

NORTHWEST FISH-CULTURAL CONFERENCE

1957

April 28, 1958

Enclosed is the Report of the Eighth Annual Conference held in the Interior Building, Portland, Oregon, on December 4-5, 1957. Summaries of each author are included as received with little or no editing. Three speakers did not submit summaries of their talks.

C. H. Ellis, Washington Department of Fisheries, 4015 20th Avenue West, Seattle, Washington, was designated the Chairman for the 1958 Conference.

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Harlan E. Johnson  
1957 Chairman

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## EVALUATION OF FEEDING TRIALS

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Some of the criteria which have been used in evaluating feeding trials are: weight gain, mortality, conversions (diet to fish, diet to fish protein, diet protein to fish protein, etc.) and biochemical and histological tests. In limited instances, such as qualitative nutrient requirements single criteria may be sufficient to define the success of an experiment. For instance, it is found that if an essential amino acid is absent from the diet, no growth will occur; the absence of an essential vitamin over a period of time will cause the appearance of specific deficiency symptoms and ultimately death. Biochemical and histological tests are useful not only as supporting data for other criteria, but may provide the only early means of detecting certain deficiencies. The use of the various types of conversions in the evaluation of feeding trials can lead to erroneous conclusions if proper controls are not exercised during the trials. The fallacy of feeding by "the table" in comparing diets having varied moisture content should be obvious for in truth, the amount of nutrient per weight of fish has not been equalized. This error then becomes compounded if the diets are evaluated on a basis of conversion of wet diet to fish.

In placing a value on a feeding trial one must first of all keep in mind what is desired. Thus, while conversion figures or cost of feed per pound of fish produced is the method of choice in evaluating production feeds for the commercial trout grower, its use by conservation agencies is valid only insofar as growth and weight can be correlated to survival in a natural environment. Marked changes in the ability to survive starvation have been shown with fish from varied nutritional backgrounds at the Western Fish Nutrition Laboratory.



## METHODS OF AMINO ACID ANALYSIS

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Protein is the major component of a salmon diet. This is true economically as well as physiologically. The protein source must contain specific levels of 10 indispensable amino acids. For example, it would appear, on the basis of our present knowledge, that lysine must constitute approximately 6.0% of the protein for optimal growth.

Methods of amino acid analysis were reviewed. Chromatograms of typical fish diets were presented which illustrated resolution of 4 and possibly 6 essential amino acids.

Enzymatic decarboxylation of amino acids as a method of analysis was discussed.

## AMINO ACID REQUIREMENTS OF CHINOOK SALMON

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Results of the direct dependence of protein requirements upon water temperatures were confirmed as 40% to 42% protein at 47° F. and 52% to 55% protein at 58° F. Feeding 40% protein with the amino acid balance similar to whole egg protein, threonine, methionine, lysine and triptophan requirements were measured at 47° F. and 58° F. In contrast to the rat, diets containing the ten essential amino acids failed to yield growth in chinook salmon. When supplemented with glutamic and aspartic acids, the fish responded with normal growth. Results of the amino acid quantitation studies indicate tentative requirements for threonine .3 percent, methionine .5 percent, lysine 2.3 percent and triptophan 0.2 percent. Requirements seem primarily dependent upon protein level fed and amino acid balance of the protein used. Current experiments testing the requirements at various protein levels and these water temperatures, are underway. In addition, supplements of glutamic acid and aspartic acid alone to the ten essential amino acids are being tested for growth responses. Arginine and histidien quantitations are included in the present feeding program.

## DIGESTIVE ENZYMES OF SALMON

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Research has been initiated to isolate and characterize the proteolytic enzymes associated with the pyloric caeca of the chinook salmon. The organs have been collected from actively-feeding fish at sea. The frozen caeca keep with no appreciable change in extractable proteolytic activity which was measured by using for substrates the synthetic esters reported to be specific for trypsin and chymotrypsin from higher animals. Because of inactivation by acids, the standard scheme for isolating tryptic enzymes could not be followed. Activity measurements on water extracts of caeca indicated that at least two enzymes were present in considerable amounts and that they probably differ from functionally similar enzymes in higher animals.

## PROBLEMS IN MANUFACTURE AND STORAGE OF MOIST PELLETS

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An economical and nutritionally adequate pelleted type fish food has long been desired by people engaged in hatchery work. Although a number of commercially pelleted fish foods are on the market a universally successful pellet has not been developed. Towards this end Oregon State College and the Fish Commission of Oregon, as one phase of their joint hatchery research program, have been working on a soft pellet particularly adaptable for salmonoid fishes. This year (1957) the feasibility of this soft pellet on a commercial scale has been explored.

To briefly review the program by which we have arrived at an economically feasible hatchery diet, we have been following this general plan:

- I. Dietary experiments which determine value of common and potential food components for use in hatchery diets.
- II. Dietary experiments to determine the value of supplemental materials being used or potentially usable in hatchery diets.
- III. Evaluation of data.
- IV. Formulation and development of production type diets.

In 1956 a promising production diet was expanded from laboratory scale to semi-pilot operation. This expansion necessitated making 2,000 pounds of food. The following pieces of equipment were used:

1. Laboratory Raymond hammer mill
2. 80 quart Hobart mixer with grinding attachments
3. A slow-speed Enterprise type meat grinder
4. Centrifugal fan

Eleven hundred pounds of vegetable and fish meals were put through the hammer mill using a screen that resulted in 90% passage through a 40 mesh sieve. Nine hundred pounds of wet components made up of turbot, tuna liver, and viscera were ground through the Hobart grinding attachment, using a plate with 1/8" openings. The meal and wet components were blended with the Hobart dough mixer. The resulting dough was pelletized with the slow speed Enterprise type meat grinder using a plate with 1/8" holes. The centrifugal fan, an 8 bladed,

1750 R.P.M., 1/4 H.P. affair beat and transformed the spaghetti-like extrusions into bite size worms which were caught in a coarse woven sack fastened to the outlet of the fan and transferred to trays to be frozen. This was the extent of the equipment and method used to make the 2000 pounds of food which we successfully fed as our 1956 Production Diet.

This year (1957) our plans were to feed 32,000 pounds of moist pellets. It would have been impractical to put through 17,600 pounds of meal through a laboratory hammer mill or pelletize 32,000 pounds of "dough" through a meat grinder with a 2" throat. For performing these tasks a custom feed mixer was contacted to grind and mix the meals and a fish cannery contacted to grind, mix, and pelletize the final combination.

Many problems arose during the transition from laboratory scale operation to commercial operation. These problems started from the initial custom compounding of the meal mix. Fish hatcheries interested in making their own pellets with their own meal mixes can avoid some of the problems we ran into by requiring that each separate batch of meal mix be coded and labeled separately. Of value also would be information as to what had been ground and mixed prior to your own custom mix, together with a report on when the mixing and grinding equipment had last been cleaned. If any reasonable doubt exists as to the condition of the mixing and grinding equipment it would probably be advisable to get down and examine the equipment personally. In general it would be advisable to have the meal mix ground as fine as possible. A fine meal will produce a better soft pellet. For our specifications we required that 75% go through a 40 mesh sieve.

Progressing to the wet components and the second phase of operation which took place in a fish cannery. Our custom pelletizers used an Autio grinder for handling and grinding the turbot, tuna, liver, and tuna viscera. Here again the finer the grind, the better the final pellet produced. For this an 1/8" plate was used. Since most of the ingredients were already available frozen for mink feed most of the wet components were ground in that state. A later attempt to grind completely thawed materials revealed that speed and effectiveness could be increased by grinding with a slight amount of frost left in the feed. For mixing the wet components with the meal mix, an Autio type mink feed mixer was used. This mixer, essentially a ribbon type mixer, was belt geared down to a 18 to 1 ratio and powered with a 1750 R.P.M., 2 H.P. motor. The capacity of the unit was supposed to be well over 100 pounds, but it was found that 100 pound loads were the most convenient. Here again the best mixing seemed to be achieved when a slight amount of frost was left in the wet components. This particular effect was noted by Wally Hublou and passed on to the custom pelletizers as a necessary step when using the Autio type mixer. For verification the cannery experimented with a completely thawed batch of wet components and found that good mixing was impossible, when thawed

materials were used. However, this is basically a mixer problem and other types of mixers would undoubtedly handle thawed as well as frosted materials. When thoroughly mixed it was found necessary to allow the dough to reach a moisture equilibrium. If an attempt was made to pelletize before the equilibrium was reached, the pellets stuck together in a doughy mass making it impossible to use or freeze properly. A minimum of not less than four hours holding seemed to produce usable pellets. Our custom pelletizers found the best system for them was to mix approximately 1,000 pounds of dough in the afternoon, let it stand overnight in a cold room, pelletize in the morning. Using this process two laborers were able to make approximately 1,000 to 1,500 pounds of pellets per day with the hurriedly assembled equipment they had on hand.

For the actual pelleting an Enterprise type meat grinder was used. A 3/8" thick plate with 1/8" holes formed the spaghetti. To achieve the slow speeds (fast speeds produced gummy extrusions with this type of grinder) necessary for pelletizing, a 2 H.P., 1300 R.P.M. motor geared down to a 14 to 1 ratio was attached to the grinder. The spaghetti-like extrusions dropped into an eight-bladed centrifugal fan which chopped them into the bite sized worms. These worms were placed on trays and frozen. The frozen pellets were then sacked in 30 pound sacks and stored until used. It was noted that the thickness of the plate and also the speed of the pelletizer or grinder greatly influenced the type of pellet formed. Thicker plates produced firm, cohesive pellets while thin plates tend to produce crumbles. Grinders operating at high speeds tend to produce sticky pellets. It was suggested that removal of the knife blade might help in producing better pellets, however, removal of the knife blade in our case made pelletizing impossible. The speed of the centrifugal fan was seemingly critical. The eight-bladed Westinghouse industrial fan rated at 1.3 H.P., 1750 R.P.M. was satisfactory while a 3450 R.P.M. fan produced much less desirable pellets.

## PROGRESS REPORT ON PRODUCTION DIET EXPERIMENTS

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### INTRODUCTION

This report covers a three year period, 1955 through 1957. During this time a total of seven production scale experiments were conducted; at three hatcheries using four species of fish.

The diet being tested in each of these experiments was an experimental moist pellet, which was composed of a meal mix and various fish products. The exact composition of the pellet varied somewhat from year to year. In all but one of the experiments the pellet diet was compared with the standard diet being used at that particular hatchery. The one exception was when the experimental pellet was compared with a dry commercial pellet.

In 1955 and 1956 the experimental pellet was prepared on a laboratory scale, in 1957 it was prepared commercially.

### 1955 EXPERIMENT

In 1955 we conducted our first production scale experiment at the Sandy Hatchery, using silver salmon in one of the regular hatchery ponds. A short summary of the results is given in Table 1. (A progress report of this experiment can be found in the report of the 1955 Northwest Fish Cultural Conference, and a complete summary of the experiment can be found in the 1956 report.)

### 1956 EXPERIMENTS

The results of the 1955 experiment were encouraging so we altered the diet somewhat, to make it easier to handle, and repeated the work in 1956; again at the Sandy Hatchery on silvers and also at the Oakridge Hatchery using spring chinook. The two experiments were conducted in a manner practically identical to the 1955 experiment. Tables 2 and 3 give a summary of the results of these two experiments.

### 1957 EXPERIMENTS

It was planned to conduct two experiments in 1957; one at the Klaskanine Hatchery using silver salmon, and the other at the Oakridge Hatchery using spring chinook. The experiments were originally started in March but due to an outbreak of "sunburn" in the pellet fed fish the



experiments were discontinued until the last of June. (For information of the "sunburn" condition refer to the report by Russ Sinnhuber, Oregon State College. Also, for a complete report on the commercial preparation of the experimental pellet refer to the report by Duncan Law, Oregon State College.) The second start of the experiments was on June 28, at the Klaskanine Hatchery, and July 1, at the Oakridge Hatchery. In both cases the fish which had originally been fed the experimental pellet, and had become "sunburned", were divided into two groups and one half of these fish were now fed the hatchery diet and other half received the experimental pellet. At Klaskanine there were two ponds of fish, at 35,000 per pond, on each diet. At Oakridge there was a pond of 55,000 fish on each diet. The experiments are still in progress at the time of this writing but a summary of the results as of November 1, for Klaskanine, and October 21, for Oakridge, are given in Tables 4 and 5.

On August 15 the Oakridge spring chinook receiving the experimental pellet were found to be getting anemic. Subsequent tests showed that the pellets being stored and fed at Oakridge were quite rancid. Fresh pellets were delivered in October and the fish seem to be improving at this time.

In September it was found that the pellets were not being used at the anticipated rate. Two more experiments were then initiated: (1) At the Klaskanine Hatchery the pellets were fed to a pond of 1957 brood steelhead trout. The comparison diet in this experiment was a dry commercial pellet manufactured by the Willis Small Company, Eugene, Oregon. This experiment was discontinued after six weeks of feeding because the commercial pellet proved unsatisfactory. The fish were reluctant to eat the dry pellets and by the fifth week the mortality began increasing quite rapidly. The food conversion for the experimental pellet was almost identical to that in Table 4 for the Klaskanine silvers. The experimental pellet diet is still being fed and the standard hatchery diet is now being used as a comparison. (2) At the Oakridge Hatchery the pellets were fed to a pond of 1956 brood blueback salmon. Another pond of blueback was fed the hatchery diet for comparison. This experiment was discontinued after eight weeks of feeding because the experimental pellets, which had been stored at Oakridge, were found to be quite rancid. As the blueback experiment was started because there was a surplus of food, and the food was now found to be unsatisfactory, the experiment was discontinued. There was no indication of anemia or other dietary troubles at the time the experiment was discontinued. The food cost to produce a pound of fish was 52.5 cents for the hatchery diet and 25.5 cents for the experimental pellet. The cost was somewhat high in both lots because of a high mortality due to bacterial gill disease.

#### SUMMARY

The food cost necessary to produce a pound of fish being fed the standard hatchery diets used in these seven experiments has been



found to vary from 44.4 to 54.0 cents. The food cost to produce a pound of fish being fed the experimental moist pellet has been found to vary from 21.1 and 25.5 cents. From the information gathered here it would appear that the use of the experimental pellets, in place of the standard hatchery diets being used at the hatcheries where the experiments were conducted, would result in a savings of close to one half the food cost necessary to produce a pound of fish.

The fish being reared on the experimental pellets have generally been found to be of very good health. However, some problems remain to be solved; mainly rancidity in the stored pellets and anemia in the fish. It is felt that in this case rancidity and anemia are closely related and that by using fresh foods and maintaining good storage procedures that these two problems can be pretty well eliminated.

Although the costs involved in rearing fish at our hatcheries are of vital concern to the people of Oregon, we must at the same time produce fish that are able to survive to adults. Most diet experiments on salmon have not been able to evaluate the returns to the fishery or to the hatchery. All of the fish in these production experiments have been marked by fin clipping, with the exception of the Oakridge blueback and the Klaskanine steelhead. The adults of the 1955-56 Sandy Hatchery silver salmon experiment are returning to spawn this fall. The returns are incomplete at this time but it appears that there will be a good recovery of marks from both groups of fish.

The marking of these fish has made possible another important evaluation; that of incidence of disease in the adults, compared to the diet history of the fish in the hatchery. The experimental pellet contains no salmon products while the hatchery diets contain salmon viscera, and in one case, salmon flesh. (For information on the incidence of disease in the 1954 brood Sandy Hatchery silver mark recoveries from the commercial fishery refer to the report by Jim Wood, Oregon Fish Commission.)

TABLE 1  
PRODUCTION DIET EXPERIMENT

SILVER SALMON - 1954 BROOD  
Species - Brood Year

SANDY HATCHERY  
Location

Experiment Started: May 23, 1955  
Experiment Terminated: February 14, 1956

DIET COMPONENTS	LOT A		LOT B	
	Experimental Pellet	Hatchery Diet	Experimental Pellet	Hatchery Diet
	Meal Mix	45%	Salmon viscera	(Composite Diet) 60%
	Turbot	40	Commercial pellets	20
	Tuna Liver	15	Beef liver	5
			Horse meat	5
			Beef lungs	4
			Beef spleen	4
			Sheep cheeks and tripe	2
POUNDS OF FOOD FED	1,841		5,029	
Moisture Content	41.2%		64.4% (Ave.)	
Pounds of Food, Dry Weight	1,083		1,792	
FOOD COST PER POUND	10.1 cents		8.1 cents	
TOTAL COST OF FOOD FED	\$185.94		\$407.35	
POUNDS OF FISH PRODUCED	801		754	
FOOD COST PER POUND OF FISH PRODUCED	23.2 cents		54.0 cents	
FOOD CONVERSION				
As Fed	2.30		6.67	
Dry Weight	1.35		2.38	
FISH MORTALITY	13.2%		3.9%	
FISH PER POUND AT TERMINATION	18		20	

TABLE 2  
PRODUCTION DIET EXPERIMENT

SILVER SALMON - 1955 BROOD  
Species - Brood Year

SANDY HATCHERY  
Location

Experiment Started: June 18, 1956  
Experiment Terminated: March 1, 1957

DIET COMPONENTS	LOT A		LOT B	
	Experimental Pellet		Hatchery Diet	
	Meal mix	55%	(Composite Diet)	47%
	Tuna Viscera	25	Salmon viscera	24
	Turbot	20	Beef lungs	18
			Commercial pellets	7
			Beef spleen	3
			Turbot	
			Salmon flesh and	
			Tuna liver	1
POUNDS OF FOOD FED	1,452		3,295	
Moisture content	39.6%		66.1% (Ave.)	
Pounds of food, dry weight	878		1,117	
FOOD COST PER POUND	9.3 cents*		8.2 cents**	
TOTAL COST OF FOOD FED	134.89		271.18	
POUNDS OF FISH PRODUCED	640		609	
FOOD COST PER POUND OF FISH PRODUCED	21.1 cents		44.5 cents	
FOOD CONVERSION				
As fed	2.27		5.41	
Dry weight	1.37		1.84	
FISH MORTALITY	5.3%		3.2%	
FISH PER POUND AT TERMINATION	20		24	

\* Includes .02 per pound for handling

\*\* Includes .01 per pound for shipping

TABLE 3  
PRODUCTION DIET EXPERIMENT

SIRING CHINOOK - 1955 BROOD  
Species - Brood Year

OAKRIDGE HATCHERY  
Location

Experiment Started: June 18, 1956  
Experiment Terminated: December 14, 1956

DIET COMPONENTS	LOT A		LOT B	
	Experimental Pellet		Hatchery Diet	
	Meal Mix	55%	(Composite Diet)	
	Tuna viscera	25	Salmon viscera	57%
	Turbot	20	Liver	11
			Commercial meal	9
			Miscellaneous meats	8
			Kidney and spleen	8
			Tuna liver	5
			Heart	1
			Water	1
POUNDS OF FOOD FED	1,204		2,371	
Moisture content	37.6%		66.6% (ave.)	
Pounds of food, dry weight	752		793	
FCOD COST PER POUND	9.3 cents*		9.7 cents**	
TOTAL COST OF FOOD FED	\$11.87		\$330.02	
POUNDS OF FISH PRODUCED	516		476	
FCOD COST PER POUND OF FISH PRODUCED	21.7 cents		48.3 cents	
FCOD CONVERSION				
As fed	2.33		5.98	
Dry weight	1.46		1.66	
FISH MORTALITY	0.9%		3.0%	
FISH PER POUND AT TERMINATION	29		31	

\* Includes .02 per pound for handling

\*\* Includes .01 per pound for shipping

TABLE 4  
PRODUCTION DIET EXPERIMENT

SILVER SALMON - 1956 BROOD  
Species - Brood Year

KLATSKANINE HATCHERY  
Location

Experiment Started: June 28, 1957  
Experiment Terminated: As of November 1, 1957

DIET COMPONENTS	LOT A		LOT B	
	Meal Mix	Experimental Pellet	Hatchery Diet	(Composite Diet)
		55.0%		
	Turbot	20.0	Salmon viscera	37%
	Tuna viscera	12.5	Tuna liver	20
	Tuna liver	12.5	Beef lungs	15
			Sheep lungs and tripe	12
			Commercial meal	10
			Beef liver	6
POUNDS OF FOOD FED	4,243		7,686	
Moisture Content	34.7%		67.2% (ave.)	
Pounds of food, dry weight	2,771		2,556	
FOOD COST PER POUND	10.5 cents*		8.3 cents**	
TOTAL COST OF FOOD FED	\$445.52		\$637.94	
POUNDS OF FISH PRODUCED	1,839		1,437	
FOOD COST PER POUND OF FISH PRODUCED	24.2 cents		44.4 cents	
FOOD CONVERSION				
As fed	2.31		5.35	
Dry weight	1.51		1.78	
FISH MORTALITY	0.4%		0.2%	
FISH PER POUND AT TERMINATION	22		25	

\* Includes .02 per pound for handling

\*\* Includes .01 per pound for shipping

TABLE 5  
PRODUCTION DIET EXPERIMENT

SPRING CHINOOK - 1956 BROOD  
Species - Brood Year

OAKRIDGE HATCHERY  
Location

Experiment Started: July 1, 1957  
Experiment Terminated: As of October 21, 1957

DIET COMPONENTS	LOT A		LOT B	
	Experimental Pellet		Hatchery Diet	
	Meal mix	55.0%	(Composite Diet)	
	Turbot	20.0	Salmon viscera	49%
	Tuna viscera	12.5	Liver	18
	Tuna liver	12.5	Commercial meal	8
			Kidney and spleen	7
			Lungs	6
			Miscellaneous meats	10
			Salmon eggs	1
			Salt	1
POUNDS OF FOOD FED	2,392		5,749	
Moisture content	34.7%		67.8% (ave.)	
Pounds of food, dry weight	1,562		1,851	
FOOD COST PER POUND	10.5 cents*		9.8 cents**	
TOTAL COST OF FOOD FED	\$251.15		\$563.35	
POUNDS OF FISH PRODUCED	1,175		1,083	
FOOD COST PER POUND OF FISH PRODUCED	21.4 cents		52.0 cents	
FOOD CONVERSION				
As fed	2.04		5.31	
Dry weight	1.33		1.71	
FISH MORTALITY	6.7%		6.9%	
FISH PER POUND AT TERMINATION	32		34	

\* Includes .02 per pound for handling

\*\* Includes .01 per pound for shipping

# 318 DAYS OF CONTINUOUS PELLET FEEDING TO RAINBOW TROUT

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On April 16, 1956, four hatchery tanks were set up and 48,552 rainbow trout weighing 11.95 pounds were placed in each tank. The fish were from eggs received from the Winthrop Hatchery and were assigned Lot No. 6-WT.

Tanks 8 and 37 were used as a control and received our regular hatchery diets and at start was 100 percent beef liver, 1 percent of salt was added after the seventh day of feeding. During the first week in May 2 percent of cod liver oil was added and at the end of the third week in May the cod liver oil was discontinued and 10 percent of distillers solubles (corn dried) was added, now making the diet 1 percent salt, 89 percent beef liver and 10 percent solubles. On June 16 the diet was changed to dough feed consisting of 2 percent salt, 74 percent beef liver, 6 percent wheat midds, 6 percent Canadian herring meal, 6 percent corn dried distillers solubles and 6 percent cottonseed meal. This diet was continued until July 15 at which time it was changed to blown Cortland consisting of 50 percent beef liver, 2 percent salt, 12 percent each of the meals, cottonseed, fish meal, distillers solubles and wheat midds. On August 1, the control diet was changed to the regular production diet of which consisted of 50 percent meals and 50 percent meats, the meats consisting of beef and/or pork livers and beef and/or pork melts, the percentages of livers and melts being determined by availability of the feeds.

Tanks 7 and 38 were fed Rangen's feeds as recommended by the company. The first feedings were 95 percent beef liver mixed with 5 percent Rangen's fry feed and gradually increased to 80 percent beef liver and 20 percent fry feed and continued to the end of April. The fish were fed alternately on the meat meal mix and straight dry feed. After May 1 the fish received only dry feed and continued until the conclusion of the experiment on February 28, 1957.

At the end of August the fish were moved outside into ponds No. 21 and 22, pond 22 being the control diet and pond 21 being fed Rangen's feed. Necessarily the fish had to be graded and thinned down and was accomplished by setting up 40,355 fish weighing 806 pounds in pond 22 and 40,347 fish weighing 838 pounds in pond 21.

From the charts and graphs maintained the following results were observed:

For the first several months less mortality and faster growth on the control group, after which time the mortality on the experimental group dropped to a point below the control group and remained so throughout

the remainder of the experiment. The overall gain on the control group remained higher but the cost per pound of fish produced was also higher.

At the conclusion of the experiment both groups were very healthy and active. More fatty deposits on the fish of the control group and they had a hemoglobin reading of 10.5 as compared to 11.5 on the experimental group.



## CUTTHROAT FEEDING EXPERIMENT LOT 7-CT

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Ennis, Montana

July 4, 1957, four hatchery tanks were set up and 20,828 fish weighing 76.0 pounds were placed in each tank. The first were from eggs received from the Creston hatchery and were assigned Lot No. 7-CT.

Tank No. 2 was used as a control and .25 grams of calcium pantothenate per 200 pounds of fish was added to diets fed throughout experimental period. We used the tank receiving calcium pantothenate as a control because previous feeding experiments proved this to be an improved diet with plenty of research behind it to base any conclusions we might make on the other diets in experiment. This tank was fed a liver Cortland diet from July 4th to July 17th inclusive consisting of 50% beef liver, 12% fish meal, 12% cottonseed meal, 12% wheat middlings, 12% distillers solubles, 2% salt. These fish did not take readily to the Cortland diet so we put on dough diet July 18th and remained on dough through August 18th, consisting of 75% beef liver, 23% meals - equal parts of fish meal, cottonseed meal, wheat middlings, distillers solubles and 2% salt. August 19th through November 20th they were fed liver Cortland.

Tank No. 1 received same diets as above but received .3 mg. riboflavin per pound of fish plus .25 gram calcium pantothenate to 200 pounds of fish.

Tank No. 44 received same diets as above but 8% flavonne, a commercial vitamin concentrate, was used.

Tank No. 43 received Rangen's dry feeds as follows: No. 2 fry feed July 4th through 17th. No. 5 fry feed or crumbles July 18th through August 14th. No. 3 fry feed fed August 15th through September 11th. September 12th through September 25th fed half No. 5 fry feed and half pellets. September 26th through October 9th fed pellets. October 10th through October 23rd fed 21 pounds No. 5 fry feed and 58 pounds pellets. October 24th through November 25th fed pellets only.

### CONCLUSION

From a study of the 14 day charts the following conclusions are made: Cutthroat trout diets supplemented with calcium pantothenate are an improvement over regular diets. Cutthroat trout diets supplemented with both calcium pantothenate and riboflavin are still better. Cutthroat trout diets supplemented with flavonne are not a success with smaller fish but seem to give very favorable results with larger fish. Rangen's dry feed appears to be the best overall diet for cutthroat trout.

# CLARK'S NEW AGE TROUT FEED FOR SOCKEYE SALMON FINGERLING DIET

Alfred C. Gastineau  
U. S. Fish and Wildlife Service  
Leavenworth, Washington

We set up June 3, 1957, an experiment to determine if Clark's New Age Trout Feed could be used as a diet in rearing sockeye salmon fingerling.

Consequently, approximately 240,000 averaging 576 per pound were set aside for this experiment, 108,000 to be used for control which received a diet consisting of 11% beef liver, 17% pork liver, 42% arrow-tooth halibut (turbot), 13% beef spleen, 5% beef lung, 5% distillers solubles, 5% seal meal and 2% salt. The remaining 132,000 to receive Clark's New Age Feed.

The fish of the control lot were in Foster-Lucas ponds at the outset but those to receive the Clark feed were kept in 11 troughs to begin with in order to have a better observation of their reaction to the diet and so a better distribution of the food could also be made.

The fish at start were fed every hour, the attendant giving them their hourly amount in two passes, in other words, he fed all the troughs half the diet then repeating gave the other half. The fish took the feed readily at the start but did not consume it entirely. However, after two weeks they were consuming all that was given. After 24 days in the troughs they were transferred to a Foster-Lucas pond and the feeding frequency reduced to 4 feeds daily, which was followed during the remainder of the experiment. The fingerling grew at a greater rate than the control and the conversion was much greater but the mortality was also much greater; also more difficulty was had in controlling Bacterial Gill disease. It is not known whether diet deficiency or metabolic products was the cause. I think a deficiency was the cause although it was observed that whenever the fish were disturbed the water became cloudy from excrement stirred up in the water from the bottom of the pond and could have been part of the difficulty.

When the experiment was closed and the fish liberated it was observed that they could not withstand handling and quite a number were lost in the process of removing them from the pond, hauling and releasing in Lake Wenatchee.

It was also observed during the latter part of the season that a portion of the mortality consisted of fish that had not been eating for some time. This was not confined to any size group.

It would seem from this experiment that Clark's new age food is nearing the requirement for sockeye salmon fingerling but still it was not adequate.

The data on the experiment is as follows:

	<u>Control</u>	<u>Clark's</u>
Number fish at start	108,423	132,573
Number per pound at start	544	608
Number fish at end	107,397	107,857
Number per pound at end	79.57	66
Per cent mortality	.95%	18.8%
Per cent gain average weight	585%	821%
Number pounds at start	199.47	217.93
Number pounds at end	1,349.7	1,643.5
Per cent gain of <u>lot</u>	577	654
Pounds feed fed	5,247	3,588.07
Cost per pound diet fed	\$.075	\$.0165
Conversion	4.56	2.51
Food cost per per pound fish gained	\$0.3431	\$0.4049
Number days on experiment	130	130

# DRY FEED FOR TROUT

Clifford Millenbach  
Washington Department of Game  
Seattle, Washington

The use of Clark's pellets for feeding rainbow and steelhead looks promising. Three hatcheries using the dry food have had good results. Growth has been excellent and the food cost of producing a pound of fish was reduced. At two hatcheries mortalities on very young fish were higher than normal. Part of the mortality may have been due to inadequate techniques rather than diet deficiencies.

Records of the two hatcheries are as follows:

	<u>Vancouver Hatchery</u>	<u>Chelan Hatchery</u>	<u>Chelan Hatchery</u>
	<u>*Wet Diet</u>	<u>Dry Diet</u>	<u>Dry Diet</u>
Number per pound at start	1,438	1,438	511
Number per pound at end	64	52.8	11½
Total number on hand at start	115,040	115,040	100,000
Total number planted or trans.	28,116	7,225	80,352
Total number on hand at end	92,762	115,322	16,200
Mortality in numbers	10,945	24,774	3,448
Percentage of mortality	9.51%	21.5%	3.4%
Weight of fish on hand at end	1,421	2,352	1,280
Weight of fish planted or trans.	-0-	409	7,560
Weight of fish at start	80	80	184
Total gain in weight of fish	1,376.5	1,882	8,656
Percentage of gain	1,720.6%	2,352.5%	4,704.3%
Pounds of feed fed	5,420.75	2,817.5	11,356
Amount fed in percent of body weight	--	--	76.3%
Cost per pound diet fed	6.45¢	16.75¢	\$1.43
Pounds of food fed per pound of fish gained	3.94	1.50	1.31
Food cost per pound of fish gained	25.41¢	22.17¢	\$.187
Food cost per ton of fish produced	\$508.28	\$443.43	\$374.00

\*Wet Diet - Liver, Lungs, Viscera, Meal

# SILVER SALMON FEEDING TEST WITH CLARK'S NEW AGE DRY TROUT FOOD

Edward B. Horn  
U. S. Fish and Wildlife Service  
Quilcene, Washington

At the start of this program we took 20 pounds of silver salmon fingerling from a pond and put them in a hatchery feeding tank and started them on the dry food. We also took another 20 pounds from the same pond and put them in another hatchery feeding tank, these were fed with the regular station meat production diet.

The first month those fish on the meat diet kept ahead in growth over those on the dry diet. The second month however, after the fish on the dry diet really took hold, they surpassed those on the meat diet and have each month thereafter.

These samples of 20 pounds at the outset represented 3,081 fish at 154 per pound.

The rearing tanks in the hatchery used for this test have a capacity of 80 cubic feet of water and we operate them with a flow of 16 gallons per minute.

This test had at the time of this report been going for 144 days. It started on July 11, 1957, and was reported on as of December 1, 1957. We had a loss of 41 during that time, 9 of which were due to mechanical causes.

The fish ran 154 per pound at start and now are 33 per pound.

Water temperatures fluctuated from 39.5 to 53.5 during the test.

There was a gain in weight of 72 pounds over the period for a conversion of 2.75. The feed cost was .44 per pound of gain. The dry feed was the crumbles and are put on the water very gently so we get a short period in which the feed floats.

We are feeding half of our silver salmon production on the dry feed 9 ponds or about 7,000 pounds of fish.

The chart furnished by the dry feed manufacturers is used for a feeding level. We have changed the quantity to those on the test in the hatchery to try and arrive at the nearest best level and after several changes the chart furnished by the feed company is about correct.

# A POSSIBLE CAUSE OF "SUNBURN" IN FISH

W. T. Yasutake

U. S. Fish and Wildlife Service  
Cook, Washington

For the past several years there have been frequent reports throughout the country, of a lesion described as "sunburn" or "back-peel". The fact that no pathogen could be found in fish with this lesion and that occasionally, shade has prevented this condition, sunlight has been correlated with this atypical condition.

The similarities between the lesions of fish and man (with pellagra) were noted with interest. Our experiment was carried out to determine if niacin deficiency will cause susceptibility to irritation by sunlight in fish as in man.

First noticeable lesion in the niacin-deficient diet fish was observed 18 days after the start of the experiment. By the 30th day, 22 fish died, all having severe lesions. At this point, the group was divided into two equal groups, one remaining on the deficient diet and the other receiving the complete diet. Fourteen days after the fish were divided, 15 of 83 had died in the former and 5 of the 82 in the latter.

Although the present study was hampered by cloudy weather which may have allowed the fish on the niacin-deficient diet to overcome the acute manifestation, we feel that the difference in mortality in the two subgroups is indicative of a possible response to dietary therapy.

## EVALUATION OF PROTEIN LEVELS IN A HATCHERY DIET

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Salmon-Cultural Laboratory  
Entiat, Washington

The 1957 diet trials, conducted at the Salmon-Cultural Laboratory, were a cooperative investigation with the Western Fish Nutrition Laboratory to determine the optimum protein level of a proven hatchery diet for chinook fingerlings.

Two groups of diets were set up with varying protein levels in each group. One group of diets was made isocaloric with the control by substituting dextrin and corn oil for a portion of the protein in the control diet mixture. The second group of diets was set up with protein levels corresponding to the first group, but not isocaloric, using Red Dog flour to reduce the protein level. This latter group of diets was designed to test a possible production diet using the much cheaper Red Dog flour. Both groups of diets were conducted simultaneously and ended after 12 weeks of feeding.

Samples of the food composing each diet were taken periodically for chemical analysis. The results of these analyses are shown in the table. Also included in the table are the results of the proximate analyses of fish sampled from each diet at the end of the experiment.

Diets 2 and 3 of the dextrin-corn oil group and diet 6 of the Red Dog flour group were statistically comparable with the control using the average weight of the individual fish as a basis for comparison.

Statistical analysis of the protein content of the fish showed that only diet 2 was comparable with the control. Both visual inspection and chemical analysis showed that the fish in diets 2, 3, and 6 had greater fat deposition than the control. This investigation clearly points out the value of using more than one criteria in comparing the efficiency of diets.

On the basis of true fish flesh produced, the control, containing 57 percent protein, is approaching the optimum protein level for this diet. These results indicate that higher protein levels will have to be tested in the future.

# EVALUATION OF PROTEIN LEVELS OF A HATCHERY DIET

Liet Number	1.	2.	3.	4.	5.	6.	7.	8.	9.
Experimental Variable	Control	Dextrin-Corn Oil Substitute			Red Dog Flour Substitute				
FOOD SAMPLE ASSAY $\frac{1}{2}$ (Percent Dry weight)									
Protein	56.7	52.8	43.9	44.3	40.1	53.5	48.3	44.9	42.1
Lipid	26.2	26.2	25.6	25.3	24.3	24.8	22.4	19.1	17.1
Ash	11.4	11.4	10.9	10.6	10.1	11.2	11.0	10.6	10.6
Carbohydrate	6.3	10.6	15.4	20.6	26.3	11.2	17.9	24.6	29.8
PROXIMATE ANALYSIS OF FISH (Percent dry weight)									
Protein	75.1	73.2	69.2	72.6	69.6	71.4	75.9	75.4	74.3
Lipid	16.6	19.2	22.8	16.9	21.2	13.7	15.8	15.8	15.6
Ash	11.7	11.2	10.4	11.9	11.3	11.3	12.2	11.9	12.4
SUMMARY									
Protein level of food sample (percent dry weight)	57	53	49	44	40	54	43	45	42
Average weight of fish at end $\frac{2}{1}$ (grams wet weight)	11.85	11.93	11.76	10.97*	10.39*	11.32	9.64*	9.98*	9.08*
Average amount of protein per fish $\frac{3}{1}$ (grams per dry weight)	2.67	2.62	2.45*	2.39*	2.28*	2.43*	2.19*	2.26*	2.02*
Control Diet:									
1/Average of four food samples for first eight weeks only.									
2/Least difference for significance at the 5% confidence level									
= 0.83 gram									
3/Least difference for significance at the 5% confidence level									
= 0.19 gram									
* Differ significantly from the control									
Control Diet:									
12.5% Hog Liver									
12.5% Beef Lung									
25.0% A.T. Halibut									
40.0% Salmon Viscera									
5.0% Seal Meal									
5.0% Distiller's Solubles									



THE EFFECT OF CERTAIN ANTIBIOTICS ON THE BACTERIUM CYTOPHAGA PSYCHROPHILA

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Seattle, Washington

In vitro studies have demonstrated certain of the antibiotics (Penicillin, Aureomycin and Terramycin) to exhibit strong inhibitory action on the mycobacterium Cytophaga psychrophila the causative agent of "Low Temperature Disease" in young silver salmon. It was found that Penicillin, Terramycin and Aureomycin inhibited growth of the bacterium, in vitro, at dilutions of  $1 \times 10^{-9}$  of the antibiotics in pure crystalline form.

Tests were conducted on silver salmon fry infected with "Low Temperature Disease" under hatchery conditions. The work was qualitative only and was incomplete. The antibiotics were introduced into shallow troughs containing infected fry by the siphon bottle drip method for one hour periods daily. Siphons were adjusted to produce a dilution of  $1 \times 10^{-7}$  of the drug in the trough throughout the period of treatment.

After three days treatment considerable improvement was noted in the treated fish for all three of the antibiotics used. Following treatment in the troughs the fry were moved outside into standard rearing ponds where the drugs were continued. No definite conclusions can be drawn from the effects of treating in ponds since the infection was complicated by infestation with Costia.

It is our intention to continue and expand these investigations in the following rearing season. Tests will include the use of Aureomycin and Terramycin in the diet after the fry reach the feeding stage.

## KIDNEY DISEASE IN RAINBOW BROOD STOCK

C. C. Green  
Oregon Game Commission  
Oakridge, Oregon

In June of 1953, in our yearling Fall and Spring Rainbow, we had quite an outbreak of kidney disease. As soon as it was determined to be kidney disease, we started the 11-day Sulfa treatment. On the 7th day after treatment was started, our mortality started dropping and by completion of the 11-day treatment, mortality dropped from the high of 60 per day to 3 out of 100,000 yearlings. In September 5,000 future brood fish were selected from the Spring Rainbow lot, which probably wasn't good practice or reasoning on my part, to be held in a cement pond 20 X 100. At 2 years no recurrence of the kidney disease and the fish were or seemed to be in the best of health and condition, as no mortality was present other than 1 or 2 now and then. At 3 years, the fish were spawned for the first time and still no sign of the kidney disease. The 1940 females were spawned and 2 3/4 million eggs were taken; a fair fertility was experienced. Some 6 weeks after spawning the kidney boil or disease made its second appearance. We tried a low level feeding of Sulmet for a period of 2 months which didn't seem to be of much help, so we gave them, for the second time, the 11-day treatment of the Sulfa. But this time not much was accomplished as our mortality continued, nothing heavy, but steady. This treatment was in July. Fish came on through until spawning which was January 9th. At this point let me say that fish were in such a way that after the spawning, fish were disposed of. This second spawning, 1,668 females were spawned; 3,560,600 green eggs were taken and 2,665,600 were eyed up eggs. I have at present some 200 of the original 5,000 left.

Summing up my experience with kidney disease, I would say our efforts were not all in vain, as we had two egg productions, plus the 200 I will spawn for the third time. From the dollar and cents operation it was costly, but the experience was valuable. My belief is that an immunity could be built in future generations and that this disease would cease to be a problem.

## A PROGRESS REPORT ON KIDNEY DISEASE AND TUBERCULOSIS RESEARCH

James W. Wood  
Oregon Fish Commission  
Oakridge, Oregon

### Kidney Disease

Kidney disease was found in adult spring chinook salmon returning in 1957 to the hatchery egg collecting stations on the Middle Fork Willamette, McKenzie, and North Santiam Rivers.

As in 1955 and 1956, controlled experiments in 1957 demonstrated that kidney disease could be transmitted by feeding to young chinook salmon. Transmission through feeding of infected food was found to be greatly influenced by water temperatures. In experiments at Oakridge the minimum incubation period was 33 days at water temperatures averaging 54.9°F. (range 50-60°F.) while no mortalities with kidney disease occurred at water temperatures averaging 46.2°F. (range 37-53°F.) until after an 81 day incubation period.

Transmission of kidney disease to fingerling chinook salmon through contact with yearling blueback salmon in a controlled experiment was found to be successful. A 50 percent mortality occurred among the chinook salmon following a 128 day exposure period.

### Tuberculosis

All of 234 hatchery marked adult silver salmon returning to Big Creek and Nehalem Hatcheries in 1956 were found through the examination of prepared smears to be tubercular. For comparison, 144 adult and 40 jack silvers of probable natural origin were examined from Tenmile Lake. None of the smears prepared from these fish were found to exhibit the presence of the characteristic acid-fast bacilli. Tuberculosis was found in 9 of 10 adult silvers examined from the South Coos River Hatchery thus clearly establishing the presence of the disease in the same general area of the state as Tenmile Lake.

In 1957, two groups of 1954 brood year marked silver salmon were examined both from the commercial fishery and upon their return to the Sandy River Hatchery. Both groups were reared for approximately 60 days on the standard hatchery diet which contained salmon viscera but no salmon carcasses. As a nutritional experiment after this time until their liberation 9 months later, one group (marked Ad-RV) was continued on the hatchery diet while the other group (marked Ad-LV) was fed a soft pellet diet containing no salmon products. In addition a third group of fish was fed the hatchery diet for a 7 month feeding period at the Sandy Hatchery and liberated into the Yamhill River (tributary of the Willamette River.). Adult returns of this mark to the commercial fishery only were

examined. The results of the examination of these three groups of marked silvers are shown in Tables 1 and 2.

From these data it would appear that tuberculosis is a hatchery caused disease. A controlled experiment is being conducted, utilizing the spring water supply at the Clackamas Laboratory, for the purpose of demonstrating possible transmission through diseased food and through the eggs of diseased females.

Weekly samples of salmon and steelhead livers were collected, when available, from the Columbia River gill net fishery landings at Astoria during open seasons from May to November, 1957. A composite of those samples examined through August 24, appears in Table 3.

Table 3. Tuberculosis in Columbia River Gill Net Landings at Astoria by Species, May to August, 1957.

<u>Species</u>	<u>No. of fish examined</u>	<u>No. of tubercular fish</u>	<u>Fish</u>
Steelhead	1,080	16	1.5
Silver	163	7	4.3
Chinook	1,818	102	5.6
Blueback	584	4	0.7

Table 1. Tuberculosis in 1954 Brood Year Marked Silver Salmon Reared at Sandy Hatchery and Recovered in the Columbia River Gill Net Fishery - 1957.

Mark	No. of Fish Released	Length of Rearing Period	Diet	No. of Fishery Returnees by Abundance of Acid-fast Bacilli in Prepared Liver Smears					Per Cent Tubercular of Total
				Neg.	1	2	3	4	
Ad-LV	14,837	11 months	Hatchery - 2 mos. Pellet - 9 "	25					0.0
Ad-RV	15,884	11 "	Hatchery - 11 "	5	2	7	2		68.8
LP (liberated into Yamhill R.)	45,406	7 "	Hatchery - 7 "	24	11	2			35.1

Table 2. Tuberculosis in 1954 Brood Year Marked Silver Salmon Reared at Sandy Hatchery and Recovered During Spawning Operations at Sandy Hatchery - 1957.

Mark	No. of Fish Released	Length of Rearing Period	Diet	No. of Hatchery Returnees by Abundance of Acid-fast Bacilli in Prepared Liver Smears					Per Cent Tubercular of Total
				Neg.	1	2	3	4	
Ad-LV	14,837	11 months	Hatchery - 2 mos. Pellet - 9 "	24					0.0
Ad-RV	15,884	11 "	Hatchery - 11 "	4	6	9	5	1	84.0

## ACID-FAST BACILLUS DISEASE OF FISH, A REVIEW

Thomas J. Parisot  
U. S. Fish and Wildlife Service  
Seattle, Washington

The first recorded observation of so-called tuberculosis in fish was made in France in 1897 by Battailon, Dubard, and Terre when this group reported it in a carp. Since this time 159 species of both salt and fresh water fish have been reported to be infected with the acid-fast bacillus causing the disease. The disease was first reported in salmonid fish in 1953 by Ordal and Earp.

The description of the disease is as follows: Externally the fish may or may not show signs. If anything, they are stunted, less active than the rest, and in general show signs of abnormal health.

Internally the liver is the best organ for obtaining the bacilli. There are often discrete lesions of varying size present in the tissue, but mostly it is in the form of the miliary type of disease. The kidney is often affected, but usually not with lesions. In this organ the disease is manifest as a softening of the posterior portion. The spleen is usually not enlarged and the organism is found throughout the entire tissue. Pockets of the bacilli can be found in or near the genitalia which are usually immature and poorly developed. Sexual immaturity is thought to be a consequence of this acid-fast bacillus disease.

## IDENTIFICATION OF ACID-FAST ORGANISMS ISOLATED FROM FISH

A. John Ross  
U. S. Fish and Wildlife Service  
Seattle, Washington

Five cultures of acid-fast organisms have been isolated from salmonid fish by this laboratory from four geographical areas. Growth has been obtained from spawning Fall Chinook salmon at the Spring Creek fish cultural station in the years 1954 and 1955. Positive cultures were also obtained from fingerling chinook salmon at the Carson fish cultural station, three-year-old steelhead from Oak Ridge, Oregon, and two-year-old steelhead originating from Chambers Creek, Washington, and held at the University of Washington.

These cultures were compared with six known species of Mycobacterium as well as unidentified strains sent us by other investigators. Existing relationships were indicated by comparative studies including morphological appearances and biochemical activities such as the ability of the organisms to utilize various carbohydrate compounds as the sole source of carbon.

The fifteen cultures were shown to fall into three well defined groups. Number 1 included M. fortuitum, one strain obtained from a neon tetra and four of the strains isolated by this laboratory. Number 2 consisted of saprophytic species while Number 3 included one strain isolated by us as well as cultures isolated from tropical, marine and fresh water fish.

M. fortuitum has been isolated from a variety of sources including human abscesses, halibut roe, soil, bovine mastitis and lizards. Since four of the cultures isolated by this laboratory are considered to be identical to M. fortuitum, the potential pathogenicity of these organisms for warm-blooded animals should receive consideration.

## CHEMOTHERAPY OF ACID-FAST ORGANISMS OF FISH

Thomas J. Parisot  
U. S. Fish and Wildlife Service  
Seattle, Washington

In the past few years, tuberculosis of salmonid fish has become a problem of major proportions. A study of treatment of the disease has been started by this laboratory with the goal of eradicating or limiting the disease among populations of fish in hatcheries. The following is a report of progress in the study.

Six isolates of acid-fast organisms have been made by members of this laboratory. These isolates are the test organisms reported upon here. Ten drugs have been obtained from commercial manufacturers that are either on the market as agents for human tuberculosis or in experimental development of the human disease.

In vitro testing indicates that none of the drugs are effective against fish tuberculosis in the ranges that affect the human disease. Two of the drugs that are in experimental stage of development have proven to be the most effective, one of the agents shows signs of stimulating the growth of the organisms and the remaining seven compounds range from very poor to fair.

Further screening, toxicity, and in vivo testing must be done before any conclusion can be drawn as to the feasibility of chemotherapy of fish tuberculosis.



## NOCARDIOSIS IN YOUNG SALMON

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Seattle, Washington

The genus Nocardia, is in the family, Actinomycetaceae, and is nearly indistinguishable from the genus Mycobacterium. Organisms of both genera are, characteristically, aerobic and acid-fast. The Nocardias, however, usually exhibit branching and they often lose their acid-fastness in laboratory cultures.

An organism with properties more like a Nocardia than a Mycobacterium was isolated from two populations (spring and fall Chinooks) of young fish at the Carson, Washington federal hatchery in the summer of 1955. The morbidity rate was unusually high (estimated 90%) but the mortality rate was quite low. In fact, the mortality rate in the fall Chinooks was nil and deaths in the spring Chinook population were attributed to other causes, probably secondary in nature.

The fish in question had never been fed any salmon products and the high incidence of infection is thought to be the result of a water-borne organism. All of the actinomycetaceae are wide-spread in nature and they are always present in large numbers where there is decaying organic matter.

It has been postulated that the organisms parasitize mucous secreting cells and mucous surfaces in general. In the fish, of course, this includes the entire body surface. The snout seemed to be a favored site of infection as well as the gills, mouth, and points of fin attachment.

## MALACHITE GREEN EQUIPMENT

Thomas B. McKee  
Oregon Fish Commission  
Clackamas, Oregon

The newly developed plexiglass malachite jug was shown to everybody in attendance. It has a capacity of 1/2 gallon and is a constant syphon jug as first developed by Roger Burrows, and will deliver 30 cc per minute of stock solution. At the 30 cc, it will operate one hour. This jug was designed by Hublou and McKee of the Fish Commission and Mr. Bruce Bettcher of PAM Co. in Portland. It was designed to hold just enough malachite for one hour treatment and would prevent any possibility of overtreatment. The tubing of the jug is acrylic, which is almost non-breakable. The jug has a 3/4" filling opening fitted with rubber washer and metal threaded plug. The jug is so designed as to fit over the end of the normal hatchery trough when in use and can be stored over a 2" x 4" board by the leg arrangement at the jug bottom. This jug was designed to eliminate breakage of glass jugs and tubing, which reaches immense proportions under normal hatchery operations. Only two Oregon Fish Commission hatcheries are equipped with pressure hatchery water supplies which lend themselves to the injection method of malachite treatment such as is used at Willard. This necessitates large numbers of malachite green jugs.

The price quoted by PAM was approximately \$15.00 per jug in single orders with substantial saving where many are purchased.

AN EXPERIMENT USING PMA AND LIGNASAN TREATMENTS AS  
A PROPHYLAXIS AGAINST BACTERIAL GILL DISEASE

Wallace F. Hublou  
Oregon Fish Commission  
Sandy, Oregon

INTRODUCTION

For the past several years the Oregon Fish Commission's salmon hatchery on the Metolius River has suffered a high mortality in the blueback and, to a lesser degree, the spring chinook salmon being reared at this station. For the last seven years of operations the total loss to liberation for the blueback has averaged 36 percent with extremes of 22 to 76 percent. For the same period the average chinook losses have been 27 percent with extremes of 13 to 42 percent.

It has been observed that the high mortality has a definite pattern, which is about the same for both species, with regard to time of year and age of fish. A review of the mortality records for the past seven years revealed that the highest mortality generally occurred during April and May when the fish had been reared between 70 and 95 days, but began to occur after about 48 days.

On July 31, 1956, during a hatchery tour by members of the hatchery biology section, Jim Wood noted the presence of the *Myxobacteria* associated with bacterial gill disease on the chinook fingerlings. No bacteria were found on the blueback examined, but the gills were badly clubbed, a condition typical of the bacterial gill infection. At the time of this examination the annual epidemic had already reached its peak and subsided.

As a result of these observations an experiment was designed for the spring of 1957 which would attempt to answer two questions:

- (1) Is bacterial gill disease the cause of the high mortality?
- (2) Can the mortality be reduced by chemical treatment?

Past experience has shown that treating during the height of an epidemic gives little or no beneficial results as by this time much of the damage has already been done. With this in mind it was planned to subject the fish to chemical treatments early in the spring as a prophylaxis against the suspected disease.

Two chemicals, pyridyl mercuric acetate (PMA) and Lignasan, were selected for use in the experiment. Both chemicals, especially PMA, have been used with good success in controlling bacterial gill disease on chinook and blueback salmon. If Lignasan would control the disease as well as PMA, it would be more desirable to use it because the cost is only \$0.82 per pound (in 125-pound drums) whereas PMA sells for \$15.04 per pound (in pound lots). Both chemicals are used at the same level for fish treatment.

## PROCEDURE

The Metolius Hatchery has five cement raceways for rearing. These ponds measure 20 x 80 feet with an average operating water depth of 19 inches. There were three ponds of blueback and two ponds of chinook. The blueback and chinook fry were ponded and fed for the first time on February 6, 1957. The stock on hand at the beginning of the experiment and the treatment data are given in Table 1.

Table 1. Basic Treatment Data on Prophylaxis Experiment for Bacterial Gill Disease on Spring Chinook and Blueback at Metolius Hatchery.

<u>Species</u>	<u>Pond No.</u>	<u>Treatment Data</u>	<u>Fish Per Pond</u>	<u>Total Stock on Hand</u>
Blueback	1	Control: No treatment	76,410	235,586
	3	Lignasan: 1 hour at 1:500,000	79,588	
	4	PMA: 1 hour at 1:500,000	79,588	
Spring Chinook	2	Control: No treatment	77,868	156,899
	5	Lignasan: 1 hour at 1:500,000	79,031	

The PMA and Lignasan were both administered by the prolonged treatment method. The pond water level was reduced to an average depth of 1 foot and the intake was shut off. The chemicals were first dissolved in warm water, then further diluted with cold water, and finally this solution sprinkled over the surface of the pond with a sprinkling can. A pump was used to recirculate and aerate the water within the pond during the treatment.

The usual mortality pattern evidenced in the past indicated that the mortality rate could be expected to start increasing sometime after 48 days of rearing. The treatments were started on March 13 after 34 days of rearing. The fish were treated weekly from March 13 to May 4 (86 days of rearing), with one exception, the chinook Lignasan group was not treated on April 17.

The experiment was discontinued on May 4 because of the very high mortality experienced by the blueback control group. From May 4 until June 15 all lots were treated periodically with PMA but the time and frequency of treatment was not the same for all lots.

## DISCUSSION AND OBSERVATIONS

It was planned to treat the fish before they contracted the suspected disease, but unfortunately the myxobacteria associated with gill disease were found on the gills of both species at the time of the first treatment on March 13.

It was tentatively planned to treat bi-weekly during March and then weekly during April and May during the usual high mortality period. However, since the mysobacteria were found on the fish on March 13 the schedule was changed and the fish were treated weekly during March also.

The chinook Lignasan group was not treated on April 17 because the weekly treatments seemed to be affecting their appetite. The fish were generally smaller, and the mortality rate was higher than that of the control group. Their appetites improved greatly after April 7 so they were again treated on April 24. No effect on appetite was observed after this treatment.

## RESULTS

The predicted epidemic took place at the time it was expected and was more severe than usual. Table 2 gives the percent mortality for each of the five ponds during the course of the experiment, February 6 to May 4, 1957.

Table 2. Percent Mortality From Bacterial Gill Disease in Prophylaxis Experiment with Blueback and Spring Chinook at the Metolius Hatchery, February 6 to May 4, 1957.

<u>Pond No.</u>	<u>Species</u>	<u>Treatment</u>	<u>Percent Mortality</u>
1	Blueback	Control	28.3
3	Blueback	Lignasan	11.9
4	Blueback	PMA	4.8
2	Spring Chinook	Control	1.9
5	Spring Chinook	Lignasan	2.2

Table 1 shows that the PMA treatments substantially reduced the blueback mortality although the frequency of treatment at the height of infection was apparently not enough to prevent an epidemic from occurring. It can also be seen that the Lignasan treatments also reduced the blueback mortality, but were not so successful as the PMA treatments.

The spring chinook mortality had not yet reached epidemic proportions at the time the experiment was terminated on May 4 (periodic treatments of PMA were given from May 4 until June 15) so the effectiveness of controlling the disease with Lignasan was not determined for this species. The control lot was treated periodically with PMA from May 4 to 24. It appeared that the severity of the disease was reduced considerably as a result of the treatments.

As a note of interest it was observed that Lignasan was a good algaecide whereas PMA seemed to encourage algae growth.

## CONCLUSIONS

The high mortality suffered by the blueback and spring chinook salmon being reared at the Metolius Hatchery in the spring of 1957 was caused by bacterial gill disease.

Weekly PMA treatments from March 13 to May 4 were successful in substantially reducing the blueback mortality. Weekly Lignasan treatments also reduced the blueback mortality, but were not so effective as PMA.

The spring chinook mortality was just beginning to increase when the experiment was terminated on May 4 so the effectiveness of Lignasan on the disease was not determined for this species.

## ICHTHYOSPORIDIUM INFECTIONS IN FISH

Robert R. Rucker  
U. S. Fish and Wildlife Service  
Seattle, Washington

My interest in appearing before you today is to seek your help in an effort to extend the known geographic range of Ichthyosporidium infections in fish. In 1953 a paper by Rucker and Gustafson titled "An Epizootic Among Rainbow Trout" appeared in the Prog. Fish-Cult. 15(4):179. The agent was reported from three localities in western Washington, a brief literature survey was given, and a photomicrograph of infected material was presented. In 1956 Gustafson and Rucker published "Studies on an Ichthyosporidium Infection in Fish: Transmission and Host Specificity" in the Fish and Wildlife Service Spec. Sci. Report Fish. No. 166. Since this time, the agent has been found in the Snake River area in Idaho. I believe if the men here from California, Oregon, Wyoming, and Montana conscientiously look for the organism, it will be found. Therefore, when examining fish, especially with white necrotic areas in the viscera, would you please place some of the material on a slide in water under low power on the microscope and look for the large spheres, Ichthyosporidium. Perhaps on occasion we are looking for bacteria and do not see the large spores. I would appreciate hearing of an extension of the geographic range of this organism and would be glad to confirm your diagnosis if you will send me some of the infected material preserved in formalin.

# LIGNASAN FOR THE CONTROL OF BACTERIAL GILL DISEASE

by  
Roger E. Burrows, Salmon-Cultural Laboratory, Entiat  
Washington

The first report of Rucker, Earp, and Burrows recommended Lignasan at 1:500,000 for the control of bacterial gill disease. Both Earp and Rucker have since encountered difficulties in treatments at that concentration. At Entiat Lignasan proved to be an effective bactericide at this concentration providing that the treatment concentration was rapidly reduced by a partial draining of the pond 15 minutes prior to the end of the hour treatment period and flushing continued for 15 minutes afterward. Such a procedure is, of course, hazardous and requires close attention.

Tests of the algicidal properties of Lignasan demonstrated wide differences in tolerance between the 1:500,000 and 1:1,000,000 concentrations. Chinook fingerling showed discomfort at the end of 1 hour and died in 5 hours at the 1:500,000 concentration but tolerated a 1:1,000,000 concentration for 20 hours with no deleterious effects. The difference in tolerance levels suggested a change in the treatment technique in which the Lignasan at a 1:1,000,000 concentration would be allowed to remain in the pond after treatment but would be diluted by the normal pond inflow after the hour exposure period.

Tests conducted in 1957 both experimentally and on a production basis have demonstrated that this treatment method is effective for the control of bacterial gill disease. Treatments at biweekly intervals have proved adequate as a prophylactic and but a single treatment is necessary if the disease is allowed to reach epidemic proportions. No mortality attributable to Lignasan has been demonstrated in chinook salmon fingerling.



## ENVIRONMENTAL CONDITIONS IN REARING PONDS

By

Roger E. Burrows, Salmon-Cultural Laboratory, Entiat, Wash.

During the past season we have been investigating the environmental conditions in salmon-rearing ponds to determine the effect of these conditions on the fish. These were preliminary investigations to determine the elements concerned in the problem. Despite the exploratory nature of the investigations some very interesting and pertinent observations were made.

To measure the environmental conditions in the ponds oxygen, ammonia, and carbon dioxide were selected as the indicators. In the course of the investigations carbon dioxide was discarded as of no consequence as an indicator during normal pond conditions.

The amount of available oxygen can be a limiting factor in the carrying capacity of ponds. The tolerance level is not the critical measure of the oxygen content when the determination of an optimum environment is desired. The tolerance level itself, which may be defined as the oxygen required to maintain basal metabolism, varies with the temperature and fish size. The tolerance level implies no activity other than that which is necessary to keep the fish alive. The oxygen level required to maintain normal activity is much higher than the tolerance level. The normal activity level varies also with water temperature and fish size. Another factor, that of acclimation to an oxygen level, affects both the tolerance and the activity levels of oxygen.

Indications that the oxygen content in a pond has dropped below the activity level are a reduction in the feeding activity of the fish and an increase in the respiratory movement. Chinook salmon fingerling three to four inches in length at water temperatures approximating 60°F. have a minimum oxygen activity level above 4 ppm. when they have been acclimated to a gradual reduction in oxygen for a period of weeks. At an oxygen level of 4 ppm. the fish are definitely off feed. This condition is accompanied by a slight increase in the oxygen content of the water due to the enforced reduction in the activity rate of the fish. It is obvious that a reduction in the oxygen content below the normal activity requirement of the fish creates an unfavorable environment, which, while not necessarily lethal, is not conducive to optimum growth.

Exploration of the oxygen levels in various types of rearing ponds have disclosed some very interesting facts. In raceway ponds a very abrupt oxygen gradient can develop. The degree of depletion depends of course on the amount and oxygen content of the water introduced, the water temperature, and the size and number of fish in the pond. By heavy stocking it is possible to create a condition whereby inflowing water at 11 ppm. of oxygen may be depleted to 3 or 4 ppm. at the outflow. Under such conditions the pond divides itself roughly into thirds. In the upper third

the oxygen content is fairly stable at from 9 to 10 ppm. In the middle third the oxygen content varies from 6 to 7 ppm. In the lower third the oxygen has dropped to 3 or 4 ppm. The demarcation lines between the several oxygen levels are quite distinct and are correlated with the hydraulic pattern of the pond.

Trout and, by inference, salmon are capable of adjusting to different oxygen levels but this adjustment is not rapid and takes place over a period of days. With three distinct zones within an oxygen gradient the fish would prefer the area of highest oxygen content with the most vigorous fish in the upper zone and the weaker fish in the lower zone. The lower third of a raceway is the critical area in such a pond when it is loaded to capacity. Here the weaker fish collect in what may be lethal or near lethal oxygen levels and certainly below the normal activity level of oxygen consumption. If the oxygen level in the lower third of the pond is below the normal activity level but above the lethal level the fish in this area will have a reduced food intake but the effect would be obscured by the actively feeding fish in the favorable environment in the upper two thirds of the pond. The tremendous difference in environmental conditions which can develop in a raceway pond do not appear to be conducive to optimum results and the loading of such ponds to the point where the oxygen content drops below 5 ppm. should be avoided.

The recirculating type of pond does not develop the marked differences in oxygen content within the pond that occurs in the raceway type. The greatest difference we have found is 2 ppm. between the water at the periphery and the outlet. This does not mean that such ponds cannot be stocked beyond the capacity of the inflowing water to meet the oxygen requirement of the fish. In fact we have tested circulars in which the water at the periphery was at 5 ppm. and at the outflow was 3.5 ppm. Under such conditions the fish concentrated at the periphery of the pond indicating that the area adjacent to the center was below the level of oxygen required for normal activity.

The lack of a sharp gradient in recirculating pond types is explained by the ability of oxygen to rapidly arrive at equilibrium and the mixing of water within the pond itself. Oxygen samples taken within three feet of a pond intake, which was delivering water at 11 ppm., contained only 5 ppm. or the same oxygen content as the remainder of the water along the periphery of the pond.

The slight oxygen gradient within the recirculating type of pond results in an even distribution of the fish throughout the pond under normal operating conditions. A uniform environment does not penalize the smaller, less vigorous fish.

The amount of available oxygen appears to be one of the primary factors which limit the carrying capacity of a pond. The inflowing water usually does not exceed the saturation level of oxygen. As water temperatures increase the amount of oxygen required for saturation decreases but the amount of oxygen required to maintain the fish increases.

The carrying capacity of a pond then decreases as the water temperature increases unless some method for increasing the oxygen content in the pond is employed.

Aeration offers one solution to this problem. Two methods of aeration are available to increase the oxygen content of the pond water. The first is to introduce the inflow as a series of jets above the pond surface. The turbulence created is quite effective for the aeration of oxygen depleted water. Turbulence has several disadvantages in that it distorts the hydraulic flow patterns within a pond and breaks up any feed which may be carried under the jets. The second method, that of aspiration, introduces both water and air through submerged jets and eliminates the disadvantages of the exposed jets. Aspirators operate on differentials in pressure and are simple to install in most pond intakes.

Neither turbulence nor aspiration are effective in waters approaching saturation hence neither of these methods offer possibilities for increasing the oxygen content in raceway ponds unless the inflowing water itself is 80 percent or below the saturation point for oxygen. Both methods are applicable to recirculating types of ponds and the more depleted the oxygen content the more effective the aeration. Aeration by means of an aspirator will increase the oxygen content in a circular pool by 10 percent when the initial oxygen is 70 percent of saturation. By aeration it is possible to increase the available oxygen without increasing the water inflow and thereby increase the carrying capacity of the pond providing that oxygen alone is the limiting factor in the carrying capacity.

The waste products of metabolism can become factors in the restricted environment of rearing ponds. Not too much is known of the effect of these waste products, other than ammonia, on the fish. Ammonia is relatively simple to measure and has been accepted as a measure of the nitrogenous products of excretion. Just how good a criterion ammonia is remains to be determined. Whether the ammonia present in the water creates a toxic condition of itself, whether high concentrations of ammonia in the pond inhibit the elimination of ammonia through the gills causing autointoxication, or whether high levels of ammonia inhibit oxygen absorption resulting in anoxemia has not been clearly demonstrated. The problem of the effect of metabolic wastes on the environment in rearing ponds certainly warrants further investigation.

Our preliminary investigations this season have produced some rather interesting data. Without attempting to more than broadly interpret the results, here are the facts:

1. In raceway ponds the ammonia concentrates at the lower end first and then develops a gradient upstream. In the recirculating type of ponds the ammonia concentrates first in the eddies if present and then, as the level increases, spreads to an even distribution throughout the pond duplicating the oxygen pattern in reverse.

2. Cleaning or flushing a pond reduces the normal ammonia concentration and the maximum level is not attained until at least six hours after the normal operating water level is reached. The ammonia content in a pond becomes stable after 24 hours and unless conditions are altered will remain constant for an indefinite period.
3. Smaller fish at comparable water temperatures excrete more ammonia per unit of body weight than do larger fish.
4. In certain pond types an ammonia build-up is correlated with the appearance of bacterial gill disease.
5. Any factor which affects the metabolic rate of the fish is reflected in changes in the ammonia content of the pond.
6. Ammonia contents up to 0.6 ppm. have been observed without any deleterious effects on the fish being noted other than the necessity of using prophylactic treatments to control bacterial gill disease.

The results of these preliminary experiments leave some doubt as to the validity of ammonia as an adequate measure of the toxicity of metabolic wastes. Next season we propose to measure the concentrations of urea, uric acid, creatine, creatinine, and amine compounds, if our chemistry is adequate, to correlate the concentrations of these compounds with ammonia.

These investigations have altered our concepts concerning the hydraulic requirements in ponds particularly with regard to short-circuiting and mixing. Flow patterns which demonstrate short-circuiting without creating extensive eddies are believed to be desirable in that they eliminate radical changes in environment within a pond. The mixing of the inflowing water with the circulating pond water replenishes the oxygen supply and prevents the concentration of metabolic waste products.

Once again the preliminary nature of these investigations must be stressed. The problem assumes more and more significance as the investigation progresses. It is certainly intriguing and should eventually supply answers to some of the problems encountered in pond rearing.

## DEVELOPMENT OF EFFECTIVE POND ALGICIDES

by  
Richard G. Bigej, Salmon-Cultural Laboratory  
Entiat, Washington

The Salmon-Cultural Laboratory has been investigating the problem of controlling filamentous green algae for the past nine years. During this time, fifteen potential algicides have been tested on two forms of algae. One is a branched form of the family Cladophoraceae and the other is an unbranched form of the family Ulotrichaceae.

The method of testing has consisted of four main steps: (1) determining the toxicity level of the chemical for chinook fingerlings; (2) determining the algicidal properties of the chemical using concentrations tolerated by fish; (3) determining the effect of repeated treatments of the chemical on fish if it is evident that the algae is being controlled; and (4) testing the chemical on a production pond using the information gathered from preceeding tests.

Only two of the fifteen chemicals tested have proven to be satisfactory algicides. These are Lignasan, which is composed of 6.25% ethyl mercury phosphate and 93.75% inert material, and Omazene, composed of 50% copper dihydrazinium sulfate and 50% wettable powder. Lignasan was most satisfactory at a concentration of 1:1,000,000 for 1-hour treatment periods administered at bi-weekly intervals. Omazene has not been given the final production pond test, but has shown that it will control algae at a concentration of 1:1,000,000 for 1-hour treatment periods administered at weekly intervals. The testing of Omazene will be completed in 1958.

## THE USE OF PARASITES IN RACIAL STUDIES

J. R. Uzmann  
U. S. Fish and Wildlife Service  
Seattle, Washington

The application of parasitological methods to the problem of distinction between races of salmon assumes the existence of consistent and measurable differences, either qualitative, quantitative, or both, in the parasite faunas of the respective stocks.

By analogy with known parasite distributions in other vertebrate species, it has been assumed that certain parasites of salmon might prove to be geographically restricted by ecological factors which are non-limiting on the distribution of potential salmon hosts. Under such conditions, parasites assume the status of "marks" and may provide evidence of the origin and migratory history of host individuals or populations.

Two aspects of the racial problem are currently under investigation. The principal problem concerns the continental origin and degree of intermingling of chum salmon stocks in the central North Pacific Ocean and Bering Sea. Preliminary results indicate that at least three species of parasites may prove useful in the identification of these stocks. A second study concerns the tributary river origin of Columbia River downstream migrant blueback salmon. Here, three parasites are found to be mutually exclusive to three of four study areas while out-migrants from the fourth area are characterized by the combination appearance of two other species.

Results to date must be considered preliminary and appraisal of methods must wait upon the results of several sampling seasons. To date, however, it appears that the method is promising and should find broad application in management studies.



## 1957 DIAMOND LAKE EGG TAKE

John D. Bliss  
Oregon Game Commission  
Hood River, Oregon

Diamond Lake is a shallow lake of three thousand acres lying in south end of Douglas County, Oregon at an altitude of five thousand one hundred and ninety feet. In September of 1954 the lake and all tributaries were treated with rotenone to remove all trash fish. The kill was successful and the lake was restocked in 1955 with spring spawning Canadian Rainbow. In 1955 the plant consisted of 146,000 yearling trout and 384,000 fry. In 1957 the three year old trout average length was 19" for females and 17" for males.

The ice went out of Diamond Lake May 6th or 7th, 1957. On May 12, employees of Oregon State Game Commission observed large groups of fish in shallow water near the shore. Water temperature at this time was 48°F. All the fish taken in the commission trap prior to May 17 were green. On May 17, six ripe females were trapped and spawned. Water temperature at this time was 52°F. By May 23, 1,000,000 eggs had been taken and it was apparent that crew was not able to keep up with the large run of fish. Water temperature at this time was 56°F. All eggs taken up to June 3 were kept at Diamond Lake hatchery and these eggs were of fair quality. There were approximately 5,000,000 eggs in this group. Water temperature at this time had climbed to a maximum of 68°F. Eggs spawned June 4, 1,357,000, June 5, 1,491,000; June 6, 1,366,000. The eggs looked good but were of very poor quality. Eggs taken June 4th, 5th and 6th were shipped green to other Game Commission Hatcheries. The eggs taken from June 7th to end of egg take were held at Diamond Lake. These eggs were of very poor quality with mortality as high as 60%. 4,983,000 of the 8,131,000 eggs held at Diamond Lake Hatchery eyed. All eggs held at Diamond Lake Hatchery were treated twice daily with malachite green for fungus control using flush treatment. Fungus did not cause any appreciable loss.

It would seem the poor quality of eggs was caused by timing of the egg take, hence quality of eggs could possibly be improved by spawning all ripe fish as soon as they are trapped.

THE EFFECT OF CONTROLLED LIGHT ON THE SEXUAL  
MATURATION OF ADULT BLUEBACK SALMON

by

Bobby D. Combs, Salmon-Cultural Laboratory, Entiat, Washington

Experiments have been conducted for the past three years to determine the effect of controlled light on the sexual maturation of adult blueback salmon. The facilities employed in these experiments include a light-tight holding pond which is illuminated by timer-controlled incandescent lamps. Fish held in this pond may be subjected to any period of light and darkness desired and the results compared with a like group held in an open pond and exposed to natural daylight.

The results of the three years' experiments may be summarized as follows:

1. Adult blueback salmon exposed to almost continuous artificial light for more than a month in the 1955 experiment spawned a month later than a comparable group held in an open pond. Although the eggs from the delayed group were smaller than those from the controls, mortalities were comparable.
2. In the 1956 experiment spawning was advanced 19 days in the lot of fish subjected to 9 1/4 hours of artificial light when compared to the control lot exposed to natural daylight. Female mortality was lower in the reduced light group but egg and fingerling mortalities were significantly higher than those of the control lot.
3. The experimental group of fish were exposed to only one hour of artificial light in the 1957 experiment. Spawning was advanced 14 days when compared to the control lot held in the open pond. A higher percentage of females died in the experimental group and green egg mortality was significantly higher than those of the control lot.
4. Light, not temperature appears to be the prime factor affecting acceleration or retardation of sexual maturation. The control fish spawned at essentially the same time each year in the three experiments although the temperature was 2.5° lower in 1955 than it was in 1956 and 1957. The experimental group spawned at a 5° lower temperature in 1955 and at a 2° higher temperature in 1956 and 1957 when compared to the control group.



THE STAGE OF DEVELOPMENT AT WHICH CHINOOK SALMON  
EGGS CAN WITHSTAND COLD WATER TEMPERATURES

by

Bobby D. Combs, Salmon-cultural Laboratory  
Entiat, Washington

Experiments at the Salmon-cultural Laboratory have demonstrated that chinook salmon eggs incubated at constant temperatures below 42.5°F. suffer higher than normal losses. Virtually 100 percent mortality results when eggs are incubated at a constant 35°F. However, experiments have shown also that if the initial incubating temperature is above 42° for a month, chinook eggs can tolerate long periods of very low temperatures with impunity. These results indicated that after certain embryological developments had occurred, low temperatures were no longer deleterious.

The 1956-57 experiment was designed to determine at what stage of development chinook salmon eggs could withstand low water temperatures. The experiment was carried out by subjecting groups of eggs to 42.5° water and transferring them, one lot at a time at 3-day intervals, into 35° water for the duration of the incubating period. One lot was held at a constant 35° and another at a constant 42.5° for the entire incubation period. Each time a group of eggs was transferred to the cold water, a 5-egg sample was removed and preserved. The embryos were dissected, stained, and examined microscopically to determine the stage of development.

The results of the experiment indicated that eggs which had developed beyond the 128 cell stage could withstand 35° water temperature with impunity. Chinook salmon eggs incubated for 6 days or longer at water temperatures of 42.5° or higher may be expected to tolerate cold water temperatures without excessive mortalities. On the other hand, chinook eggs spawned naturally in cold headwater streams late in the fall when water temperatures are 40° or lower probably suffer high losses. Virtually 100 percent mortality undoubtedly occurs when eggs are spawned at 35°F.

## A POSSIBLE DIET PROBLEM AT THE EAGLE CREEK FISH-CULTURAL STATION

John R. Parvin  
U. S. Fish and Wildlife Service  
Estacada, Oregon

The first dietary problem involved in a new station is what to feed. Considerable difficulty had been experienced by other hatcheries concerning the incident of kidney disease and tuberculosis. It was felt that a possible source of these infections could be salmon products. A decision was made to eliminate all salmon products from the station diets. Turbot was substituted as the fish constituent of all diets fed at this hatchery.

At this time this decision appears wise as no kidney disease has been diagnosed up to this time. However, other problems may have been initiated by this action. As far as the available literature is concerned very little is available concerning the properties of turbot. It is possible that there is information which was not readily available to this station.

Turbot was introduced into the diets of fall chinook salmon on April 12, 1957, and was fed as part of the diets of spring chinook salmon, silver salmon, and steelhead trout as they became sufficiently advanced. This product was fed at approximately 35% of the diet during the entire feeding season. The diets were composed of ingredients similar to diets fed at Leavenworth, Washington, Coleman, California, and possibly other stations. The diet composition was as follows: 15% beef liver, 20% hog liver, 20% beef spleen, 35% turbot, 3% distillers corn solubles, 7% meal. The meal was either Tyrrell's or Dynafish.

No difference in results in respect to the following condition was experienced by the fish fed either meal.

The procedure used in diet preparation was to prepare and mix the feed for a 24 hr. period at one time. Food from the previous day was fed at the first feed. During the entire time the fish were fed four times daily.

During the latter part of July after a feeding period of approximately 12 weeks when the average size of the fish was approximately 100 per pound the silver salmon began to show the following syndromes: Nervousness, scoliosis, lordosis, accompanied by a very light mortality. In fact there was no noticeable increase in mortality. The loss during July, 1957, amounted to 2.1%. The curvature of the spinal column was present in only about 5% of the stock. The spring chinook may have been effected because their growth was uneven with some pin heads apparent. The fall chinook had previously been liberated. Steelhead were not involved.

The staff of this hatchery conferred with individuals who might be sources of information and combed the available literature. A paper by Phillips indicated that the silver salmon might possibly be suffering from a thiamine deficiency.

In order to combat this possible nutritional deficiency changes in procedure were initiated. The procedure used in the handling of the food was altered so that all food fed was ground the same day and mixed within two hours of feeding. A purchase of thiamine HCL was made from American Cyanimid Corp. This was introduced into the diet at three times the published daily requirements as a therapeutic measure.

Subsequent observation of the progress of the condition showed that with the steps taken it was not progressive. After corrective measures were taken very few additional fish became involved. As the affected fish succumbed the percentage of cripples became less and at the present time very few are apparent.

The silver salmon on hand at this time are being held for spring release. They are in very good condition, averaging 25 per pound. The mortality for the month of November was 27%, mostly individuals showing residual syndromes.

In discussing the apparent deficiency there are several aspects to be considered. This was not set up as an experiment with proper controls. It is possible that the condition was not diagnosed correctly, that if no steps had been taken similar results would have occurred. Previous experience has shown that all is not as it may seem. I know of instances in which disease was treated with apparent good results but when a control was introduced into subsequent similar situations the progress of the fish under treatment and the controls were the same. They both recovered.

It is not known if any tests have been made of the incident of thiaminase in turbot. If the stock involved did have a thiamine deficiency it is thought that the method of food preparation and holding could have been a contributing factor.

It is possible that water conditions at this hatchery may be responsible for variations in results from other stations. Electrolysis tests of the station water supply show that its purity approximates that of distilled water. The effects of the mineral content of water upon the fish held therein is understood to be at this time a controversial subject.

This paper is submitted with the following purposes in mind. The subject might possibly be of passing interest to those present. It might also alert someone who might anticipate using turbot to its possible dangers. And last but not least the staff of this station is seeking information on the subject. We would be happy to have anyone familiar with the conditions which we have experienced to volunteer his opinion.

## NOTES ON IMPROVEMENTS FOR THE MORTON FISH GRADER

Kenneth E. Morton  
Oregon Game Commission  
Camp Sherman, Oregon

A new and improved water spray pipe has been developed which permits a much larger volume of water to pour in a continuous sheet over the grading slots. With the larger volume of water the grader can be operated with less slope and increased efficiency.

A new plastic extrusion has been designed which will eliminate the necessity for fabricating the plexiglass dividers out of sheet and tube. The new extrusion will permit an opening adjustment of zero inches to one inch and will also permit the more accurate grading of smaller sizes. The diamond shape of this new extrusion will be available shortly after the first of the year; anyone desiring further information on either of the above two items can write to the manufacturer listed below.

PAM Co.  
1951 N. W. Wilson Street  
Portland 9, Oregon

# THE DEVELOPMENT OF A GOLDEN TROUT BROOD STOCK IN OREGON

Kenneth E. Morton  
Oregon Game Commission  
Camp Sherman, Oregon

In order to further develop the successful introduction of Golden trout in Oregon, the Game Commission decided to attempt the rearing of Golden brood stock at Wizard Falls.

On September 19, 1954 - 1,097 advanced Golden fry were received. The fry were started on a diet consisting of 50 percent beef liver and 50 percent pork liver. Approximately two months later the diet was changed to 15 percent beef liver, 15 percent pork liver, 30 percent beef lungs, 38 percent Alaskan salmon offal with eggs and 2 percent salt. One year later, on September 30, 1955, the fish averaged 8.2 per pound. The total mortality for this first year was 24 percent.

The following spring, in late April (19 months from the date the fry began feeding) some of the males were mature. No mature females were present. A strange development occurred as the males approached maturity, this was the sudden appearance of a deep corrugated roughness along the back, from head to tail. None of the females were so affected and the condition disappeared from the males soon after the spawning season. A few side swimmers were also present during the second year of growth, the cause of either malady is unknown, there was no serious mortality from them.

Most of the females matured as they approached three years of age, maturation was four months earlier than the parent stock, apparently due to our higher water temperature.

The first eggs were taken on April 4, 1957. Of the 393 females on hand, 248 spawned over a five week period and averaged 595 eggs per female. There were 145 or 37 percent of the females that failed to mature in their third year.

A total of 147,515 eggs were taken. Only 63,780 or 43.2 percent reached the eyed stage. The mortality during the eyed stage was 24.4 percent. Mortality during the fry stage was 10.5 percent. Of the total eggs taken, only 28.3 percent or 43,120 advanced fry were received.

While the golden trout eggs appeared to be normal in all respects, the low percentage of fertilization and the poor viability of the fry were probably due to the very small amount of thin, watery, sperm that was available from only a limited number of bucks. There were a considerable number of males that failed to produce sperm of any kind.

There were 339 males on hand at the start of the spawning season - 54 less in number than females.

A serious loss of 193 males resulted when an employee miscalculated a malachite green treatment and gave all bucks (held in a separate raceway) a triple dosage. Prospects for the egg take next spring are not too bright, with approximately 360 females on hand and only 75 males (some of which may be sterile) we cannot be overly optimistic about results.

Approximately one year before the first spawning date we received a small shipment of fresh frozen whole shrimp. The diet was changed at that time to include 4 percent shrimp and the salmon offal reduced by 4 percent. While we do not have a control lot it is our feeling that the addition of shrimp to the diet was very effective in producing a highly colored Golden trout. The fish are certainly the most highly colored hatchery reared fish that we have ever seen.

From last spring's hatch (1957) we selected 4,000 of the strongest fry to be reared as replacement brood stock. Since the fry were ponded we have only lost 7.1 percent, by far the greater loss occurred during the first six weeks, only five fish have been lost in the last three months. It is very interesting to note the second generation is averaging 64.8 per pound after six months of growth compared with 115.0 per pound for the parent fish at the same age.

If the present good health, low mortality and fast growth of the second generation is any indication of their future development, it appears entirely possible that through selective breeding and acclimatization we may yet develop a successful Golden trout brood fish.

# PRELIMINARY RETURNS OF MARKED FISH FROM COLUMBIA RIVER BARGING EXPERIMENTS

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Starting with fish from the brood year of 1954 and continuing through brood year of 1957, the Washington Department of Fisheries has marked and liberated 1,000,000 to 1,250,000 fall chinook salmon each year within the Columbia River watershed.

These annual liberations from the Klickitat Hatchery have been made in four or five separate locations and have been accomplished by dual means of transportation, namely trucking and barging. Such experiments have become known as the barging experiments and were originally described in Fisheries Research Papers, Washington Department of Fisheries Vol. 1, No. 4, March 1956.

Two and three-year-old marked fall chinook from the initial Columbia barging and hauling experiments returned to the Columbia and its tributaries in 1956 and 1957. These returns of immature fish can only be considered as furnishing grossly preliminary information. However, some interesting patterns of returns developed such as may be observed from the table attached.

Even though no conclusions can or should be drawn from these preliminary returns, some aspects seem quite obvious; namely, if fish are to return to a particular hatchery or stream they must be liberated in that stream, and there is apparently less homing instinct and/or survival from barging fish downstream than there is from hauling them by truck. It also appears that releases of salmon from the Klickitat Hatchery make excellent contributions to the commercial and sports fisheries; percentagewise Klickitat releases appear to make the best contribution of any hatchery operated on the Columbia system.



STATE OF WASHINGTON DEPARTMENT OF FISHERIES  
PRELIMINARY MARKED FISH RECOVERIES  
OF COLUMBIA RIVER BARGE EXPERIMENT  
(Two and Three Year Old Fish From 1954 and 1955 Broods)

		FIN MARK AND LIBERATION POINT				
AREA & YEAR OF RECOVERY		AdLV	Ad&RV	Anal	LV	RV
		Main Col. River at Skamokawa by Barge from Lyle	Mouth of Klickitat by truck	Elokomin Riv. at Hatchery by truck	Klickitat Riv. at Hatchery	Main Col. Riv. at Skamokawa by truck
Col. R Gillnet (1)	1956	32	8	8	16	8
	1957	144	96	68	164	244
(2) Col. River Sport	1956	16	4	8	4	24
	1957	8	8	0	32	16
Eloko- min Hatch.	1956	1	0	21	0	13
	1957	2	3	36	0	8
Kalama	1956	0	0	5	0	1
Hatch.	1957	0	0	13	1	2
Klick. Falls #5	1956	0	3	0	2	0
	1957	0	7	0	96	22
Little White FWLS	1957	2	2	1	2	1
Spring Creek FWLS	1957	0	0	0	2	1
Big White FWLS	1957	0	3	0	1	0
Big Creek OFC	1957	10	0	41	0	11
Bonneville Hatch. OFC	1957	10	6	0	0	0



Herman Cr. Hatch. OFC	1957	3	2	0	0	0
Sandy Hatch. OFC	1957	1	0	0	0	0
TOTAL		229	142	201	320	351

- (1) Col. Riv. gillnet and sport catch recoveries are estimated calculations of total recoveries.
- (2) Ocean troll & sport catch data are unavailable but indications are that the total will represent a total **several** times as large as the above listed river catch.

# HATCHERY RECOVERIES OF MARKED FALL CHINOOK SALMON

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Cook, Washington

A number of marking experiments have been conducted at Columbia River Hatcheries of the Fish & Wildlife Service to determine the optimum time for release of fall chinook salmon.

Four groups of 1951 brood fall chinook salmon fingerlings were marked and released at the Little White Salmon Hatchery and adults were recovered at that hatchery in 1954, 1955 and 1956. The release and recovery data are given below:

Mark	Number Released	Date Released	Days Reared	Adults Recovered			
				1954	1955	1956	Total
D-LP	105,000	May 15, 1952	58	0	0	0	0
D-RP	108,000	July 11, 1952	115	0	1	0	1
An-RP	102,000	July 11, 1952	115	2	3	2	7
An-LP	103,000	Sept. 5, 1952	171	11	24	2	37

The marked adults were of normal size and there was little difference in weight of those returning from the July and September releases. Liver samples were not taken for disease studies but there were no gross lesions or indications of infection with acid fast bacteria.

Two groups of 1953 brood fall chinook salmon were released from the Willard Hatchery into the Little White Salmon River and adults were recovered at Little White Salmon in 1955, 1956 and 1957. The release and recovery data are given below:

Mark	Number Released	Date Released	Days Reared	Adults Recovered			
				1955	1956	1957	total
D-RV	224,000	June 20, 1954	112	0	2	5	7
An-RP	109,000	March 7, 1955	370	2	14	36	52

At all ages the returning adults from the yearling release were considerably smaller than those from the June release. Livers were taken from all marked fish recovered in 1957 but they have not yet been examined for disease organisms. Two females recovered from the yearling plant had abnormal ovaries that often accompany infection with acid fast bacteria.

A more intensive marking program with the same objectives is now underway. 1956 brood fall chinook salmon at Little White Salmon were marked and released in May, July, September, October and February (yearlings). We plan to continue this marking experiments using the same marks and release dates for five successive brood years.

We have another problem at our Spring Creek Hatchery that we hope to solve with a marking experiment now in progress. Spring Creek has had excellent results from annual releases of both unfed fry and 60 to 100 day fingerlings. Two groups of 1956 brood fry were marked just before they were ready to start feeding. One group was released immediately and the other group reared until May 10. We plan to continue this experiment for five brood years.