for 4 days, that the effects of the Chlorabutanol and/or impact on the ground was a cause for the fish to regorge their food and all the fish that apparently survived the drop died within 3 days.

So from the foregoing, some other method had to be devised to plant these inaccessible areas, than by the old teadice method of packing fish on your back or the method of pack animals, cans and the constant changing of water, of course even today the pack animal whether he be animal or man, can not be done away with but the method and operation can and was surely improved by methods later described.

By use of the plastic bag which is used by the Forestry Service to line garbage cans in the camp grounds and in that the source of supply was handy, was the reason that this type of bag was used in the

air-drop that I wrote the article in the July 1960 Progressive Fish-culturist. If we were to use a greater number of bags, it would be far better to secure a bag of a little better design, that is a more of a straight sided bag with a rounding bottom. This bag is not the strongest bag as the bottom seam is weak and leaks; so it is necessary to secure the seam with several wraps of a rubber band.

With two gallons of water, two pounds of fish and the excess air was exuded from the bag and replaced with oxygen until the bag was nearly full. If too full there is no room for a hand hold; again care must be taken that the operator does not punch the bag with a finger nail. It is amazing the strength of a plastic bag and then in the same thought how weak this material is under limited stress or strain.

In dropping the bags from the aircraft, altitudes of 140 feet the bag was opened near the top by use of a sharp pocket knife. Upon impact the bag could be seen as it split further, thus allowing all the fish to escape. Two weeks later an observation was made of the lake and no mortality was seen and fish appeared in good condition. One week short of a year later another observation was made and the fish were then running 3.3 per pound or about 9 inches in length and of deep body. A good number was seen from the shore.

This past summer distribution was made by the use of the old pack cans and transportation was the mule. Rainbow trout at 140 per pound, six gallons of water in plastic bag, 2½ pounds of fish and filled the bag tight with oxygen to conform with the design of the can. Cans were loaded into a 3/4 ton pickup and hauled for 2 hours and 15 minutes over a mountain road a distance of 29 miles. A raise of altitude of 5,200 feet. In that the pack-string was not ready to take-off, it necessatiated a 2 hour delay, of which the cans were left setting in the pickup. Loading time at the hatchery was 4:30 A.M., arrival at the pack string was 6:45 A.M. Loading onto the pack string and departure was at 9:00 A.M. The first of the fish were planted at 12:45 P.M. and the last at 1:45 P.M.; total time lapse from being placed in the cans until being unloaded at point of distribution was 8 hours and 15 minutes and the last of the fish were planted after a period of 9 hours. The temperature varied from 48° at the hatchery to 63° at the point of liberation. Time was taken to temper the water from 63° to between 53° and 55° and the fish were liberated in 50° water. Upon the return trip points of liberation were noted and fish were seen swimming contently in the same area.

Another trip was made from Winthrop to Mt. Adams Lake, a distance of 285 miles. One hundred eleven pounds of Rainbow trout, 80 per pound were placed in our small distribution tank at 3:30 A.M. hauled to where the road crosses the Klickitat River. Transferred them into 15 milk and/or pack cans, at approximately 7 pounds per can. The procedure was to first place a couple gallons of water in the can, insert the plastic bag, fill bag in can just over half full with water, add a pound or so of ice and then put in the fish. Displace air and fill with oxygen and tie off bag with rubber band.

These cans were loaded into 4 wheel drive vehicles and one hour and 10 minutes later were liberated into lake. The crew ate their lunch taking about 35 to 40 minutes and no mortality was experienced. This method used in transporting fish in the lake was the most successful ever made in this area.

In conclusion the use of the plastic bag and oxygen has made distribution of fish possible into all the high country area, that not long ago were deemed absolutely inaccessible. By the use of our large and small distribution tanks and the methods described above were are able to plant fish anywhere in our area.

REARING POND ENVIRONMENTS

Salmon-Cultural Laboratory
Entiat, Washington
Roger E. Burrows

In 1960 the work of the Salmon-Cultural Laboratory on rearing pond environments has consisted of the measurement of cyclic changes in environment within a 24-hour period and during a summer rearing season, exploration of the physiology of excretion, and the effect of the accumulation of excretory products on the animal. Work has been confined to chinook salmon fingerling and concentrated on the raceway type of rearing pond.

Patterns of Expulsion in Raceway Ponds

Previous experiments on various pond types have demonstrated that the excretory products expelled from rearing ponds vary in composition during a 24-hour period. The intent of this experiment was to determine how the environment changes as the weight of fish per gallon per minute of inflow increases.

Four raceway-type rearing ponds were sampled at hourly intervals for three, 24-hour periods spaced 6 weeks apart from the forepart of June until the last of August. The amount of ammonia and urea present in the outflowing water was determined from the hourly samples.

The experimental raceways were 4 feet wide and 40 feet long with an average water depth of 2 feet. Two of the raceways were of the conventional type and two were of the recirculating type. One of each composed a pair. In one pair 125 gpm was introduced per pond and in the second, 62.5 gpm. All ponds were stocked with equal poundages of exactly comparable chinook salmon fingerlings at the start of the experiment.

The first series of samples was taken on June 6. The lightly stocked ponds contained 1.6 pounds per gpm. In these ponds no measurable amount of ammonia was present. Urea, on the other hand, was present for 22 or 23 hours of the 24 in amounts in excess of 0.2 ppm. In the heavier stocked ponds, 3.2 pounds per gpm, the patterns were similar except that measurable amounts of ammonia occurred for 1 hour of the 24 and urea was never absent.

The next series of samples, taken on July 20, showed a build-up of ammonia until it was dominant over urea in the heavier ponds, 5.7 pounds per gpm, and the first appearance of ammonia in the lighter ponds carrying 2.9 pounds per gpm.

The final series of samples, taken on August 31, showed ammonia present for from 20 to 23 hours in the heavier stocked ponds carrying from 8.2 to 8.5 pounds per gpm and approaching dominance in

the lighter ponds carrying 4.4 pounds per gpm. The reversal of the ammonia-urea relationship as the poundage of fish increases is of extreme interest in that an inhibition of urea excretion in the presence of ammonia is indicated.

Physiology of Excretion

In order to further explore the change in dominance encountered in the excretory products of salmon fingerling, blood analyses were made to determine the presence and amounts of ammonia and urea in the blood. Because the techniques of analyses had to be established only determinations from the latter part of the season were possible. In these samples the ammonia levels were relatively constant varying from 27 to 38 ppm with a mean of 31 ppm on 24 determinations. In contrast the urea varied from 0 to 98 ppm with a mean of 15 ppm on 24 determinations. The relatively constant level of ammonia in the blood indicates a limited tolerance and an effective method of excretion by salmon fingerlings. The tremendous variation in the urea levels encountered indicates a wide tolerance to urea by the fish and very possibly an inhibition of excretion. Whenever a high level of urea was encountered in the blood, high ammonia levels were present in the pond water. We are just beginning to explore this problem and do not have enough data to draw any definite conclusions, but it appears as though ammonia in quantities above 0.2 ppm in the water inhibits the excretion of urea and eventually its formation.

Effect of Excretory Products on Salmon Fingerlings

The analyses of the pond waters indicated that only urea and ammonia were excreted in significant amounts by salmon fingerling. This year three experiments were conducted to determine the effect of these excretory products on the fish. Two experiments measured the effect of fixed ammonia concentrations at two water temperatures and the third the effect of urea at a single temperature.

In the first ammonia experiment, sufficient ammonium hydroxide was introduced by means of constant siphons into small troughs supplied with 2 gpm of inflow to create constant concentrations of 0.3, 0.5, and 0.7 ppm. The experiment was continued over a 6-week period with samples of 5 fish, each, withdrawn at weekly intervals and the condition of the gills compared with a comparable sample from a control group which had been exposed to no measurable amount of ammonia. Water temperatures were maintained at approximately 43° F.

The concentrations selected were the maximum and average concentrations encountered in ponds approaching their carrying capacity. Semi-weekly measurements of the ammonia concentrations within the experimental troughs indicated a mean variation of not more than 0.05 ppm throughout the experiment.

Ammonia was demonstrated to be a gill irritant at all the concentrations tested. The epithelial cells of the gill lamellae proliferated first, followed by clubbing of the tips of the filaments and then a hyperplasia of the filaments. This condition developed in

all concentrations during the first three weeks. The fish from the 0.7 ppm concentration showed the effects of exposure after the first week and continued to show a higher incidence of extensive affection. Beyond the first three weeks no further deterioration of the gill tissue was noted, in fact, the proliferated cells consolidated to form a thickened epithelial layer. No significant mortality was experienced in any of the lots throughout the 6-week experimental period and no gill bacteria were observed.

The second ammonia experiment duplicated the first except that the water temperatures averaged 57° F. The results were practically identical except that the proliferated epithelial cells did not consolidate to form the thickened epithelial layer encountered in the first groups.

The degree of recovery of both lots of affected fish was noted when removed from the ammonia concentrations. In the first experiment no discernable recovery was observed after 6 weeks. In the second group the fish were showing marked recovery after 3 weeks.

In the third experiment, identical procedures were followed except that urea at 0.2, 0.3 and 0.4 ppm concentrations were substituted for the ammonia concentrations. These levels were higher than those normally encountered in rearing ponds. No effect was noted on the gills at the end of 3 weeks of exposure and the experiment was abandoned.

These experiments demonstrate that ammonia, not urea, is capable of creating an unfavorable environment in rearing ponds. One demonstrable condition is the gill irritation produced in the controlled experiments. We hypothesize that, while the fish can tolerate the ammonia concentrations, the proliferated epithelium provides an avenue of infection for the waterborne bacteria associated with bacterial gill disease and possibly protozoan parasites as well. Prolonged exposure to relatively high concentrations of ammonia is believed to be the precursor of bacterial gill disease.

Reduced stamina and inferior growth rates are also the result of exposure to high ammonia concentrations. The stamina tunnel tests indicate that fish subjected to prolonged exposures of ammonia have significantly inferior performance ratings. Reduced growth rates due to prolonged ammonia exposures are indicated from the experiments conducted in the conventional experimental raceways. After 19 weeks of feeding, a reduction of 10 percent or 41 pounds, in the total gain of the fish occurred in the lot subjected to the higher ammonia concentrations.

All the evidence indicates that ammonia is the principal excretory product responsible for creating an unfavorable environment in rearing ponds. The effects of this environment are insidious and difficult to ascertain. If the condition of the fingerling at time of release is critical then the practice of carrying rearing ponds at or approaching capacity at this time must be avoided if maximum survivals are to be attained.

RECOVERIES OF MARKED ADULT FALL CHINOOK SALMON AT SPRING CREEK AND LITTLE WHITE SALMON

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Spring Creek

Fall Chinook Salmon of the 1956, 1957 and 1958 broods were marked and released at Spring Creek National Fish Hatchery to compare survival from releases of unfed fry and 90 day reared fingerlings. Both groups of fish were marked before they started to feed and were about 1,100 per pound at the time of marking. Each year the group marked Adipose-Left Pectoral was released on February 5 immediately after marking. The second group was marked Adipose-Right Pectoral and then reared for about 90 days and released on May 7. Approximately 240,000 fish of each mark were released each year.
Marked adults from this experiment were recovered in 1959 and 1960. Data on recoveries at Spring Creek are given in the following table:

Mark	Ad-Lp	Ad-Rp
Date Released	February 5	May 7
Days Reared	0	90
Fish Per Pound	1,077 - 1,178	121 - 143
Adults Recovered 1959	3	107
Adults Recovered 1960	10	84
Total Recovered	13	191

These data indicate a much higher survival of fingerlings released than of unfed fry. No fry will be released from Spring Creek in 1961.

Little White Salmon

Fall Chinook Salmon of the 1956, 1957 and 1958 broods were marked and released at Little White Salmon National Fish Hatchery to determine survival after several periods of rearing. The fish were marked in May and June when they were 500 to 200 per lb. Releases were made in May, July, September, October and February (yearlings) with about 200,000 marked fish in each group. Two marks were released in October to compare returns from Dorsal and Anal marks.

Marked fish from this experiment were recovered in 1958, 1959 and 1960. Recoveries at Little White Salmon are shown as follows:

Mark	LP	RP	D-LP	D-RP	An-Rp	An-Lp
Date Released	May	July	Sept.	Oct.	Oct.	Feb.
Days Reared	70	125	190	230	230	350
Fish Per Pound	272-387	134-155	87-96	76-88	72-88	59-70
Adults Recov'd '58	1	2	0	0	0	0
Adults Recov'd '59	12	32	4	7	20	53
Adults Recov'd '60	18	46	6	4	31	71
Total Recovered	31	80	10	11	51	124

The greatest returns were obtained from fish reared for almost one year. I believe that one of the reasons for the low returns of the Dorsal-Left Pectoral and Dorsal-Right Pectoral marks is that removal of the dorsal fin had an adverse effect on survival after release. Almost five times as many adults marked Anal-Right Pectoral were recovered than thos marked Dorsal-Right Pectoral, although both groups were released at the same time.

All of the marked fish from Little White Salmon were hauled in tank trucks and released directly into the Columbia River about one-half mile below the mouth of the Little White Salmon River. The September plants were made from hatchery water at about 47°F into river water at 64 to 67° F. This 20° difference in temperatures may have caused some mortality. For the July releases the temperature difference was about 15°F and these plants produced the second highest returns.

Final analysis of the results of both of these marking experiments can not be completed until the last of the 1958 brood return as 5 year-olds in 1963.

ABSTRACT

THE SEAWARD MIGRATION AND RETURN OF HATCHERY-REARED STEELHEAD TROUT IN A COASTAL RIVER OF OREGON

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The characteristics of the downstream migration of wild and hatchery-reared steelhead smolts and the subsequent survival and return of adult hatchery fish were investigated on the Alsea River from 1956 through 1960.

The objectives were to provide knowledge concerning the survival of steelhead trout from Oregon's hatcheries and supply criteria for efficient stocking of hatchery fish. Survival of adult hatchery steelhead was computed from the estimated catch in the sport fishery and escapement counts from traps operated on tributaries.

The downstream migration phenomenon in wild fish is apparently dependent upon size. Those steelhead with a rapid growth rate are able to attain the physiological state enabling movement into saltwater in one year, while other fish of the same brood year require a longer time. Two-year-old fish are the predominant group moving out of the stream during the spring migration of wild fish and might be considered normal in respect to growth.

The mean length of hatchery smolts recovered in the lower river trap for any given release was larger than the mean length of fish released. The mean length of fish recovered in the upper river by electrofishing was considerably less than the mean length of the same group released, indicating the residualism of subsmolt size steelhead.

The size of fish released had a greater influence upon survival to adults than time of liberation within the time limits tested (April and May). A size somewhere below 10 fish per pound appears to have a critical effect upon survival. Release times may become more important in their influence on survival as the dates become more extreme in relation to the Spring migration.

The contribution of hatchery fish to the sport fishery was assessed and the cost of producing the returning adult hatchery fish was computed.

NUTRITION AND DISEASE IN TROUT

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At the request of several state agencies, the College of Fisheries of the University of Washington has agreed to develop and coordinate a research program to evaluate the role of nutritional, environmental and physiological factors as they relate to the development of certain diseases of trout. The College of Fisheries has received support from the National Cancer Institute. In order to organize the research effort, meetings have been held with representatives of the various Western States, including Washington, California, Idaho, Utah, Colorado and Oregon, and with trout feed manufacturers, to determine some of the diet constituents that may bear upon the nutrition and the health of fish. The extensive material collected in the course of these meetings has suggested that some of the trout diets may not be entirely adequate to support growth to maturity without development of diseases, particularly hepatoma. A program has been developed to test the relationships of nutritional imbalance, strain susceptibilities, hatchery management and water conditions to the prevalence of hepatoma in trout.

Without the active investigations of the staffs of State and Federal public health departments and of governmental and commercial fish cultural groups in the Western States, the proposed research program would not be possible. In our own state of Washington, Dr. Earnest Ager, Washington State Department of Health, and his staff have surveyed the commercial trout hatcheries and have examined a great number of tissue samples. The results from the histological examination of these samples by Dr. Kenneth L. Partlow have provided an insight into the prevalence of the disease. We are grateful to these research workers and all the others who have provided the information so important in giving insight into the etiology of hepatoma.

The major consideration in the proposed program designed to determine causes and to suggest remedies for the occurrence of hepatoma in trout are:

1. That a nutritional imbalance may result from either the dietary combinations or may result from the relative availability or unavailability of dietary constituents to the digestive, absorptive and metabolic processes normally present in trout.

2. That strains of trout may be more susceptible or predisposed to the development of the disease.

3. That diet, diet and strain of fish, and the physical environment, including water quality and hatchery practices, may interact to induce the development of hepatoma.

This investigation was supported by a P.H.S. contract SA-43PH 3804 from the National Cancer Institute, Public Health Service.

Other research efforts related to the hepatoma problem have been developed and will provide valuable data. Dr. Howard Tanner and his associates at Colorado State University will attempt to determine whether a viral agent is involved in the disease. They plan to feed a diet that has been suspected of producing hepatoma in susceptible strains of fish, and then they will challenge these trout with preparations of infected liver tissues.

Earlier this afternoon, Dr. Halver of the Fish and Wildlife Service ably described the program under way at Hagerman, Idaho and at Cook, Washington to determine response of trout to the action of known carcinogens and to test regression diets. Many of the concerned states have feeding trials already under way, which will provide valuable data when it has been assembled and evaluated.

It is clear from the data now available that when some dry ingredients are added to wet diets or that when all dry pelletted diets are fed to trout, there is an increased incidence of hepatoma. The production of the disease in trout fed Cortland #6A diet reduces the number of ingredients that must be tested in the planned research program. The Cortland diet consists of a mixture of frozen fresh meat, blended with an equivalent amount of a dry meal containing equal parts fish meal, cottonseed meal, wheat middlings and distiller's solubles, and 3 percent added salt. It is not clear from the information available whether a toxic agent is present in the dry meals or whether a dietary imbalance results when these diets are fed to trout.

Dr. John Halver, U. S. Fish and Wildlife Service, and Dr. Russell Sinnhuber, Oregon State College, in earlier discussion have remarked upon the aspects of the fat and fat oxidative processes as they concern the nutrition of animals. They have indicated that these oxidative processes and the formation of peroxides can interact and destroy vitamins and other susceptible food constituents, and that these oxidative processes may have a direct effect upon the body tissues and upon important biochemical processes in the body tissues. The work of Dr. Halver and his associates has been amply discussed previously and this aspect of the problem will not become a part of our program. However, we must take into consideration some of the ingredients commonly incorporated into trout foods and processes used in producing these ingredients.

Fish meal is a major ingredient in commercially produced, pelletted trout diets. The great variability of commercial fish meals results from the normal production methods, which from a practical standpoint can not be precisely controlled. The meal itself is subjected to heating during the drying process. Even in storage the individual sacks or stacks of meal will change in composition, due to internal heating. The damage to the meal will vary, depending upon the initial quality of the fish, the drying process, packaging methods and storage conditions. All of these variables must be taken into consideration in planning the program, although they are difficult or impossible to evaluate individually.

Flame dryers are commonly used in the commercial process, and during the drying process oxidative changes do take place, vitamines are destroyed and fats are oxidized. As Dr. Sinnhuber points out, the airlift drying process used to produce some types of fish meals frequently included in trout diets may result in the formation of a varnish around the protein particles. In itself this varnish has no nutritional value and may actually interfere with the full utilization of the protein substance. At present the cumulative effect of these processes, acting to change the biological value of protein for fish, can not be determined except by feeding trials.

The use of cottonseed meal for fish diets is equal in importance to that of fish meals. We must recognize that on occasion feeding cottonseed meals has met with difficulty. In the case of pigs, direct toxicity to liver metabolism has been reported for gossypol, which is found in a high proportion in some cottonseed meals. Gossypol is believed to interfere with the liver processes and result in deteriorative changes. A few years ago, in an eastern hatchery, an imported cottonseed meal was found to have a deleterious effect when fed to hatchery fish. This toxicity was not found when American produced meal was included in the diets. To select a cottonseed meal, fish meal or other materials for use in fish diets, it is necessary to define the sources and production methods used to prepare these meals and to devise tests to determine their suitability in trout diets. Such tests must be of a practical nature, in order to provide a basis for realistic specifications for commercially produced fish diets.

At present, a testing method is used in California to determine the suitability of cottonseed meals for chicken feeds. This method is based upon the fact that the egg is noticeably affected by the gossypol which may be present in cottonseed meals. Such a testing program has as its objective to determine biologically whether a meal has a low gossypol value and is suitable for inclusion in chicken diets. It is likely that a similar series of tests can be devised to provide a measure of the quality of various components included in a fish diet.

The situation with regard to the safety of ingredients such as wheat middlings, cottonseed meals and alfalfa is complicated by the presence of residuals from chemicals used to improve yield or to preserve the product during storage Such chemicals may be found at a later stage of processing in a material such as distiller's solubles. Hence, we have a number of biologically active contaminants which we must take into account but which are difficult to analyze, evaluate or trace to their original sources.

Apart from the raw materials in the diet and possible contaminants, we must consider the hatchery environment and the genetic strains of fish used in hatcheries. At this time the effects of genetics, disease, feeding practices or water quality can not be adequately evaluated. However, these factors must be taken into account during the development of research effort.

The field research program we have devised has as a primary goal the reduction of the number of factors involved in the etiology of trout hepatoma in order to provide a basis for critical testing of the more probably variables. The specific aims in our studies are:

1. To determine the most probable combinations of dietary ingredients and processing treatments of those food substances that may result in development of hepatoma in trout.

2. To determine the relationship of dietary imbalance and diet utilization which may be implicated in the etiology of trout hepatoma.

3. To determine the physiological ability of salmonid fishes of various ages to digest and utilize protein, carbohydrate and fat, and then to evaluate the availability of nutrients from various combinations of dietary ingredients.

4. To correlate growth, appearance, condition factor, food conversion, histopathology, hematology and enzyme activity of trout with experimental diet combinations which may be expected to induce hepatoma development.

5. To develop a rapid test to determine long-term nutritional or toxic effects of food ingredients or their synergistic actions that may induce or increase the susceptibility of a rapidly maturing warm water fish (Tilapia sp.) to spontaneous tumor development.

6. To determine the potential importance of factors including age, sexual maturity and other humoral changes, disease, temperature regimen, and physiological stress upon the development of hepatoma in fish.

To accomplish these aims, we have set up an experimental program for the various hatcheries who are cooperating in the field research program. The experimental procedure is designed to test not only diet combinations, but to evaluate the genetic factors and environmental conditions. The general procedure to be used in the field experimental program follows:

1. Shasta strain eggs, shipped from California, are to be hatched in the test hatchery.

2. Simultaneously, eggs obtained from the brood stocks locally available, are also to be hatched in the test hatchery.

3. Feed a control diet to both the Shasta fish and a group of local fish from the swim up stage until the experiment is terminated.

4. Divide the bulk of the hatch of local fish into two groups and feed each group the same experimental diet. These fish will be handled as a part of the normal hatchery production except for detailed records to be kept and samples of fish to be taken for detailed examination.

The test diets are designed to test either protein quality or total available energy. To achieve the required experimental combinations, the protein sources, carbohydrate sources and inert materials will be varied.

For these nutritional studies, each hatchery will supply a minimum of two small raceways or nursery ponds for the control experiments, while duplicate raceways will be supplied for the test diet fish. These ponds or raceways should be supplied with water that has not previously been used in the hatchery.

In addition to the effects of dietary imbalance, we are concerned with the potential effects of normal hatchery operations on the the development of the experimental fish and require detailed information about various factors normally associated with each of the test hatcheries. These data will include: Water chemistry and temperature, methods and frequency of disease treatments applied to eggs and fish, sanitary practices, feeding procedures and methods for weighing and measuring the fish and determining food allowances.

Another facet of the hepatoma problem is, of course, to determine the history of the stocks of fish used in the various hatcheries. This information will include original sources of brood stocks, period of inbreeding, fertility of females, methods of handling brood stock, hatchability and survival of fry. Although the genetic history of the fish does not appear to be a major factor at the present time, such information may be found to be more important at some later stage in the program.

Apart from the diet and environmental studies, it is apparent that more knowledge is needed concerning the enzyme capability of the trout as it develops; that is, what foods can a trout digest and at what rate can he digest these foods? The objectives of our laboratory studies are:

1. To determine at intervals from hatching to maturity the proteinase, carbohydrase and lipase activity of tissue preparations from the intestinal tract and pancreas.

2. To determine the rate of action of digestive tract preparations upon the various food constituents normally found in trout diets.

3. To compare the results obtained from enzyme studies for trout with enzyme studies for a warm water species, Tilapia, which grows rapidly in aquaria and will permit repeated generation studies.

4. By use of the Tilapia, we can study factors such as age, sexual maturity, humoral change, temperature conditions, metabolic products and physiological stress which may play a large part in the etiology of hepatoma. Since in aquaria these conditions can be controlled closely, the use of Tilapia, which grows and matures rapidly, is felt to be valuable.

In summary, it is believed that diet or dietary imbalance is a major factor in the development of hepatoma in trout. It is probable that other factors such as strains of fish and environmental conditions may be involved or that all of these may interact to produce the liver change. The required study program is now being developed and we wish to encourage all groups who are interested to express their views on the program and any of the related problems, so that they can be taken into account in determining the etiology of the disease. Our primary role is to insure that unnecessary duplication will not occur, that information will be made fully available to all interested parties and that information which will lead to corrective measures will be given at the earliest possible time.

The coordination group consists of Dr. Richard Van Cleve, Dean of the College of Fisheries, Dr. Max Katz, Dr. Melville Dollar and Mr. Raymond Simon, who are located at the College of Fisheries, University of Washington, Seattle 5, Washington. We look forward to frequent contacts during the coming months and we welcome any assistance you can provide in determining the causes of this trout disease.

Carcinogenesis in Trout

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Western Fish Nutrition Laboratory

U. S. Fish and Wildlife Service

Cook, Washington

Conclusive evidence has been accumulated that trout fed certain rations for periods up to one year will develop high incidence of primary hepatoma. Specific causative agents for the development of these liver tumors have not yet been determined. In order to classify clinically, histologically and physiologically primary hepatoma of this type and relate it to tumors found in other experimental animals, eleven classical mammalian carcinogenic materials have been selected for study and observation of physiological symptoms in rainbow trout. These materials have been incorporated over a 256 fold range in a highly purified basal ration which has been demonstrated satisfactory for rearing rainbow trout for at least three years without the appearance of any detectable nutritional deficiency syndrome. In addition, fat, protein, carbohydrate and ash fractions of various suspect commercial rations have been substituted for major nutritional components in other studies.

Surgical examination and classification of stages of development of primary hepatoma have been completed on 200 rainbow trout. Regression diets will be fed to portions of this affected population and periodic three month examinations of the state of the development of the hepatoma in each fish will be tabulated. The affects of certain hormones on the rate of tumor regression will also be measured.

ABSTRACT

THE ROLE OF HISTOPATHOLOGY IN FISH NUTRITION STUDIES WITH REMARKS ON TROUT HEPATOMAS

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The Role of Histopathology in Fish Nutrition is:

1. To determine what is the normal histology of Salmonid tissues and organs and to describe and illustrate this in monographic form. Initial efforts are directed at the hemopoietic system in order that results may soon be correlated with the findings on Salmonid hematology.

2. To describe the histopathology of quality control samples collected from various production and experimental feedings of Salmonids.

3. To trace the gross and microscopic development of trout hepatomas and other pathologies from their incipient stages through all subsequent stages of growth and degenerative breakdown or possible regression.

4. To describe equivalent findings, if any, in young trout subjected to hepatoma induction experiments and in mature trout from hepatoma regression experiments.

5. To describe the microscopic course of liver changes in affected rainbow trout as traced from normal livers through fatty degeneration, cirrhosis and several phases of hepatoma development. These changes are illustrated with a series of twelve photomicrographs in color.