proceedings of

The Northwest Fish Culture Conference

December 4-6, 1968

Boise, Idaho

THE NORTHWEST FISH CULTURE CONFERENCE

Northwest Fish Culture Conferences are informal meetings for exchange of information and ideas concerning all areas of fish culture. Current progress reports of management practices and problems, new developments and research studies are presented. Active discussion and constructive criticism are encouraged and furnish highlights of the conference. All persons interested in or associated with fish husbandry are invited to attend and to participate. Subject material is limited to topics that have direct application to fish culture.

The PROCEEDINGS contain unedited briefs of oral reports presented at each conference. Much of the material concerns progress of incompleted studies or projects. THESE INFORMAL RECORDS ARE NOT TO BE INTERPRETED OR QUOTED AS A PUBLICATION.

Proceedings of the Nineteenth Annual Northwest Fish Culture Conference December 4, 5 and 6, 1968

The Nineteenth Annual Northwest Fish Culture Conference was held in Boise, Idaho, at the Boise Hotel. About two hundred men attended the conference and fifty men participated in a tour of Hagerman Valley commercial, federal and state fish hatcheries on December 6.

A planning committee composed of Dr. John Halver, Bureau of Sport Fisheries and Wildlife; John Johansen, Washington Department of Game; Bud Ellis, Washington Department of Fisheries, Dr. Lauren Donaldson, University of Washington; Dr. John Fryer, Oregon State University; Chris Jensen, Oregon Game Commission; and Paul Cuplin, Idaho Fish and Game Department, functioned during the year under the chairmanship of Wally Hublou, Fish Commission of Oregon. The planning committee met on the evening of December 3 in Boise to prepare final recommendations to the membership regarding funding and direction of the conference. The recommendations were presented to the membership by Wally Hublou, pages 18 and 19, Planning Committee Report. All recommendations were accepted. A statement of the objectives of the conference was presented by the Planning Committee to the membership. This statement will appear as the first page of each proceedings beginning with this issue.

We take this opportunity to thank Lorrie Spiker, Carolyn Walker, and Vicki Rehwalt for correspondence, programs, membership address list and preparation of the text for the proceedings. Thanks to Worth Stevens, Printing Supervisor, who set up and printed the proceedings.

Special thanks to the session chairmen, William Alvord, Ron Goede, and Dave Erickson. Thanks also go to Walt Bethke for helping with planning the conference and planning the hatchery tour.

The 1969 conference will be at Tumwater, Washington under the chairmanship of John Johansen; Chris Jensen was selected chairman for the 1970 Conference.

Paul Cuplin, 1968 Chairman

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TESTING OF VEGETABLE AND FISH OILS AND PASTEURIZED HAKE AS OREGON PELLET INGREDIENTS

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In 1967 we conducted an experiment for 20 weeks with spring chinook fingerlings to determine if salmon and herring oils were as good as soybean or corn oil in the Oregon Pellet. Also, in the same experiment, pasteurized hake was evaluated for use as an alternate wet-fish ingredient.

The nutritional value of the fish oils for salmon should be superior to the vegetable oils. The cost of salmon and herring oil is reported to be less than soybean or corn oil.

Pasteurized hake was tested because hake should be available in good quantities and should be relatively cheap.

The oils were tested both in the Oregon Pellet and the Oregon Test Diet (a semi-purified ration).

Pasteurized hake was evaluated as the complete wet-fish portion of the Oregon Pellet. The natural fat level in hake is lower than the other wet-fish components used in the pellet so additional fish oil was added to make the total fat level comparable to the regular Oregon Pellet with tuna viscera-turbot.

The fish oils produced significantly greater weight gains, better conversions and higher hematocrits than the vegetable oils.

Pasteurized hake produced results not significantly different from the Oregon Pellet with tuna viscera-turbot.

Salmon oil, herring oil and pasteurized hake all show promise for future use in the Oregon Pellet.

Advances With The Abernathy Diet

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Since the development of the Abernathy moist pellet in 1966, several changes have taken place:

1. Vitamins C and E have been added to the vitamin mix.

2. Protein content (wet weight) of the pellet has been increased to 32.5 percent.

3. Either herring or hake meals can be substituted for salmon meal if done so on an isoprotein basis.

4. Dried skim milk has been eliminated and replaced with dried whey (MNC).

5. Holding the pellet under frozen storage for periods of up to 1 year did not decrease the nutritional quality.

The persent formula for the moist pellet is as follows:

Fish meal - - - - -	30.0	percent
Dried whey- - - - -	17.5	"
Cottonseed meal - -	10.8	"
Wheat germ meal - -	9.4	"
Vitamin mix - - - -	1.0	"
CMC - - - - - - - -	1.0	"
Soybean oil - - - -	4.4	"
Water - - - - - - -	25.9	"

Our most recent as well as our future endeavors are being directed toward dry pellets. We have obtained a Dravo pelleting disc with a variable speed feed control, a dryer, and a screener. During 1969 we will be testing the Abernathy dried pellet on a production basis at the Abernathy Salmon - Cultural Laboratory and the Spring Creek and Little White National Fish Hatcheries.

CHANGES IN FISH CULTURE NECESSARY TO IMPROVE QUALITY OF CHINOOK FINGERLINGS

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Results of adult survival experiments indicate that the average chinook fingerling at time of release should have higher stamina, larger size, increased protein and energy reserves and a lower incidence of disease if increases in adult survivals are to be expected. In order to accomplish this objective, certain changes in fish-cultural procedures must be initiated.

Stamina is affected by environment, diet, and disease and may be increased by exercise, adequate diets, the elimination of overcrowding in ponds, and the control of gill infections.

Increased fish size attained by optimum rearing temperatures, balanced diets, and longer rearing periods. Optimum temperatures are available only through spring or well supplies, artificial heating or cooling systems, or reuse systems. Artificial control only practical for reconditioning and reuse system. Diets must contain at least 50 percent of the available calories as protein of high quality if optimum growth is to be attained. Longer rearing periods to reach greater size only practical if release coincides with favorable estuarine conditions.

Increased protein and energy reserves developed by diets. Body composition should be approximately 15.5 percent protein and 8.0 percent fat at time of release. Oils appear to be most efficient energy source for deposition of fat reserve. Amount fed should not be reduced in excess of 24 hours before release.

Disease control may best be accomplished through treatment or immunization of the fish or sterilization of water supply. Water sterilization may be accomplished through combined filtration and ultraviolet radiation. Again, sterilization is only practical for reuse systems.

In summary, to increase survival of hatchery-reared fall chinook salmon, the environment and diet must be more closely controlled. The indications are the greater productivity of the improved facilities will more than compensate for the cost of alterations.

WOUND HEALING IN ASCORBIC ACID DEFICIENT SALMONIDS

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Last year, we reported growth anomalies in ascorbic acid deficient coho salmon. We found cases of severe scoliosis, lordosis, and other skeletal anomalies that appeared to result from an inhibition of collagen formation in deficient fish. These and supporting experiments indicated an ascorbic acid requirement of some 20 mg percent of dry diet for coho salmon. Since collagen is essential to wound repair, a series of experiments were completed this year to determine the effects of ascorbic acid deficiency on this important physiological process in salmonids.

Twenty-five gram rainbow trout and coho salmon in duplicate 100 fish lots were reared in 15° C spring water at our Hagerman station. Test diets which included ascorbic acid levels of either 0, 5, 10, 20, 40, or 100 mg percent of the dry diet were fed for 24 weeks at which time 10 fish from each lot were mildly anesthetized with MS-222 (1/25,000) and given two puncture wounds with a sharp taper-pointed scalpel. One wound pierced skin and skeletal muscle in the dorso-lateral region near the dorsal fin. The other pierced the entire thickness of the belly wall near the mid-line. Each wound was promptly closed with a single, tied gut suture and the fish was allowed to recover for three weeks after which the wounded fish from each lot were preserved in Bouins fixative for histological examination.

The 100 mg percent Vitamin C group, fed the complete test diet, served as controls and showed the greatest amount of healing. All zero level rainbow trout died within two weeks after wounding. Their unhealed wounds bled and sometimes became infected. Zero level cohos lived but their wounds failed to heal. The gaping wounds contained poorly clotted blood and granulation tissue. At the 5 and 10 mg percent Vitamin C levels, minimal healing usually occurred, depending upon the amount of trauma and of misalignment of the edges of the wound. Healing was even very poor in some wounds in trout on 40 mg percent of the vitamin when the surface of the wound was depressed. When little or no wound depression occurs, and alignment is good, healing is better in 40 mg percent trout. Belly wounds in 100 mg percent controls had very little more healing than did those on 40 mg percent ascorbic acid.

Dorso-lateral body wall wounds usually had much more muscle damage with signs of muscle atrophy and regenerating small sized muscle fibers. This type of wound in a zero level coho showed no healing but hemorrhage and granulation tissue closed the space between the original gaping wound tissue. Wounded trout fed 5 mg percent Vitamin C may show good healing of epidermis even though the wound is somewhat depressed. Collagen is seldom united and is not healed in these fish. Both subepidermal and hyprodermal melanin is absent or nearly so. A deep wound in a 10 mg percent trout shows extensive muscle atrophy, gaping collagen layer, and sloughed epidermis. Scales and pigment are scarce or absent in these unhealed wounds but when good alignment of the edges occurs, subepidermal pigment is rapidly restored. Some trout fed only 10 mg percent Vitamin C heal faster than others although signs of delayed healing can still be seen in thicker epidermis, dermal collagen (immature), lost or regenerating scales, and muscle atrophy. Trout fed 20 mg percent Vitamin C have but slightly more healing than those getting 10 mg percent but with good skin alignment most layers and scales are quite well repaired. However, the deep muscle bundles show many atrophic and regenating muscle fibers. Even in fish fed 100 mg percent of Vitamin C healing is delayed in the same layers as in fish getting only 10 to 20 mg percent of the vitamin. Muscle atrophy is still very evident in the controls also and it is again obvious that healing is dependent on both Vitamin C adequacy and on the severity and condition of the wound.

In conclusion, trout lacking dietary Vitamin C for 24 weeks and then inflicted with two puncture wounds as described above die within one or two weeks following wounding while coho salmon identically treated survive but their wounds do not heal within three weeks--if at all. Trout and salmon fed 5, 10, 20, 40, or 100 mg percent of Vitamin C appear to show improved healing with added Vitamin C up to about 40 mg percent. Coho salmon appear to heal faster or more completely than trout within three weeks after wounding when fed minimal amounts of Vitamin C. Fish fed 40 or 100 mg percent of the Vitamin appear completely healed upon gross examination after three weeks but microscopic study reveals incomplete healing of dermal collagen, skeletal muscles, and damaged or lost scales. Trauma, misalignment, and infection are factors which may retard or even prevent normal wound healing which appears to require about 20 mg percent of ascorbic acid for cohos and about 40 mg percent for rainbow trout.

HEAT PRODUCED BY LIVING FISH

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After measuring the metabolizable energy of a feed, one must know how much of this energy is lost as heat in order to estimate the amount available for growth. Ingested feed energy which is not excreted is deposited as tissue or other products or it is oxidized to $CO_2 + H_2O$. The energy of all oxidized feed appears as heat. Therefore, the equation, ME = Growth Energy + Heat Production, holds true for fish as for all other organisms.

Heat production may be determined indirectly by feeding at several levels or energy intake, plotting the growth data and extrapolating the growth line to zero growth. Heat production can also be determined by measuring directly the heat produced by the living organism.

A bomb calorimeter was adapted to hold living fish. One hundred gram groups of coho salmon (average weight, 1.14 g) were placed in the calorimeter. The heat produced by the fish was measured for 24 hours. Fish which were fasted 48 hours produced about 70 cal/100 g body weight/hour. Fish fed just before they were put into the calorimeter produced about 104 cal/100 g body weight/hour.

SPRING CHINOOK ENERGY STORES STUDIES

by
Daniel B. Romey

This experiment was conducted to determine: (1) how a modified Oregon Pellet formula, containing selected levels of corn oil and/or dextrose, affected fat deposition and glycogen storage in spring chinook fingerlings; and (2) if increased dietary fat and carbohydrate would cause increased mortality or detrimental histological changes.

A modified Oregon Pellet formula containing 30% wet fish and no supplemental oil or carbohydrates was used as the basal ration. In one series of diets, the basal ration was modified by adding 4, 8, and 12% corn oil; another series contained 4, 8, and 12% dextrose; and one diet contained both 8% corn oil and 8% dextrose. In all diets the corn oil and dextrose were added in place of an equivalent weight of cottonseed meal. A control diet consisted of the regular Oregon Pellet containing 40% wet fish.

The test diets were fed to duplicate lots of 500 spring chinook fingerlings held in spring water at approximately 48 - 52 F. All rations were fed on an iso-protein basis, based on feeding rates in the Oregon Pellet feeding chart. The fish were an average size of 445 fish/lb at the start of the experiment and they received the diets for 29 weeks until they reached approximately 35 fish/lb.

Addition of 4% and 8% corn oil improved growth. Growth rate with the diet containing 8% corn oil was equal to or better than the control. The ration using 12% corn oil seemed to depress growth. Addition of corn oil up to 12% of the diet did not cause increased mortality. Incorporation of corn oil in 4% increments appeared to increase total carcass fat in approximately 2% increments. The addition of up to 12% corn oil to the diet had no obvious affect on total liver fat as determined by proximate analysis.

Addition of 4% and 8% dextrose improved growth. The ration containing 8% dextrose produced growth comparable to the control diet. Further addition of dextrose up to 12% of the diet did not appear to improve growth. Feeding dextrose at levels as high as 12% of the diet did not appreciably affect total carcass fat. Fish, fed pellets without dextrose, had liver glycogen levels ranging from 6-7%, and muscle glycogen concentrations ranging from 0.25-0.31%. Addition of 4, 8, and 12% dextrose to the basal ration appeared to produce liver glycogen concentrations averaging 8, 9.5, and 10.75%, respectively (increases of about 20, 45, and 60% compared to the basal ration). Muscle glycogen rose to a maximum of about 0.40-0.45% in fish fed diets with 8% and 12% dextrose.

The ration containing both 8% corn oil and 8% dextrose produced the best growth of any diet tested, and mortality was very low. This diet produced approximately the same rate of fat deposition as the diet containing only 8% corn oil, and liver glycogen concentrations comparable to the ration containing only 8% dextrose.

Mean hematocrit values ranged from 40-44% and were comparable in all diet groups.

Preliminary histological examinations of liver tissue indicated there was considerable variation between fish receiving the same treatment. However, the results suggest that there were some degenerative changes in cell structure associated with glycogen storage and/or possibly fat deposition. We plan to conduct further experiments to determine whether these changes if detrimental, are permanent or reversible.

CHELATED MINERALS IN IDAHO TROUT DIETS

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INTRODUCTION

Open-formula dry fish feed diets have been used in Idaho Fish and Game Department fish hatcheries since 1963. The Idaho Fish and Game Commission requested that an open-formula fish feed diet be formulated for bid specification. Dr. A.M. Dollar, then Associate Professor at the University of Washington, College of Fisheries, was consulted and a diet was formulated. A large number of diets have since been tested. Diets are tested at production hatcheries by production personnel. Ingredient substitution to test the efficiency of various feed ingredients has been the major investigation. The diet formula is altered as new information warrants a change.

Knowledge of the trace mineral requirements for trout is incomplete. The herring meal and other dry meals added to dry diets are believed to provide adequate amounts of trace minterals for fish reared in moderately hard water. To supplement the mineral supply, kelp meal at the rate of three percent has been added to most of our dry fish feed formulas.

It was believed that additional minerals were necessary for trout reared in extremely soft water in Northern Idaho. Little information was available as to the amount of minerals to add and the efficiency of compounds, such as manganese sulphate, manganous oxide, zinc oxide, and copper oxide in the diet. It was thought that chelated minerals might be more readily assimulated by trout than the sulphates and oxides.

Chelated minerals MF type manufactured by Key Minerals, Inc., Salt Lake City, Utah, were tested. The MF type designation refers to the approximate mineral composition of whole milk.

Chelated minerals are relatively new in animal feeding but have been commonly used with plants for many years. The Association of American Feed Control Officials defines "metal proteinate" as the product resulting from the chelation of a soluble salt with amino acids and/or partially hydrolyzed protein.

It must be declared as an ingredient as the specific metal proteinate, e.g. copper proteinate, zinc proteinate, etc. In this paper, chelated mineral will be used interchangeably with the term "metal proteinate."

The mortality of cutthroat trout fed a diet with 2.5 percent chelated mineral was twice that of the other seven diet groups being tested in 1964 feeding tests. During 1965, tests were repeated with cutthroat trout with a reduction of chelated mineral MF type to .25 percent of the diet. Again, diets with MF type chelated minerals added indicated mortalities double those of any other diet tested. During this same period of 1965, Clark Fork Hatchery in Northern Idaho was experiencing difficulty with starting cutthroat trout fry on dry feed. The cutthroat trout fry diet consisted basically of 69 percent herring meal, 17 percent fat in the form of lecithin and herring oil and the MF type chelated minerals added at the rate of .25 percent. Although no comparative feeding tests were carried on at Clark Fork Hatchery, the .25 percent MF type mineral has been used each year in the starter fry feed with good success in this extremely soft water which has only 14 ppm total dissolved solids.

Tests commenced in 1968 to evaluate the performance of MF-C chelated minerals (Table II) in rainbow trout diets. The MF chelated mineral formula was altered and four percent calcium substituted for four percent magnesium; otherwise, the formula is the same.

In addition to the common methods of evaluating feed efficiency, such as feed conversion, mortality and general health, chemical analysis of the fish was included. Chemical analysis of fish water and feed samples was performed by Albion Laboratories, Ogden, Utah, under the direction of Dr. Harvey Ashmead. No grading of fish was carried on during the experiment. The amount of ration fed daily was calculated from the New York State feeding chart by percent of body weight and water temperature. The test diet was used when fish were large enough to receive size 2 fry feed. The test diets are listed in Table I. The present production diet is provided to the Department at a rate of $7.89 per 100 pounds of feed which includes 69 cents for .5 pounds of vitamin concentrate.

The chelated minerals tested were added at a leval of five pounds per ton of feed at a cost of 80 cents per pound.

Method of Testing Fish Feed and Fish Samples

Samples of fish feed and fish were each weighed and dissolved in a mixture of 17-3 nitric acid and perchloracetic acid, then made up to volumes giving a standard concentration of sample (example, 100 mg/ml). Appropriate dilutions were made in distilled water for endpoints. Samples were aspirated into an acetylene flame and compared with known standard solutions in a Model 290 atomic absorption spectrophotometer.

Fish Chemical Analysis

Fish samples were collected and analyzed on a monthly basis. The last month of analysis is reported for each station in Table V. Analysis of fish fed for a short period did not indicate a trend in metal deposition in the fish. One hundred fifty-five fish samples have been analyzed to date with no evident relationship between size of fish and amount of mineral deposition in the fish body.

Test Diets

Test and production diets were manufactured by a commercial fish feed producer. Fish feed chemical analysis for mineral content (Table III) indicates that MF-C minerals were added and reasonably well distributed in the test diets.

Diet Tests

The results of the diet testing is presented in Table IV. The feed conversion and mortality are reported along with the number of fish in each test and the duration of the test.

DISCUSSION

Chemical analysis of diets indicated that commercial preparation of feeds does satisfactorily present the chelated trace minerals in the diet at the .25 percent level.

Deposition of zinc in the fish fed the MF-C diet was evident after a few months of feeding. No other metals appeared in greater quantities in fish fed the MF-C diet than in fish fed the diet without chelated minerals added. No relationship between metals contained in the water supply and metals deposited in fish was indicated.

In a rapidly growing fish, metal content in tissue may be manifested as a lowering of metal levels rather than an increase in metals due to cell division, water intake, and generally more active body metabolism.

A relationship between a greater amount of zinc deposition in the fish and more efficient conversion of kilograms of feed to kilogram of fish appears to be evident in the American Falls and Eagle Fish Hatchery tests.

Some chelates may be useful while others may be harmful. Further testing will continue to determine the effects of prolonged use of MF-C chelated minerals in rainbow trout feeding.

CONCLUSIONS

The MF-C chelated minerals added at the level of .25 percent promoted more rapid growth in rainbow trout in some of the water supplies where it was tested. The relationship of water mineral content to the feeding results was not evident.

ACKNOWLEDGEMENTS

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Thanks are also extended to Dr. Harvey Ashmead and Paul A. Little, Albion Laboratories, Ogden, Utah, for the chemical analysis of fish, fish feed, and water samples.

TABLE I

1968 Idaho Test Diet Ingredient Formula

Ingredient	Percent Production Diet	Percent MF-C
Herring meal	31	31
Blood flour (spray dried)	10	10
Soybean flour meal	15	15
Whey	8	8
Wheat middlings	19	19
Brewer's yeast	5	5
Kelp meal	3	3
Condensed fish solubles	1	1
Herring oil	2	2
Salt (iodized)	3.5	3.25
Minerals MF-C[1]		.25
Vitamin concentrate	.5	.5
Lignin sulfonate	2	2

[1] MF-C Formula

Calcium	4.00%
Cobalt	0.02%
Copper	0.06%
Iron	0.80%
Magnesium	4.00%
Manganese	0.04%
Zinc	0.80%

TABLE II

Average Mineral Composition of Fish Samples During the Last Month of Feeding Test

Station	Diet	Feeding period in months	No. of samples	Fe	Mg	Cu	Mn	Zn	Ca
				Milligrams per 100 grams					
American Falls	A	5	(6)	1.0	39.0	0.1	0.1	1.2	521
	MF-C	5	(6)	1.0	37.0	0.15	0.1	1.6	537
Eagle	A	5	(2)	1.5	32.0	0.07	0.1	1.3	490
	MF-C	5	(2)	1.2	46.0	0.09	0.1	2.0	510
Grace	A	7	(6)	1.0	43	.08	0.1	1.1	660
	MF-C	7	(6)	1.5	51	.11	0.3	1.1	553
Hagerman	A	1	(8)	3.0	33.0	0.1	0.1	1.12	560
	MF-C	1	(6)	3.0	34.0	0.4	0	1.16	500
Hayspur	A	4	(6)	2.0	32.0	0.25	0.033	1.05	558
	MF-C	4	(6)	1.66	32.0	0.26	0.050	1.31	571
Mackay	A	4	(6)	1.2	38	.04	0.3	1.0	820
	MF-C	4	(6)	1.0	18	.02	0.1	1.1	1000
Twin Falls	A	5	(2)	7.7	55	0.11	0.4	1.3	525
	MF-C	5	(2)	2.1	55	0.11	0.2	1.3	495

TABLE III

Composition of MF-C Chelated Mineral Formula and Average Mineral Content of A and MF-C Diets

Diet	No. of samples	Fe	Mg	Cu	Mn	Co	Zn	Ca
		Milligrams/100 grams						
MF-C Formula		2.0	10.0	0.015	0.010	0.005	2.0	10.0
A	(6)	8.5	278.0	1.0	2.8	0.06	5.5	2,300
MF-C	(10)	11.3	306.0	1.1	3.3	0.10	6.8	2,030

TABLE IV

Feed Conversion, Mortality and Number of Rainbow Trout in Each Test by Station and Duration of Test for Idaho Production and MF-C Diets

Station	Diet	No. of fish at beginning of test	No. months tested	Feed* Conversion	Lbs. fish feed per lb. of fish	Mortality in percent	Percent Hematocrit
American Falls	A	7,840	5	.84	1.85	6.4	30
	MF-C	7,840	5	.73	1.60	4.5	32
Eagle	A	16,000	5	.81	1.79	3.1	--
	MF-C	16,000	5	.62	1.36	3.6	--
Grace	A	10,160	7	.60	1.33	.4	31
	MF-C	10,160	7	.65	1.43	.5	33
Hagerman	A	14,658	5	.72	1.58	5.0	37
	MF-C	14,658	5	.67	1.48	5.5	32
Hayspur	A	15,030	7	.55	1.22	.6	33
	MF-C	15,030	7	.59	1.30	.8	35
Mackay	A	15,000	4	.84	1.86	1.9	36
	MF-C	15,000	4	.86	1.89	2.1	30
Twin Falls	A	1,400	6	.66	1.45	2.0	35
	MF-C	1,400	6	.64	1.42	2.2	36

*Kg of feed per kg of fish.

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PLANNING COMMITTEE REPORT

Wallace F. Hublou
Fish Commission of Oregon
Clackamas, Oregon

At last year's conference, a planning committee was appointed to review some problems I suggested should be given attention. The committee is composed of the most recent chairman from each of the participating agencies and institutions. The gentlemen appointed were: Dr. John Halver, Bureau of Sport Fisheries and Wildlife; John Johansen, Washington Department of Game; Bud Ellis, Washington Department of Fisheries; Dr. Lauren Donaldson, University of Washington; Dr. John Fryer, Oregon State University; Chris Jensen, Oregon Game Commission; Paul Cuplin, Idaho Fish and Game Department; and Wally Hublou, Fish Commission of Oregon. Four problems were considered by the committee:

1. Due to a large increase in number of persons attending and participating in the conference, we have a potential problem of too much material to handle within a two-day meeting schedule. The questions asked the committee were: "Should the conference chairman have authority to control the amount and kind of material presented? If not, should the conference by lengthened?"

We recommend that the chairman be given authority to limit material, if necessary, by assigning special topics. He should not have authority to determine what is acceptable information on assigned topics. We further recommend that we should not hold simultaneous or night sessions nor should we lengthen the conference.

2. The cost of the conference is now so high that it is difficult for the host agency or institution to pay the entire bill. The question asked the committee was, "Should we pay for holding the conference and printing the Proceedings by having a formal registration fee and/or charging for each copy of the Proceedings?"

We recommend that the conference chairman be given authority to charge a registration fee of up to three dollars per person if he finds this necessary to meet expenses. We further recommend that cost of processing the Proceedings be held to a minimum by annual revision of the mailing list by the chairman.

3. Should the Proceedings be considered a publication? The committee recommends that we continue as in the past by considering the Proceedings an informal, unedited record of the conference.

4. It would be nice from the standpoint of planning, budgeting, etc., to know two years in advance who is going to host the conference. The committee unanimously recommends that the conference chairman be appointed two years in advance.

In addition to the above recommendations, the committee suggests that the purpose or objectives of the Northwest Fish Culture Conference should be printed on the inside front cover of the Proceedings. We propose the following statement be accepted by the membership for this purpose:

THE NORTHWEST FISH CULTURE CONFERENCE

Northwest Fish Culture Conferences are informal meetings for exchange of information and ideas concerning all areas of fish culture. Current progress reports of management practices and problems, new developments and research studies are presented. Active discussion and constructive criticism are encouraged and furnish highlights of the conference. All persons interested in or associated with fish husbandry are invited to attend and to participate. Subject material is limited to topics that have direct application to fish culture.

The PROCEEDINGS contain unedited briefs of oral reports presented at each conference. Much of the material concerns progress of incompleted studies or projects. THESE INFORMAL RECORDS ARE NOT TO BE INTERPRETED OR QUOTED AS A PUBLICATION.

All recommendations of the planning committee were adopted by the members on December 5, 1968.

MORTON'S MONSTER IN ACTION

THE BULK MOVEMENT OF HATCHERY FISH--MADE EASY

KENNETH E. (GENE) MORTON
OREGON STATE GAME COMMISSION
CAMP SHERMAN, OREGON

A film and narration of a live fish pumping device, previously described in detail at the 1963 NFCC, Tumwater, Washington, showed some minor improvements and the ease of piping fish during grading and transferring operations. The device permits positive control over the rate of discharge of fish on to the grading bars for maximum grading efficiency.

Improvements on the Morton Fish Grader permit the direct discharge of each size group into sliding, reversible trays. The trays can be connected directly to four-inch aluminum irrigation pipe for transferring the three size groups, automatically, to their respective size group pond.

A new diffusion chamber, attached to the head of the grader, has proven highly successful in distributing fish equally to all grading bars.

The device is also used for loading fish tankers, requiring about 20 minutes to load one ton of fish. The fish are weighed by displacement and are never touched by hand, dip net, or seine.

THE SORTING FACILITY AT THE COWLITZ TROUT HATCHERY

Cecil L. Fox
Kramer, Chin & Mayo
Consulting Engineers
Seattle, Washington

The benefits and desirability of fish grading are generally acknowledged as an aid in better hatchery management and programming. However, most hatcheries do not have a convenient or suitable method for moving or handling fish and therefore are unable to grade fish as often as would be desirable for good management purposes. Because both the city of Tacoma and the Washington Department of Game were concerned and interested in providing as efficient operation as possible in the Cowlitz Trout Hatchery, we as the engineers for the project were instructed to design a fish handling facility that would enable the hatchery personnel to handle fish with as much ease as possible. After conferences with Cliff Millenbach, Quentin Edson, Chris Jensen and others, we developed the design that was built on the site of the Cowlitz Trout Hatchery.

The facility generally consists of a combined drain and fish transport pipe system, the graders, sorting tanks and loading facility. In addition to the sorting operation, provisions were also made to hold and load out the adult scrap fish from the holding pond by moving them through a buried 12 inch plastic pipe to the crowding channel. Also, measurement of quantities of fish in the sorting tanks by use of displacement and a float indicator system was also provided. More specifically, the fish are moved from the raceways through a 12 inch round drain plug through an underground flume and pipe system to the sorting facility. The flow enters by gravity into a channel which is 8 feet wide by 45 feet long. The water depth is approximately 2½ feet deep and is controlled by a weir overflow at the drain. When all the fish are in the crowding channel, the entry pipe is closed and a second drain gate is closed. The water is then introduced through a separate pipe into the channel from the lift end. This attracts the fish and raises the water level providing the additional height required for the gravity operation. The crowding and grading operation can now be done.

At the time of crowding, the crowder lowers its screen at a point to collect a lift load of fish and moves toward the fish lift. The crowder is an electrically powered, track mounted piece of equipment that can have the carriage travel speed adjusted to meet the conditions. The crowder screen is the probe type which allows the carriage to move to any point with the screen raised. The fish lift is a platform type lift with a sloping top powered by a water driven hydraulic cyclinder similar to that of a service station lift. After the crowder has moved to the stops allowing the lift to be operated, the fish are lifted up and onto the graders. The grader picks two sizes which fall into sorting tanks and the jumbos go to the end sorting tank. The three sorting tanks each have been sized for 1,000 gallons to provide one tank truck load per grade of fish. Each sorting tank has its own separate supply and circulation pump to enable each to be drained without disrupting the process. The pumps are provided with float switches to turn them off when a tank is drained.

The grader assembly consists of two graders with a stainless steel false weir, entrance apron, spray headers, and adjustment wheels and levers. Levers are provided to raise the upper sections of the graders to clear fish and to provide a six inch clear passage for moving the adults through the facility. Four handwheels with lock levers are used to set the grading bar spacing. It might be noted here that the spray headers are separated to isolate the spray water into its individual sorting tank. This is to provide for the displacement measuring when used. A float well with adjustable scale and indicator is provided for each sorting tank.

Until last month, the only use of the facility has been that of moving adults for loading purposes which is very satisfactory. However, the success or failure of this facility depended upon its ability to grade fish. The outcome of the first full scale run of grading was very gratifying to all. Four raceways of fish of about 4,000 pounds each were graded in about six hours with three men performing all the necessary work. The fish damage was considered less than would have occurred if graded by hand.

We believe that in today's larger hatcheries, the money spent on this type of facility is more than justified and is returned in better programming, lower labor costs, and more effective utilization of food budgets.

If any are interest in having a similar facility, we would be more than happy to design one for your hatchery.

PROGRESS REPORT ON RAPID RIVER SALMON HATCHERY

Burton D. Ainsworth Jr.
Idaho Fish and Game Department
Riggins, Idaho

A report on Rapid River Salmon Hatchery construction and operation was given to the Northwest Fish Culture Conference in 1964 by Bob Quidor, the superintendent of the hatchery at that time. This report will update the progress that has been made since the original construction.

A brief summary on the background of the hatchery. Rapid River Salmon was constructed in 1964 by Idaho Power Company as a part of its Snake River Fishery Maintenance Program. The hatchery is operated by the Idaho Fish and Game Department with funds provided by Idaho Power Company. The purpose of the hatchery was to relocate the Spring Chinook Salmon run that normally migrated up the Snake River above the Idaho Power Company Dams of Brownlee, Oxbow, and Hells Canyon. Efforts of Idaho Power Company failed in the original attempt to continue the migrating run of fish past these dams.

The original construction of the hatchery in 1964 consisted of an adult holding pond, twelve concrete raceways, hatchery building, two residences, and water intake system. The adult holding pond is 80' x 25' x 6' and has a holding capacity of 800 adult salmon. The twelve cement raceways are each 100' x 6' x 4' and this year they will handle 5,750,000 swim-up fry. Automatic fry feeders are being installed at the present time on the raceways. The hatchery building houses an incubation room that contains 28, 16-tray Heath Incubators, a 30,000 pound freezer unit for moist pellets, a shop and office. The main water supply flows into a thirty inch pipe line screened through two electric, rotating screens that are operated on a time clock and float mechanism. A water pump spray system is used to wash the debris from the screens as they rotate. Water for the incubators is obtained from Rapid River through a six inch pipe line.

Because of increased production, two earthen ponds were constructed during the winter of 1966 and 1967. One of these ponds was an adult holding pond, (150' x 40' x 6') with a capacity of 1,900 adults. The second pond was a rearing pond, (200' x 80' x 3') with a capacity of approximately 1,000,000 fingerlings (4½ - 5"). The rearing pond is equipped with four Neilsen moist pellet feeders.

Additional egg incubation space was needed this year and a temporary building was constructed that houses 22, 16-tray Heath Incubators, bringing the total number of incubators to 50.

There are two routes that upstream migrating salmon take to reach the hatchery. The first and original route was up the Snake River to Oxbow Dam trapping facilities and after construction of Hells Canyon was started the fish were trapped there. The fish are trucked from the trap to the Oxbow Hatchery Holding Ponds where they are sorted and held until enough fish are available for a load to Rapid River Hatchery.

The return to Rapid River of adult salmon from released smolts is the second and is the main source of spawners at the hatchery. The trapping facilities on Rapid River are located two miles below the hatchery. The fish are transported by truck from the trap to the hatchery holding ponds.

Table 1 indicates the number of fish and eggs handled at the hatchery during the time it has been in operation. The first return of adult salmon was in 1967 with 1,039 jacks returning to Rapid River. These fish were from the 1964 brood year. The following year 1968, a successful return of 3,416 adults and 740 jacks from the 1964 and 1965 brood years respectively were trapped on Rapid River. Of the 1968 return 2,402 adults plus the 351 adults from the Snake River were held for spawning. All of the fish in excess of 2,753 were transported to other holding areas or to streams nearby in the Salmon River Drainage.

The average monthly growth from swim-up fry to release size is shown on table 2. The flucuation of water temperature has the greatest affect on growth of the fish. The water temperature varies from a minimum of 34 degrees F. during the winter months to a maximum of 60 degrees F. during the summer months. The average yearly water temperature is 45 degrees F.. The size of the smolts at release time (latter part of March) is about 23 per pound and 4½ to 5 inches in length.

The type of feed used is Oregon Moist Pellets from the starter mash through to 1/8 inch pellets.

Prior to the introduction of Spring Chinook Salmon into Rapid River by the hatchery, there had not been any known spring Chinook spawning run in the river. There was, however, a Summer Chinook Salmon run that usually start entering the river about the 1st of July. The springs start entering the river near the 1st week of May.

We are encouraged by the progress made in the past four years and hope that this progress will continue in the years to come.

TABLE 2 - AVERAGE MONTHLY GROWTH (From Swim-up to Release)

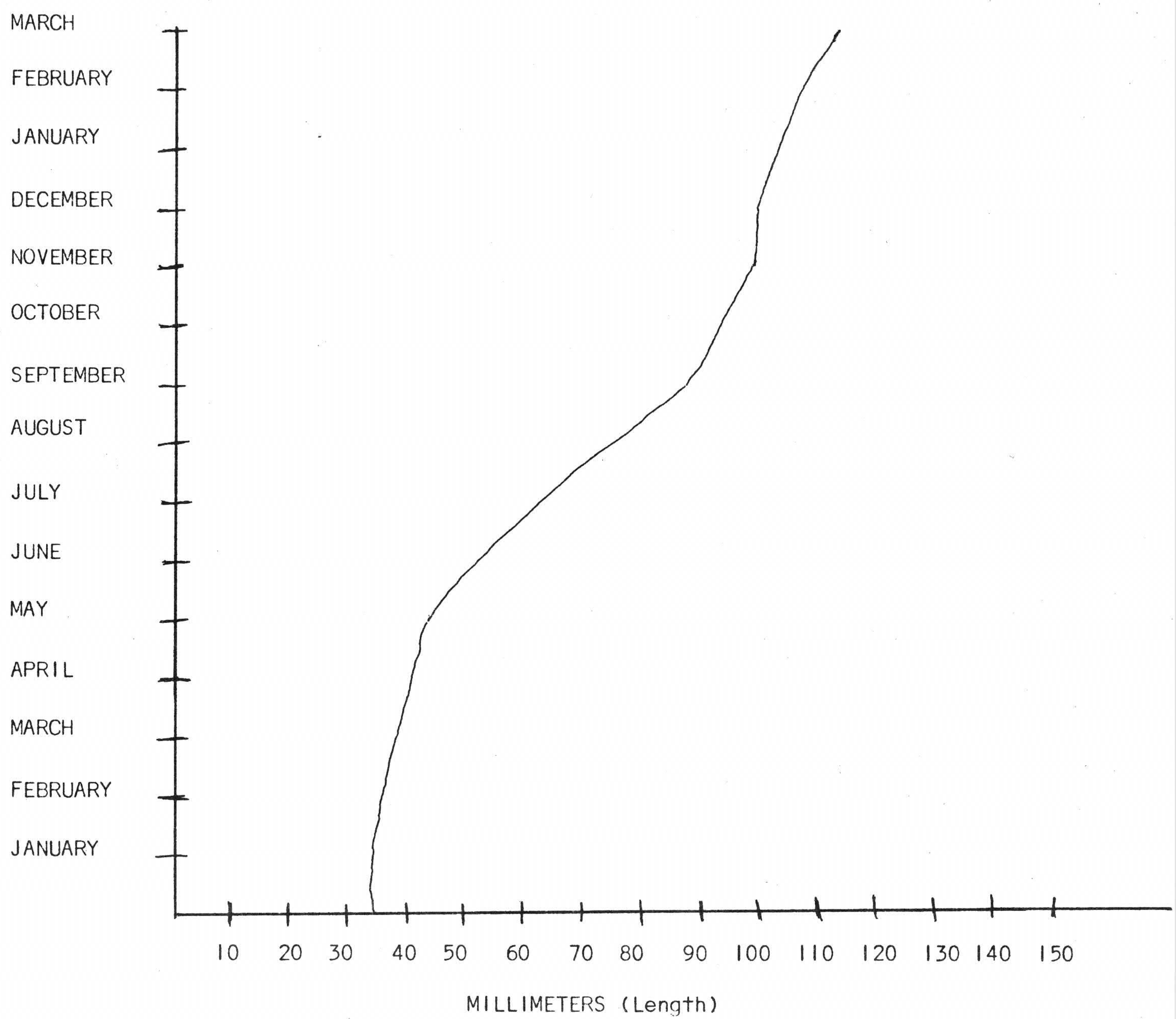

TABLE I

YEAR	RECEIVED FROM SNAKE RIVER	RECEIVED FROM RAPID RIVER TRAP	% MORTALITY PRIOR TO SPAWNING	FEMALES SPAWNED	EGGS OBTAINED	SMOLTS RELEASED	YEAR SMOLTS RELEASED
1964	349		16%	182	887,000	580,000	1966
1965	408		21%	133	604,000	480,000	1967
1966	1,511		18%	621	2,296,000	1,460,000	1968
1967	974	1,039 (Jacks)	11%	518	2,055,000	900,000[1]	1969
1968	351[2]	3,416[3] 740 (Jacks)	2%	1,809	6,640,000		

1-Fingerling on hand for release in 1969
2-All fish held for spawning at Rapid River Hatchery
3-Capacity of hatchery holding ponds is 2700 adults so 2,402 of these fish held for spawning

CARBON DIOXIDE ANESTHESIA FOR ADULT SALMON

Harlan E. Johnson
U. S. Bureau of Sport Fisheries and Wildlife
Cook, Washington

Carbon dioxide was used as the anesthetic agent for calming adult chinook and coho salmon before checking the fish for sexual maturity at Little White Salmon Fish Hatchery in 1968.

Compressed carbon dioxide in 50 pound cylinders was used. A special regulating valve and flow meter were attached to the cylinder. The gas passed from the meter through an air hose to perforated one-half inch metal tubing lying on the bottom of the anesthetic tank. The tank contained about 225 gallons of water at a depth of 12 inches. The flow meter was adjusted to deliver about 150 cubic feet per hour of carbon dioxide gas and introduction of the gas into the tank was started about 15 minutes before the fish. Efficiency could probably be increased by breaking the carbon dioxide into smaller bubbles with carborundum stones.

Twenty-five to fifty adult salmon were placed in the anesthetic solution and held there for about three minutes. These fish displayed some hyper-activity when they first entered the tank but this was not a serious problem and the fish became relatively quiet in 10 to 15 seconds. After three minutes, the fish were delivered to a table and were checked for sexual maturity.

The concentration of carbon dioxide in the anesthetic solution was about 200 to 400 ppm. The cost of the regulator and meter was $90. The carbon dioxide cost $7.85 for a 50 pound cylinder and approximately two cylinders were used during a spawning day. Sixteen cylinders ($126.00) were used during the 1968 spawntaking season for handling 11,300 coho and 1,800 chinook salmon. It was necessary to drain and refill the anesthetic tank several times during a full day of spawntaking.

The carbon dioxide solution calmed the adult salmon but did not entirely immobilize them. It did not produce as complete anesthesia as tricane methane sulfonate previously used but was considered satisfactory by the hatchery employees handling the fish.

Carbon dioxide anesthesia caused no apparent harm to the adult salmon when held in the solution for three minutes or to the eggs taken from these fish. Many of the eggs have hatched and the fry appear normal.

PRACTICAL APPLICATIONS OF WATER REUSE SYSTEMS IN REGION ONE HATCHERIES

Kenneth R. Higgs
U. S. Bureau of Sport Fisheries and Wildlife
Cook, Washington

First, I should probably describe the type of water reuse systems that I am referring to. These are not systems that simply reuse water either by gravity flow through a series of ponds, or by pumping used water back through a pond, but a system that actually "reconditions" the water before it is reused.

"Reconditioned" water is water that has been used for fish production but will have the oxygen replenished and the excess carbon dioxide and ammonia removed before it is used again.

While many of you are probably familiar with this type of a reuse system, I will briefly try to explain the basic operation for those of you who are not.

This reuse system basically consists of an aerating unit, a water recirculating pump and an oyster shell and crushed rock filter bed. In addition, it may also have a heating system as well as a sterilization unit.

The method used to recondition water is relatively simple. Used drain water from production ponds has the lost oxygen replenished and the excess carbon dioxide removed by aspiration and violent agitation. This is generally accomplished in an aspirator tower. Conversion of ammonia to nitrates is achieved by the action of nitrifying bacteria which build up on the surface of the crushed rock in the filter bed. The oyster shell maintains water quality and supplies calcium and trace minerals to the system.

This type of a reuse system is capable of operating as a semiclosed water recirculating system. By this, I mean that only a small percentage of the system's water is required as supplemental water to operate satisfactorily.

In the July, 1968 issue of the PROGRESSIVE FISH CULTURIST, there is an article entitled "Controlled Environments for Salmon Propagation" by Roger Burrows and Bobby Combs. This article explains in detail the operation and design requirements of a reuse system.

Our Bureau now has reuse units in operation at the Little White Salmon and the Coleman National Fish Hatcheries. Units are also under planned construction at the Dworshak and Spring Creek National Fish Hatcheries.

Now that you have some ideas of the type and extent that reuse systems are being used in Bureau hatcheries, I will try to explain how they have benefited production at the Coleman and Little White Salmon National Fish Hatcheries.

At the Coleman Hatchery in California, Sacramento River Chinook Disease, SRCD, has been a very serious endemic problem. This disease causes considerable fish mortalities at that station. It was found experimentally that by increasing the rearing water temperature several degrees Fahrenheit, this disease could be controlled.

On a production basis, operating costs would be prohibitive to heat all of the station's water to control this disease. But, by installing a reuse system, only a small percentage of the water would need to be heated. This was felt to be practical.

On a production scale test using five 8' x 80' raceways requiring 1,800 gallons of reconditioned water per minute, only 180 gallons of supplemental water was needed. This entire unit was maintained at 59° F with warm supplemental water. Supplemental water was a combination of mixed 64° F well water and sterilized creek water that had a temperature range of 48° F to 56° F.

To my knowledge, this increased water temperature completely controlled this virus disease at a reasonable operating cost by using a reuse system.

The reuse system at the Little White Salmon Hatchery in Washington is the one that I am the most familiar with. This hatchery has fairly cold water which is often muddy when the small fry are starting to feed. As a result, we have had an extremely difficult time rearing our fry larger than 150 fish per pound at release time. There is fairly strong evidence indicating that a size of at least 100 fish per pound at release time is desirable if a hatchery wants to maintain a reasonable return of adult fish.

As it is not practical to release chinook fry into the Columbia River later than mid-June (because of unfavorable water temperatures) we would somehow have to produce larger fish by this date.

In the past, our incubation time for eggs has been nearly 120 days. By heating our egg incubation water, we could reduce our incubation time by as much as six weeks. This increased feeding time would probably allow us to produce this desired size fry.

A reuse system with two heaters was installed for egg incubation only. But, due to leakage problems in our system, we were only able to start our fry feeding three weeks earlier than normal.

We did not, however, realize any expected size gain on most of these early feeding fish. As luck would have it, our river water supply was muddy for 14 days when these fry were starting to feed. This muddy water prevented our fry from feeding normally. While we realize an overall average of 124 fish per pound, as opposed to 140 fish per pound--our previous record, we lost nearly 21 percent of our fry that were started to feed in this muddy water.

This extra gain we received was realized in only five million of our 12 million fry production. These fry were started feeding with our limited spring water supply.

As it is extremely difficult to start swim-up chinook salmon fry feeding in cold, muddy water, we decided it would probably be to our advantage to incubate only six million eggs for early feeding. We could then get half of our fry feeding early in our available spring water for six weeks and then move them out into the river water. Our other six million fry could now be started feeding in our spring water. By staggering our fry feeding dates, we could now start them all on spring water, eliminating this annual gamble of fry losses from muddy water.

At our hatchery at least, once the fry start to feed properly, the muddy water does not affect them as badly.

Our reuse system is now working as planned. We will be able to start one-half of our fry six weeks earlier than normal. If all goes well, we should be able to reach this magical size of 100 fish per pound this season. If so, benefits from this reuse system could increase our adult run considerably.

Effect of Stocking Location of Juvenile Steelhead Trout, *Salmo gairdnerii*, on Adult Catch

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ABSTRACT

Marked yearling hatchery-reared steelhead smolts were stocked at varying distances from tidewater in two Oregon coastal streams in April of 1964 and 1965 and the numbers of returning adults caught in the sport fishery were determined.

Objectives of the study were to determine the preciseness of the homing mechanism in directing the returning adult fish to particular locations in a stream and the contribution to the sport fishery of adult fish of different release origins.

Homing imprint was demonstrated to be influenced by stocking site within a stream. A contribution to the fishery of a particular release group was found to be dependent on a delay in upstream migration in the geographic area of stocking and on the fishing effort in the area.

SURVIVAL OF INTER-HATCHERY TRANSFERS OF FALL CHINOOK SALMON

Fred Cleaver
Bureau of Commercial Fisheries
Portland, Oregon

My purpose is to talk on the effect of transferring fall chinook salmon between hatcheries. It has been long recognized that inter-hatchery transfers are frequently not successful but the knowledge has not always been applied.

The returns of fall chinook to the lower Columbia River hatcheries from the 1961 and 1962 broods provide an example. There was a strong correlation between survival back into a hatchery and the percent of the eggs which originated at that station.

The lower returns from transferred eggs can be ascribed to a variety of reasons. Where there are differences in egg quality, the donor station ordinarily retains the best. It is evident that fish from transferred eggs may survive in some instances without returning to the hatchery. There is also the possibility that they are not genetically adapted to the new station. It is this latter possibility that I would like to discuss.

C. H. Ellis of the Washington Department of Fisheries has remarked that more often than not the first few years of introduction of fall chinook to a new station produces poor returns but subsequently the returns become much better. This suggests that something is present in the later generations that was not dominant in the first fish released.

I propose that there are inherent differences in stock that partially account for the success of fingerling releases. Many gross differences in chinook stocks are evident. For example, there are hereditary differences in time of migration from ocean, in time of spawning, and in age of downstream migration. There are also differences in growth rate and ocean distribution. It is likely that there are more minute differences as well. These may include the behavior of fry from wild stock versus fry from a hatchery stock, adaptations to a temperature regine or to water chemistry, adaptations to local diseases, and adaptations to food. Although most stations now use similar diets, this was not true with the 1961 and 1962 broods. I am sure many of you have had experiences where one species did well and another poorly on the same diet.

The Washington Department of Fisheries has provided some good experiments that show that survival of adult fall chinook back to the hatchery is strongly dependent upon race. In one series of experiments on Willapa Bay, fish from Puget Sound and the Klickitat River provided much poorer returns than did the native fish. However, hybrids between native males and Puget Sound eggs survived about as well as the local fish did. This suggests that inherited factors were responsible for survival of fish at Willapa. In other experiments at Capitol Lake near Olympia, eggs of the Little White salmon stock had much lower survival to maturity than did the local stock. However, survival of the second generation of Little White salmon fish was comparatively improved.

The differences that affect survival are probably strongest before July of the first year. Most fall chinook salmon enter the sea in late spring or early summer and given the same size and condition, presumably would survive equally well. The ease with which coho and steelhead are transferred may rest largely on the fact that the smolts are of migrant size or larger when released in spring. Perhaps fall chinook fingerlings would react much the same if they were released at 10 or 15 to the pound. Roger Burrows has approached this with releases at 25 to the pound, and it will be most interesting to observe the survival he obtains.

Thus, it appears there are differences in hatchery stocks that make transfers of fall chinook unattractive and to be avoided if possible. Wild fish from a hatchery stream may also do poorly for the same reasons. Curiously enough, the best fall chinook salmon hatcheries in the Northwest seem to be on streams that have not supported appreciable natural runs. Soos Creek on Puget Sound and Bonneville and Spring Creek Hatcheries on the Columbia River are examples. This may imply that the presence of a wild run handicaps the success of a station.

If transfers cannot be avoided, the best approach appears to be a cross of local males with imported eggs. As soon as a local stock is present in significant numbers, import should be discontinued. Where no local stock at all is available, the introduction will need to be completely alien to the stream. The best procedure then is to take seed from a station with similar physical conditions and preferably nearby. Returns from a first cycle should be kept separate and used to breed the new stock. This evidently underlies Ellis' remark that the first several years' introductions provide little return and then the station begins to produce. Initial adaptation seems rapid. I have mentioned the improved returns from the second generation of Little White salmon stock at Capitol Lake. Another example might be the Washington Department of Fisheries station on Hood Canal. For the first four years, returns were fewer than 100 fish each year. After the fifth year, returns leaped into the thousands. Again, it appears wise to keep the first returns separate, if possible, and prevent them from being overwhelmed by continued introductions.

THE EVALUATION OF COHO AT 10 PUGET SOUND HATCHERIES

Harry G. Senn

WASHINGTON DEPARTMENT OF FISHERIES

An evaluation of smolt coho production from ten Washington State Salmon hatcheries was initiated in 1966 using the 1964 brood and continued with two successive broods. This study followed and was patterned after a Columbia River hatchery evaluation program (Worlund, Wahle, Zimmer 1967) formulated in the early 1960's. The annual production from the ten study stations approximated 7.5 million migrant coho averaging 20-25 fish per pound. (In addition a chinook rearing program was conducted at each station).

Marked coho from the 1964 brood entered the 1967 fisheries as adults.1/

1/ Because the final catch statistics will not be available until early 1969 a complete report cannot be given at this date. It will be available later.

Benefits to cost ratios will approximate other studies ($2 or 3:$1) as do the catch to excapement ratio (2.5 or 3:1).

THE INFLUENCE OF STOCK SELECTION OF COHO ON TIME OF ARRIVAL AT COLUMBIA RIVER HATCHERIES

Richard T. Pressey
Bureau of Commercial Fisheries
Portland, Oregon

For several cycles a number of Columbia River hatcheries have received a run of adult coho salmon which starts in late August, peaks around the 15th to 20th of September and is complete the first week of October. Notable among the stations is the Little White Salmon National Fish Hatchery at Cook, Washington. The managers of this station find the timing of this run causes difficulties as their fall chinook run is also arriving during this period. Problems are encountered in separating coho salmon from chinook and the adult holding space is overburdened.

The Little White Salmon hatchery prior to 1954 was dedicated primarily to the incubation rearing of chinook salmon. In 1954 it was decided that coho would be part of the station's program. Since this coho introduction is of recent date it was easy to examine the origin of the run. Practically the entire group of eggs moved into Little White from 1954 through 1956 to initiate a coho run came from the Toutle River hatchery. In examining the arrival time of the parent Toutle run to the hatchery rack at this station we find that it also appears in late August, peaks in mid-September and is nearly complete by the first of October. Considering the heritage of the Little White Salmon coho stock it is not surprising to find this group of fish matching the migratory timing of the donor stock.

In reviewing shipments of coho eggs from the Toutle hatchery it is noteworthy to find this station has contributed large quantities of eggs to Bonneville, Washougal, Kalama, Cascade and Eagle Creek (Estacada) hatcheries. All of these stations have at least part of their run arriving from late August to the first of October. This phenomena certainly suggests the use of Toutle stock could be the reason.

The Eagle Creek hatchery at Estacada established its coho run in 1956 with two principle donor stocks from the Toutle and Sandy River hatcheries. The Sandy River stock does not appear at the hatchery until mid-October and the run extends through November. It is interesting to find that the return of fish to Eagle Creek from the Toutle and Sandy plants produced a run starting in late August and ending in November. The early segment probably was from Toutle stock and the later segment from Sandy. Since early arriving coho face a holding space and a warm water problem at Eagle Creek, eggs from the August-September run were sent to other stations. As a result of this transfer most of the early coho have now been eliminated from the Eagle Creek run, so "mother" Toutle no longer influences the timing of the Eagle Creek run which now starts in mid-October and extends to early December.

A quantity of Toutle eggs were shipped to the Elokomin hatchery shortly after the opening of this station. These were matched in number with ova from the Big Creek hatchery, a later run. The stock from Toutle suffered heavily from disease and only a few smolt were liberated. The Big Creek stock faired well and a large quantity of smolts was released. The timing of the coho run at the Elokomin hatchery now resembles the Big Creek migratory period. On record, this is the only miscarriage suffered by the very viable, early migrating Toutle stock of coho.

A RETURN OF ADULT COHO SALMON DEMONSTRATING A HIGH DEGREE OF SELECTIVITY IN HOMING

C. H. Ellis
Washington Department of Fisheries
Olympia, Washington

Juvenile Releases

In the Spring of 1967 a total of 1,288,359 yearling coho of the 1965 brood, were released from the Simpson Hatchery which is located immediately adjacent to the junction of the Bingham Fork and the East Fork of the Satsop River in South-west Washington. (Refer to accompanying diagrammatic chart for installation location and planting sites.)

These coho had been reared approximately fourteen months in the Simpson Hatchery facilities and liberated in the following locations, numbers and sizes, namely:

(A). Unmarked Fish

On March 2, 1967, 121,175 unmarked coho at size 25 fish per pound were liberated at hatchery site in the East Fork; on March 15, 1967, 121,700 unmarked coho at size 20 fish per pound were liberated at hatchery site in East Fork; on May 2, 1967, 644,336 unmarked coho at size 16 fish per pound were also liberated at the hatchery site in the East Fork. There was a total of 887,211 unmarked yearling coho liberated in the East Fork at the hatchery site. All 1965 brood unmarked hatchery reared coho were released in the East Fork at the hatchery site.

(B). Marked Fish

All marked fish numbering 200,862 fish at size of 18 fish per pound were liberated on April 5, 1967, in the Bingham Fork, approximately one mile above the Bingham Trap. Of the total reared 1965 brood yearling coho released in both forks of the Satsop River, 18.46 percent were marked.

Adult Returns

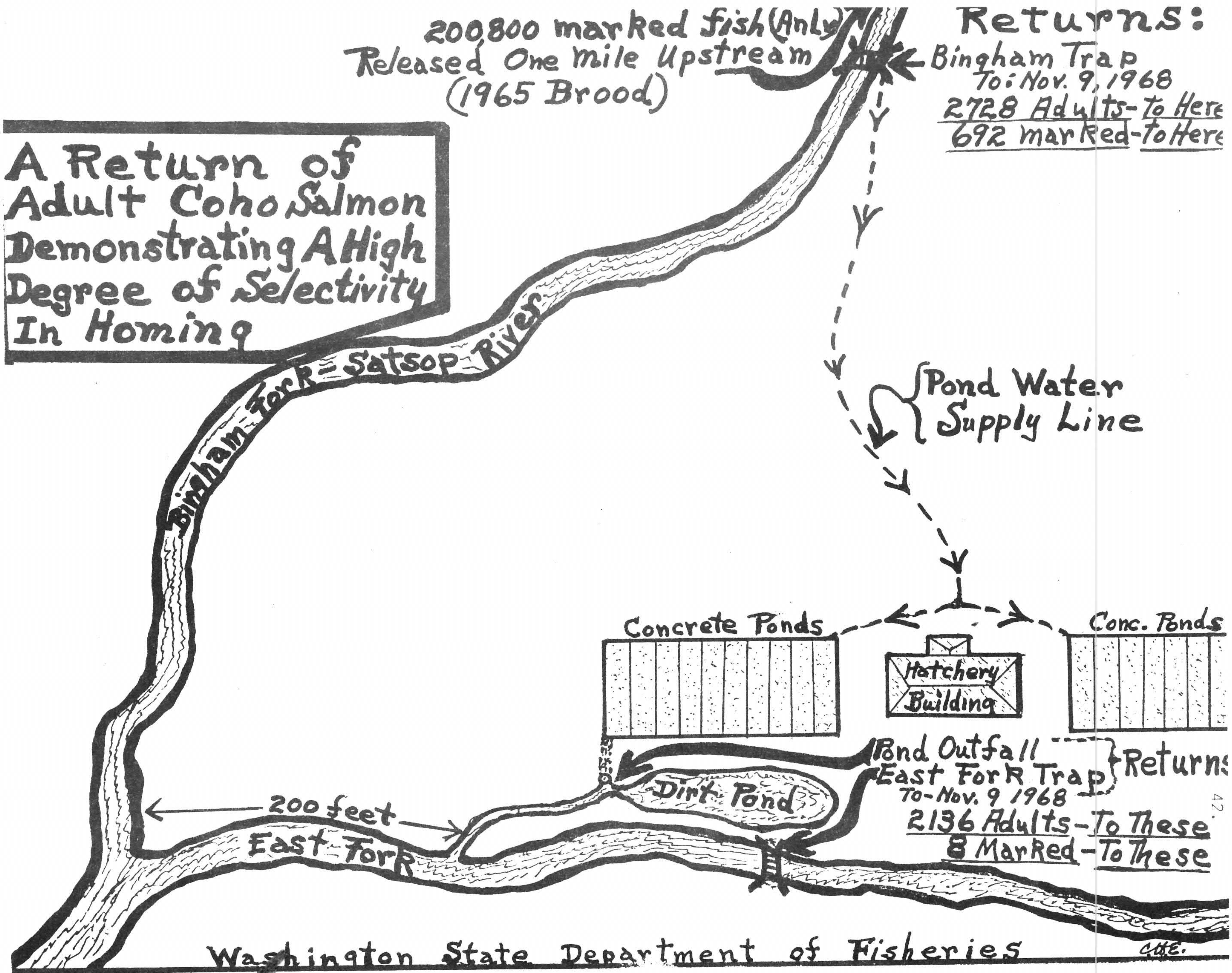
During the Fall of 1968, marked adults started returning to the Simpson Hatchery and to November 9, 1968, the following numbers of marked adults had returned to the two traps (Bingham Fork and East Fork) of the Simpson station, namely:

Bingham Trap to November 9, 1968 - 2,728 adults of which 692 bore the AnLV mark.

East Fork Trap - 2,136 adults of which 8 bore the AnLV mark.

To November 9, 1968, the total number of adults returning to both traps amounted to 4,864 of which 700 or 14.4 percent were marked. This is compared to the total of 18.46 percent of all releases of yearlings that were marked. However, 100 percent of the marked releases were liberated in the Bingham Fork while 100 percent of the unmarked releases were made in the East Fork. It appears that the marked fish have homed very specifically while the unmarked fish have divided themselves between both forks of the river. The close proximity of the release point of the unmarked fish (only 200 feet) to the Bingham Fork probably contributed greatly to this reaction. Bingham Fork water (8 to 10 cfs) being discharged into the East Fork through the hatchery water supply system could be a contributor.

All releases of yearling coho from the 1965 brood which were released into both forks of the Satsop River were progeny from eggs taken in the East Fork trap.


A Return of Adult Coho Salmon Demonstrating A High Degree of Selectivity In Homing
200,800 marked fish (Only)
Released One Mile Upstream
(1965 Brood)
Returns:
Bingham Trap
To: Nov. 9, 1968
2728 Adults - To Here
692 marked - To Here
Bingham Fork - Satsop River
Pond Water Supply Line
Concrete Ponds
Hatchery Building
Conc. Ponds
Pond Outfall
East Fork Trap
Returns
To - Nov. 9 1968
2136 Adults - To These
8 Marked - To These
Dirt Pond
200 feet
East Fork
Washington State Department of Fisheries
C.H.E.

SKAMANIA
STEELHEAD HATCHERY
RT 2 BOX 464 WASHOUGAL, WASH. 98671
Marvin Hull

Increase in size of returning summerrun adult steelhead

Returning summerrun steelhead to the Skamania hatchery usually peak in July the same as the counts from Bonneville dam upstream frôn this Washougal river tributary to the Columbia river. The adults have a choice of entering the holding pond or going on up the North Fork. Seasonal fluxation of the river flow varies the attraction of the ladder to the fish. Only those trapped in the holding pond are counted.

The hatchery started operations fall of '56 with the earliest measurements record for the first marked return in 59-60. Eighty one no marks were not measured but visually compared to the marked which averaged 24 inches, 26 inches and 15 inches from three respective plants of 9,500 or 1060 pounds, 28,500 or 3364 pounds and 17,500 or 1260 pounds. All the hatchery smolts were marked to 1963 exclusive of the stub dorsal hatchery characteristic.

The 60-61 assorted marks with respective plants: Table 0

Adult return	Longest	Average	Smolt plant	Weight	Date	Age
166	32	26.6	-	-	-	-
8	26	25	9,500	1060	5/57	2-1
7	26	25	9,500	1267	11/57	1½
90	29	24	28,500	3364	3/58	2
3	-	21	17,600	1260	5/58	2
97	24	22.2	34,500	4814	10/58	1½
88	-	22.4	22,500	2810	3/59	2

With the increase returns for the 61-62 adult summerrun steelhead measurements were not averaged. The total was 598. See Table 1. To compare only the difference in size of this 598 with the adults for 67-68 each size group of this 598 was multiplied by five. The red bars thus show a stimulated return of 2990. The green bars illustrate the actual mumbers of each size for 67-68 totaling 3177 also summarized in Table 2.

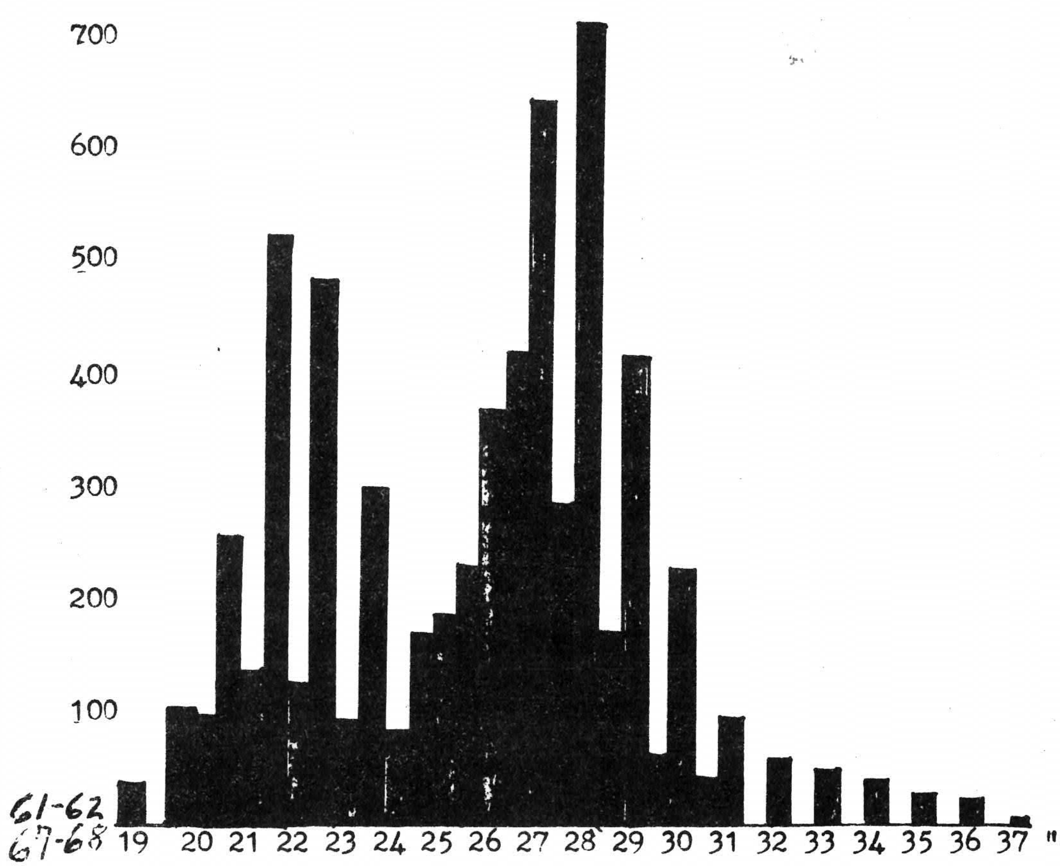
The average size for 61-62 was 23.1 inches long which in field checks by our game fish biologists is considered to average three plus pounds. The average size for 67-68 was 27.1 inches or seven pounds using the department rule for four pounds at 24 inches and a pound per inch to 32 inches for winterrun steelhead. Summerrun will not stand weighing when they enter and it is meaningless after 4-8 months of starvation. Thus the length has increased by 18% and the weight perhaps doubled. Such increase is much greater than anticipated and a closer look is attempted.

It is a first that the 990 group didnot reappear áfter one year or on a 1-1 cycle. Here to fore a successful plant has always been well represented percentage wise on the following year or a 2-1 cycle. Further the 35 and 33 groups are a definite return for three years or on a 1-3 cycle. We apparently had a return as a 2-3 cycle in the 19 group table 1 which is probably a confusion with subsequent marks using one of the same fins and sloppy fin clipping with regeneration. At least the size is not there for fish three years in salt water today. Of course some overworked individual is eventually goihg to say how about group 8 table 0. This hatchery skeleton was erratic all the way. It was our first plant and on 5/22 the fish averaged 11 and 7.5 for the 1 and 2 year smolts about equal in numbers. They dominated in the trout catch above the hatchery all summer with two missing fins obvious. There was no return the next year, 5 the second year and 8 the third year which proves why the competition in clearing marks for fish and although it is the only plant where the third year is the best return it shows lots of need for improvement. We have since kept the earliest eggs to stretch out the growing year, used only thelargest males which are fast growing and slow maturing, utilized the improved fish feeds and had mild winters which has resulted in reaching size with all our smolts in one year averaging 7.5 with these last spring.* This has increased their relative life cycle time in the ocean resulting in a much larger individual size average for adult summerrun steelhead in two generations.

Adult return	Length range	Smolt plant	Weight	Date	Age
		Table 1	61-62		
93	20-31	-	-	-	-
19	21-30	28,500	3364	3/58	2
85	25-30	34,500	4814	10/58	1½
106	23-31	22,500	2810	3/59	2
47	20-28	22,500	5270	10/59	1½
248	19-28	38,700	5975	4/60	2
		Table 2	67-68		
105	23-33	-	-	-	-
2014	20-37	54,000**	9555	4/65	2
990	21-34	45,000	6635	4/65	1
35	25-36	16,000	2130	4/64	1
33	27-35	16,000	2440	3/64	1

* Fry are now thinned to station capacity usually about 10% in fall.

** These stub dorsal hatchery fish were not marked otherwise.



SELECTED LIBERATION SITES AS AN AID TO INCREASED SALMON PRODUCTION

Richard E. Noble
Washington Department of Fisheries
Olympia, Washington

The salmon culturists of the past have not concerned themselves to a large degree on the relative importance of a release site for their hatchery product. Typically, the liberations have been at the hatchery site or to specific streams designated by the area biologist.

The station managers would not give much thought to the economics of production as it might relate to individual release sites.

The significance of this relationship was brought to the attention of the Department of Fisheries Hatchery Division when biologists and administrators started recommending closure of specific hatcheries because of poor returns.

Our analysis of the juveniles released from these hatcheries give no indication of sub-clinical or physiological conditions which would indicate abnormal survivals.

The stations receiving the most criticism were located above areas suspected of carrying pollution loads that could be detrimental to salmonids. Experiments were designed to measure the differential survival between groups of salmon liberated at different sites; i.e., the hatchery stream versus a stream or area that would allow the migrant to bypass the suspected pollution block.

The first station involved (1967 brood Kalama stock) fall chinook fingerlings being reared at the Department's Washougal station.

During the period of June 3 to 14, 1968, a single population of 320,000 fingerlings were divided into four equal lots by using a four place sampler. The resulting groups were differentially marked by freeze branding. The fish were checked by the pathologist and tests run by the Abernathy fish culture laboratory indicated the quality to be excellent.

The four differentially marked groups were liberated as follows on June 17, 1968.

	LIBERATION SITE	NO. FISH	AVG. SIZE
1.	Washougal Hatchery	77,924	77/lb.
2.	Trucked & liberated into Col. Riv. 2½ mi. below mouth Washougal River.	77,693	77/lb.
3.	Trucked & liberated in Washougal Riv. just above river mouth.	76,538	77/lb.
4.	Trucked for one hour then liberated at hatchery same as 1.	78,694	77/lb.

The recovery effort indicates differential survival rates for the groups as related to release sites and with an obvious advantage for those liberated below the mouth of the Washougal River. The distance from the Washougal Hatchery to the mouth of the river is 20 miles and from the mouth of the Washougal to the sampling site is approximately 100 miles.

If the sampling is indicative of total survival, the survival of fall chinook reared at Washougal can be more than doubled by hauling below the mouth of the Washougal River.

Another thermal marking study involved fall chinook reared at Ringold, a rearing site located near Pasco, Washington. Two equal groups of chinook fingerlings were given identifying brands and one lot liberated at the Ringold site and the other hauled by truck past the four dams and liberated below Bonneville. The recovery effort at Puget Island indicated a differential catch of 15:1 in favor of the Bonneville release.

The third experiment of interest which relates to release sites is one in progress at the Department's Simpson Station located on the Satsop River, Grays Harbor drainage.

Coho of the 1964 and 1965 brood years were used in the study with two comparable groups of fish from each brood identified by fin clip, then one lot from each year was liberated into the hatchery stream and the comparison group hauled and released into the Humptulips River. The Humptulips drains into the lower part of Grays Harbor and fish emigrating would miss the major part of the area of questionable salmonid habitat.

Distance to salt water, truck time, fish quality and mark effect was comparable between the two test groups.

The preliminary marine catch of both broods show a differential contribution favoring the Humptulips release.

Initial calculations for the 1964 brood show a 70 percent increased harvest for the Humptulips release over the companion lot liberated in the hatchery streams.

The 1965 data indicates even a greater difference favoring the same group, possibly in excess of $2\frac{1}{2}$ times greater contribution.

A finalized report will be available when the catch and sampling data are completed for 1967 and 1968.

The recoveries by the Bureau of Commercial Fisheries, Puget Island Seining project were as follows:

DATE	LAn * HATCHERY	LDn * HATCHERY TRUCK	RAn * WASHOUGAL RIVER MOUTH	RDn * COLUMBIA RV. 2 1/2 Mi. below WASHOUGAL RV.
6-17	--	--	--	--
6-18	--	--	--	--
6-19	--	--	--	10
6-20	--	1	15	27
6-21	4	5	12	30
6-22	11	10	17	14
6-23	20	17	21	32
6-24	10	11	11	14
6-25	7	7	12	11
6-26	5	3	5	4
6-27	3	4	11	11
6-28	4	4	7	6
6-29	4	7	9	8
6-30	5	7	4	14
7-1	--	2	1	9
7-2	1	2	1	6
7-3	4	2	4	5
7-4	4	4	2	4
7-5	1	2	--	4
7-6	1	1	3	3
7-7	2	2	--	4
7-8	5	5	3	2
7-9	1	--	--	3
7-10	1	--	2	7
Sub-Total	93	96	140	228
7/10 thru 9/10 Final Totals	103	103	145	236

* Thermal cold brands

WHAT HAS ARTIFICIAL PROPAGATION DONE FOR THE UMPQUA RIVER PROGRAM

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Oregon State Game Commission
Roseburg, Oregon

Various artificial propagation programs continue to play an integral part in maintaining or increasing anadromous fish runs in the Umpqua River.

Table 1 illustrates the increase of spring chinook in the North Umpqua as measured at the Winchester Dam counting station, and the contribution from hatchery production.

Table 1

Spring Chinook Counts at Winchester Dam, 1946-68

Period	Average Run	Hatchery Contribution	
		Number of Fish	Percent of Run
1946-50	2,745	---	---
1951-55	5,908	929	15.7%
1956-60	5,355	822	15.3%
1961-65	8,671	1,911	22.0%
1966-(68)	8,522	2,074	24.3%

An attempt was made with fish from the 1965 brood to correlate visual smoltification characteristics with previously approved size and time of release data. The 88,000 fish were released in 1967 on time but were somewhat larger than desired (4.8 fish/pound). Visual smoltification characteristics indicated that the release was approximately four weeks late.

A return of 1,640 fish from the 1965 brood was recorded the same year they were released (f-sS) and 1,137 returned in 1968 (f-s-sS). These two life history patterns would normally make up thirty percent of the returns.

Smolt characteristic examinations began in November, 1967, with fish from the 1966 brood. As smolting progressed, the fish were hand-graded and released. A total of 171,000 fish were released in this manner (130,000 @ 7.4/lb. in Dec. - 20,000 @ 8.9/lb. in Jan. and 21,000 @ 9.4/lb. in March). It is interesting to note that only 1,256 fish were recovered in 1968 (f-sS). Table 2 shows the transformation of the 1966 brood prior to release.

Table 2

Rate of Parr-Smolt Transformation for 1966 Brood Umpqua Spring Chinook

Date	Average Fork Length	Average Condition Factor	Percent of Fish Intermediates or Smolts
Dec.22, 1967	5.61"	1.161	48%
Jan. 3, 1968	5.81"	1.137	50%
Jan.10, 1968	5.91"	1.111	52%
Feb. 7, 1968	5.56"	1.181	38%
Feb.21, 1968	5.63"	1.127	46%
Mar. 6, 1968	6.38"	1.126	80%

Problems in obtaining brood fish has delayed the approved expansion of the spring chinook program. Brood fish were collected from the pool below Soda Springs Dam on the North Umpqua River at the beginning of the program. In 1967, additional brood fish were collected from the Winchester Ladder and trucked to Soda Springs. The fish did not hold well in the pool and some of them drifted down into the river. The type of dip-net, and size of hauling vehicle were the primary cause of handling and delayed mortalities.

In 1968, spring chinook were again collected from the Winchester Ladder but were transported to the McLeod holding ponds on the Rogue River. By October 15, no eggs had been taken from the fish held at McLeod although the egg-take below Soda Springs was completed by October 11.

The adults held at McLeod were returned to the Rock Creek Hatchery on the Umpqua and eventually eggs were taken from less than ten percent of the females. The remaining fish did not ripen.

A thirty-five acre rearing pond (Whistler's Bend) located on the Umpqua River, 20 miles upstream from Roseburg was constructed in 1960. From 1961 through 1966 attempts were made to naturally rear summer steelhead or spring chinook. Both programs were unsuccessful for lack of a good quality year-around water supply. Fall chinook fry however, (800-1,024/lb.) were stocked into Whistler's Bend Pond in 1967, at a rate of 7,300 fish per acre. The pond was drained on the first of June, a total of 177,000 fish or 70.2 percent of the number stocked were recovered. Without supplemental feeding, this was considered an excellent survival. The fish averaging 145.7 per pound at draw-down represented a production figure of 29.1 pounds of fish per acre.

The stocking rate into the pond was increased to 15,700 fish per acre in 1968 and a supplemental feeding program was initiated. At drawdown, June 3, a total of 454,000 fingerling was recovered or 82.8 percent of the number stocked. The fish averaged 72.5 per pound, representing a production of 158.7 pounds of fish per acre.

The food conversion factor was computed at 1.41 pound of feed to produce 1.0 pound of fish.

The average runs of summer steelhead passing Winchester Dam from 1946 to 1948 is presented in Table 3. In an earlier report, the heavy decline during 1955-59 was discussed. The artificial propagation program began in 1957, with recent excellent results, based on production of smolts reaching 7.0 per pound in March.

Table 3

Summer Steelhead Counts at Winchester Dam, 1946-68

		Artificial Propagation Contribution	
Period	Average Run	Number of Fish	Percent of Run
1946-50	3,149	---	---
1951-55	3,439	---	---
1956-60	2,395	822	34.3%
1961-65	3,874	1,339	34.6%
1966-(68)	5,501	2,834	51.5%

A release of 92,200 summer steelhead in March 1966 has resulted in a return of 2,135 one-plus ocean fish (2.3% return) and 1,805 two-plus ocean fish (2.0%). Of interest here is the high return (46%) of two plus fish. The normal return of two-plus fish has been less than 20 per cent.

The winter steelhead program on the Umpqua River was carried on for six years. It's main purpose was to measure seasonal contributions to the fishery in relation to the summer steelhead program. It was found that a larger portion of the summer stock returned in the winter than did the winter stock in the summer.

A release of 24,500 winter steelhead (8.0/lb.) in March 1966 and 12,200 fish (8.6/lb.) in April 1966 have resulted in returns of 2,515 adults (10.3%) from the March group and 631 adults (5.2%) from the April group. The different rates of return in the lots of fish having nearly identical factors of size and smoltification indicates the high importance of having the yearling steelhead ready to stock in March.

A relatively new program showing promise, is the one utilizing sea-run cutthroat. Table 4 illustrates the cutthroat runs as measured at Winchester Dam over the past 22 years.

Table 5 presents a history of the stocking and rate of return. Of particular interest is the 1966 run where the March release returned 26 to 1 over the May release. Returns for 1967 decreased possibly because of the delay in stocking to allow completion of treatment for kidney disease.

Table 4

Sea-Run Cutthroat Counts at Winchester Dam, 1946-68

Period	Average Run	Hatchery Contribution Number of Fish	Percent of Run
1946-50	715	---	----
1951-55	1,090	---	----
1956-60	437	---	----
1961-65	256	114	44.5%
1966-(68)	1,787	1,087	60.8%

Table 5

Sea-Run Cutthroat Stocking and Rate of Return, 1963-68

Number of Fish Stocked	Size at Stocking	Date of Stocking	Returns	Percent Return
10,000	4.0/lb.	June, 1963	11	0.11
10,000	3.6/lb.	Apr., 1964	217	2.17
10,200	3.6/lb.	Mar., 1965	146	1.46
10,000	3.1/lb.	Mar., 1966	1,450	14.50
10,000	2.1/lb.	May, 1966	59	0.59
20,000	2.7/lb.	Apr., 1967	1,606	8.03

TEMPERATURE INCUBATION ANALYSIS AT RINGOLD STUDY AREA

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Ringold Springs Natural Rearing Studies began in 1962. The emphasis of these studies has been on the capabilities of the station for rearing fall chinook. It has been the practice to rear coho salmon during the remainder of the year (from June through December).

The natural rearing capabilities were investigated during the first five years of operation. A hatchery-type rearing program with an O.M.P. diet was begun in 1967 and investigations along this line are continuing.

Ringold Pond Study Area is located adjacent to the Columbia River in eastern Washington approximately 25 miles north of Pasco. The water supply at Ringold is unique among the salmon rearing facilities of the Washington Department of Fisheries. The springs are the result of irrigation in the Columbia Basin Project. The springs, which issue from a bluff one-quarter mile from the Columbia River, began flowing about 14 years ago and have increased in volume gradually each year. The water temperature remains near 60°F. throughout the year. The water is very high in dissolved solids (636.6 ppm in September, 1967) and is somewhat alkaline. pH readings usually fall within the 8 to 8.4 range. The water is a good producer of aquatic life, both vegetable and animal and has given good results with the rearing of fall chinook with and O.M.P. diet.

In the Fall of 1964, 95,000 eggs were taken at Ringold and hatched in shallow troughs at the pond outlet. The temperature of water from the pond averaged 58°F. for two weeks and then gradually cooled to 47°F. during the incubation period. Survival from spawning to ponding was 60 percent.

In 1965, only 11 percent of 363,000 eggs developed into fry. After a loss due to a pump failure, 4 percent of the eggs taken were ponded as fry.

In 1966, Heath incubators and a water chiller were installed. With water cooled to 58°F. survival of eggs taken at Ringold was 60 percent identical to results in 1964. Also in 1966, eyed eggs from the Klickitat Hatchery had high losses with 68 percent survival of the fry at ponding.

In the Fall of 1967, studies were undertaken at Ringold Pond Study Area to determine if the water quality is suitable and which water temperatures may be used successfully to hatch and hold fry prior to ponding. Another purpose of this study was to determine whether eggs could be incubated successfully in the warmer spring water at Ringold after eyeing at another station. These studies were carried out under the auspices of the U. S. Bureau of Commercial Fisheries and are reported at length in "Salmon Egg Incubation and Columbia River Emigration Studies, June 1968".

Others have shown that 57°F. is the upper temperature limit at which fall chinook eggs can be incubated successfully. However, the need for determining the combined effect of the local water quality and warm temperatures upon the embryos at various stages of development made this study desirable.

For this study, green fall chinook eggs were brought from Klickitat Salmon Hatchery as soon as they were water hardened. Eggs were from a single egg take and were mixed for a random sample.

Control lots were incubated at Klickitat Hatchery in 49°F. spring water which has given good results in the incubation of salmon eggs.

At Ringold, eggs were incubated in Heath incubators with water temperatures controlled by use of a refrigerated water chiller.

Losses were recorded at 24 hours after spawning and throughout the period from the time eggs were eyed until the fry were released. Accurate counts of the eggs were made at the eyed stage and the fry were again counted at release. Percentages of survivals and mortalities were calculated from this information.

One control lot was incubated at Klickitat Hatchery with 93 percent survival. A second control lot incubated at Klickitat was held in a cream can while eggs were being transported to Ringold. These eggs had a survival of 90.6 percent (See Figure 1).

At Ringold eggs were incubated in uncooled spring water (62°F. decreasing to 60°F.) and cooled water of 58°F., 55°F., and 52°F.

Mortality was extremely high in the 60°F. water. Only 19.1 percent of the eggs survived as swim-up fry at ponding time. In 58°F. water survival was 57.8 percent to ponding time. Survival in 55°F. and 52°F. water was nearly identical with 79.2 percent and 79.1 percent, respectively. However, fry incubated in 52°F. water were in better condition at ponding time than any incubated in warmer water.

Prior to hatching, the eggs became extremely weak-shelled. Pink spots appeared on the eggs. Coho eggs exhibiting this same condition were examined under a microscope. They actually had holes through the outer shell and it is believed that the chinook eggs did also. The weak-shelled condition was worse in the warmer waters than in the cooler waters. This condition apparently was due to a combination of the warm temperatures and the water quality (probably water hardness).

A portion of the eggs were held in 52°F. water at Ringold until they were eyed and then placed in the warmer waters for the remainder of the incubation period. One group was newly eyed while a second group was nearly ready to hatch. Eggs from these groups gave better results in 60°F. water than those incubated full time in the uncooled water, but eyed eggs placed into 58°F. and 55°F. water to hatch showed little or no improvement over the original groups.

Eyed eggs were brought from Klickitat on two subsequent dates. These eggs were from the same broodstock spawned on the same date as were the original eggs.

One lot was received as newly eyed eggs. Survivals were better than with any of the previous lots. Eggs incubated in 52°F. water had 94.6 percent survival from the newly eyed stage to ponding. Those in 55°F. water had 92.7 percent survival. Those in 58°F. water had 88.8 percent survival. Those in 60°F. water had 71.1 percent survival.

A second lot of eyed eggs was received from Klickitat just before they were due to hatch. Except in 60°F. water, survivals were barely higher than with the newly eyed eggs. Those hatched in 60°F. water had slightly lower survival than the previous 60°F. group.

SUPPLEMENTAL COHO INCUBATION EXPERIMENT

During the early stages of the study, the water chiller developed trouble and shut off on two occasions. This allowed the water to the cooled incubators to return to the uncooled temperatures of 61°F. or 60°F. There was concern that this rapid temperature change might harm the developing embryos.

A small-scale experiment was initiated with coho eggs to determine the effect of rapid temperature changes upon developing embryos. Eggs from a female coho were fertilized and 1300 eggs were divided and placed in two trays in 52°F. water. Each day until hatching, both trays were drained, Tray #1 was removed from its place and put into the incubator with 60°F. water. Tray #2 was removed in the same manner and immediately returned to its place in 52°F. water to duplicate the mechanical action upon Tray #1. After 15 to 30 minutes, both trays were again drained, removed, and replaced in their respective places in 52°F. water. Thus, the eggs in Tray #1 were changed from 52° to 60°F. and back again each day until hatching.

It would be expected that survival would be lower in Tray #1 if the embryos suffered from "thermal shock." However, survival was nearly identical for both trays. Total loss for Tray #1 was 321 and for Tray #2, 319. Losses for both trays were fairly comparable throughout the experiment. All surviving fry appeared healthy and vigorous.

There was no evidence that thermal shock has any effect on the developing embryos and it is assumed that the chinook eggs of the incubation study were not harmed by the failure of the water chiller on the occasions mentioned above.

SUMMARY

As anticipated, use of uncooled spring water for incubation purposes proved unsatisfactory in every case. Water 52° to 55°F. gave best results with eggs incubated full term at Ringold. Results, however, were still mediocre with survivals of 79 percent from spawning to ponding time. Weak spots in the egg shells were also a serious problem in eggs incubated full term at Ringold.

Best results were obtained with eggs brought from Klickitat after they were eyed. Survivals were good to fair in waters 52° to 58°F. (84 percent to 90 percent from spawning to ponding). Eggs did not develop weak spots in the shells and fry were in better condition at ponding time. Eggs did best in 52°F. water, and well eyed eggs did slightly better than those received when newly eyed.

If eggs are to be incubated full term with Ringold Springs water, temperatures not over 55°F. may be used with best results. With 58°F. water fry of poorer quality may be expected.

If eggs are to come from another source they should be held until eyed before being shipped. Water at 58°F. may be used with fair success, but again best results will be secured with water not over 55°F.

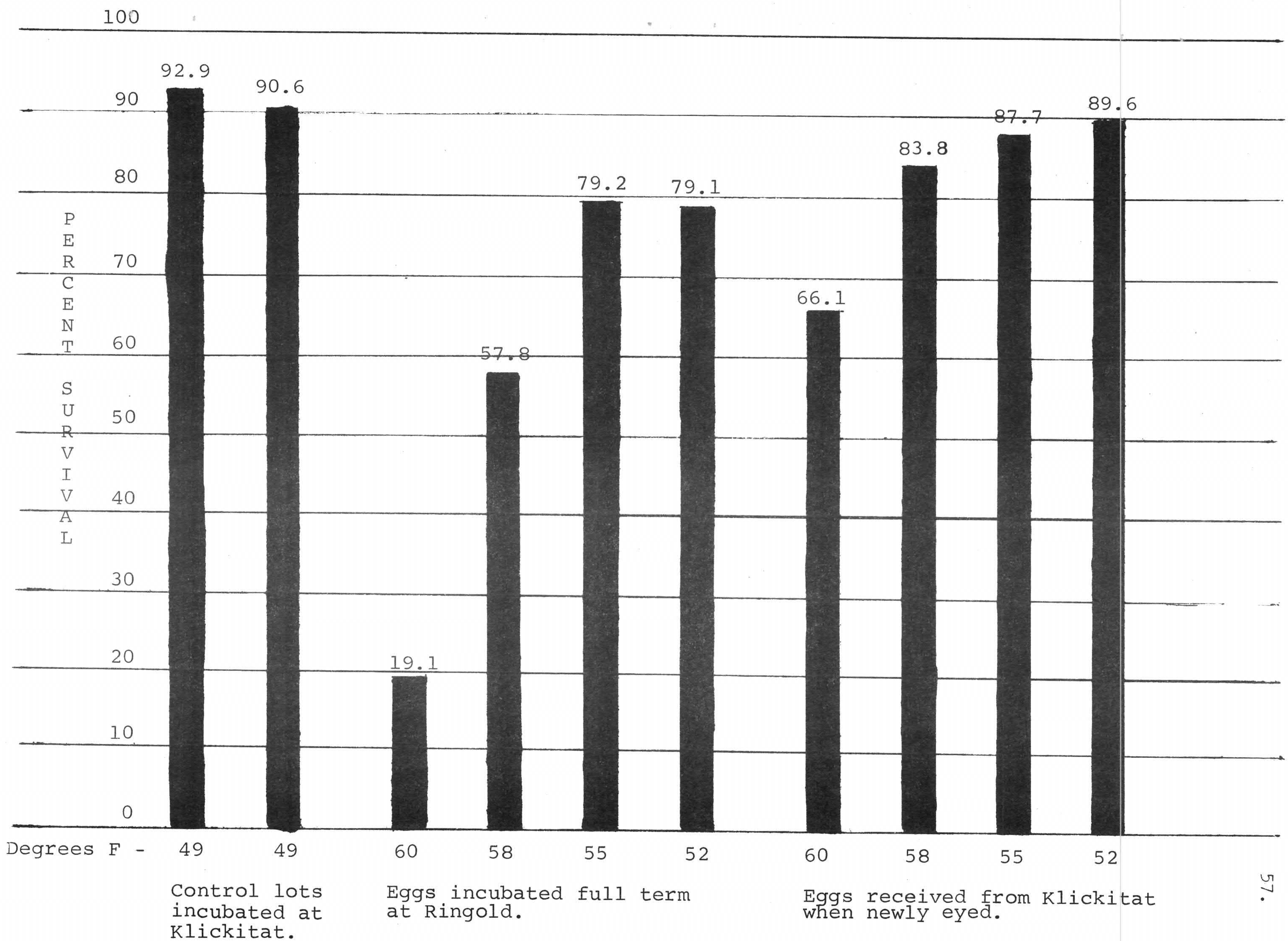

Figure 1. Comparison of survival from spawning to ponding time.

Changes in chemical composition, coefficient of condition, and body morphology associated with the parr-smolt transformation in steelhead trout

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Smolting in anadromous salmonids is associated with changes in physiology, coefficient of condition, chemical composition, morphology, and behavior which precede downstream migration and transform a parr into a migratory smolt. The two main reasons for studying smolting are to understand the early life history of the juvenile steelhead trout and aid fish culturists and biologists in recognizing potential steelhead migrants.

The objectives of the study were to measure and evaluate changes in chemical composition, coefficient of condition, and body morphology for juvenile steelhead trout from pre-migratory through post-migratory periods.

Winter-run yearling steelhead trout from Big Creek and the Sandy River were used for the study. The eggs were incubated and fish reared under identical conditions at the Oregon Game Commission's Gnat Creek Hatchery. Prior to sampling the fish were starved 24-36 hours. Fork length and standard length were taken prior to fixation and the coefficient of condition was computed from these measurements.

Each month fish were selected for proximate analysis from two size categories, those which were migrant size (16 and 19 cm in fork length) and those which were not (13 cm fork length). Size categories for migrants and non-migrants were based on length frequencies of migrants on the Sandy River. Three sub-samples ($>$ 3 fish on each sample) were taken for each size group and the results of the three were averaged. All chemical analyses were made on samples of homogenate according to approved methods of the Association of Agricultural Chemists.

The study of relative growth of certain external body parts was based on specimens from Big Creek stock collected at the hatchery from February, 1964 through July, 1964. Monthly collections consisted of 10 fish in each available one centimeter size group. Nine morphological measurements were made on each specimen. All measurements were made to the nearest millimeter.

Results from proximate analysis showed that the amount of moisture and ash remained relatively constant for the three size groups (13, 16, and 19 cm) throughout the sampling period. The amount of protein remained constant for the 13 cm group whereas the 16 and 19 cm groups showed a slight decline during the migratory period (May). Lipid showed a more pronounced decrease for the 16 and 19 cm groups during the migratory period. Lipid and protein increased in the post-migratory period (June-July). Hatchery and native migrants were captured migrating downstream and chemical composition was similar for both groups.

A stable coefficient of condition (1.000-1.020) was observed for the non-migrant groups throughout the sampling period. The migrant-sized animals displaced a reduction in condition from 1.000 in March to 0.890 in May followed by an increase to 0.980 in July.

The morphology study showed that migrant-sized animals had a decrease in body and caudal peduncle depth during the migratory period, whereas non-migrants did not. Smaller non-migrants tended to have larger heads than migrant-sized animals. The other morphological characters did not differ greatly for the two groups of fish.

Changes in lipid levels, body depth, and coefficient of condition are closely related and appear to be good indices of smolting in winter steelhead reared at Gnat Creek Hatchery.

VARIATION IN QUALITY OF CHINOOK SALMON EGGS AND FRY

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During the 1968 spawning season at the Salmon-Cultural Laboratory, 350 female chinook salmon were spawned individually and the eggs held in separate incubator trays for the entire incubation period. In an effort to improve chinook fingerling quality, selection of individual lots of eggs was made on the basis of egg size and egg and fry survival. The progeny of 230 females were selected for rearing and those of 120 females were rejected.

Great variation in the quality of eggs and fry was evident in the 350 lots and the experiment pointed out the advantages of holding the eggs of individual females separately for ease in selection. First, egg size varied from 79 to 183 per displacement ounce and the eggs of 32 females smaller than 130 per ounce were rejected. Second, egg and fry mortality varied from 0.6% to 100%. By eliminating all lots of eggs with a green egg mortality greater than 5%, about 30% of the total mortality was found to be sustained by less than 8% of the females. Third, by further eliminating all lots with total egg and fry mortality over 10%, the total mortality of the select was 3.7% as contrasted to 19.4% for the rejected lots.

It appears that whenever possible, salmon should be spawned individually and the eggs held separately. A considerable saving in the manpower required for egg picking would be realized if obviously bad lots of eggs were eliminated. An improvement in the quality of the fish produced by the retention of only high quality eggs appears likely.

RESULTS OF SELECTIVE BREEDING OF RAINBOW TROUT AT ROARING RIVER HATCHERY

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The brood fish program at Roaring River Hatchery is aimed at improving the present strain of rainbow trout both genetically and economically.

Location and History. Roaring River Hatchery, started in 1924, is presently the major egg-taking station for fall rainbow trout in Oregon. It is located in the west drainage of the Cascades, 18 miles east of Albany.

The first record of rainbow brood stock at the hatchery indicates that the fish were obtained in March, 1937 from W. S. Meader Trout Farm in Idaho. Eggs were first taken from brood fish at Roaring River in 1940. Production records for the past 28 years indicate that the station is well adapted to rainbow egg production.

Objectives. The selective breeding program started at Roaring River Hatchery approximately eight years ago with the following objectives in mind.

1. To increase number and size of eggs per female.
2. To improve color, spotting, and body conformation of fish.
3. To select for three-year-old spawners in order to promote maximum growth prior to maturity.
4. To select for more productive fish by ridding population of barren fish.
5. To develop a faster growing fish.
6. To develop disease-resistance in the stock.
7. To increase the productive ability of the male stock.
8. To determine at what age production declines in both male and female.

Methods. Some early selective work was carried on from 1944 to 1956, but no concerted effort was made from 1958 through 1961.

From old production records, a minimum size and weight for fish to be selected was established. Only those females (all ages) producing in excess of 2,500 large eggs were saved for brood stock. Other qualifications consisted of deep blue and green body coloration, proper spotting above lateral line, firm flesh, and suitable body conformation.

Using the above criteria, brood fish were selected in 1962 from a group of fish (1959 brood year) that had originally been hand graded from fast growing production fish at the hatchery.

All fish were sorted at two years of age to remove two-year mature spawners. Barren or non-productive fish at three years were also eliminated.

Eggs from not less than 100 females were selected. Fish from these eggs were graded down to 100,000 to be kept for yearlings. From the yearlings, 20,000 of the fastest growing fish were saved. At sixteen months and again at two years, the fish were hand-selected to remove any mature spawners or off-colored fish. At spawning time the brood fish were once more hand-graded for size, quality and general health.

Brood fish were terminated when production decreased.

The feeding rate was stabilized at 1.5 percent of body weight after the fish reached two years of age.

Progress. Average egg production per female from 1959 through 1968 has increased approximately 165 percent in three-year-old fish, 206 percent in four-year-old fish, and more than 350 percent in five-year-old fish. Table 1 shows the increase for each progeny year.

Table 1. Increase in Fecundity among Roaring River Hatchery Broodstock.

	Average Number of Eggs Per Female			
Age Class	1959	1963	1966	1968
Three	1,800	3,050	4,250	4,800
Four	2,450 1/	3,800	5,600	7,500
Five	2,400	3,500	6,100	10,960
Six	3,100 2/	4,250	5,500 2/	

1/ Records started 1960.
2/ Six year brood discontinued after 1967 - poor production and quality.

Egg size has not changed to any great extent in the three-year-old spawners. Four and five year females show some size increase in their eggs. Table 2 depicts increases by year for each age group.

Table 2. Increase in Egg Size of Female Rainbow Trout at Roaring River Hatchery from 1962-1968.

Age Class	Average Number of Eggs Per Female						
	62	63	64	65	66	67	68
Three	335	-	330	332	335	320	330
Four	290	250	-	275	251	245	226
Five	-	240	235	-	216	234	209
Six	-	200	200	200	-	200	-

Gains in growth over the past six years have been good. Whereas the brood fish averaged 18 inches in 1962, they presently range from approximately 21 to 27 inches (fork length). Table 3 shows the gain in length and pounds from 1962 to 1968.

Table 3. Increase in Length and Weight of Broodstock at Roaring River Hatchery from 1962-1968.

Age Class		Average Length in Inches and Weight in Pounds						
		62	63	64	65	66	67	68
Three	Inches	17.5		21.5	19.5	22.5	21.5	22.3
	pounds	3.5			4.5	5.0	5.0	6.0
Four	"		19.5		22.5	24.0	25.5	25.6
	"			7.5		7.5	12.0	10.6
Five	"	18.3		23.5		25.0	26.5	28.6
	"	5.5				9.0	10.3	15.3
Six	"	18.3	24.3	23.5	24.3		27.0	
	"	6.0				9.5	10.0	

Color and body conformation has improved from the acceptable level of only 40 percent of the brood fish in 1962 to a high of 95 percent of the fish in 1968.

In 1962, 20 percent of the two-year-old fish matured and 5 percent of the four-year-old and older fish were barren or non-productive. In 1968, only 2 percent of the stock matured at two years, while barren and non-productive fish in the older stock numbered less than 1 percent.

The effects of selective breeding on disease resistance of broodstocks and production fish have not yet been determined. It has been found, however, that brood fish lose condition after their sixth year and eggs are sometimes of poor quality. This may be related to diet.

From the limited data available, it appears that selection may provide some benefit for the offspring making up the production fish at Roaring River Hatchery. Table 4 compares the growth and conversion of yearling trout from 1961-1968.

Table 4. Results of Selective Breeding on Succeeding Generations at Roaring River Hatchery.

Periods	Number Days Fed	Fish Per Pound	Food Conversion
1961-62	401	4.7	1.93
1963-64	382[1/]	4.7	1.81
1965-66	403	3.0	1.84
1967-68	378	3.2	1.64

1/ Oak Springs Hatchery fingerlings.

Summary. The past eight years of selective breeding of fall rainbow at the Roaring River Hatchery has demonstrated that egg production can be tripled without a decrease in egg size.

Lengths and weights of brood fish can be increased, while color and body conformation can be improved.

Progeny of selected fish grow faster and are more efficient in food conversion.

EFFECT OF YOLK SAC ABSORPTION ON THE SWIMMING ABILITY OF FALL CHINOOK SALMON

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Tests were made during the winters of 1965-66 and 1966-67 of the swimming ability of fall chinook salmon (Oncorhynchus tshawytscha) during and after yolk sac absorption. The reason for the initial tests was to obtain swimming speed information for small fish to be used in the design of fish collecting facilities, particularly at the Tehama-Colusa Spawning Channel in California.

Two sources of fish were used: hatchery fish incubated and reared in constant-temperature well water (53° F) and migrants from the Abernathy Incubation Channel supplied with creek water of variable temperature. A number of authors have shown that a straight line relationship exists between the water temperature and the rate of yolk sac absorption. This relationship enabled comparisons between groups of fish by the amount of yolk sac present at each testing period.

The 1965-66 tests measured the ability of fish to escape impingement on a fixed screen placed at a 90 degree angle to flow. Individual tests involved 50-fish samples submitted to a constant water velocity for 20 minutes. Velocities used varied from 0.35 to 1.10 fps.

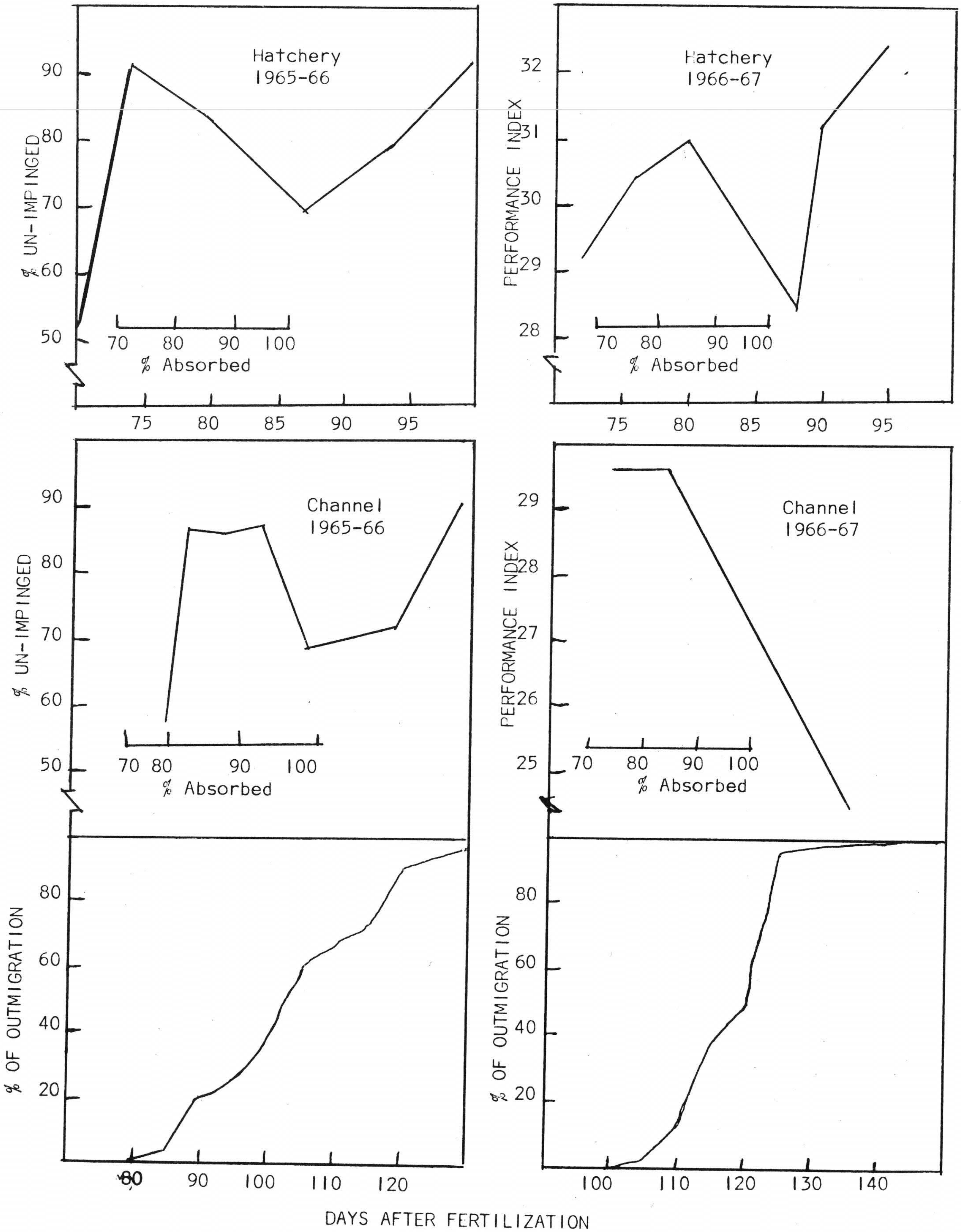
The testing period extended from when about 60 percent of the yolk sac was absorbed until about 3 weeks after absorption. In all tests, a slump or period of reduced swimming ability occurred shortly before complete sac absorption with the lowest point occurring just after complete absorption.

The 1966-67 tests were designed to more closely evaluate the period of reduced swimming performance and involved a stamina tunnel testing procedure developed for the first or lowest gear ratio. Each testing period involved 5 tests of 100 fish each from the hatchery source and 3 tests each from the channel. Reductions in swimming ability were found at about the same percentage of yolk sac absorption as in the impingement studies. The performance index calculated from the tests normally increases with growth in tests with larger fish. Recovery after complete sac absorption was not demonstrated in the channel fish due to insufficient numbers of migrating fish for testing. However, the few fish migrating at this time had obvious stamina improvement.

The figure presented shows results of the 1965-66 impingement tests at 0.8 fps and the 1966-67 performance index tests using the stamina tunnel first gear procedure. Effects of colder water temperature during the 1956-66 channel tests is shown in the slower rate of yolk sac absorption after 85 percent absorption was reached.

A decrease in swimming ability during yolk sac absorption may be of little importance in the survival of hatchery fish which are normally reared under protected conditions to a much larger size before being released. However, during both years of testing, the lowered performance coincided with the period of peak migration from the channel. This reduced capacity may be an important factor in the time of migration and the survival of migrants from channels.

EFFECT OF YOLK SAC ABSORPTION ON THE SWIMMING ABILITY OF FALL CHINOOK SALMON



ADVANCES IN THE CRYO-PRESERVATION OF SALMONID SPERM[1]

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INTRODUCTION

Since 1853, biologists have studied and attempted to preserve viable fish sperm (de Quatrefages, 1853; Ellis and Jones, 1939; Rucker, 1949; Sneed and Clemens, 1956; Mitchum, 1963). The most notable success was achieved by Blaxter (1953) who froze the sperm of herring (Clupea harengus) in 80% sea water and 12.5% glycerol at -79 C (dry ice). The sperm was thawed six months later and used to fertilize 80 and 85% of fresh herring eggs. Until recently, attempts at cryo-preservation[2] of salmonid gametes have failed or yielded inconclusive results. This paper summarizes our research in cryogenics which has resulted in 59% fertilization of steelhead trout (Salmo gairdneri) eggs with sperm frozen at -196 C (liquid nitrogen). Current research of other investigators is discussed briefly.

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[1]Supported by funds from the U. S. DEPARTMENT OF THE INTERIOR, Bureau of Commercial Fisheries.

[2]Cryo-: From the Greek "kryos" meaning cold or frost; cryogenics referring to the science that deals with the production of very low temperatures and their effects on the properties of matter.

Our investigations began in July, 1966, with the principal objective of developing methodology for the cryo-preservation of salmonid sperm. To date we have developed: two methods for collecting semen; standard tests of viability of spermatozoa; a best rate of freezing and thawing spermatozoa; and an extender[3] and protector[4] which have given encouraging results. Details of the above techniques have been reported by Horton, Graybill and Wu (1967), Graybill (1968) and Graybill and Horton (1969).

The benefits to fishery biology that would result from the successful development (90+% fertilization) of cryo-preservation techniques for fish sperm include: (1) A reduction in hatchery costs, since few males would need to be reared (in a system of hatcheries) and their sperm could be stored for several years. (2) Species of fish maturing at different seasons of the year could be crossbred. (3) Facilitation of disease control by making it convenient to cross disease susceptible stocks with those having an inherent resistance to the pathogen. (4) Reduction of the genetic variability in research by using the stored semen from a single male to fertilize the eggs of a series of females spaced over time. (5) Provision of a genetic tool for selective breeding, i.e., father to generations of daughters, or "odd" to "even" year runs of pink salmon (Oncorhynchus gorbuscha).

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[3]Extender: A solution of salts, sometimes including organic compounds which help maintain the viability of cells during refrigeration.

[4]Protector: An organic compound which protects the viability of cells during the freezing and thawing processes.

Pertinent literature on the cryo-preservation of salmonid sperm is sparce. Mitchum (1963) attempted unsuccessfully to store the sperm of rainbow trout (Salmo gairdneri) in Ringer's solution, glycerated yolk-citrate, skimmed milk and homogenized milk extenders using dry ice or liquid nitrogen (LN_2) refrigerants. Hodgins and Ridgway (1964) recovered motile and morphologically intact chinook salmon (Oncorhynchus tshawytscha) and pink salmon spermatozoa which had been frozen 7 days with LN_2 in a citrate-dextrose-sodium chloride solution containing dimethyl sulfoxide (DMSO). Sperm motility was low and the fertilizing ability of the thawed spermatozoa was not tested. The first successful fertilization of salmonid eggs with cryo-preserved sperm was reported by Graybill (1968). He obtained up to 18% fertilization of steelhead trout eggs with sperm extended and protected with a modified Cortland salt solution containing 5 or 12.5% DMSO and frozen in the vapor of LN_2 (-196 C). Fertilization (to 12%) of Atlantic salmon (Salmo salar) eggs with sperm frozen ½-hr at -25 C was reported by Hoyle and Idler (1968). They used a modified Cortland medium containing 2.5% lactose with 5% ethylene glycol.

METHODS

Salmonids were obtained at state and federal fish hatcheries in Oregon (see Acknowledgements). Mature salmon were killed, whereas mature trout were anesthetized in M.S. 222 (tricaine methanesulfonate). Semen was collected by either the dual tube suction device described by Graybill and Horton (1969) or by manual stripping. With the latter technique, the area of the genital pore was cleaned and wiped dry, and a small quantity of semen was expressed as waste before the experimental sample was collected into sterilized test tubes. The test tubes were filled about one half full of semen, stoppered and immersed in ice water (4 C).

At the laboratory, the semen was mixed 1:4 (in progressive steps) with an extender equilibrated to 4 C and containing either 9.6 or 12.8% DMSO (the protector). The extended and protected semen was placed in 1 ml glass ampoules, sealed by heat,[4] and frozen at a rate of 1 C/min[5] or 30 C/min (LN_2 vapor). The ampoules of frozen semen were stored in a LN_2 refrigerator.[6]

Determinations of percent motility[7] and percent relative activity[8] of spermatozoa were made to obtain an estimate of viability of thawed gametes. For all practical purposes, however, the only useful measure of viability is the ability of spermatozoa to fertilize eggs.

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[4]Ampoule Sealer, Model 161, Kahlenberg-Globe Equipment Co., Box 2803, Sarasota, Florida.

[5]Freezing Chamber, Model BF-4B, Union Carbide Corp., 2770 Leonis Blvd., Los Angeles, California.

[6]Biostat, Model 1000-X, Cryogenic Engineering Co., 4955 Bannock St., Denver, Colorado.

[7]Percent motility: An estimate of the percent of cells observed in a sample that were motile.

[8]Percent relative activity: The degree of activity of motile cells relative to the highest degree of motility ever observed (100%).

To test the fertilizing capacity of cryo-preserved semen, fresh eggs were collected from incised salmon or stripped from anesthetized trout. Ampoules of frozen semen were thawed in a 4 C water bath and mixed immediately with about 150 fresh eggs. Fresh semen was used as a control. Eggs were then incubated to the eyed stage, shocked (Leitritz, 1959) and examined for percent fertilization.

RESULTS

Tests were conducted to improve on our Extender 48 (Graybill, 1968; and Graybill and Horton, 1969), which was developed from the Cortland salt solution (Wolf, 1963). Based on our experimental results, the concentration of sodium bicarbonate ($NaHCO_3$) was set at 500 mg/100 ml (Fig. 1). The minimum effective concentration of lecithin was determined to be 750 mg/100 ml (Fig. 2). Fructose was used in place of glucose at a concentration of 100 mg/100 ml, the minimum quantity tested (Fig. 3). Mannitol, an osmotic antagonist of DMSO (Barner, 1965), was added to Extender 48 at a concentration of 100 mg/100 ml (Fig. 4). A comparison of the chemical components of the Cortland salt solution to our modified Extender 48 is presented in Table 1.

Sperm frozen in Extender 48 in combination with two concentrations of protector (DMSO) and two rates of freeze were used to fertilize fresh eggs of steelhead trout. Results of this experiment indicated that rapid freezing of sperm in Extender 48 containing 12.8% DMSO was superior to slow freezing in Extender 48 containing 9.6% DMSO (Table 2). The 30.2 and 59.1% fertilization in two lots indicated that our overall techniques were improved over our earlier efforts

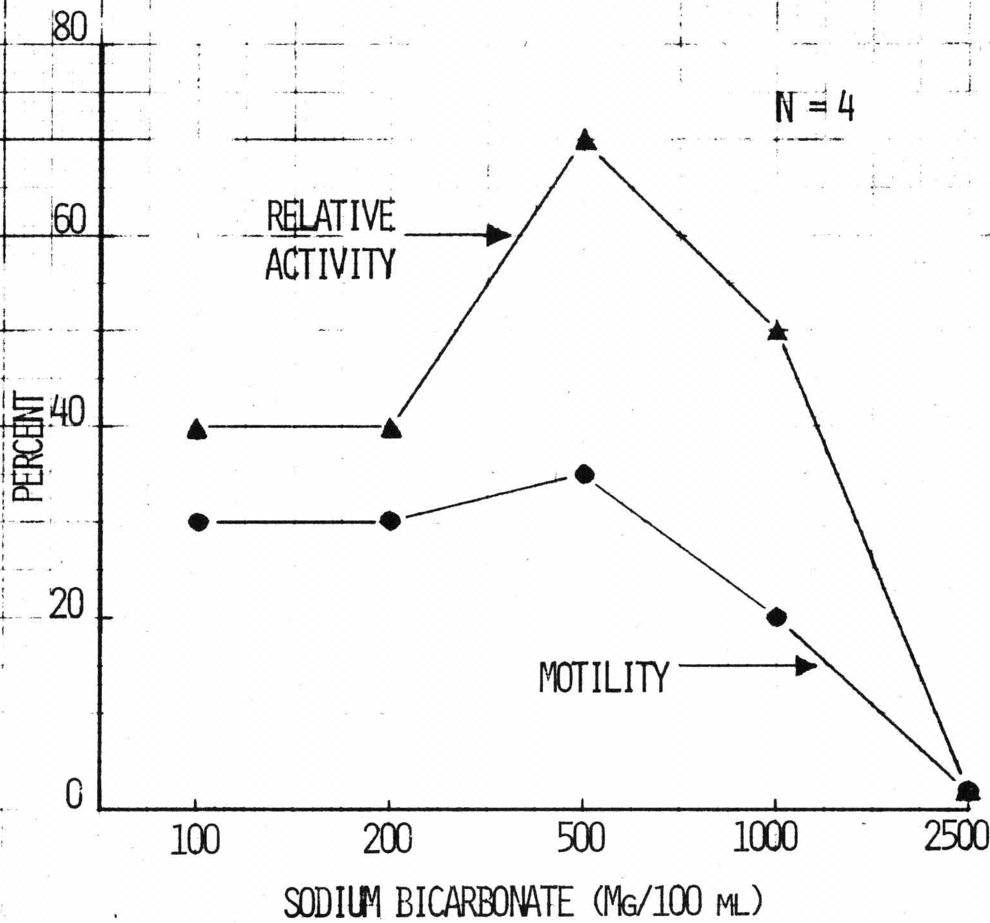

FIGURE 1. EFFECT OF VARIOUS CONCENTRATIONS OF SODIUM BICARBONATE ($NaHCO_3$) ON THE PERCENT MOTILITY AND PERCENT RELATIVE ACTIVITY OF THAWED STEELHEAD TROUT SPERM.

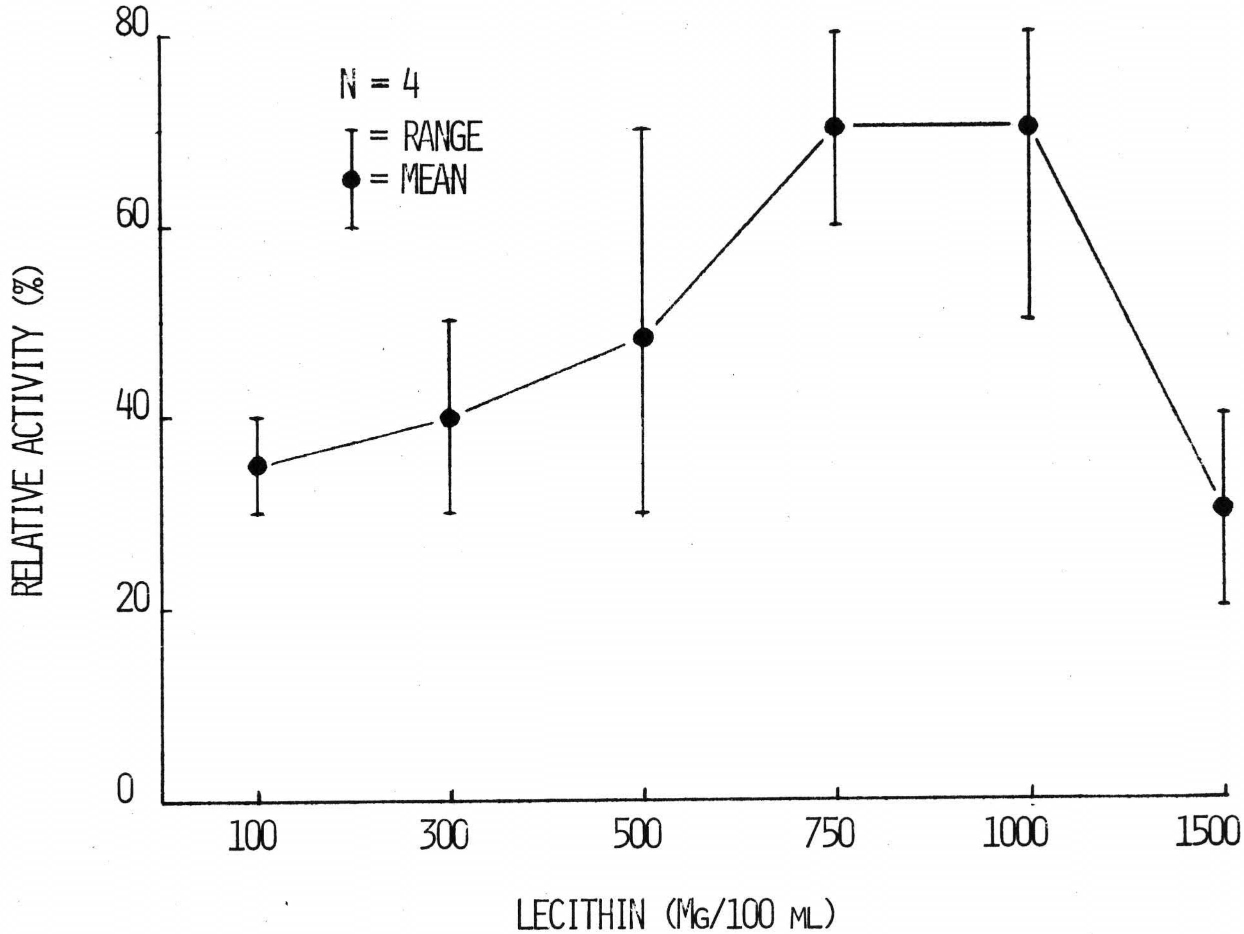

FIGURE 2. THE EFFECT OF VARIOUS CONCENTRATIONS OF LECITHIN ON THE PERCENT RELATIVE ACTIVITY OF THAWED STEELHEAD TROUT SPERM.

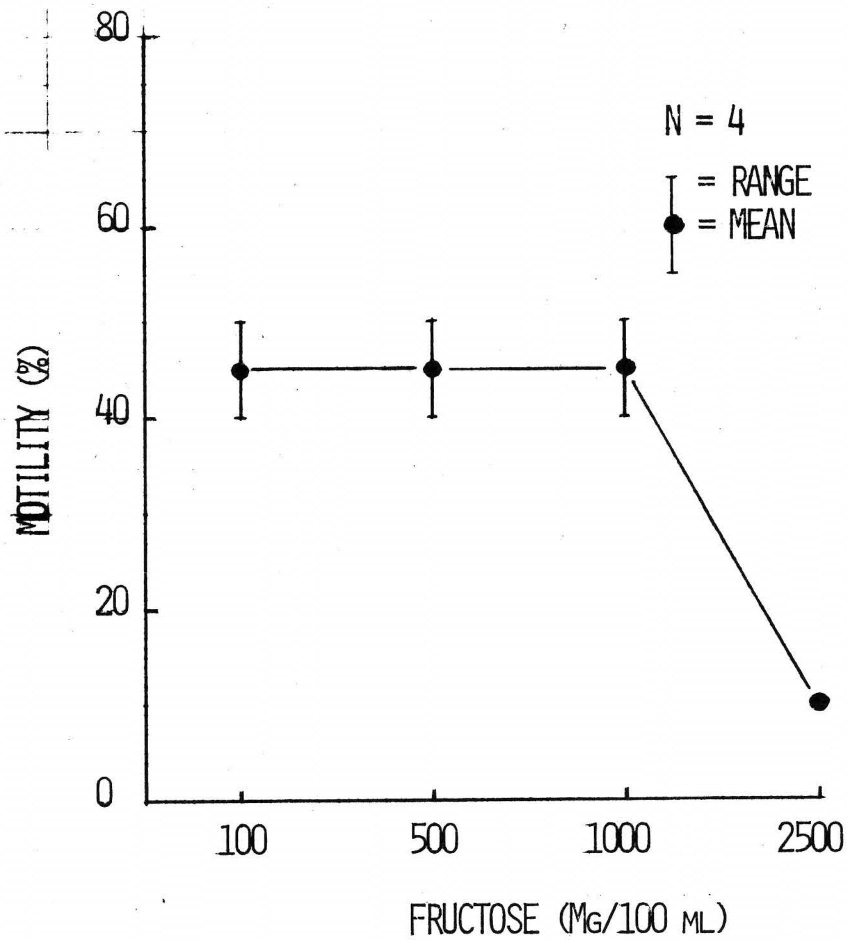

FIGURE 3. THE EFFECT OF VARIOUS CONCENTRATIONS OF FRUCTOSE ON THE PERCENT MOTILITY OF THAWED STEELHEAD TROUT SPERM.

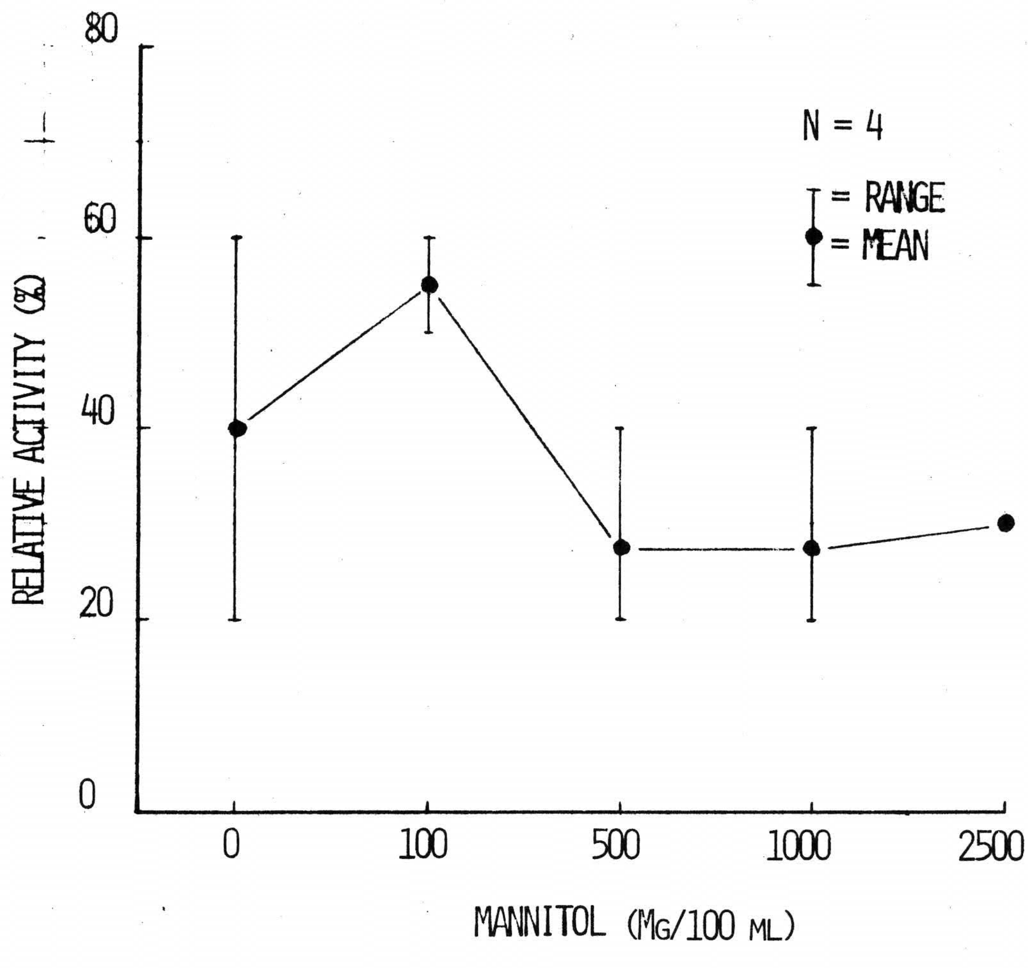

FIGURE 4. THE EFFECT OF VARIOUS CONCENTRATIONS OF MANNITOL ON THE PERCENT RELATIVE ACTIVITY OF THAWED STEELHEAD TROUT SPERM.

TABLE 1. MODIFIED EXTENDER 48 COMPARED TO THE CORTLAND SALT SOLUTION.

CHEMICAL COMPONENT	CORTLAND SALT SOLUTION (MG/100 ML)	EXTENDER 48 (MG/100 ML)
$NaCl$	725	730
$CaCl_2 \cdot 2H_2O$	23	23
KCl	38	38
$NaH_2PO_4 \cdot H_2O$	41	41
$NaHCO_3$	100	500
$MgSO_4 \cdot 7H_2O$	23	23
GLUCOSE	100	---
FRUCTOSE	---	100
LECITHIN	---	750
MANNITOL	---	100

Table 2. Percent fertilization of steelhead eggs with cryo-preserved sperm, 5/7/68.

Extender Number	Protector (Percent)	Freeze Rate	Days Sperm Frozen	Number of Eggs	Percent Fertilization
48	DMSO 9.6	-1 C/min	4	132	0.0
48	DMSO 9.6	-1 C/min	4	157	0.0
48	DMSO 9.6	-1 C/min	4	182	0.0
48	DMSO 9.6	-1 C/min	4	122	0.0
48	DMSO 9.6	-30 C/min	4	50	2.0
48	DMSO 9.6	-30 C/min	4	110	0.9
48	DMSO 9.6	-30 C/min	4	110	0.0
48	DMSO 9.6	-30 C/min	4	138	0.0
48	DMSO 9.6	-30 C/min	4	52	0.0
48	DMSO 12.8	-30 C/min	7	173	16.8
48	DMSO 12.8	-30 C/min	7	154	10.4
48	DMSO 12.8	-30 C/min	7	120	10.8
48	DMSO 12.8	-30 C/min	4	152	8.6
48	DMSO 12.8	-30 C/min	4	116	30.2
48	DMSO 12.8	-30 C/min	4	110	59.1
Control	---------	---------	0	84	100.0

(Graybill and Horton, 1969).

Varying performance (percent motility and percent relative activity) of sperm between different males of the same species suggested a difference in quality of semen. To test the effect of time after first maturation on the vitality of sperm, semen was collected from spring chinook salmon at varying intervals, stored in vials at 4 C and used to fertilize fresh eggs from a single female on the same day. The results indicated that while percent motility of sperm was initially high for all samples, the apparent vigor of sperm collected later in the season diminished more rapidly than that of sperm collected when the fish first became mature. Furthermore, the percent fertilization of eggs was higher for early collected sperm than for gametes collected 5 or 10 days later (Fig. 5).

DISCUSSION

Lecithin was included in our Extender 48 because as a lipid it is thought to be an important source of intracellular energy for sperm motility (Gregory, 1968). Fructose was used in place of glucose in Extender 48 because fructose is the naturally occurring sugar in the seminal plasma of cutthroat (Salmo clarkii **[sic]**) and brook (Salvelinus fontinalis) trout (Gregory, 1968).

Other investigators are studying the chemical components of seminal fluids of fishes as guides for the preparation of extenders. Cruea[9] has determined the concentrations of components found in the

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[9] D. D. Cruea. Personal communication, September 24, 1968.

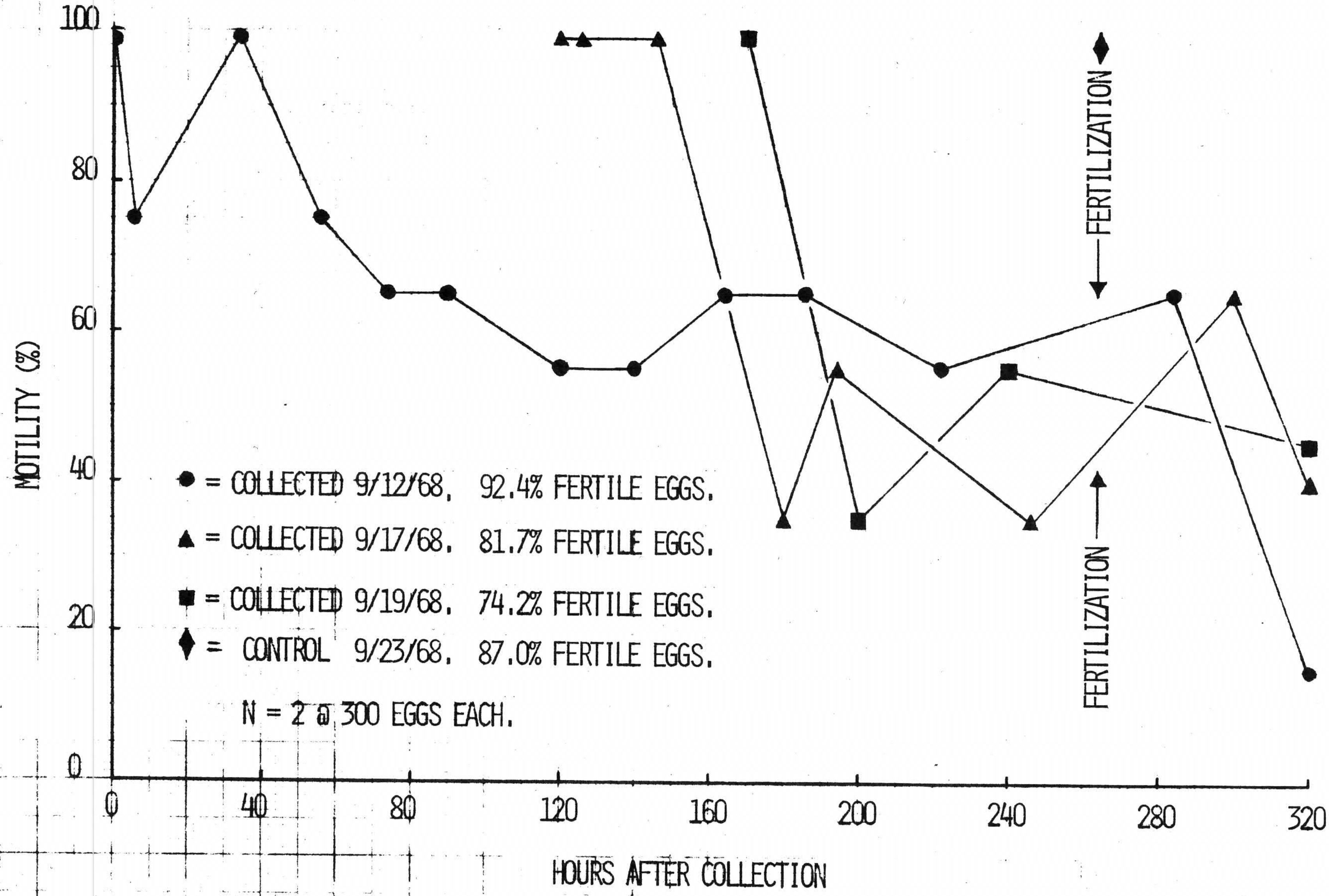

FIGURE 5. THE EFFECT OF COLLECTION DATE AND STORAGE TIME ON THE PERCENT MOTILITY AND FERTILIZING CAPACITY OF CHINOOK SALMON EGGS HELD AT 4 C.

seminal fluids of cutthroat and rainbow trout, carp (Cyprinus carpio) and northern pike (Esox lucius). Hwang and Idler (1969) have studied the seminal components of Atlantic salmon.

The long term storage of chilled, but not frozen, semen offers many practical uses. In our work we have obtained 92.4% fertilization of fresh chinook salmon eggs with undiluted sperm held 11 days at 4 C. Truscott, et al. (1968) held semen of Atlantic salmon in a modified Cortland medium with 5% DMSO for 28 days at -4.5C (unfrozen) with 81% fertilization resulting when the sperm was added to fresh eggs.

We plan to continue our search for a freezing and thawing technique that will yield viable sperm capable of fertilizing 90+% of fresh salmonid eggs. In our quest, we will look closely at sperm quality, preferred thawing and fertilization temperatures and possible new components for our extender. We believe that the practical application of cryogenics in fishery biology is imminent.

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The Status of Oral Immunization of Juvenile Coho Salmon Against Furunculosis

by

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Following the synthesis of a promising immunizing agent against furunculosis in 1965, several attempts have been made to prove its efficacy in the production hatchery. This report is a summary of the trials conducted at coho salmon hatcheries in Oregon and Washington.

The 1966 trials at Issaquah, WDF and Siletz, OFC hatcheries were very heartening. Good immunity against furunculosis was indicated. At Issaquah the initial outbreak occurred about 10 days after the last feeding of FSA. At Siletz, the FSA was fed at low levels through the first weeks of the outbreak.

The 1967 trials were disappointing. From follow-up studies we deduced that the commercially prepared FSA consisted of particles too large to cross the gut wall of the fish -- an essential requirement of an oral vaccine. Also, the follow-up studies provided evidence that we must take strain differences in the organism into account. For example, Issaquah FSA will not protect Quilcene fish.

The 1968 trials started out to be the biggest ever. There were 4.75 million coho salmon plus an equal number of controls at eight national and state hatcheries in the study. The FSA had been carefully prepared and tested antigenically, physically, and chemically. As they say at Cape Kennedy -- "All systems are GO" -- we thought!

Of the eight hatcheries in this year's trial, three had no detectable furunculosis. One had a rather severe mixture of bacterial gill disease and furunculosis so the efficacy of the FSA was obscure. Of the four having outbreaks of furunculosis, only one had a weekly mortality in excess of 1.0%. And in no case was there any indication of protection even though circulating antibodies were detected prior to the start of the outbreak.

It puzzled us that the FSA checked out so closely to the original FSA we used in 1966 and that antibodies were detected in the fish fed the FSA but there was no evidence of protection. When all the data were plotted one glaring variable was not controlled -- we had fed the FSA in the early stages of the outbreaks.

In 1964, during a redmouth disease vaccination study at Hagerman NFH, we found that if immune fish were challenged shortly after being fed the vaccine they were not protected against the disease. Many authors have recorded this phenomenon in mammals and have called it "Immune paralysis"-- some have called it "interference". What is thought to have occurred is the tying up of the available antibody by the vaccine thereby leaving none to inactivate the infecting organism. This effect is reported to last for a few hours to days.

We have no definite proof that immune paralysis occurred in this year's FSA trials -- but we do have enough evidence to warrant a trial to prove or disprove its effect. We are also investigating the possibility that there has been antigenic alteration of the commercially made FSA through loss of virulence of the organism.

So, we stand a little further along than we did last year. We still firmly believe that oral immunization will work -- just when, we cannot say.

PROLONGED FORMALIN TREATMENT FOR FISH PARASITES

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Formalin is usually recommended as one method of treating for external parasites without moving the fish from the pond. Treatments of from 1:4000 to 1:6000 for one hour is the usual dosage. There always seems to be the exception where either the species of fish or the hatchery water supply does not lend itself to this treatment with a resulting heavy loss of fish.

Ray Allison reported in the PROGRESSIVE FISH CULTURIST, Volume 19, April, 1957, the use of Formalin in the treatment of fingerling speckled bullhead (*Ameiurus nebulosus marmoratus*) that were heavily infested with Gyrodactylus. He used concentrations of 5, 10, and 15 ppm Formalin for a 72 hour period with an average reduction of infestation of 92 percent to 99 percent.

Wayne A. Willford of the Fish Control Laboratory, Bureau of Sport Fisheries and Wildlife, LaCrosse, Wisconsin, reports in INVESTIGATIONS IN FISH CONTROL, Report No. 18, that among 22 therapeutic chemicals of which 16 were toxic to fish, Formalin rated along with Nickel Sulfate and Quinacrine Hydrochloride as the least toxic with a safety margin of at least sixfold at recommended use levels over a 48 hour period.

With this information in mind, we set up experiments using winter steelhead (*Salmo gairdneri*) in standard hatchery troughs with 50 fish to a basket and a water flow of 10 gpm. Samples were taken to verify that the fish were infected with Gyrodactylus. Formalin was mixed with water to give a stock solution more readily metered into the trough. A constant flow syphon was used to meter the stock solution into the trough at 13.5 cc per minute. At the end of each experiment, the fish were placed in fresh water and held for observation. Water temperatures ranged from 44^{o} F to 47^{o} F with a pH of 6.5 and water hardness of 34 ppm.

Concentrations of 5, 10, 15, 30, and 50 ppm were tested. At the end of each experiment, samples were taken in the control fish to verify that parasites were still present. Each experiment was repeated three times.

Parasites were still present at the end of the 72 hour treatment in both the 5 and 10 ppm groups but checks 10 days later showed the fish to be free of parasites. In one of the 15 ppm groups there were parasites present at the end of the 72 hours but none were found three days later.

On the first tests of the 30 and 50 ppm groups, the first check for parasites was made at 18 and 19 hours and both proved free of parasites. On the additional groups, checks were made every two hours. Indications are that Gyrodactylus can be killed at concentrations of 30 ppm Formalin in eight hours with at least 72 hours without danger of killing fish. Fifty ppm Formalin will rid fish of Gyrodactylus within six to eight hours but the danger of fish loss is considerably greater. The first losses in the 50 ppm groups started showing at 18 to 20 hours. One interesting observation was that there was only one instance of a delayed loss and this occurred in one of the 30 ppm groups. This particular fish was showing signs of distress at about 60 hours of treatment. At the end of 72 hours, it was placed in fresh water but did not die for another 18 hours.

In summary, I feel that either the 15 ppm treatment for 72 hours or the 30 ppm treatment for eight hours is very satisfactory in treatment of Gyrodactylus.

A word of caution--every hatcheryman should run a sample test before treating an entire pond of fish.

	Hours of Treatment					
	24		48		72	
PPM	Parasites Present	Fish Loss	Parasites Present	Fish Loss	Parasites Present	Fish Loss
	p	0	p	0	p	0
5	p	0	p	0	p	0
	p	0	p	0	p	0
	p	0	p	0	p	0
10	p	0	p	0	p	0
	p	0	p	0	p	0
	p	0	0	0	0	0
15	p	0	p	0	p	0
	p	0	p	0	0	0
	0	0	0	0	0	0
30	0	0	0	0	0	0
	0	0	0	0	0	0
	0	4	0	2	-------	-------
50	0	3	0	6	0	1
	0	2	0	4	0	1

Experimental Control of Columnaris Disease with a New Nitrofuran Drug (P-7138)

by

Donald F. Amend and Avron J. Ross

ABSTRACT

P-7138 is a new vinylogous nitrofuran drug especially developed for treating fish diseases. Using coho salmon (*Oncorhynchus kisutch*), we tested the drug to determine: (1) the efficacy in controlling experimentally induced columnaris disease (*Chondrococcus columnaris*), (2) the tissue retention rates, and (3) to test the toxicity. P-7138 effectively controlled the disease with a single 1-hour treatment at 1.0 ppm or with two 1-hour treatments 24 hours apart at 0.5 ppm. Treatments at 0.25 ppm were ineffective. The drug was eliminated from most tissues in less than 24 hours, from all tissues within 48 hours, and it was not toxic. The advantages in using this drug are discussed.

CONTROL OF ICHTHYOPHTHIRIUS UNDER UNFAVORABLE CONDITIONS AT OAK SPRINGS HATCHERY

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The Oak Springs Hatchery, located on the Deschutes River near Maupin, is a spring fed, constant temperature station where diseases and parasites are generally not a problem. In early October of 1966, however, an outbreak of "Ich" was discovered in the summer steelhead yearlings.

The fish were immediately thinned from two into three ponds and later into a fourth pond to be treated with formalin. Treatment began on October 6 and continued daily through November 25, except for six days when the fish were not treated.

Formalin was sprayed into the pond with a pond pump (equipped with a fine nozzle) in five to ten minutes, with the treatment time beginning after the formalin was introduced. The pumping was continued for circulation and aeration for forty-five minutes. Treatment was accomplished using 100 pounds or more of water per pound of fish in the pond. At the end of the treatment, each pond was flushed while maintaining the water level at a depth of approximately one foot. The ponds measured 40 feet wide by 50 feet long by 6 feet deep and had one outlet in one corner. The shape of the pond resulted in a circular water pattern even at low water levels.

Each pond was treated the first day with formalin at a concentration of 1:6000 for one hour. The concentration was decreased by 500 parts of water each following day until a concentration of 1:4000 was attained on the fifth day of treatment. As formalin had not been used previously in Oak Springs Hatchery water, it was deemed advisable to proceed cautiously until it could be determined that the fish would tolerate the recommended concentration of 1:4000.

Five days after the formalin treatment began, large lesions up to 3/4-inch in diameter appeared on the sides of the fish, generally in an area between the dorsal fin and the tail section. Material from these lesions was examined microscopically, cultered, and found to contain numerous Aeromonas bacteria. Sulfamerazine, at the rate of 5 grams per 100 pounds of fish, was then introduced into the diet. Subsequent examination after the treatment revealed that many fish with small lesions had recovered.

On October 27, twenty-two days after the first formalin treatment, examination of a few fish indicated that an effective kill of "Ich" was not being accomplished. The length of the treatment was then increased to one hour and fifteen minutes at a concentration of 1:4000. This concentration was applied through November 25, at which time the "Ich" infestation had been eliminated.

During the forty-four days of treatment, the larger group of steelhead fingerling increased from 58 to 43 fish per pound and the smaller fingerling from 82 to 65 fish per pound. Mortality amounted to 23,000 or 13.1 percent of the number of fish treated (175,000) in October and 3,000 fingerling or 1.9 percent of 152,000 fish treated in November.

The above data indicates that large steelhead fingerlings can be safely treated daily with formalin for prolonged periods of time at the Oak Springs Hatchery.

While the steelhead were undergoing treatment, rainbow fingerlings in an adjacent pond to the infected steelhead were examined many times, but "Ich" was not found. The rainbow were nevertheless given preventative treatment with formalin at 1:4000 for four consecutive days in early November and then once a week for the remainder of November.

In early December, the rainbow fingerlings were separated into seven ponds (six adjacent to each other). "Ich" was detected in all of the ponds on January 5. The six ponds in series had small 8-inch outlets requiring a minimum of one hour to drain. Because it appeared risky to use the standard formalin treatment, mal-green was chosen for treatment. The chemical was introduced daily for one hour by the siphon method at a concentration of 1:2½ million. The pond water retained the green dye coloration from six to eight hours each day. Treatment continued from January 6 through January 31. Pond number 7 (40 feet by 50 feet) was treated daily with formalin at a concentration of 1:4000 from January 5-27. The chemical was introduced into the pond with a pond pump and allowed to remain for one hour.

At the end of January, examinations indicated that the rainbow trout in the seven ponds were free of "Ich". The mortality for the month totaled only 200 out of 208,000 fingerlings being treated. The fish continued to grow at a normal rate during the prolonged treatment.

The experiments indicate that mal-green can be used successfully for treating rainbow trout for "Ich" in ponds having poor outflow patterns.

Use of Temperature to Control a Salmonid Virus Disease (IHN)

by

James A. Servizi, Robert W. Mead and Donald F. Amend

ABSTRACT

Elevated water temperature was used to control the Infectious Hematopoietic Necrosis (IHN) virus in sockeye salmon at the International Pacific Salmon Fisheries Commission salmon hatchery near Cultus Lake. In troughs where IHN epizootics occurred, the mortality reached 100 percent if water temperatures were maintained at 50 to 53° F, but if the temperature was raised to 66 to 68° F immediately after symptoms of the disease developed, mortality was held to 9 percent or less. However, if temperature treatment was delayed for 5 days or more, mortality ranged between 50 and 75 percent. Treatment at these elevated temperatures had no apparent adverse effect on the fish.

Laboratory results showed that experimentally induced IHN disease in sockeye salmon could be completely controlled if treatment at 68° F commenced within 24 hours after infection. Partial control was obtained when treatment was delayed up to 3 days, and no control was observed if treatment was delayed for 4 days or more. In addition, it was found that treatment at 68° F must continue for a minimum of 4 days to be effective. Reinfection and subsequent high mortalities occurred

when the fish were re-exposed to the virus immediately following treatment. The fish became progressively resistant to reinfection if re-exposure was delayed for up to 30 days.

We concluded that IHN could be controlled if: (1) treatment between 64 and 68° F was initiated prior to or immediately following appearance of disease symptoms, (2) treatment lasted a minimum of 7 days, and (3) the fish were not re-exposed to the disease following treatment.

BRIEF COMMENTS REGARDING FALL CHINOOK AND COHO SALMON HATCHERY EVALUATION STUDIES--COLUMBIA RIVER

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Comments regarding initial development and progress of the fall chinook salmon hatchery evaluation study have been presented at earlier Northwest Fish Cultural meetings and I assume most of you have a pretty fair knowledge of its operation. Basically, it is a study designed to measure contribution of hatchery-raised fall chinook to the commercial and sport fisheries.

A manuscript covering contribution of the 1961 brood-year fall chinook salmon to the commercial and sport fisheries both in the ocean and the Columbia River is now in Washington for publication. The results of the study are very encouraging. Using calculated production costs at the hatcheries and estimates of contribution resulting from the marking program, it is now estimated that for 12 hatcheries on the Columbia River raising fall chinook salmon, the average benefit-cost ratio is about $2.30 to $1.00. During the period of marking, 1961 through 1964 brood years, 213 million fall chinook were released from the study hatcheries, of which about 31 million were marked. Total estimated value of the catch of 1961 brood fall chinook originating from all Columbia River hatcheries is about $2,000,000.

The sampling season of 1969 will terminate all of the mark-recovery effort. As to ocean distribution of Columbia River fall chinook salmon, greatest numbers of marked fish were taken to the north of the Columbia River. Some were recovered in Alaska and as far south as California. Heaviest concentration of marks was found in the fishery off Vancouver Island. The estimated average catch to escapement ratio for the study hatcheries was about 6:1.

In order to take advantage of the mark-recovery effort underway for fall chinook salmon, an evaluation of hatchery-produced coho salmon from the Columbia River was initiated with brood year 1965. Approximately 10 percent of production of yearling fish at each of 17 hatcheries was marked in 1966 and 1967. Early returns of marked coho showed predominantly in the Washington and in the Columbia River mouth sport fisheries as two-year-olds or jacks and some appeared at the hatcheries. Mark returns of three-year-old coho this year, through the end of July (last reporting date), give some indication of the general distribution of Columbia River coho. As an example, through July 27 the following numbers of marked three-year-old coho were recovered in the troll fisheries: Oregon, 5,583; Washington, 2,199; British Columbia, 403; and California, 1,517--showing that coho of Columbia River origin were taken farther south along the coast than were the fall chinook salmon.

In the course of the fall chinook hatchery evalution study, a separate mark was used to identify some individual hatcheries. In the coho study, however, the 17 stations involved were too numerous to identify individually. Instead, three groupings were made of the hatcheries, one for the stations above Bonneville Dam (upper), and the second for the stations between Bonneville Dam and the Cowlitz River (middle), and the third for the stations from the Cowlitz to the mouth of the Columbia (lower). For some reason as yet unknown, far more marks attributed to the middle portion of the Columbia River have shown in the ocean and Columbia River area (adipose only) as have been attributed to either the upper (Ad-LM) or the lower area (Ad-RM). Releases of fish in the three areas were quite comparable in number. As with the fall chinook hatchery evaluation study, the coho study will be completed also in calendar year 1969.

As with the fall chinook hatchery evaluation study, groups of marked coho have been held to study fin regeneration. A recent examination of these groups of fish revealed that while in fresh water there was little or no regeneration of either the adipose or the maxillary.

One additional interesting fact that has come from the fall chinook hatchery evaluation study is that it now appears a fin-clipping mortality of about 30 percent can be expected when using the fin combination as employed in this study. In our sockeye hatchery evaluation study program at Leavenworth National Fish Hatchery, the mortality due to fin-clipping was estimated to be in the neighborhood of 40 percent.