Harry Wagner

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The reports presented at the Northwest Fish-Cultural Conference held in Longview, Washington, December 4 and 5, 1962, are enclosed. No attempt has been made to edit the summaries presented by the authors except to correct obvious mistakes in spelling. These summaries of the progress reports are not available for publication or citation without the express permission of the individual authors concerned.

There were 187 in registered attendance which is, I believe, the largest of these conferences on record. I was very much pleased with the diversity of the program and the high quality of the reporting. I would like to thank the participants for their excellent cooperation both at the meetings and in promptly submitting their summaries.

Mr. C. H. (Bud) Ellis, Washington Department of Fisheries, is the chairman of the 1963 confererence which, probably, will be held in Olympia, Washington.

Roger E. Burrows
Chairman

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A MYXOSPORIDIAN, Ceratomyxa sp., INVOLVED IN LOSSES OF ADULT SALMON

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The Myxosporidia are exclusively parasites of cold-blooded vertebrates, especially fish. A number of species of these protozoans are commonly found in Pacific salmon. Damage to salmon by most myxosporidians is usually negligible in terms of observed fish losses although there is no doubt a certain amount of stress placed on the host by the parasites.

A species of myxosporidian belonging to the genus Ceratomyxa was first observed in Pacific salmon in 1955. Observations between 1955 and 1960 and associated fish losses were discussed at the Eleventh Annual Northwest Fish-Cultural Conference (1960). In 1962, this parasite was found to be involved in losses of adult chinook and silver salmon on the Columbia River and its tributaries. The protozoan was found in all groups of adult chinook and silver salmon examined in Washington between the Elokomin River, approximately 35 miles from the mouth of the Columbia, and Rocky Reach Dam, approximately 450 miles from the mouth. Losses of chinook were thought to be low or negligible in the groups observed at hatchery holding facilities or in artificial spawning channels. Various silver runs, however, suffered substantial losses, up to 25 per cent, by comparison. The highest observed losses were sustained by runs entering the Klickitat and Washougal Rivers. Losses, due to Ceratomyxa, were generally lower in the silver runs entering tributaries further downstream.

All observed occurrences of Ceratomyxa in Pacific salmon have, to date, been in adult chinook and silvers in the Columbia River drainage, although search for the parasite outside the Columbia drainage has been diligent. Also, Ceratomyxa has not been found in young salmon in the Columbia, only the adults. Present evidence indicates salmon contract the disease upon entering the Columbia as adults and mature Ceratomyxa spores appear approximately one month later. It is possible, however, that the examination of adults entering the Columbia has been inadequate and the parasite has remained unnoticed by the examination methods employed. These methods depend on the appearance of mature spores in prepared liver smears as part of the examination for acid-fast bacteria. It is, therefore, possible that the disease is contracted by the young salmon migrating from the Columbia, carried throughout their marine growing period, and rapidly develops when the salmon enter fresh water on their spawning migration.

First evidence of the disease is usually found in the lamina propria layer of the posterior intestine. A budding form of trophozooite, reproducing new individuals by asexual means, is first observed in this layer. These are carried by the blood and as the disease advances, trophozooites in various stages of development may be found in nearly all tissues. In advanced stages, white necrotic areas in the gut, liver, and kidney may be seen. In silver salmon the gut wall may thicken and all recognizable tissue layers destroyed. Perforated lesions in the gut appear common in chinook salmon dying from the disease. Trophozooites are observed in gill lesions of infected adults but the presence of fungus and numerous bacteria tend to obscure the true etiology

of these gill lesions. Water temperatures appear to influence development of the disease, colder temperatures retarding and warmer temperatures speeding up its development.

Myxosporidian taxonomy is generally based on spore morphology and size. Measurements of live spores and fixed and stained spores were made from tissues of adult silver salmon from the Columbia. A comparison with Ceratomyxa shasta (Noble, 1950) was made (Table 1). It may be seen in Table 1 that the fixing of the spores results in considerable shrinkage. The average size of the Bouin's fixed spores from the Columbia River is almost identical to Ceratomyxa shasta from California rainbow trout. The description of the infection and spore morphology also appears identical. It is thought that the myxosporidian infecting adult Columbia River silver and chinook is identical to Ceratomyxa shasta. Ceratomyxa shasta is the only one of 67 described species of Ceratomyxa found in fresh water.

Table 1. Ceratomyxa spore measurements.

Spore Source	Method of Preparation	Average Measurements (microns) Length	Width	Polar Cap. Diam.	Number of Spores Measured
Columbia River:					
Silver Salmon	Live	17.5	6.6	2.3	104
Silver Salmon	Schaudin-Giemsa	16.6	5.7	2.1	10
Silver Salmon	Bouin's-Iron Hem.	14.1	5.9	-	50
California:					
Rainbow Trout (Ceratomyxa shasta) (Nobel, 1950)	"Fixed and Stained"	14	6	-	-

An experiment is presently being conducted at the Washougal Hatchery to determine if the disease can be transmitted to yearling silver salmon by the feeding of infected tissues of adults.

The method by which adult salmon in the Columbia contract Ceratomyxa is unknown. It appears unlikely that the adults become infected through the ingestion of spores, the commonly accepted route of myxosporidian transmission, since adult salmon do not actively feed after entering fresh water.

Until more is known on the life history of Ceratomyxa in the Columbia River, the transfer of fish from the Columbia to other drainages should be looked upon with suspicion. This disease has a very real potential as a killer of fish and is presently beyond treatment with known therapeutic agents.

Literature Cited:

Noble, Elmer R.
1950 On a myxosporidian (protozoan) parasite of California trout. Journal of Parasitology 36 (5):457-459.

Wood, James W.
1960 Progress report on fish disease research. Eleventh Annual Northwest Fish Cultural Conference, pp. 34.

CONTROL OF DISEASES IN ADULT SALMON, A PRELIMINARY REPORT

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Introduction

The need for methods to control diseases in adult salmon has become increasingly important in recent years. The beneficial effects of developing such disease control measures are quite obvious. The major problem appears to be in keeping the females in a sound physiological state until the eggs can be harvested.

This is a preliminary report of research undertaken during the first few months of a fish-passage research program on disease control. Three phases are being considered--drug, chemical, and immunological investigations.

Results of Drug Experiments

A series of experiments were carried out at the Dexter Dam (Middle Willamette River) holding ponds to determine the toxicity and blood levels of four sulfonamides in adult spring chinook salmon. The compounds tested were Sulmet (sulfamethazine), S.E.Z. (sulfaethoxypyridazine), Gantrisin (sulfisoxazole), and Bactrovet (sulfadimethoxine).

In no instance was significant toxicity to the fish noted after an injected dose of 100 milligrams per pound of fish, or up to 250 milligrams per pound in the case of Gantrisin and Bactrovet. Toxicity of S.E.Z. was significant, though not excessive, following the 200 and 250 milligram per pound of fish injection. The greatest toxicity was found to occur after Sulmet injections of 200 and 250 milligrams per pound--six out of eight fish dying after this treatment. It is important to note that at concentrations which result in therapeutic levels comparable to and, in most cases, higher than those found in other animals, there is no detectable toxic effect. Therapeutic levels are reached and maintained up to 72 hours after a single injection of 100 milligrams per pound of fish.

Table 1 shows the levels of sulfonamide obtained in milligrams per 100 milliliters of blood, or milligrams per cent. It can be seen that 12 hours after the 100 milligram dose all compounds have reached a blood level slightly higher than those thought to be therapeutic for other animals. These experimental values are in a range of from 17.0-22.2 milligrams per cent. All the compounds maintain levels which are still within or near accepted therapeutic limits for up to 72 hours as indicated by the values in the range of 4.5-16.5 milligrams per cent.

Drug	Dosage Level	Ms. Per 100 Ml. of Blood at 12 Hr.	24 Hr.	48 Hr.	72 Hr.
Sez	50	10.2	7.8	5.3	4.2
	100	17.0	13.7	9.6	7.5
	150	27.9	24.6	21.7	16.1
	200	30.8	24.6	17.5	20.3
	250	20.2	19.0	17.0	14.2
Sulmet	50	6.2	7.2	3.4	3.8
	100	20.3	17.2	10.8	9.5
	150	29.0	22.0	14.7	14.4
	200	52.0	38.0	27.6	27.0
	250	58.0	33.0	18.0	26.0
Bactrovet	50	9.0	8.2	6.5	5.4
	100	20.2	18.4	12.8	16.5
	150	39.3	32.1	21.7	21.7
	200	49.5	46.5	42.0	29.5
Gantrisin	50	12.5	8.9	4.7	2.2
	100	22.2	15.9	8.2	4.5
	150	42.0	26.1	21.2	11.3
	200	53.5	36.6	36.0	27.3
	250	57.0	43.8	37.2	28.4

Table 1. Absorption of four sulfonamide drugs after a single interperitoneal injection (dosage level in mg. per pound of fish)

The ability to maintain these drugs in the blood system of adult salmon is indicated in Figure 1 which shows the per cent loss of the highest concentration obtained in the blood after an injection of 50 milligrams per pound of fish. In the series of concentrations administered the ultimate order of magnitude was consistently the same; i.e., Bactrovet maintained the highest level, Sulmet the next, then S.E.Z., and Gantrisin last. Peak blood concentrations were found to occur 12 hours after the injection of the sulfonamide indicating a rapid dispersion of the compound from the peritoneal cavity into the blood. Figure 2 shows the elimination of the sulfonamides after an injection at the 100 milligram level. The low value obtained at 48 hours with Bactrovet has been attributed to a condition of noninflammatory edema caused by hemorrhaging from the dorsal aorta, the site of blood removal. Although this was a pooled sample from four fish, the effect is obvious. It can be seen that the slopes of the respective lines are essentially the same as those at the 50 milligram level.

The absence of toxicity after injection of 100 milligrams per pound of fish was reiterated in another experiment at Dexter Dam. One hundred fish, 20 per group, were injected with Sulmet, S.E.Z., Gantrisin, Bactrovet and saline (the latter as a control group), and the animals observed for

DRUGS ADMINISTERED AT
50 MG. PER POUND OF FISH

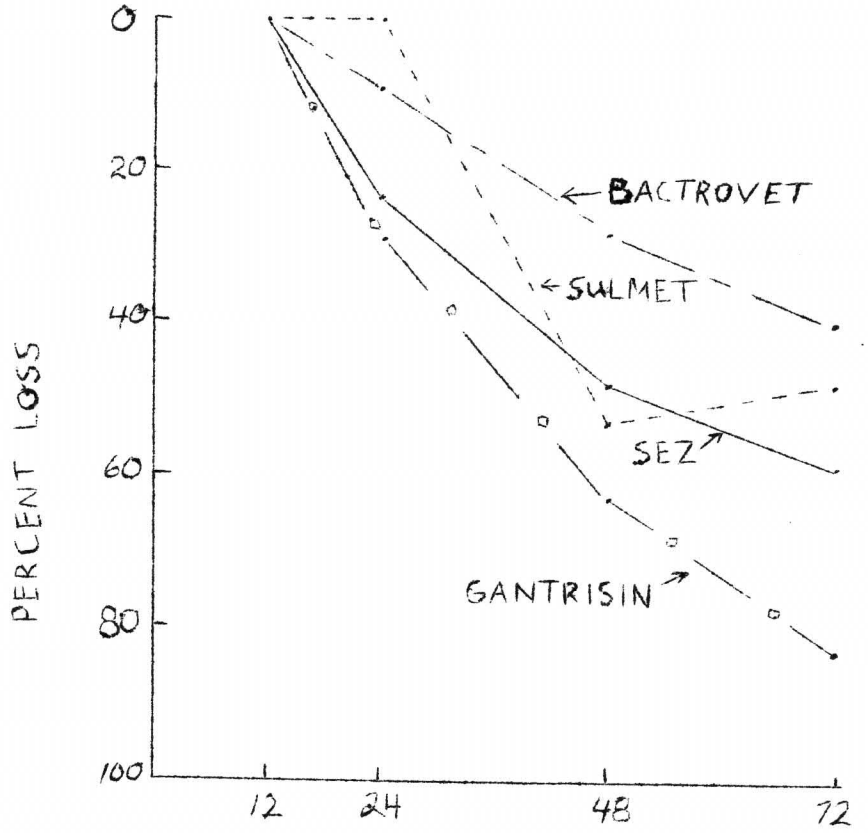

FIGURE 1

DRUGS ADMINISTERED AT
100 MG. PER POUND OF FISH

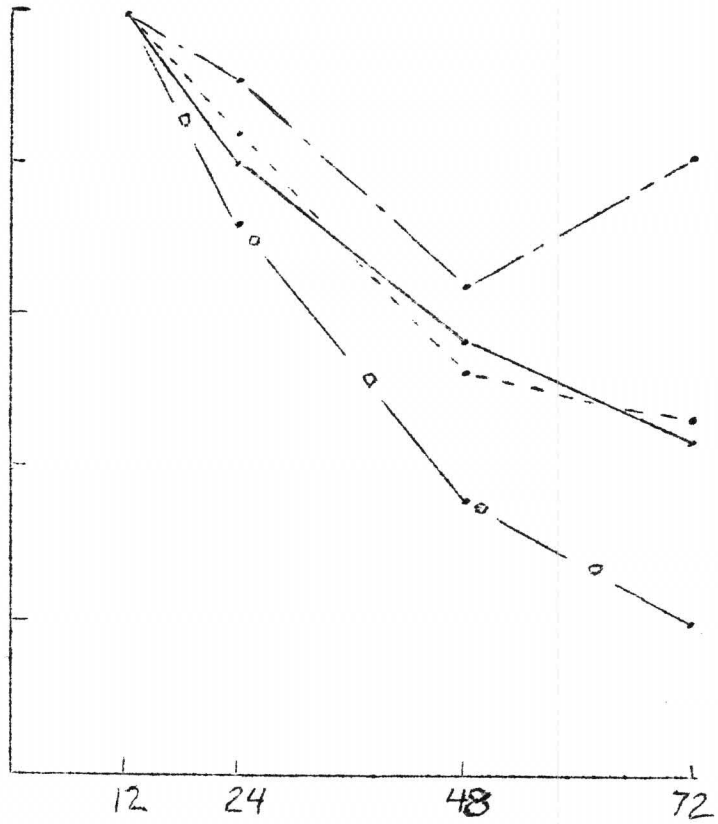

FIGURE 2

ELIMINATION OF SULFONAMIDE DRUGS FROM THE BLOOD OF ADULT CHINOOK SALMON EXPRESSED IN PERCENT LOSS

mortalities over a 6-day period. There were no mortalities in this experiment, indicating again, as in the previous experiment, that the 100 milligram dosage level was not acutely toxic to the adult salmon.

It might be mentioned that both the intraperitoneal and intramuscular injection of Bactrovet gave essentially the same results.

Chemical Studies

Chemical investigations have been directed toward establishing the tolerance limits of adult spring chinook salmon to four chemicals--Lignasan (ethyl mercury phosphate), P.M.A. (pyridalmercuric acetate), formalin, and malachite green. Limited results have indicated that malachite green, now applied commonly at a concentration of 1:1,000,000 may if further experiments support the original findings, be applicable at concentrations considerably higher than those presently employed. Concentrations of 1:500,000 malachite green have been consistently employed successfully both on adult salmon at Dexter Dam and in the laboratory at Clackamas. Since the toxicity of a compound varies with the physio-chemical character of the water, a concentration of 1:500,000 may not give favorable results at other than these study sites.

The results of preliminary investigations with P.M.A. are shown in Table 2. Toxicity studies with this compound have been extensive enough to indicate its effects more fully. As can be seen, P.M.A. assumes toxic proportions near a concentration of 1:200,000 and becomes fatal to all fish when increased to a concentration of 1:25,000. These results are all based on a 1-hour exposure at temperatures from 53.5-56.0° F (average of 54°).

Encouraging results have been obtained using topical application of malachite green. One group of fish concurrently involved in an immunity experiment developed extensive fungus infections. It was found that weekly application of 20% malachite green directly on the lesions eliminated the parasitic growth from this area. By the end of the 6-week experiment all the original lesions were still free of fungus and advanced healing was evident. Similar, though less dramatic, results were obtained when fish in the holding pond were treated topically with 20% malachite green.

Table 2. Results of bioassays to determine the toxicity of pyridalmercuric acetate (PMA) to adult chinook salmon

Exposure Time	Concentration	Number of Fish Tested	Number of Fish Surviving	Percent Survival
1 Hour	1:400,000	12	12	100
"	1:300,000	12	12	100
"	1:200,000	12	9	75
"	1:100,000	12	10	83
"	1: 50,000	12	7	58
"	1: 25,000	12	0	0

Immunity Studies

Attempts to detect the presence of antibodies in adult chinook salmon have not yet proved successful. Several methods have been employed to detect the presence of these substances including precipitin and agglutinating techniques. It is not believed that experimentation has been extensive enough to draw any conclusions at this time since the investigation of several important factors is still pending. It has been found in other salmonids that certain criteria, if fulfilled, insure maximum antibody response. These include the maintenance of an optimum water temperature, a sufficient interval for antibody formation after injection of the antigen, and the utilization of the best route of inoculation. All three apparently vary depending upon the species of fish. Experimentation at this laboratory involved the use of temperatures in the range of 53.5-56.0° F. with both intravenous and intraperitoneal injections weekly over a period of 4 weeks, and in a later experiment, 2 weeks.

Another factor of great importance must be considered in immunological experiments with adult salmon. The fish are no longer feeding and are therefore depending entirely upon stored supplies of proteins, fats, and carbohydrates for all their metabolic needs. It is reasonable to suspect that this may limit the amount of protein which can be shunted into the antibody forming mechanisms. There is not, however, sufficient information to conclude that antibody formation does not take place during this period in the salmon's life cycle.

Future Studies

There are many investigations presently considered as important to this program, some of which have already been initiated. Considering each phase of the program separately:

1. Chemical and drug studies are to be extended using not only the compounds presently in use, but also new compounds which are being received as part of a cooperative program with Eli Lilly and Company's research laboratories. These experiments will involve sensitivity tests designed to detect the antimicrobial spectrum of each chemical and the feasibility of treating adult salmon with the screened compounds. In order to make the administration of effective drugs more practicable, various methods of treatment are being outlined and investigated. The chemicals used in topical and general application experiments will be more intensively investigated to determine the specific toxicity levels of each compound for adult chinook salmon.

2. Immunity studies will be continued with emphasis on detecting bacterial agglutinating antibodies and, if possible, the detection of precipitins. An experiment is now being designed to attempt passive immunization of silver salmon by administration of immune serum from adult rainbow trout.

3. Bacterial physiological studies are now being initiated to elucidate the biochemical aspects of fish microbial pathogenicity. These studies will be concentrated on Chondrococcus columnaris and Aeromonas salmonicida.

"BAD EGG" STUDIES CONTINUED

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This report presents information derived from a fall chinook salmon "bad egg" study conducted at the Oregon Fish Commission Oxbow Hatchery during the 1961-62 spawning and rearing seasons. The experiment supports the 1960-61 bad egg study findings reported at last year's conference and, in addition, measures the extra labor required for the care of diseased eggs.

To orient new participants and refresh the memories of those who were present last year, I will briefly describe the term "bad eggs".

Bad eggs are characterized in the fish by a milky-grey or bloody discharge from the vent when the females are tested for ripeness. Internally some or all of the following symptoms are usually present: (1) few to many enlarged white (dead) eggs are scattered in small groups or located individually on the surface of the ovarian mass immediately beneath the mesentery; (2) a cluster of diseased ova is located centrally within the ovary at the anterior origin or point of attachment; and (3) abnormal sexual maturation is evidenced by the presence of loose free-flowing eggs in only one ovary, or in portions of either or both ovaries, or the eggs of both ovaries are impacted. Sometimes one or both ovaries are "blood shot" and are blackish purple. This is thought to be the result of a severe bruise, and is not considered a symptom of the bad egg condition described here.

Diseased ova have been described in steelhead trout as well as silver and spring chinook salmon at OFC hatcheries but the highest incidence has always been in fall chinook salmon. The condition has also been observed in fall chinook at Astoria fish canneries and as far inland as Oxbow Dam on the Snake River.

The seasonal incidence of bad egg fall chinook returning to OFC Columbia River hatcheries has varied from minimal to approximately 20% of the females.

The 61% survival to liberation obtained in the small scale 1960-61 bad egg study indicated the need of similar studies to determine if comparable survivals could be obtained on a hatchery production basis. Accordingly, a study involving larger numbers of eggs was scheduled at the Oxbow Hatchery during the 1961-62 season.

The experimental animals were 1961-brood Oxbow fall chinook. Control and test groups of eggs and fry were incubated at Oxbow according to accepted hatchery techniques. Test fingerlings were reared in hatchery troughs and control fingerlings were reared in outside ponds. All experimental work was done by Oxbow Hatchery personnel.

Bad egg fall chinook were scarce at all Fish Commission Columbia River stations during the 1961 spawning season. Only 30 fish, or 4.6% of the total females spawned at Oxbow, were diseased. The total diseased egg take, 90,353 eggs, was too small for a practical hatchery production test (Table 1).

Table 1. Results of 1961-1962 Diseased Egg Study, Oxbow Hatchery.

	Abnormal Group	Normal Group
Total Females Spawned	30	626
Total Egg Take	90,353	3,404,504
Average Number of Eggs Per Female	3,012	5,438
Average Number of Eggs Handled Per Man Hour	3,346	30,950
Per Cent Total Mortality	42.9	7.3
Per Cent Fingerling Survival	57.1	92.7

The average fecundity of the diseased females was 3,012 eggs compared to 5,438 eggs per female in the normal fish. The number of eggs handled per man hour was 3,346 in the abnormal lot, and 30,950 in the normal lot. This comparison shows a ratio of about 1:9, and illustrates the amount of extra labor required for the care of bad eggs. The total loss in the abnormal group was 42.9% compared to a total loss of 7.3% in the normal group. Most of the excessive loss in the abnormal group occurred early in the experiment, and was comprised primarily of eggs and newly hatched or premature fry. Fingerling survival was 57.1% in the diseased group and 92.7% in the normal group. Percent mortalities and survivals for both lots were calculated only through April 16, 1962 when it became necessary to clear the hatchery troughs.

Results from both the 1960-61 and 1961-62 bad egg studies indicate that over 50% of the eggs spawned from "bad egg" females may survive to healthy fingerlings. However, the 1:9 labor differential experienced between the two lots may prohibit the spawning of bad egg females except possibly during those years of limited egg takes.

PROGRESS REPORT - COLEMAN VIRUS PROBLEM

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Coleman National Fish Hatchery

The Virus problem continues to be with us at Coleman. Last year I reported on an attempt at re-circulating warmed up water by means of a pumping system so that a large share of the seasonal egg take could be handled. We had extreme difficulty in maintaining what we believed to be the virus controlling temperature of around 56° F. In the process of circulating the water, something was generated in the system which caused considerable mortality among fry and showed up in the eggs taken at a later date. The system failed and we had to re-arrange the piping tanks and pumps in the middle of the work to provide a system that would permit us to go on. There was some question in the minds of some that we should drop the warm water and give the Virus study up as a lost cause. The heavy losses we experienced were enough to give added thought before anything further was done.

We had reason to believe the original system would function as we wanted it to. There was no record of any previous failure along this line, but once the breakdown was apparent, we obtained many stories of where somewhat similar work was attempted and had failed but never was mentioned.

The system salvaged and re-arranged, finally produced a good many fish and while all records of last year are confused, we believe the general idea can be utilized and probably control the Virus. The resulting fish from the final work done last year showed a noticeable lack of the Virus. This gave us further hope that we are on the right track.

We now have a system set up which will utilize the warm water on a one time incubation, hatching, and sac absorption period. This water will be reused to rear feeding fish. The program now will be to subject eyed eggs to the 56° F. temperature water, hatch them and pass through the sac at the 56° temperature. Some of the groups will continue in 56° water upon feeding for a period of 30 days, others will be reared in the regular Battle Creek water at it's normal temperature.

There is some reason to believe that if eyed eggs are placed in 56° water, allowed to hatch and resulting fry develop out of the sac stage in this water that the Virus is controlled, and these fish can then be placed in our regular water of colder temperature. To further study this, some groups will be held in 56° water beyond the sac stage. The current program is based on these indications. We expect to come up with some worth while results this year.

METHODS FOR THE PREPARATION OF TISSUE CULTURES FROM SALMONID FISHES

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and
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INTRODUCTION

Tissue culture is a term applied to those techniques by which tissues and cells of higher organisms are maintained in a viable condition and in some cases grown after having been excised from their source. The in vitro cultivation of tissues and cells is a relatively new innovation in biology and has gainedwide-spread use in a variety of disciplines such as virology, immunology, histology, cytology, and cancer research. Its role in a modern fish pathology program has been well established by Wolf and his co-workers in America and by Grutzner in Europe. Three major factors have led to recent interest in tissue culture:

1. Recognition by virologists, about 1950, that during multiplication many viruses produce degenerative changes in culture cells which can be easily distinguished.

2. The development of antibiotics which can be added to the tissue culture medium for the control of contaminating microorganisms.

3. Development of the enzyme dispersed cell method employing trypsin for the preparation of monolayer cell cultures.

Two basic techniques have been investigated in this study in order to develop methods for the preparation of primary cultures of salmon and steelhead tissues.

FRAGMENT METHOD

This method has been used to culture a variety of fish tissues (Table 1). This was the first technique tested and appears to be the most reliable. The following steps are believed to be required in order to establish these cultures:

1. The fish is killed and washed with a 1:1 million Roccal solution, then bled by cutting the tail at the caudal peduncle.

2. The desired organ is excised using absolute aseptic technique and rinsed in a sterile cold, balanced salt solution at pH 7.4. It should be noted that when the source of tissue is an external organ, yolk-sac fry, or embryonic material, the first step becomes the rinse in cold balance salt solution.

3. The tissue is cut into fragments approximately 2-5 mm square and transferred to roller tubes or other culture containers as desired.

4. The tubes containing the fragments are allowed to stand for a period of 3 to 4 hours in order to allow the fragments to become firmly stuck to the glass.

5. At the end of this period the desired nutrient fluid is added and the tubes placed in an 18° C incubator.

6. The nutrient fluid should be changed 48 hours after incubation is initiated. Fragments can be observed for growth by examination with the compound microscope. The newly grown cells can be seen extending out in a monolayer sheet around the fragments.

Table 1. Cells Cultured by Fragment Method.

Species	Tissue	Growth 1/
Silver salmon	Yolk-sac fry	++++
	Embryonic	++++
	Embryo heart	2/
Sockeye salmon	Fin	+++
	Yolk-sac fry	++++
Chinook salmon	Tumor	++
	Gill	+
	Yolk-sac fry	++++
Steelhead trout	Yolk-sac fry	++++
Rainbow trout	Hepatoma	+++
Squawfish	Fin	+++
Guppy	Tumor-like growth	+
	Young fish	+

1/ + = slight
++ = fair
+++ = good
++++ = excellent
2/ Kept beating 5 days after being excised from animal

ENZYME DISPERSED METHOD

Enzyme dispersion has been used to prepare a variety of tissues for cultivation with varying results. Table 2 indicates tissues successfully cultured by this method. Growth was indicated by the development of a confluent monolayer sheet of cells over the surface of the glass culture container. The procedure developed for use in this work is as follows:

1. The desired tissue is obtained as already described and minced into small fragments. These fragments are transferred to a standard trypsinizing flask equipped with a magnetic mixing bar. The tissue is washed twice in the nutrient fluid to be used during cultivation.

2. After washing, trypsin is added at a concentration of 0.25% and the pH of the suspension adjusted to 7.4. The flask containing the tissue and enzyme is then agitated slowly by means of a laboratory magmix for 15 minutes at which time the fluid fraction is removed and discarded. This operation is termed the pre-enzyme treatment.

3. An equal volume of fresh trypsin is added and the agitation continued for 30 minutes. Again the fluid fraction containing cells and small aggregate of cells is removed and washed three times in cold nutrient fluid to remove the trypsin from the preparation.

Table 2. Cells Cultured by the Enzyme Dispersed Method Using Trypsin at 15° C.

Species	Tissue
Sockeye salmon	Connective tissue of liver Gonad Kidney Air bladder Yolk-sac fry Fin
Chinook salmon	Yolk-sac fry Connective tissue of liver Kidney Air bladder Fin Gill
Silver salmon	Embryo

4. The cells are resuspended in the desired nutrient fluid and cell counts made by means of a hemocytometer. The concentration of cells is adjusted to 500,000 per cc and planted in tubes or bottles.

5. The cultures are placed in an 18° C incubator and left undisturbed for 48 hours. The nutrient fluid should be changed at the end of this 48 hour period and the cultures may also be examined at this time.

Precautions taken when using the above method are:

1. It is believed the temperature should be maintained below 20° C (15-20° C) when preparing cultures and during incubation and examination.

2. Absolute sterile conditions must be maintained as rigidly as possible at all times.

3. Glassware and instruments must be washed and sterilized according to standard method for the preparation of these materials.

4. The pH of solutions used for the preparation of cell cultures should be in the range of 7.2-7.4.

MEDIA

Lactalbumin hydrolysate-yeast extract medium (LY) and Eagles medium have been employed successfully in this work. Animal serum must be added to these preparations (20% by volume) in order to insure growth. Human and agamma calf serum seem to be best. Fish serum has produced very poor growth in all preparations tested.

Antibiotics are added directly to the nutrient fluid at a concentration of 500 units per ml penicillin, 500 micrograms per ml streptomycin, and 100 units per ml mycostatin. Penicillin and streptomycin can be added at twice the concentration indicated without apparent harm to the cells. Mycostatin at concentrations above 100 units per ml of medium should be avoided.

CONTINUOUS CULTIVATION OF CELLS GROWN FROM PRIMARY CULTURES

A number of primary cultures have been transferred to observe which groups of cells survive best under conditions of continuous cultivation. Table 3 indicates the source of tissue, number of transfers, and the age of each culture at death. Embryonic and tumor tissue seem to offer a good source of cells for survival under these conditions.

Table 3. Results of Experiments to Develop a Call Line of Fish Tissues.

Species	Tissue	Number of Transfers	Age of Culture at Death (Days)
Sockeye salmon	Yolk-sac fry	3	67
	Kidney	1	49
	Gonad	2	61
Steelhead trout	Yolk-sac fry	1	70
Chinook salmon	Tumor	1	84
Rainbow trout	Hepatoma	4	86
		1	18
		2	25
		2	67
		2	115
		1	96
Silver salmon	Embryo	14	95
		13	240 1/

1/ Still living.

INFECTIOUS PANCREATIC NECROSIS

John Kincheloe

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A short movie depicting gross symptoms of Infectious Pancreatic Necrosis was presented. The movie was filmed by Dr. Ken Wolf and Mr. Lyle Pettijohn at the Eastern Fish Disease Laboratory.

The film shows moribund fish and mortalities in a hatchery raceway. Some moribund fish were then placed in a trough for closer examination. Dissection of the affected fish is also included in the movie. These dissections portray the internal gross pathology associated with this disease.

A short discussion after the film indicated high mortalities which have occurred in the East as a result of this disease. It was also mentioned that gross symptoms may be ambiguous and must be confirmed by laboratory methods.

INFECTIOUS PANCREATIC NECROSIS IN THE WEST

Thomas J. Parisot
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Western Fish Disease Laboratory
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Infectious Pancreatic Necrosis in rainbow trout (Salmo gairdneri), brook trout (Salvelinus fontinalis) and cutthroat trout (Salmo clarki), has been experimentally substantiated for the first time in the western United States, the latter representing a new host. Brook trout fin-tissue culture inoculated with bacteria-free filtrate from the diseased fish tissue showed marked degenerative changes after 24 hours. Chinook salmon (Oncorhynchus tshawytscha), kokanee (O. nerka) and silver salmon (O. kisutch) were not susceptible to the virus when inoculated. Histologically, extensive pancreatic necrosis was observed in the original and experimental materials, however striated muscle hyalinization was detected only in the original material.

Experimental Effects of *Nanophyetus salmincola* Metacercariae in Rainbow Trout

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Abstract

A fourteen week controlled experiment was conducted to determine the effects on juvenile rainbow trout of varying levels of infection with *Nanophyetus* metacercariae.

Pathogenesis was measured in terms of effects upon growth response, mortality rates, and stamina. 1 X, 2 X and 4 X levels of infection were established by maintaining known snail carriers in direct association with previously unexposed test fishes. The 1 X level of infection was represented by a snail to fish ratio which yielded an accumulating infection intensity in test fish approximating that observed in wild silver salmon juveniles from Abernathy Creek, Longview, Washington. Results (table 1) indicated that infection intensities prevailing in Abernathy Creek wild silvers are non-pathogenic in rainbow trout; 2 X and 4 X levels, however, induced significant mortality differences when compared with 1 X and control lots. Morbid effects of this parasite appear to be the "all or none" type inasmuch as no significant differences were observed between lots with respect to growth response, or stamina (as measured by the stamina tunnel test of Abernathy Salmon Cultural Laboratory).

Aug. 10 - Nov. 19, 1962

Table 1.--Experimental Effects of Nanophyetus salmincola Metacercariae in Rainbow Trout

TREATMENTS:

	1 X				2 X				4 X				CONTROLS	
Time in Weeks	Mean Weight (Grams)	Cum. % Mort.	Mean # Larvae Post. Kidney	Mean # Larvae per Gram of Fish	Mean Weight (Grams)	Cum. % Mort.	Mean # Larvae Post. Kidney	Mean # Larvae per Gram of Fish	Mean Weight (Grams)	Cum. % Mort.	Mean # Larvae Post. Kidney	Mean # Larvae per Gram of Fish	Mean Weight (Grams)	Cum. % Mort.
0	1.52	-	-	-	1.46	-	-	0	1.59	-	-	-	1.54	0
2	2.07	-	1	0.5	2.04	-	3	1	2.13	-	7	4	2.12	0
4	2.26	-	16	6	2.26	0.36	22	11	2.32	-	32	16	2.28	0
6	2.75	-	25	10	2.82	0.36	75	23	2.91	-	102	40	2.83	0
8	3.23	-	77	23	3.30	0.72	168	60	3.39	0.72	203	60	3.32	0
10	3.87	-	111	30	3.88	2.14	348	89	3.91	2.86	396	117	4.02	0
12	4.53	-	298	60	4.47	3.21	358	85	4.52	3.93	478	101	4.73	0.36
14	5.40	-	258	50	5.32	4.64*	422	88	5.40	4.64*	470	99	5.67	0.36

* Highly significant, P < .001

CONTROL OF TREMATODE CERCARIAE IN HATCHERY WATER SUPPLIES

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Abernathy Creek, like many streams in western Washington and Oregon, supports a large population of snails of the species Oxytrema silicula. This snail is the primary host of the trematode Nanophyetus salmincola, the salmon-poisoning fluke. During the late spring, summer, and fall months, repeated generations of Nanophyetus cercariae are released into the stream and are introduced into the rearing ponds of the Salmon-Cultural Laboratory through the creek water supply.

During the 1961 rearing season much of the fall chinook fingerling mortality was attributed to cercarial invasion. Losses due to cercarial action may have been caused directly by gill and other tissue damage or indirectly by providing easy access for bacterial infections. In 1962, during a furunculosis infection, moribund fish showed a metacercariae incidence 50 percent greater than survivors. Both the susceptibility and virulence of other diseases is probably increased due to cercarial invasion.

Some method of reducing the incidence of cercariae in the water supply appeared mandatory if over-summer rearing of fingerling salmon was to be successful. Four methods of control were explored in 1962. The first method was dilution. Approximately 1000 gpm of cercariae-free well water was available to supply from 20 to 50 percent of the total amount of water required, thus reducing the numbers of cercariae by a like percentage.

The second approach was the development of a snail migration barrier. It was assumed that large numbers of snails were displaced downstream during winter freshets and that upstream migration occurred during low water conditions in the summer. Laboratory tests indicated that snails would not cross a 9-inch wide copper plate in water at a pH of 7.2, the pH of Abernathy Creek. A strip of copper flashing 14 inches wide was installed on the face of Abernathy falls immediately downstream of the hatchery intake. Snails did not cross the copper as long as it was bright but did cross it readily when the copper became corroded. To be effective then, a copper barrier would have to be maintained in a clean, shiny condition.

A third method for controlling cercariae that was considered was that of microstraining the water. Microstraining equipment is available commercially with 23, 30, or 60 micron screens. A sample of the 60 micron screen was tested and found to remove all the cercariae from the filtered water. Although this equipment would be effective, it is expensive and it was believed that other more economical means of cercariae control should be investigated first.

The fourth control method explored was that of electrocution. Laboratory tests indicated that cercariae could be electrocuted with 60 cycle alternating current at 240 volts per inch with a one second exposure period. An electrically-charged grid was constructed and installed in the hatchery water supply in May, 1962.

A significant reduction in the incidence of cercarial invaders was accomplished although all of the cercariae were not destroyed in the water supply. The numbers of metacercariae found in the posterior third of the kidney from fish samples withdrawn weekly from the rearing ponds was compared with the numbers found in cercariae-free target fish held in the creek for consecutive 7-day periods. From June 15 until September 18, the average number of metacercariae in the pond fish increased from approximately 50 to 90. During the same period, the 7-day accumulation of metacercariae in the target fish often exceeded the total accumulation in the pond fish. As is evident from the figure, population explosions in the creek were not reflected in the pond fish samples. While no direct comparison can be made between the two groups of fish due to dilution with well water in the ponds for a portion of the time and to variations in potential exposure, it is evident that the electrical grid was effective in reducing the incidence of cercariae in the creek water supply.

Laboratory experiments were carried out during the summer of 1962 to determine the most efficient voltage for electrocuting the cercariae. Voltages ranging from 230 volts per inch to 440 volts per inch with varying exposure periods were tested. The most efficient voltage tested was 310 volts per inch when both rate of kill and economy of operation were considered. At this voltage, 80 percent of the cercariae were killed or disabled with an exposure time of less than .5 second. High frequencies, up to one megacycle per second were found to be ineffective when operated at practical voltages. Based on the results of these experiments, a new electrical grid operating at 310 volts per inch will be constructed and installed in the hatchery water supply for operation during the 1963 rearing season.

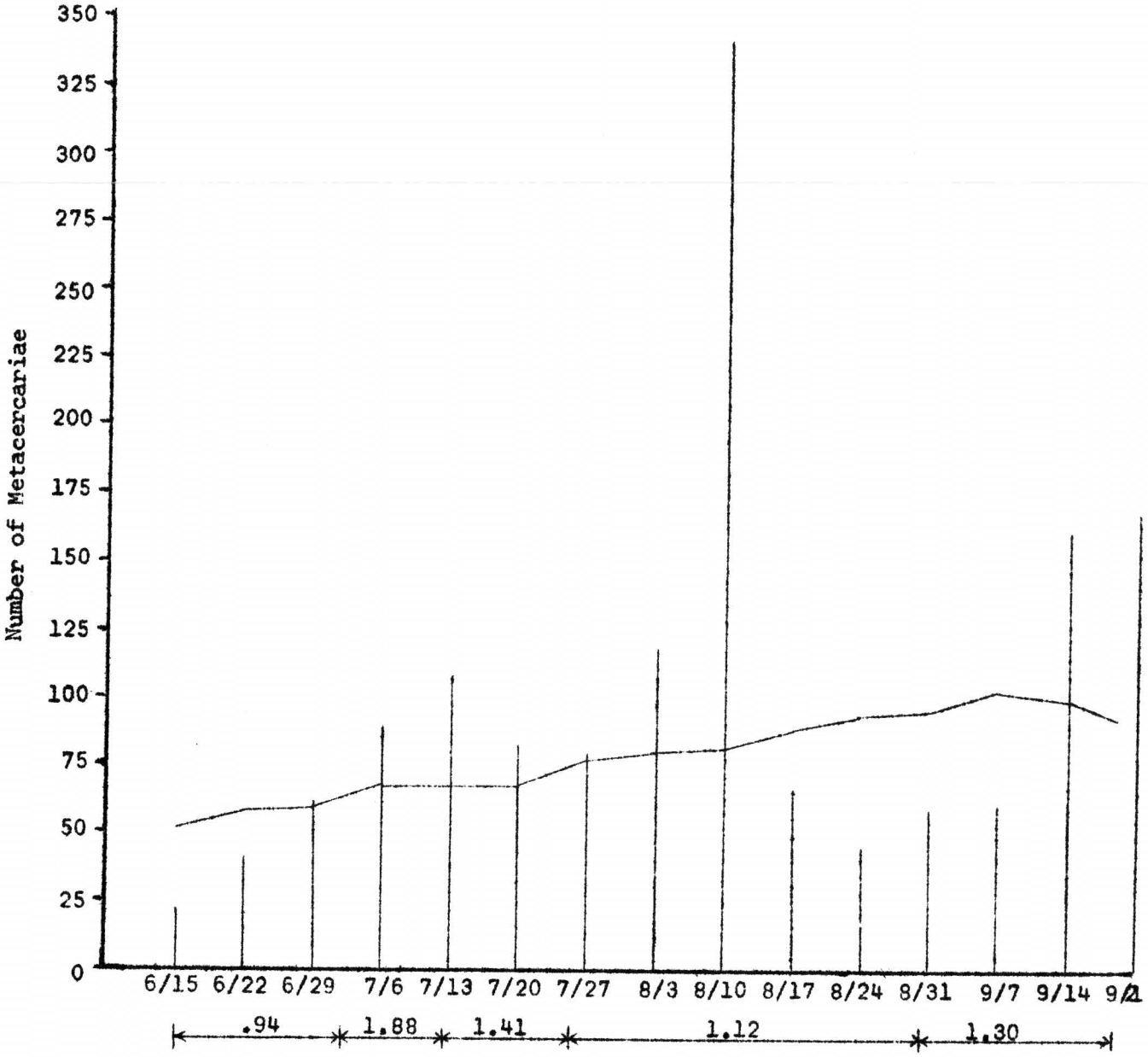

Figure 1:--Incidence of metacercariae in hatchery-reared fish (horizontal line) as compared with incidence of metacercariae in metacercariae-free fish after a one-week exposure to creek water (vertical bars).

FORMALIN IN THE HATCHERY

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Formalin is used extensively in hatcheries for the control of external parasites on fish. There are reports that it is toxic at some hatcheries, especially to rainbow trout.

Formalin was probably first used about the turn of the century for the control of parasites on fish. In 1909, Léger suggested the use of a 15-minute bath in a 1:2,500 solution for the control of Costia necatrix on trout. Kingsbury and Embody in 1932 suggested the use of a constant flow device to introduce formalin into the inflowing water of a trough or pond for an hour treatment. They suggested concentrations of 1:2,000 at 50° F., 1:2,500 at 55° F. to 70° F. and 1:3,000 at temperatures above 70° F. Schäperclaus in 1942 concluded that formalin at 1:2,000 for 30 minutes or 1:4,000 for one hour were satisfactory for treating fish, and that Costia, Cyclochaeta and Gyrodactylus were killed in 16 minutes even in the more dilute concentration of 1:5,000. Dr. Fish introduced the use of formalin in the West in the 1930's recommending a single one-hour exposure of 1:4,000 concentration, but suggested a 20-minute exposure on three consecutive days if the fish were exceptionally crowded or if the oxygen concentrations were abnormally low.

Formalin and formol are common names for the standard aqueous solution of 37% by weight of formaldehyde (CH_2O). Formalin commonly contains sufficient methanol (8% to 15%) to prevent precipitation under ordinary conditions of transportation and storage of a polymer, paraformaldehyde $(CH_2O)_nH_2O$. When the precipitate forms in a formalin solution, the upper layer of solution will be under-strength and the lower layer will be over-strength, but not of significance generally for fish cultural use.

Tests of one-hour in different types of waters with various species of fish indicated that a formalin concentration of 1:500 killed the majority of the fish tested; 1:1,000 about 10%, while there was an occasional mortality at 1:2,000. Dilutions of 1:3,000 and greater proved non-toxic under these conditions. Rainbow trout were the most sensitive of the fish tested.

Formalin at a concentration of 1:4,000 for one hour is suggested for the control of most external protozoan and trematode parasites on most salmonid fish.

It is suggested that first a small group of fish be treated experimentally under the same conditions to be used for treating the production fish. The efficiency of the treatment can thus be determined before subjecting large numbers of fish to a possible lethal or an ineffective formalin bath.

GILL PATHOLOGY DUE TO FORMALIN TOXICITY

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Preliminary study was initiated to determine histologically the toxic effects of formalin on the gills of rainbow trout and silver salmon. Two concentrations, 1:500 and 1:4000 were used. At the the 1:500 level the fish, in groups of five, were treated for 10, 20, 30 minutes and 1 hour. At the 1:4000 level the rainbows were treated for 1, 1-1/2, 2, 3 hours, 2 hours in formalin plus 24 hours in fresh water and 3 hours in formalin plus 24 hours in fresh water. Silvers at this level were, in addition to the above, treated for 5 hours and for 5 hours plus 24 hours in fresh water.

Histologically, the area of the gill tissue most affected appeared to be in the lamella epithelium. Two atypical conditions were observed: one was hypertrophy and the other, separation of the epithelial cells from the supporting cells. At the 1:500 level rainbows started to exhibit some toxic effects after 10 minutes of treatment and after 1 hour showed extensive distention of epithelium. At the 1:4000 level some pathology was observed after one hour. Although recovery appeared complete in fish treated for 2 hours in formalin then transferred to fresh water for 24 hours, the group which was treated for 3 hours and then transferred to fresh water for 24 hours still showed mild epithelial hypertrophy and distention. Silvers exposed at both levels manifested little or no pathology except for the fish treated for 1 hour at the 1:500 level.

THE ABSORPTION OF SULFAMETHAZINE BY SPRING CHINOOK SALMON WHEN INCORPORATED IN FISH-MEAT AND OREGON PELLET DIETS

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and
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INTRODUCTION

Past experimentation with sulmet (sulfamethazine) in the Oregon pellet diet involved toxicity studies and absorption tests at relatively high dosage levels. Since the Oregon Fish Commission incorporates sulmet in both fish-meat and Oregon pellet diets for disease control at concentrations considered to be prophylactic or therapeutic, it was necessary to determine the degree of absorption at the various dosage levels used. It was also desired to compare the efficiency of absorption for sulmet when fed in the two diets.

MATERIALS AND METHODS

The experiment was conducted at the Clackamas Research Laboratory with yearling North Santiam spring chinook salmon. The fish averaged 12.0 grams each (approximately 40 fish per pound) and had never received sulfa drugs.

There were seven experimental lots: Three received Sulmet in the pellet diet at the 1-, 2-, and 5-gram (per 100 pounds of fish) levels; three were fed fish-meat with Sulmet at the 2-, 5-, and 10-gram levels; and one control received the pellet diet without medication. The pellet-fed fish received 1.6% of their body weight per day, the meat-fed fish 3.5%. The blood sulfa levels were measured at 24-hour intervals for a total of 5 observations. The average water temperature was 53° F.

The pellets were prepared at the OSU Seafoods Laboratory, Astoria, Oregon. The fish-meat diet consisted of equal parts beef liver, pork liver, and pasteurized salmon viscera. The beef and pork livers were obtained fresh and ground through a 1/8-inch plate. The pasteurized salmon viscera was obtained from the production food supplies. The diet ingredients and sulmet were blended with a mixer. The diets were then weighed out into daily rations, frozen, and thawed upon use. When the meat diets were thawed the ingredients were bound with 2% salt and fed immediately. It is believed the fish-meat diets were fed under optimum conditions as care was taken to insure the food was well bound and properly fed.

RESULTS AND DISCUSSION

The experiment was designed to follow blood-sulfa levels until peak values were obtained in all lots. After the fourth blood sample was analyzed, it was believed that all lots were at or near peak concentrations, therefore the experiment was terminated after the fifth sample had been taken. Figure 1 shows that the Oregon pellet diets required only about one-half as much drug to attain approximately the same blood concentration as did the fish-meat diets. This indicates the pellet diet was about twice as efficient in getting the sulfa in the fish.

Figure 1 also shows that the 10-gram level in the meat and the 5-gram level in the pellet, the presently recommended therapeutic levels, probably meet the requirements for a therapeutic dosage. However, the 2-gram level in the meat and the 1-gram level in the pellet, the presently recommended prophylactic levels, may not attain high enough blood concentrations to offer complete prophylaxis for certain fish pathogens. The 5-gram level in the meat or the 2-gram level in the pellet seem to be more realistic blood concentrations for prophylactic use.

The 5-gram level of sulmet in the pellet diet has been used in previous feeding experiments, and was again used in this experiment for comparative purposes. This concentration has now been tested under the following conditions: (1) three sizes of fish, (2) two locations, (3) two brood years, (4) two brood sources, and (5) three water temperatures. In each case blood-sulfa absorption has followed approximately the same curve.

INFLUENCE OF SULMET ON MORTALITY WHEN ADMINISTERED ON A DECLINING WATER TEMPERATURE

In January 1962 juvenile spring chinook salmon at the Trask River Salmon Hatchery were experiencing losses due to Aeromonas salmonicida, the causative agent of furunculosis. The fish were receiving a fish-meat diet when a therapeutic level of sulmet (10 grams per 100 pounds of fish) was initiated. The water temperature at the start of therapy averaged 45° F., however, 5 days after medication started the temperature dropped to an average of 38° F. The mortality increased abruptly with the drop in water temperature. A sulmet toxicity was suspected and the treatment promptly withdrawn. With the elimination of sulmet in the diet mortalities returned to the pre-treatment level. Since sulmet has been fed many times before at this dosage level in a fish-meat diet with no apparent toxicity, the question arose as to the cause of the mortality. One apparent factor which was different from other treatments was the rapid lowering of water temperature.

It was decided to try and duplicate this condition experimentally. A controlled temperature apparatus was available at the Sandy Laboratory and temperature ranges could be obtained to approximately duplicate the situation at the Trask River Hatchery.

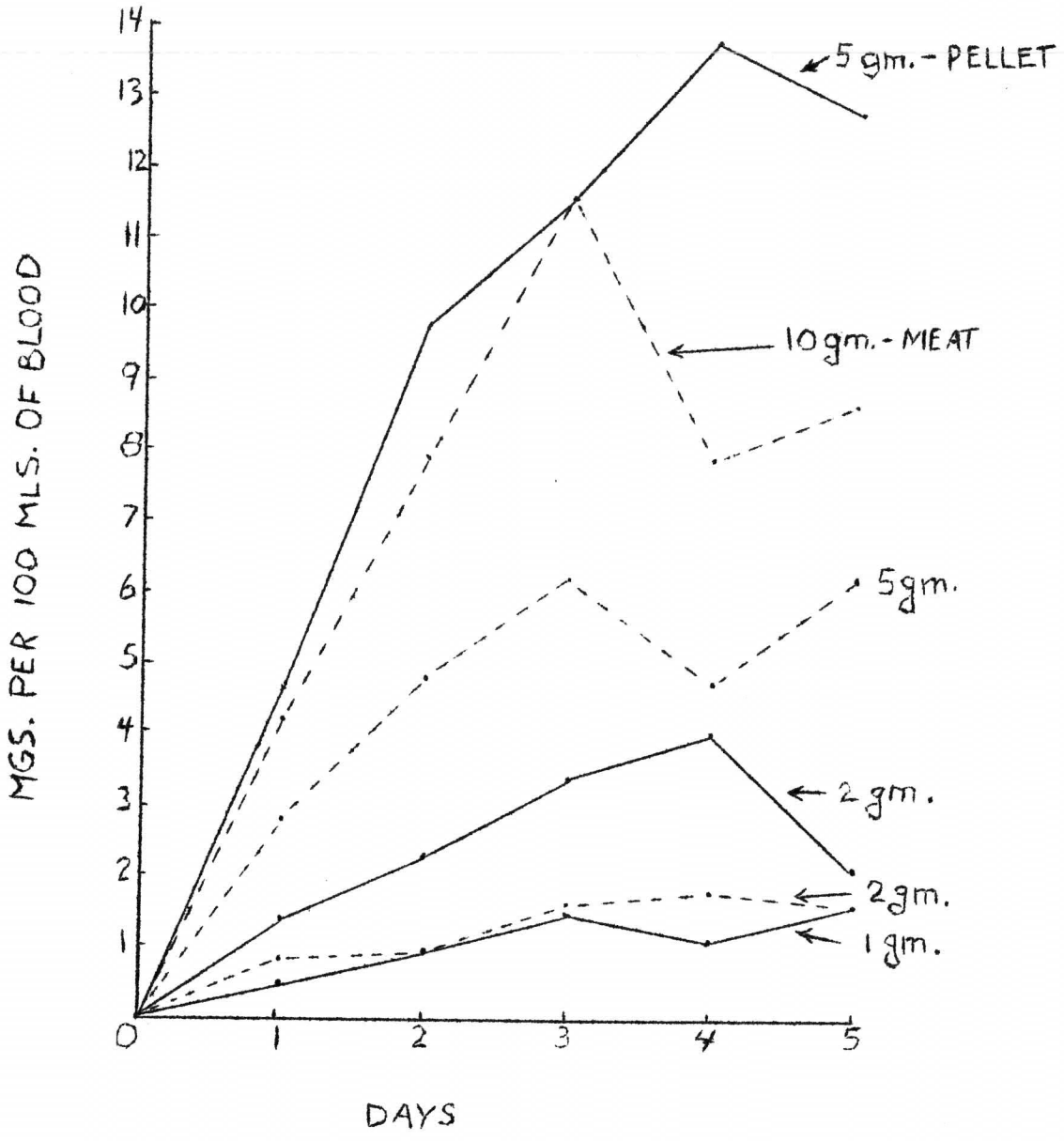

FIGURE 1. ABSORPTION OF SULFAMETHAZINE WHEN INCORPORATED IN A MEAT AND PELLET DIET. (DOSAGE IN GRAMS PER 100 POUNDS OF FISH.)

Following the sulfa absorption phase of the experiment previously discussed the remaining 15 fish in each lot continued to receive the medication. The fish received the last dosage 18 hours before they were transported to the Sandy Hatchery. At that time all lots, except the 1-gram pellet, were placed in separate rectangular tanks in the controlled temperature apparatus. The water temperature was then dropped from 53° F. to 38° F. in approximately 3 hours. The fish were observed for 14 days and not fed during that period. The first signs of distress were observed in the 10-gram fish-meat diet 3 days after the experiment started. The symptoms were noted when the fish appeared to be having trouble maintaining their horizontal equilibrium. They remained head down on the bottom of the tank with the tail at an approximate 45-degree angle from the bottom. There was a gradual loss of lateral equilibrium and the fish began sporadically swimming about the tanks. Death usually followed about 8 hours after the first symptoms appeared. Examination of Figure 2 shows that the mortalities were highest in the fish-meat diets, and the mortalities increased with the concentration of sulfa. Mortalities were experienced in the 5-gram pellet, but none were observed in the 2-gram pellet. One fish died in the control at the last of the experiment about 4 days after the mortalities ceased in the other lots. Most mortalities occurred between the 3rd and 9th days. It therefore appears that mortalities can be induced while feeding sulmet in a fish-meat diet, and to a lesser extent in Oregon pellets, when the water temperature is dropped from above 50° to below 40° F.

SUMMARY

The Oregon pellet diet appears to be approximately twice as efficient as the fish-meat diet in delivering sulfamethazine to salmon. The presently recommended therapeutic dosage for both the fish-meat and Oregon pellet diets appears to be satisfactory, however the prophylactic dosages may require an increase for control of certain fish disease.

Feeding sulfamethazine on a declining water temperature may cause increased mortalities. Treatment under these conditions should possibly be avoided.

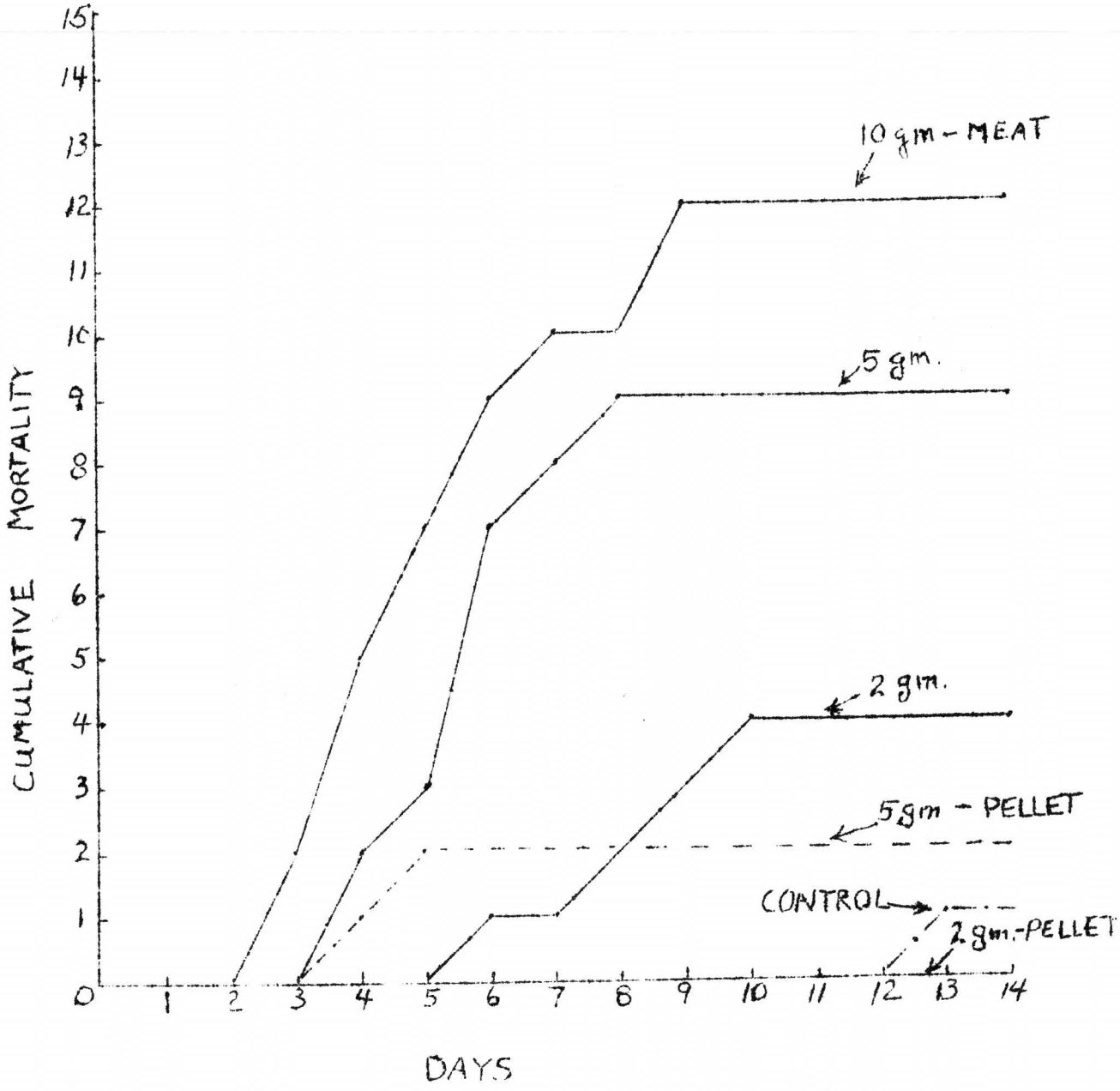

FIGURE 2. EFFECT OF FEEDING SULFAMETHAZINE AT VARIOUS CONCENTRATIONS ON A DECLINING WATER TEMPERATURE.

TRIALS WITH CRUMBLED OREGON PELLETS AND A PROGRESS REPORT OF OTHER 1962 NUTRITION STUDIES

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Clackamas, Oregon

TRIALS WITH CRUMBLED OREGON PELLETS

At the 1961 Northwest Fish-Cultural Conference, a progress report was presented of a feeding experiment with crumbled Oregon pellets. Final results indicated that the crumbles could be fed successfully to chinook and silver salmon when these fish reached a size of 700 per pound. Growth almost equalled a fish-meat control, food conversions were very good, mortality was low, and blood condition was satisfactory.

The 1961 studies were conducted because approximately 30% of the food used annually by the Oregon Fish Commission was fed in fish-meat diets before the fish were 300 per pound, the minimum size for pellet feeding at that time. This minimum fish size for pellets precluded their use for fall chinook because the fish are liberated at 200-400 per pound after 90 days rearing. Since the food cost to produce fish with pellets is much lower than with the fish-meat diets, it appeared desirable to get all fish on pellets as early in their life as possible. However, feeding pellets to fish smaller than 300 per pound was not possible until smaller sizes of pellets were made available. The crumbles used in the 1961 studies were prepared in the laboratory. Later in the year, the manufacturer of Oregon pellets was contacted as to the feasibility of producing crumbles. He advised that this could be done and that a limited amount could be made for more extensive studies in 1962.

Six pilot production trials with commercially produced crumbled Oregon pellets were conducted this past spring and early summer utilizing fall chinook at Bonneville and Oxbow hatcheries, spring chinook at Willamette and Marion Forks hatcheries, and silver salmon at Cascade and Sandy hatcheries.

All tests were started when the fish reached approximately 700 per pound in weight. The fish received a fish-meat starting diet prior to crumble feeding. Two sizes of crumbles were used: 16 mesh (specified particle size 0.045 to 0.034 inch) fed to fish 700 to 400 per pound, and 12 mesh (specified particle size 0.060 to 0.045 inch) fed to fish 400 to 300 per pound. Regular sized pellets were fed after the fish reached 300 per pound.

In these pilot trials some 11,100 pounds of crumbled pellets were fed, producing 7,036 pounds of fish, for an over-all food conversion of 1.6. Table 1 shows a breakdown as to hatchery where the pilot trials were conducted, species used, numbers of fish in the trial, beginning and ending weight in fish per pound, food conversions attained, and blood condition as measured by hematocrit values.

From the results of the pilot trials, crumbles will be put in full production in 1963.

Table 1.--Summary of Results, "Crumbled" Oregon Pellet Production Feeding Trials, 1962.

Hatchery	Species	Number of Fish	Ave. Fish Wt. (Fish/lb) Start	End	Food Conversion	Mortality %	Average Hematocrits at End %
Bonneville	Fall Chinook	772,000	705	300	1.9	1.2	33
Cascade	Silvers	298,000	701	238	1.3	0.3	31
Marion Forks	Spring Chinook	172,000	628	332	1.8	0.8	38
Oxbow	Fall Chinook	793,000	697	165	1.5	0.6	44
Sandy	Silvers	318,000	700	254	1.6	0.6	36
Willamette	Spring Chinook	107,000	684	236	1.5	0.8	36

A PROGRESS REPORT OF OTHER 1962 NUTRITION STUDIES

A cooperative diet experiment with the Astoria Seafoods Laboratory of Oregon State University was initiated at the Clackamas Laboratory in early August 1962. The study is still in progress at this time, Plans call for termination sometime after mid-January 1963. The following information is preliminary and is presented only as a progress report.

The objectives of the study are to rank the nutritional quality of pasteurized salmon viscera, tuna viscera, turbot, and dogfish shark with and without livers when fed to spring chinook salmon at the 40% level (the total wet ingredient) in the Oregon pellet. The wet ingredients presently used have not been ranked according to their nutritional quality when fed in this diet and the supply of presently used items is somewhat unstable making it important to test other potential components.

Prior to the start of the diet trials, hematocrits were taken, fish were randomly distributed and lots weighed, and 25% of each lot was measured. All diets are replicated (2 lots per diet). The fish are being fed on a timed appetite basis. The lots are weighed and hematocrits taken at 4-week intervals.

When the test is completed each lot will be weighed and counted and 25% measured. Proximate analyses of fish samples and hematology measurements including hematocrits, hemoglobins, differential cell counts, and erythrocyte measurements will be made. Overall food conversions will be calculated, general health observations made, and mortality figures summerized.

As of November 28, the trials have been underway 16 weeks. At this time growth has been good in all lots and quite comparable and those fed tuna viscera appear to be somewhat heavier than those fed pasteurized salmon viscera. Food conversions for all groups have been very good, mortalities negligible, and blood quality, as measured by hematocrits, satisfactory. No obvious deficiency symptoms have been observed at this time.

OREGON PELLET FEEDING PRODUCTION SUMMARY

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The Oregon Fish Commission has reported on nutritional studies at each of the Northwest Fish Culture Conferences since they began in 1950. One of the results of these studies, conducted in cooperation with personnel from Oregon State University, is the development of the Oregon Pellet. As a result of this new diet our feeding program has changed drastically. The only "wet diet" now being fed is the starting diet and only for a comparatively short time. Mechanical problems involved in producing Oregon Pellets made it impractical to feed fish smaller than 300 per pound when this program first started. Tom McKee has just reported on the results obtained by starting fish on pellets when the fish were 700 per pound after these mechanical problems were solved.

Our pellet feeding program started out on a very small scale. It was not until the year 1959 when the 1958 brood silvers were to be fed, that we fed the Oregon Pellet to all silvers after they reached a size of 300 fish per pound. Spring chinook during this year were only being fed experimentally, a few ponds per hatchery.

All of our feeding experiments have produced encouraging results. Most of our difficulties have been associated with refrigeration problems either in transporting or storage of the pellets.

One measure of the success of a salmon feeding program is to compare the egg-take with the liberation that produced the respective runs. A better measure would be the number of fish that returned to the fisheries and to the stations from which they were liberated. This tabulation is not available because of the many marking and other problems encountered in such a program. The egg-take will be used as an indicator of the success of our pellet feeding program.

There were 3,778,000 yearling 1958 brood silvers liberated in 1959-60. The resulting egg-take was 32,000,000 eggs plus a counted escapement of enough females to produce at least 3,000,000. The average silver egg-take for the previous 10 years was 10.7 million.

There were 6,087,000 yearling 1959 brood silvers liberated into hatchery streams. The 1962 egg-take is not complete at this date but the results are gratifying.

The excellent returns last year and this of silvers may be due to better conditions in the ocean or to other factors above which we have no control - natural escapements was also good in 1961 and appears to be this year - however, we believe our changes in feeding and other fish culture techniques to be a big factor in these returns.

The results of feeding Oregon Pellets to steelhead and spring chinook is not as apparent so far, as those from feeding silvers. Of course, springs are usually four or five year fish. Only the four year fish have returned to date.

Our total spring egg-take this year was 6,608,600 which is about one million more than the prior ten year average. Most of the fish returning were four-year-olds. We should expect a larger egg-take next year when both four-and-five year olds will be returning. The Willamette Hatchery took 4.3 million eggs of this season's total. This is the largest egg-take at this station since 1942.

Fall chinook have been fed a comparatively small amount of pellets, because pellets small enough were not available. This next year all falls will be fed pellets when the fish reach the size of 700 per pound.

The price of pellets has dropped from about 15¢ to a little over 12¢ per pound. Some of the factors that have influenced this price drop are: other agency purchases, our increased demand, development of specialized equipment and improved techniques in manufacturing.

We expect to feed about 1.5 million pounds of pellets to the 1962 brood fish and to get a conversion of 2.1 to 2.2 pounds of pellets to produce a pound of fish. We no longer consider our feeding program an experiment, however, experiments are being conducted that we hope will lead to a cheaper and better product.

DIET TRIALS INVOLVING THE USE OF OREGON MOIST PELLETS FOR FEEDING FINGERLING FALL CHINOOKS, STEELHEAD, AND KAMLOOPS TROUT AT COLEMAN STATION

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Introduction

As most of you know, present day fish hatcheries often resemble modern production factories. Studies, constantly being conducted in both types of production, sometimes ensure a better product at lower cost.

Numerous attempts at this station to convert fall chinook fingerlings to dry diets in the past, have met with indifferent success. This past summer diet trials using the Oregon Moist pellets in two sizes were tested against production diet controls, wherever possible, on the three species of fish reared at Coleman. Information obtained from several sources indicated that a 50 - 60% level of pellets might be adequate.

Since Kamloops trout are the most voracious eaters, at this station, all three ponds of Kamloops were started on 1/16" pellets, when approximately 241 fish to the pound. Four ponds of steelhead (2 ponds at 360/lb. and 2 ponds at 430/lb.), plus one pond of fall chinooks at 84/lb. were also started at this time. While the two different chinooks had several ponds of similar sized fish as controls. Control groups received the production diet of beef liver, beef spleen, wheat shorts, and salt.

The primary objective was

1) To determine if all three species reared at Coleman would readily accept Oregon Moist pellets throughout the entire growing season.

In addition, other important objectives were:

2) To determine if growth and survival rates of pellet fed fish would compare favorably with control groups on standard production diet.

3) To compare the general well being of each group by periodic hematocrits, hemoglobins, and internal examinations.

4) To determine the ability of pellet fed fish to withstand the rigors of handling and truck hauling for long distances down the Sacramento River.

5) To compare conversions, cost to produce a pound of fish, and labor costs of each pelleted group against it's designated controls.

I will comment on each objective in the order listed.

First to determine if all three species reared at Coleman would readily accept Oregon pellets throughout the entire growing season: While the Kamloops and Steelhead accepted pellets immediately, the one pond of chinooks tested at this time, required about five days to adjust to this food, which we considered satisfactory.

Secondly to determine if growth and survival rates of the test fish would compare favorably with control groups on standard production diet. The following figures represent gains and mortalities for the 14-week trial period.

	Final months' gain	Total % gain	Total % mortality
Fall chinooks on OMP @ 84/lb.	17%	330%	12.0%
Fall chinooks on wet diet @ 84/lb.	28%	489%	9.2%
Steelhead on OMP @ 360/lb	63%	735%	5.7%
Steelhead on wet diet @ 360/lb.	57%	629%	6.3%
Steelhead on OMP @ 430/lb.	100%	812%	9.4%
Steelhead on wet diet @ 430/lb.	75%	760%	9.8%
Kamloops on OMP @ 241/lb.	45%	967%	5.1%

Fish in all these groups experienced excessive mortalities, during July, that required a week of Terramycin therapy. Probably the addition of Terramycin for so short a time did not greatly influence the overall percent gain

The third objective was to check the general well being of each group, by periodic hematocrits, hemoglobins, and gross examinations. Upon termination of the diet trials I found the figures for each OMP group and its control group were so similar they were averaged and listed below.

	Hematocrit average	Hemoglobin average
Chinooks on OMP @ 84/lb.	38.7%	7.8 grams
Chinooks on wet diet @ 84/lb.	37.0%	7.6 grams
Steelhead on OMP @ 360/lb.	43.2%	8.0 grams
Steelhead on wet diet @ 360/lb.	40.4%	7.0 grams
Steelhead on OMP @ 430/lb.	41.8%	8.5 grams
Steelhead on wet diet @ 430/lb.	These were mixed during grading so a reading was not attempted	
Kamloops on OMP @ 241/lb.	43.7%	8.2 grams

The fourth objective was to test the ability of pellet fed fish to withstand the rigors of handling and truck transport to distant release sites. We found that the normal precautionary measures, such as refrigerated tankers and 72 hours of starvation prior to hauling, ensured the successful release of all groups.

The fifth, and final, objective discloses that the success or failure of this investigation rests on the costs of food, conversion, transportation and availability of the diets being considered.

SUMMARY OF FEEDING TRIALS

	Final months conversion	Overall conversion to date	Fish per lb. at start	Fish per lb. at end	Cost diets used in final mo.	Cost lb. of fish prod.
Chinooks on OMP @ 84/lb.	1.1	2.2	84	18	18.8¢	37.5¢
Chinooks on wet diet @ 84/lb.	1.2	4.3	84	37	10.2¢	36.5¢
Steelhead on OMP @ 360/lb	2.0	2.2	360	22	34.2¢	37.5¢
Steelhead on wet diet @ 360/lb.	3.1	4.6	360	31	27.2¢	39.1¢
Steelhead on OMP @ 430/lb.	2.1	2.5	430	34	35.8¢	42.7¢
Steelhead on wet diet @ 430/lb.	3.2	4.8	430	33	27.2¢	40.8¢
Kamloops on OMP @ 241	2.1	2.4	241	134	35.8¢	40.9¢

Since the figures presented represent only a few ponds of fish and for only a 14-week period, it is not my intention to conclude that Oregon Moist pellets constitute a diet superior to the present production diet, but merely to determine that all three species would accept this food throughout the rearing season.

I might add in closing that I don't believe that this was a fair trial for chinooks, since they were started on pellets at 84/lb., but we did find that chinooks of this size, after a short transition period, readily accept this food.

The scope of the diet trials is being greatly enlarged for the 1963 rearing season, for additional ponds of fall chinooks will be offered pellets at an earlier age, and more adequate controls will be maintained.

1962 FEEDING TRIALS
SALMON-CULTURAL LABORATORY

Laurie G. Fowler
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Longview, Washington

The 1962 feeding trials conducted at the Abernathy Salmon-Cultural Laboratory, Longview, Washington, were designed to test three protein levels, 20 percent, 25 percent, and 27.5 percent, and four caloric levels, 1300, 1650, 2000, and 2350. The objective was to determine optimum protein and caloric levels and the degree of meat and/or vitamin supplementation necessary in variations of a reconstituted composite meal diet. The diets were reconstituted with water which served to control the protein intake and to provide a mush-type feed, similar in consistency to a raw products diet. The composition of the diets are shown in table 1.

Fall chinook fingerlings were utilized as the test animals and supplied with well water at a constant temperature of 53° F. The six-foot circular rearing tanks used in the experiment were inside and consequently received no appreciable amount of natural food either from air-borne terrestrials or from the water supply.

The results of the feeding trials are as follows:

1. After 16 weeks of feeding, the low-calorie, low-protein diets started showing symptoms of a nutritional deficiency believed to be a hypovitaminosis. The deficiency symptoms then spread progressively into the high-protein, high-calorie diets until, at the end of 24 weeks, all of the diets, with the exception of the all-meat control diet, showed some fish exhibiting the deficiency syndrome. Affected fish were injected intraperitoneally with a 10-day supply of the B-complex vitamins, separately and in groups. The group receiving the complete supplement was the only one in which a response could be detected.

2. The 25 percent protein diets proved to be the most efficient diets as measured by protein fed and protein utilized.

3. At the 25 percent protein level an increase in the caloric level from 1650 calories to 2350 calories per kilogram of diet by the addition of peanut oil, resulted in a reduction of mortalities, a significantly higher protein deposition, a more efficient protein utilization, and a lower vitamin requirement in the fish. A sparing action on both the protein and vitamin requirements at higher caloric intakes is indicated.

Table 1

COMPOSITION OF DIETS OF THE

1962 FEEDING TRIALS

Salmon-Cultrual Laboratory

Diet #	Percent Protein	Calories per kg. of diet	Meat-Meal Ratio
1*	19.3	1306	90:10
2	20	1308	50:50
3	"	1300	30:70
4	"	"	20:80
5 1/	"	"	10:90
6 1/	"	"	10:90
7 1/	"	1650	10:90
8 1/	"	2000	10:90
9	25	1650	50:50
10 1/	"	"	30:70
11 1/	"	2000	30:70
12 1/	"	2350	30:70
13	"	1650	20:80
14 1/	"	"	10:90
15 1/	"	"	10:90
16 1/	"	2000	10:90
17 1/	"	2350	10:90
18 1/	"	2000	100% Meal
19 1/	27.5	2000	30-70
20 1/	"	2000	10-90
21 1/	"	2350	10-90

Leavenworth Production Diet (Control)	100.0%
Beef Liver	20.0
Hog Liver	20.0
Beef Spleen	20.0
Salmon Viscera	30.0
Salmon Carcass Meal	5.0
Distiller's Solubles	5.0
A-1 Meal Mixture	100.0%
Salmon Carcass Meal	35.0%
Dried Skim Milk	30.0%
Cottonseed Meal	20.0%
Wheat Germ	15.0%
Meat Mixture	100.0%
Beef Liver	50.0%
Hog Liver	50.0%

* Control diet

1/ Vitamin Supplement added

The Control diet was bound by the addition of two grams of salt per 100 grams of diet.

The experimental diets were bound by the addition of 2 grams of salt and 2 grams of CMC per 100 grams of diet.

Peanut oil was used to regulate the fat content of the reconstituted dry meal diets.

4. Proximate analysis of samples of fish taken at 12 weeks and 24 weeks showed a decrease in the percentage of body fat during the second 12 weeks as compared to the same diet taken at 12 weeks, while the percent protein remained approximately the same. These changes in body composition indicate an increased energy requirement in the larger fish. This additional requirement was also shown in comparable diets with different caloric intakes. At the end of 12 weeks there was no difference in total gain and/or protein deposition in diets with caloric intakes of 2000 per kilogram and 2350 per kilogram. But from then on and until the end of the experiment, the high caloric diets had better gains, fewer mortalities, and better protein utilization.

5. The addition of a complete B-complex supplement of crystalline vitamins to certain diets throughout the experiment at levels designed for maximum storage, proved ineffective in preventing the hypovitaminosis, did not promote growth, and did not reduce mortalities. High meat supplementation also proved ineffective in preventing the nutritional deficiency.

6. Stamina tests were run on the diets at the conclusion of the experiment and results revealed that stamina indices were higher in diets which had marginal vitamin deficiencies than in diets where the deficiency was extensive.

7. An all-meal diet devoid of meat supplementation and fed at the 2000 calories per kilogram level proved to be capable of maintaining fish for a 24-week period with gains, protein deposition, performance, and general condition comparable to its meat-supplemented counterparts.

We are quite enthusiastic about the results of the all meal diet and it appears that with a little additional work, this diet may be developed into a satisfactory diet for chinook salmon to be used in production feeding. Future experiments are proposed in which the vitamins will be increased by fortifying the diets with high vitamin food supplements and at varying levels of caloric intakes.

MEASUREMENT OF PROTEIN QUALITY

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Western Fish Nutrition Laboratory
Bureau of Sport Fisheries and Wildlife
Cook, Washington

The objective of our section of the laboratory program has been to determine the simplest, most reliable methods for measuring protein quality. Previously the nitrogen balance technique had been shown to provide a quantitative measure of protein quality by determining the minimum amount of a protein required to maintain the animal in nitrogen equilibrium.

In the current series of experiments further evaluations of Protein Efficiency Ratio were reported. A level of 20% protein was shown to give maximal efficiency with the basal ration employed (maximum gain was not obtained at this level). A sharp rise in P.E.R. up to 20% level and then a slow decline was noted. This was considered consistent with known protein requirements of salmonids.

The growth rate of silver salmon on several diet ingredients, including cottonseed meal (C.S.M.) was discussed. C.S.M. as a single source of protein proved inadequate to support growth.

A third series of experiments designed to measure supplementary value of protein was discussed. Four levels, 15 - 30% of casein, a reference protein, were fed ad libitum to silver salmon. Test proteins were fed at a 10% level combined with 20% casein, giving a total protein content of 30%. Gelatin was shown to have a supplemental value since the weight gain exceeded the 25% level of casein. Results with C.S.M. were equivocal due to the high mortality. The addition of 10% salmon meal and salmon eggs produced greater gains than obtained with 30% casein indicating a definite supplemental value. These observations were considered to be consistent with previous evaluations of protein quality.

DESIGN AND USE OF EQUIPMENT TO FEED EXPERIMENTAL DIETS CONTAINING TOXIC MATERIALS TO SMALL LOTS OF FISH

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Hagerman, Idaho

Kitchen graters and garlic presses are commonly used to break solid experimental diets into particles small enough to be used by the fish. With the introduction of Hepatoma Research it became necessary to devise equipment that would reduce the exposure of the personnel to feed containing known chemical carcinogens. Feeders were constructed using lucite tubing and cartridge type calking guns which in addition to preventing exposure to carcinogenic materials had the following advantages over equipment previously used: (1) More efficient feeding; (2) Reduced danger of contamination or mixing of different feeds; (3) Reduce chance of spreading disease; (4) More fish fed per man hour; (5) Reduced oxidation and spoilage.

PROGRESS REPORT ON MARKING OF PACIFIC SALMON WITH TETRACYCLINE ANTIBIOTICS

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Seattle, Washington

Among the various methods of marking being developed to assist in evaluation of hatchery production, we have been experimenting with tetracycline antibiotics as a marking device. The tetracycline series (oxytetracycline, chlortetracycline, tetracycline) are broad spectrum antibiotics, each of which possesses the phenomena of localizing in areas of bone proliferation. Upon entering the blood stream, tetracyclines are apparently deposited at any area within the organism where calcification is occurring. The deposited antibiotic is detected by its fluorescent property, emitting a visible yellow color after excitation with ultra-violet illumination. The observed result is a yellow line depicting zones of bone proliferation at time of tetracycline administration. The width of the line is dependent upon duration of administration, and the intensity, within limits, is dependent upon dose given. Also, multiple lines may be formed by administration at different time intervals.

Our studies have been directed toward marking by incorporating tetracycline antibiotics in the diet, with emphasis on optimum dose, duration of administration, palatability of diets when mixed with tetracycline, and use of potentiators. A dose of approximately 1 to 2 grams of oxytetracycline or chlortetracycline per kilogram body weight is adequate for obtaining a mark which is easily detected. This amount is 15 to 25 times greater than doses of these antibiotics given for disease treatment or control. As for duration of administration, just one feeding is sufficient. However, individuals often appear to be off feed, and 100 percent marking is not achieved unless feeding is continued for several days. Also, some commercial forms of the antibiotic are unpalatable when they constitute a large proportion of the diet, as would be necessary if giving the recommended dose in one feeding. We have achieved best results with a pelletized diet fed over three to four consecutive days. The dose required for adequate marking is quite large, compared to that needed to mark mammalian skeletal tissue, possibly indicating poor absorption from the intestine. However, through use of proper potentiators, or elimination of chemicals in the diet which form complexes with tetracyclines, it may be possible to obtain higher blood levels of antibiotic with lower doses, thus minimizing costs.

We wish to present here two studies we are currently conducting on Pacific salmon, using tetracycline antibiotics. These studies are designed to gain information on 1) possibility of differential survival of unmarked and tetracycline-marked fish, and 2) permanence of tetracycline-induced marks.

During the past 18 months, we have fed over three-quarters of a million fish tetracycline antibiotics without observing any distress or mortalities attributable to the antibiotics. We have also fed smaller groups of salmon up to 12 times the dose needed for marking without any adverse effects being noted. On the basis of these studies, there is no evidence of short-term mortality involved with feeding tetracyclines. Also, none of our studies indicates any possibility of a long-term mortality effect.

In August 1962, we fed one-third (627,833 fish) of the Leavenworth hatchery sockeye salmon production 2 grams per kilogram body weight of oxytetracycline hydrochloride mixed with glucosamine and vitamin D in a low-calcium, pelletized diet. This diet was fed for three and one-half consecutive days. A sample of 544 fish taken two months after feeding indicated that 100 percent of the sockeyes fed antibiotic were marked. Concurrently with the tetracycline marking study, one-third of the Leavenworth hatchery production was also fin clipped. Knowing the proportion of fish, both tetracycline-marked and fin-clipped to fin-clipped only, at time of release, we expect the same ratio to exist from samples taken at time of out-migration from Lake Wenatchee, and at time of return as adults. A lower proportion of tetracycline-marked and fin-clipped fish to fin-clipped only fish would indicate either less survival of tetracycline-marked sockeye or disappearance of the mark. If it is evident that application of tetracycline antibiotics do not affect survival, then a difference between ratios of tetracycline-marked only fish to fin-clipped fish, at release and at recovery will estimate fin-clipping mortality.

It was recently demonstrated in man that tetracyclines remain evident in the bone nine years after administration. Sockeye salmon and rainbow trout held at the Seattle Biological Laboratory show excellent marks 10 months after administration of tetracyclines. To test retention and ease of detection of deposited tetracyclines in fish after considerable growth has occurred, we fed, in March 1962, one-sixth (204,941 fish) of the 1960 brood of silver salmon at the Klaskanine hatchery. The silvers were given a total of one gram per kilogram body weight of oxytetracycline hydrochloride with glucosamine mixed in a standard wet diet and fed during two consecutive days. A sample of 196 fish taken two days after feeding indicated approximately 95 percent were marked.

The marked silver salmon were the last group released from the hatchery, at which time they weighed 15 per pound, the same weight as those released during the preceding six weeks. A fraction of the 1960 brood of releases have been returning as "jacks" since October 1962 after seven months ocean life and a 45-fold increase in weight. An average of one out of every 10 "jacks" returning were sampled for the tetracycline mark.

The size frequency of marked and unmarked fish in our sample is shown in figure 1. Noting that the two size frequencies are similar in distribution, it does not appear that the antibiotics given adversely affect growth.

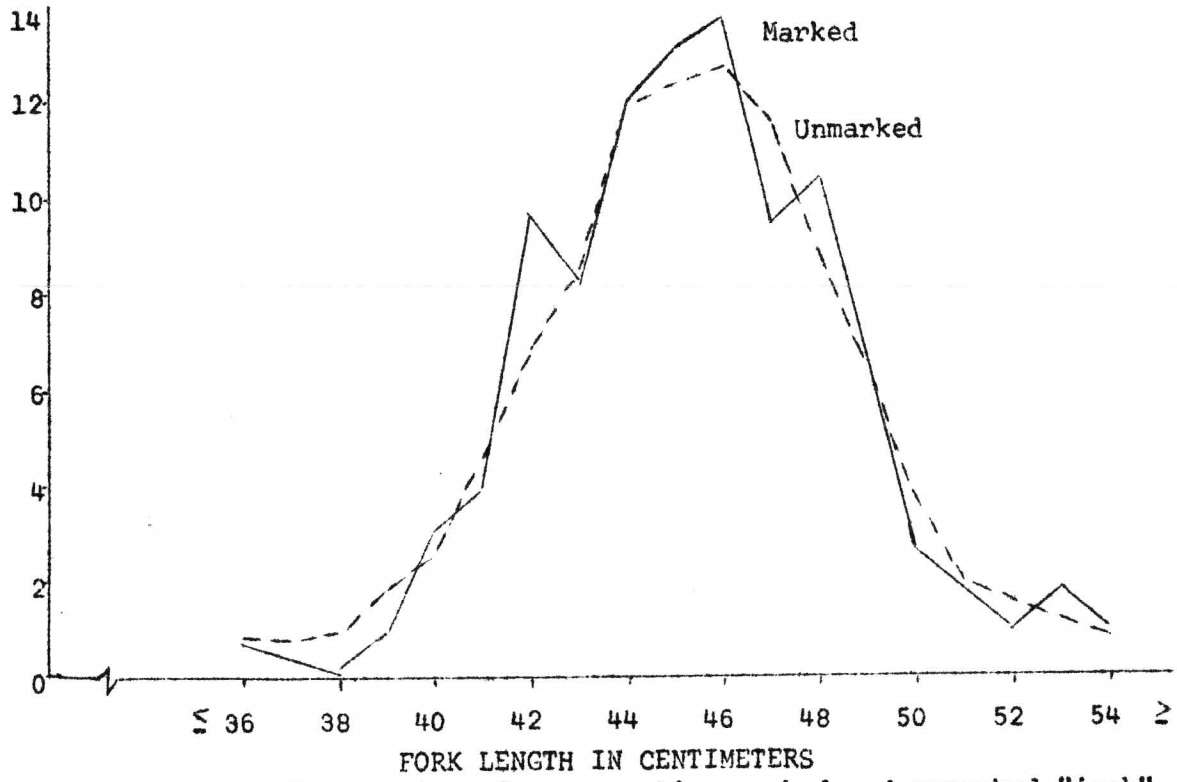
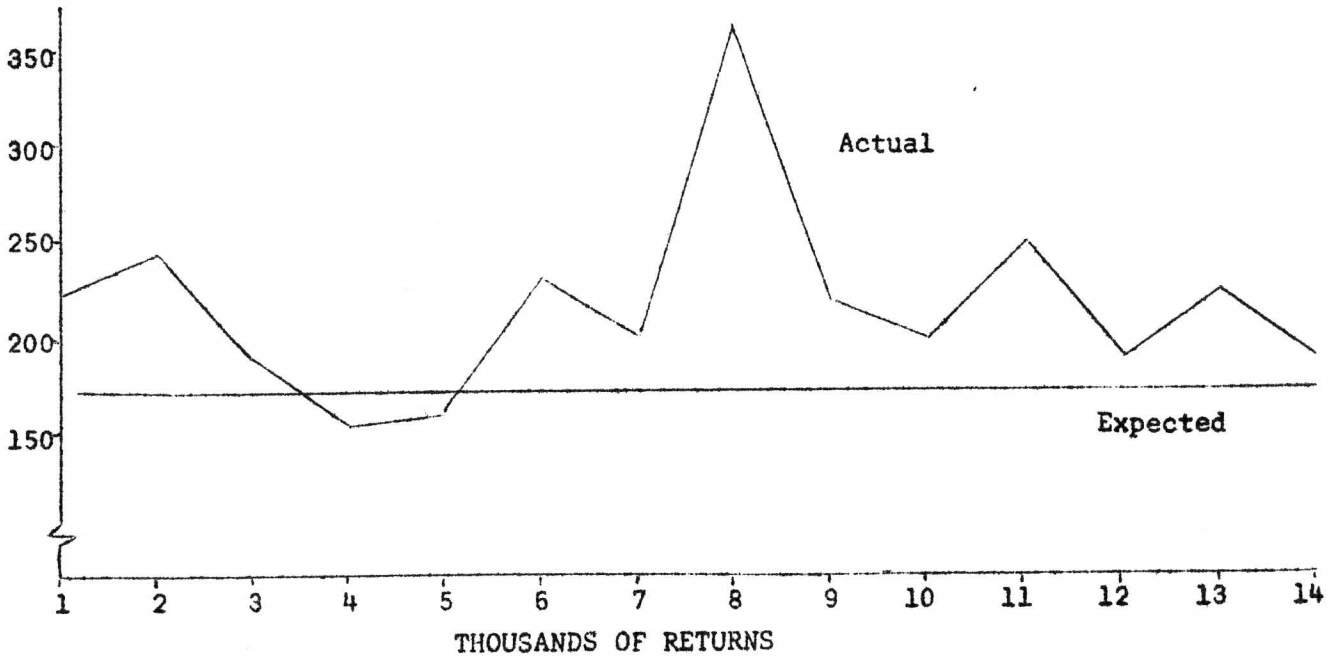


Figure 1.--Size frequencies of tetracycline marked and unmarked "jack" silver salmon returnees to Klaskanine hatchery. Data from sample of 1602 fish.



Sample Size	202	202	202	107	100	71	56	56	91	100	100	100	100	108
Date Return	October 8-9	10	10	11	11	12	12	13-15	16-22	23-5	November 6	7-9	12-14	15-27

Figure 2.--Expected and actual returns of tetracycline marked "jack" silver salmon per thousand returnees.

Considering that 95 percent of the silver salmon fed tetracycline were marked, the percentage marked for all 1960 brood silver releases was 17.24 percent. If the deposited antibiotic is retained, survival of marked and unmarked fish is equal, and straying minimal, then we would expect 17.24 percent of all 1960 silvers returning to the Klaskanine hatchery to be marked. Our sampling indicates that 21.20 percent of the returning "jacks" are marked.

Figure 2 portrays the number of marked "jacks" per thousand "jack" returns. The number marked per thousand is based on our sample size as indicated at the base of figure 2, and expanded to the expected value if all "jack" returns were sampled. The horizontal line indicates the expected return of marked silvers (17.24 percent, or 172 per thousand). The variation in sample size is due to our adjusting the ratio sampled by the quantity of fish returning at any one time. It is seen from the dates of return shown in figure 2, that even though the marked silvers were the last group released from the hatchery, they tend to return in a fairly constant manner throughout the duration of the run.

Several principal advantages in the use of tetracycline antibiotics as a marking tool, as compared to the presently available methods are: ease of marking entire hatchery populations; apparent permanence of mark; and probable equal survival between marked and unmarked fish. Also, through use of multiple marks and differences in chemical properties of the tetracycline series, a number of marking combinations may be developed.

RECOVERIES OF MARKED ADULT SALMON RELEASED FROM SPRING CREEK AND LITTLE WHITE SALMON NATIONAL FISH HATCHERIES

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Cook, Washington

This is the fourth year that I have reported on recoveries of marked fall chinook salmon released at Spring Creek and Little White Salmon National Fish Hatcheries and the second year on recoveries of marked silver salmon from Little White Salmon.

Spring Creek

Fall chinook salmon of the 1956, 1957, and 1958 brood years were marked and released at Spring Creek to compare survival from releases of unfed fry and 90 day reared fingerlings. Both groups of fish were marked just before they were ready to feed when they were about 1,100 fish per pound. Each year the fish marked Adipose-Left Pectoral were released February 5 immediately after marking. The second group was marked Adipose-Right Pectoral and then reared for about 90 days before release on May 7. The following are the data on releases:

	Ad-LP	Ad-RP
	Fry	Fingerlings
Total Marked	732,000	733,000
Date Released	February 5	May 7
Days Reared	0	90
Fish Per Pound	1,077 - 1,178	121 - 143

Marked adults from this experiment were recovered in 1959, 1960, 1961 and 1962. Data on recoveries at Spring Creek are given in the following table:

Year Recovered	Age	Ad-LP	Ad-RP
		Fry	Fingerlings
1959	2 - 3	3	107
1960	2-3-4	10	84
1961	3-4-5	0	82
1962	4-5-6	0	4
Total		13	277

Marked fish from this experiment were recovered in 1958, 1959, 1960, 1961, and 1962. Recoveries at only Little White Salmon are shown below:

Year Recovered	Age	LP May	RP July	D-LP Sept	D-RP Oct	An-RP Oct	An-LP Feb	Total
1958	2	1	2	0	0	0	0	3
1959	2 - 3	12	32	4	7	20	53	128
1960	2-3-4	18	46	6	4	31	72	177
1961	3-4-5	24	55	8	8	74	64	233
1962	4-5-6	11	24	5	10	71	60	181
Total		66	159	23	29	196	249	722
Adjusted		30	70	10	12	84	100	

In contrast to Spring Creek, the mark recoveries at Little White Salmon in 1962 were comparable to those in previous years. In order to compensate for different numbers of fish marked with each mark I have inserted in the above table and in the next table adjusted numbers based on a recovery of 100 fish marked Ad-LP.

Recoveries of Little White Salmon marked adult fall chinook salmon in 1960 and 1961 as reported by the Oregon Fish Commission were as follows:

Place Recovered	LP May	RP July	D-LP Sept	D-RP Oct	An-RP Oct	An-LP Feb
Little White Salmon	42	101	14	12	105	136
Other Locations	119	246	8	7	114	161
Total	161	347	22	19	219	297
Adjusted	61	127	8	7	78	100

The data on all recoveries to date at only Little White Salmon indicate the highest survival from fish released as yearlings in February. Total reported recoveries in 1960 and 1961 are greatest in the group released in July. Starting in 1960, fall chinook at Little White Salmon have been released in late June, about six weeks later than the previous normal release time.

I am unable to explain the sudden decline in the number of marked fish recovered in 1962. Spring Creek had a "normal" run of about 14,000 adult fall chinook but only 4 of these were marked fish from this experiment.

The Mark Analysis Section of the Oregon Fish Commission compiles lists of marked fish recovered by all agencies. The following data on marked fall chinook salmon from Spring Creek recovered in 1960 and 1961 are taken from their reports:

	Ad-LP	Ad-RP
Place Recovered	Fry	Fingerlings
Spring Creek	10	166
Other Locations	25	245
Total	35	411

All of these data indicate that fall chinook salmon released from Spring Creek after 90 days of feeding survive at a much higher rate than those released as unfed fry. Releases of unfed fry at Spring Creek were discontinued after the 1958 brood.

Little White Salmon

Fall chinook salmon of the 1956, 1957, and 1958 broods were marked and released at Little White Salmon to determine survival after several periods of rearing. The fish were marked in May and June when they were 500-200 fish per pound. Releases were made in May, July, September, October and as yearlings in February. Two marks were released in October to compare returns from Dorsal and Anal marks.

The following are the data on releases:

Mark	LP	RP	D-LP	D-RP	An-RP	An-LP
Total Marked	615,000	635,000	646,000	652,000	653,000	694,000
Date Released	May	July	Sept.	Oct.	Oct.	Feb.
Days Reared	70	125	190	230	230	350
Fish Per Pound	272-387	134-155	87-96	76-88	72-88	59-70

The fish marked Dorsal-Right Pectoral were recovered in much smaller numbers than those marked Anal-Right Pectoral released at the same time. We have had similar results with other groups of Dorsal marked fall chinook salmon in the past and I do not feel that the data from Dorsal marks are of any value.

Silver Salmon

Silver salmon of the 1958, 1959 and 1960 broods were marked and released at Little White Salmon. Each year a group of silvers marked Left Ventral were released in November and another group marked Right Ventral were released in February as yearlings as shown below:

	LV	RV
Total Marked	776,000	778,000
Date Released	November	February
Days Reared	250	350
Fish Per Pound	46 - 70	37 - 46

Marked silver salmon from this experiment were recovered in 1960, 1961 and 1962. I have divided the marked fish into two groups, jacks and adults. Jacks are defined as maturing male silver salmon less than 20 inches, total length. Numbers of fish recovered are given below:

Year Recovered	Left Ventral November		Right Ventral February	
	Jacks	Adults	Jacks	Adults
1960	22		27	
1961	27	70	55	141
1962	41	71	33	170
Total	90	141	115	311

These data indicate a much greater survival of silver salmon released in February than of those released in November. All silver salmon reared at Lower Columbia River National Fish Hatcheries are now released in February or March.

THE USE OF STAMINA AS A MEASURE OF FINGERLING QUALITY

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Longview, Washington

Two experiments were conducted during 1962 in an attempt to measure fingerling quality using differences in stamina as the criterion. These experiments were: (1) to test the effect of size of fish on the performance and (2) to induce stamina differences between two lots of fish by subjecting them to different environments.

Stamina differences were determined by testing 100-fish samples in the stamina tunnel. The fish are counted as they leave the tube, and the numbers recorded at minute intervals. In comparing the performance of various groups of fish, a performance index is calculated by adding the time the first 25 percent of the fish leave the tube to the time when 75 percent are gone.

Increased stamina with growth

During the spring, a study was conducted to determine the effect of size of fish on the performance. Samples of fish from a single raceway pond were tested over a three-month period, usually at weekly intervals. The tests were run using well water at a constant temperature of 53° F. The fish were placed in a trough of well water for 16 to 24 hours before the tests to allow temperature acclimatization and to eliminate the effect of recent feeding. Testing fish immediately after feeding had previously been found to result in poor performance. The diet varied slightly during the study period but was basically a meat mixture of liver, spleen, and turbot combined with small amounts of salmon meal.

Results of the study are shown in figure 1. During the study period, the average weight of the fish increased from 1.04 grams to 3.64 grams. The performance index increased from 11.4 to 23.8.

A number of tests using creek water were also run on these same fish. The temperature of the creek varied from 44° F. to 49° F. during this period. While further studies will be needed to determine the effect of temperature on performance, the cooler water tests showed a similar curve which averaged .8 to 2.3 performance points below the constant temperature curve.

This study will be repeated during the spring of 1963 to establish the reliability of the curve. If time permits, stamina tests will also be run on fish from the same stock, but being reared in different pond types.

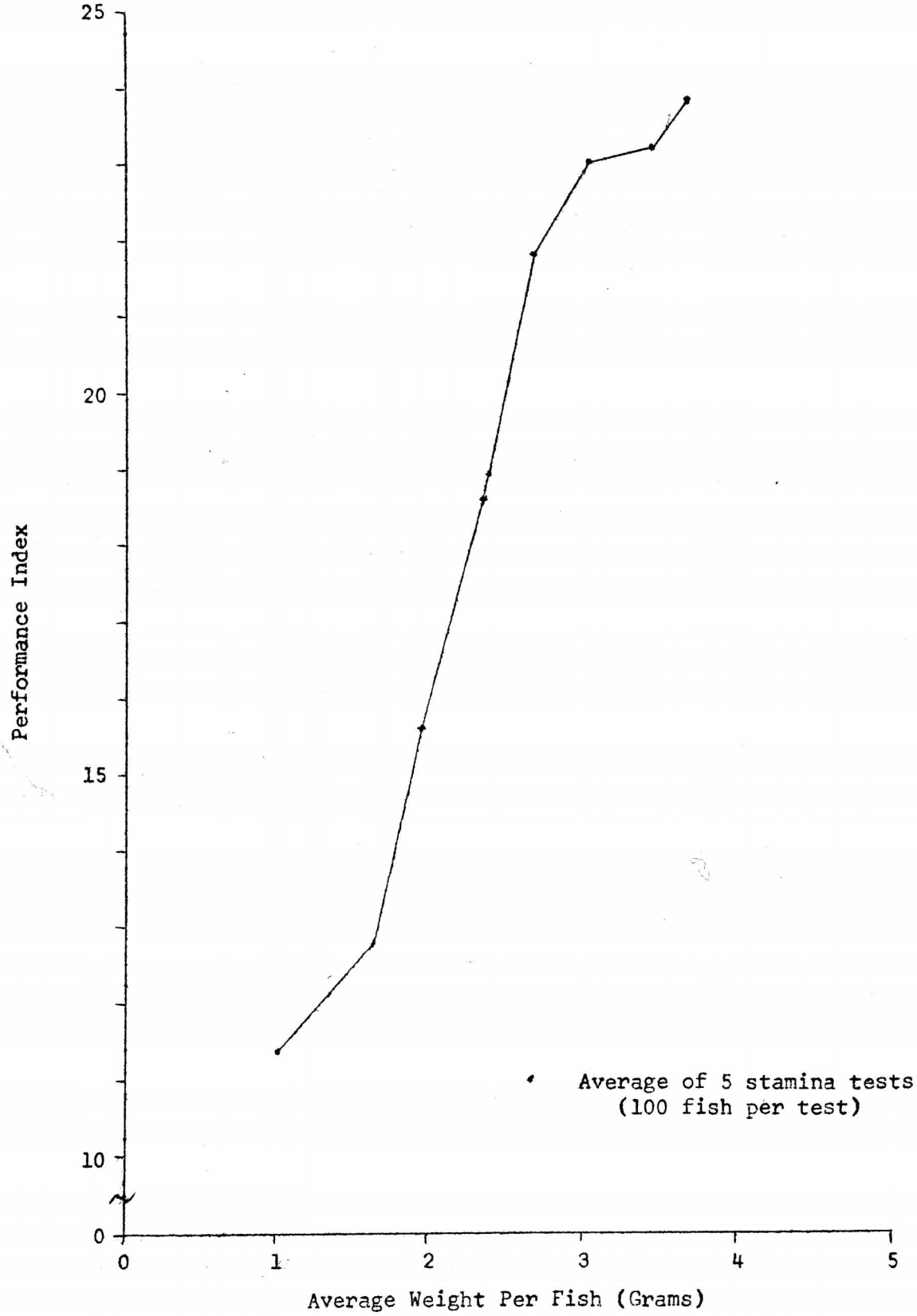

Figure 1.--Increased stamina with growth in fall chinook fingerlings

Effect of environment on stamina

In previous tests, it was found that performance is effected by environment. An experiment was begun on May 24 to induce stamina differences by means of different rearing pond environments. Two groups of fall chinook fingerlings, about 200,000 each, received identical diets and stocking rates, but were raised in different pond types. The ponds used were the conventional 8 x 80 raceway having a maximum water velocity of less than .1 fps and the rectangular-recirculating pond with velocities ranging from .2 fps to 1 fps.

Samples from each pond type were tested over a 17-week rearing period, usually at bi-weekly intervals. Figure 2 shows the results of this testing. Randomized samples from the rectangular ponds showed an average performance index of 41 while the average of the samples from the raceways was 30. A difference of 3 performance points was a significant difference. Actually the fish from the raceways showed no significant increase in performance during the rearing period as the initial performance index was 27.

In August, each group of fish received a different single-fin mark; the raceway-reared fish were marked by the excision of their right pectoral fin and the fish reared in the rectangular pond were marked by removal of their left pectoral fin. The fish were released into Abernathy Creek on September 18 and 19.

The final phase of the effect of stamina differences on survival will be the comparison of the numbers of marked adult fall chinook from each group returning to Abernathy Creek. Since we assume that fish with good stamina will have higher survival, we expect a greater return from the fish reared in the rectangular-recirculating ponds over those raised in the conventional raceways.

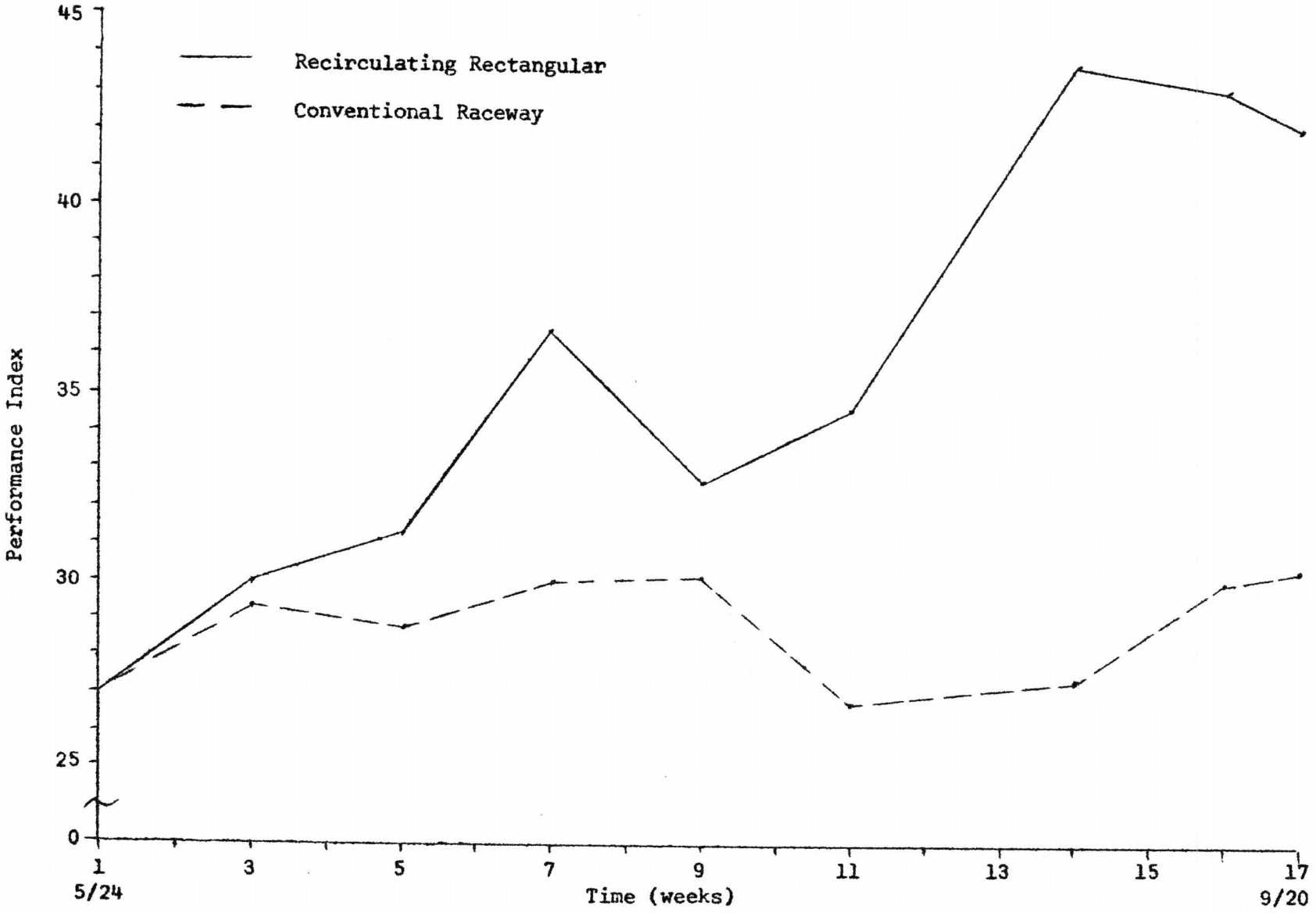

Figure 2.--Stamina differences induced by environment

THE ROLE OF FISH BODY CHEMISTRY IN CHARACTERIZING PHYSICAL CAPABILITY OF CHINOOK SALMON FINGERLING

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The role that fish body chemistry may serve for establishing desirable indices of quality is under study. Three-month-old fingerling chinook salmon from twelve hatcheries were characterized by chemical analysis of body and blood plasma components. The carcass was analyzed for total glycogen, water, lipid, protein and ash content. Components measured in the blood plasma were total protein, glucose, total cholesterol, creatinine, uric acid, urea, phosphorus, calcium and chloride. The relationship of each of the chemical entities to physical performance (performance index) of the fish was studied.

Attempts to correlate the body chemistry with physical performance were only partially successful. Correlation studies of ten lots of fish showed that there was a direct relationship between percent body protein and performance index with a measured correlation coefficient of 0.84. The regression graph of performance index on body protein is shown in figure 1. The amount of usable data was not sufficient to show any correlation between performance index and blood plasma components or body components other than percent protein. Data from six lots of fish was not included in the analysis because in some cases there was extensive gill proliferation and in some cases the marked fish were far superior to the unmarked fish in physical performance which is unlikely and indicates a sampling error. In the values which were used there was no difference in performance index of the marked and unmarked fish. Fish from later releases of three of these hatcheries did not show the damaged gill condition and were included in the correlation studies.

We hypothesize that any measurement that will serve to characterize fish may be correlated with the number of returning adult salmon. Any chemical measurement that can be related to physical performance could conceivably yield advance information as to the return potential. Some chemical components of the blood plasma are present in amounts too low to be measured by available analytical methods. There is such a dearth of information on fish chemistry that screening of values and development of procedures can proceed simultaneously.

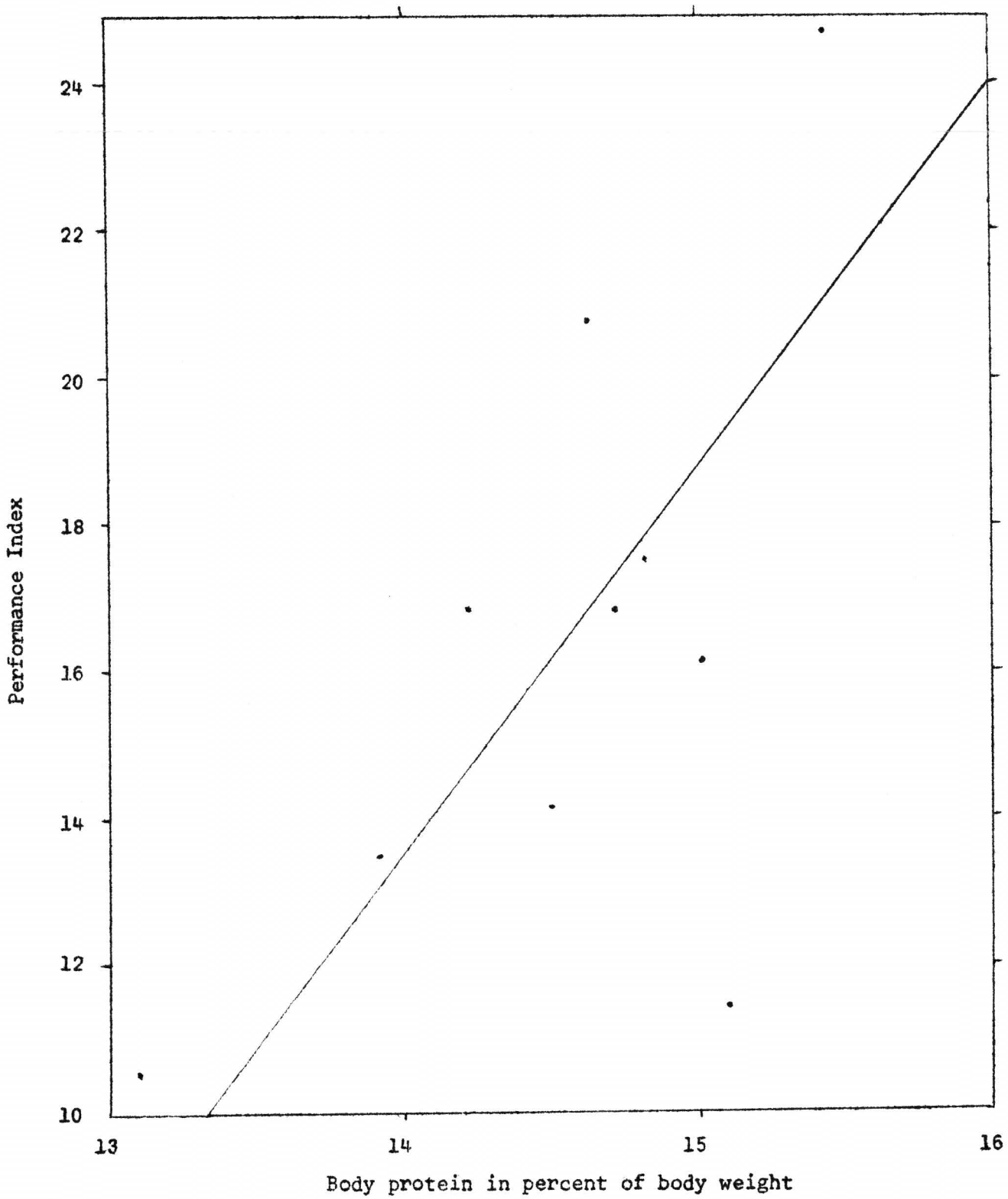


Figure 1.--Regression graph of performance index on body protein.

SEX RATIO CONTROL IN HATCHERY-REARED RUNS OF CHINOOK SALMON (O. TSCHAWYTSCHA)

J. Howard McCormick
Bureau of Sport Fisheries and Wildlife
Longview, Washington

In the artificial propagation of salmon a large percentage of the returning fish serve no useful purpose. These are the males which return in excess of those required to fertilize the eggs taken.

If we assume that one male is used to fertilize the eggs of two females, then in a normal run of fish with a 1:1 ratio of males to females, 25% of the returning fish will be of no value to the spawn-taking operation. It thus becomes evident that the same number of eggs could be derived from a reduced number of returning adults if females returned as an increased percentage of the spawning run. The result of such an alteration in the sex ratio of a returning run would be to reduce the fish-handling time in the spawn-taking operation. Such an alteration would allow an increased harvest by the fishery in a direct relationship to the reduced number of fish which must return to provide the required number of fertilized eggs. Both of these advantages could be derived through converting a given percentage of the males in the original release to females, thus eliminating a surplus of males.

The possibility for such an accomplishment was reported in the work of Toki-O Yamamoto who has published several papers in recent years wherein he presents the accomplishment of just such sex ratio alteration by feeding sex hormones as a portion of the diet of young developing medaka, a small oviparous aquarium fish. The sex reversals accomplished by Yamamoto with medakas were complete, lasting, and resulted in fertile adults.

The success of Yamamoto in accomplishing sex-reversal in the madaka and our recognition of the problem of more male salmon returning to the hatcheries than are needed has led us to explore the possibility of applying the principles developed with the medaka to salmon-cultural procedures.

Our first approach was to seek a hormone administration method which would require a minimum of time to apply and yet would have the desired sex-reversal effect. The method of administration used in our experiments was to prepare an aqueous suspension of the hormone and then to place freshly fertilized and rinsed eggs in this mixture for water-hardening.

Two female hormones were used in our initial experiment, diethylstilbesterol and estrone. The results obtained with diethylstilbesteril were unsatisfactory and its use has been discontinued for the present. The

results obtained when estrone was used, however, were very encouraging, though not conclusive. High mortalities have obscured clear-cut findings. To keep the small treatment lots separate the incubating trays, used in hatching the eggs, were specially compartmented. This compartmenting resulted in poor water circulation and excessive mortality ensued. At the present time we are repeating the estrone experiment to confirm the results of last year's work, taking precautions to avoid the circulation problem previously encountered.

The sex of the fingerlings produced was determined when the fish reached the mean size of about two inches (49.1 mm.). The method employed in the determination of the sex of the fingerlings involved dissection of the fish and removal of the gonads for whole wet-mount preparations.

The criteria used in distinguishing the two sexes were the septa and oocytes of the female gonad, and the lack of these structures plus the granular appearance of the male gonad. Examination of the slides was with the low power compound microscope.

The results of this exploratory experiment using estrone treatment of Chinook salmon eggs are as presented in table 1.

Points of particular interest are the 100% female populations at concentrations 10-12 ppm estrone, and the highly significant divergence from an expected 1:1 male to female ratio, determined by the x^2 method, in estrone concentrations 7.0 ppm through 40 ppm. It is also important to note the successive decreased survival rate in proceeding from concentrations 7.0 ppm through 40 ppm.

There may be an optimum treatment concentration level indicated here, suggesting something in the vicinity of 8.0 ppm concentration even though higher percentages of females were produced in some of the other treatment concentrations. The females produced per unit treated is at a maximum at the 8.0 ppm of estrone concentration.

It should be pointed out that the high mortality occurring in some of the treatments with the highest percentage of females makes it difficult to completely discount the possibility of sex-selective mortality. However, we have taken into consideration the location of the mortalities in relation to time of occurrence and location in the incubator and, in addition to this, applied chi-square tests of the sex ratios of the various populations assuming sex-selective mortality rather than sex-reversal. The results of this critical examination of our data indicate that sex-reversal is more likely than the possibility of sex-selective mortality.

To telescope the time factor and reduce the number of specimens required, rainbow trout will be used in an experiment this year to evaluate the fertility of females produced through hormone sex-reversal of fish of the male genotype.

Table 1.--Results of estrone treatments of chinook salmon eggs (O. tshawytscha)

Estrone Concentration	Percent Mortality			Fingerling Produced		
	Eggs	Fry	Total	Females	Males	% Females
Control	85.0	1.0	86.0	8	6	57.1
Control	5.2	3.0	8.2	51	59	46.4
0.4 ppm	14.9	9.9	24.8	42	34	55.2
2.0 ppm	16.3	2.1	18.4	38	42	47.5
3.0 ppm	12.9	2.1	15.0	37	41	47.4
4.0 ppm	63.0	2.0	65.0	12	23	34.3
5.0 ppm	100.0		100.0	0	0	0.0
6.0 ppm	11.8	12.7	24.5	40	36	52.6
7.0 ppm	20.8	8.9	29.7	49	22	69.0**
8.0 ppm	14.9	28.7	43.6	48	7	87.3**
10.0 ppm	12.9	49.5	62.4	34	0	100.0**
12.0 ppm	21.8	39.6	61.4	35	0	100.0**
20.0 ppm	18.0	60.0	78.0	21	1	95.4**
40.0 ppm	15.2	70.7	85.9	13	1	92.8**

** Highly significant difference

Should we be successful in developing this technique to a practical point, so as to be able to introduce it into the routine spawn-taking operation, one precaution must be taken; that is, to identify by some mark thos fish which have been derived from hormone treated eggs. The reason for this is that the apparent sex of the returning fish will not be a reliable indication of their sex genotype.

The fish which have received hormone treatment must be so identified so as to make it possible to retreat all eggs derived from these fish. The importance of this retreatment lies in the fact that sex-reversed females possess a sex gene composition which would result in offspring with a sex ratio favoring males which is entirely contrary to the end desired. The genetic implication of a hormone treatment program such as has been discussed are presented in Figure 1.

In summary, first we believe that greater efficiency in the spawn-taking operation could be obtained if it were possible to control the sex ratio of returning adults so as to provide a higher percentage of females than are in the natural runs. Second, we also believe that this can be accomplished through hormone treatment of a pre-determined percentage of the egg take. Third, when such a procedure is introduced, marking of all treated fish will be necessary so as to avoid genetic complications which would result from masked sex-gene compositions. Fourth, the work which we have done to date is very promising but needs considerably more verification before it can be applied with confidence.

Figure 1.--GENETIC IMPLICATIONS OF SUCCESSIVE MATINGS OF FEMALES FROM ESTRONE TREATED EGGS TO NATURAL MALES[1/]

Mating of Natural Adults

P-1 Generation

50 Females

50 Males ♂ \ ♀	X	X
X	XX	XX
Y	XY	XY

Progeny 2 ♀ : 2 ♂

Total Population Sex Ratio 1 ♀ : 1 ♂

Matings of Adults from Untreated Portion of the Population

F-1 Generation 50% of eggs from P-1 mating estrone treated. Returning run thus treated, 3 ♀ : 1 ♂

25 Females

9 Males ♂ \ ♀	X	X
X	XX	XX
Y	XY	XY

Progeny 2 ♀ : 2 ♂

Matings of Males from the Untreated Portion of the Population to Females from the Treated Portion

25 Females

8 Males ♂ \ ♀	X	X
X	XX	XX
Y	XY	XY

Progeny 2 ♀ : 2 ♂

25 Females

8 Males ♂ \ ♀	X	Y
X	XX	XY
Y	XY	YY

Progeny 1 ♀ : 3 ♂

Total Population Sex Ratio Without Retreatment 1 ♀ : 1.4 ♂

Matings of Adults from Untreated Portion of the Population

F-2 Generation Retreatment of all egg taken from ♀s with previous estrone treatment. Returning run thus treated, 3 ♀ : 1 ♂

25 Females

9 Males ♂ \ ♀	X	X
X	XX	XX
Y	XY	XY

Progeny 2♀:2♂

Matings of Males from the Untreated Portion of the Population to Females from the Treated Portion

19 Females

6 Males ♂ \ ♀	X	X
X	XX	XX
Y	XY	XY

Progeny 2♀:2♂

25 Females

8 Males ♂ \ ♀	X	Y
X	XX	XY
Y	XY	YY

Progeny 1♀:3♂

6 Females

2 Males ♂ \ ♀	Y	Y
X	XY	XY
Y	YY	YY

Progeny 4♂

Total Population Sex Ratio Without Retreatment 1 ♀ : 1.7 ♂

1/ Working with an assumed constant return run of 100 fish

GROWTH ACCELERATION IN A COLD WATER HATCHERY

John K. Susac
Oregon State Game Commission
Bend, Oregon

The Fall River Hatchery water supply is a spring-fed stream providing water at the intake close to 42° at most times of the day and year, with the exception that temperatures increase up to 56° for short periods daily during warm spring and summer days.

Ponds are 100x20x3-1/2', hold 7,000 cubic feet of water, and, with normal operation, the water flow is approximately 1 second foot per minute.

Fish growth is very slow, so consideration has been given to methods of increasing the temperature to obtain better growth.

The objects of the experiment to be covered here were to determine if inflow could be shut off daily when the maximum temperature was reached and how long fish could be held successfully in the static warmer water.

Methods

On March 28, two ponds of eastern brook were started, 207,000 (32 pounds) in experimental pond 4 and 255,000 (38 pounds) in control pond 5.

Growth counts were made every ten days. (Figure 1) Oxygen samples were taken periodically.

From March 28 to April 10, the water flow was shut off in pond 4 from 7 p.m. until 3 p.m. the following day. The water was left running for four hours per day.

From April 10 to May 10, the water was shut off from 8 p.m. until the next noon. The water was on eight hours a day.

From May 11 to July 20, the water was shut off from 8 p.m. until about 8 a.m. the next morning. The water was on twelve hours a day. During this period, three one-half inch pond sprays were in operation at all times.

A second experiment was started on April 21 in experimental pond 1 and control pond 2. Each pond contained 283,000 kokanee at 5,760 per pound. The two ponds were operated on the same schedule as ponds 3 and 4 in experiment B.

Control pond 2 exhibited increasing losses beginning June 21 up to 300 per day.

Beginning June 29, the control pond (2) water flow was shut off in the same manner as experimental pond (1) of kokanee, which had suffered negligible mortality.

Mortality in the control pond decreased to nothing by July 7, but the same schedule was continued for both ponds until July 20.

Group C was started on April 21, with 275,000 eastern brook in experimental pond 7 and 273,000 eastern brook in control pond 8. Both groups were 3,500 to the pound at the beginning of the experiment. Water flow manipulation was on the same schedule as the other two experiments during the period involved.

There were periods after May 10 varying in length from one to four days during which water in the experimental ponds was not shut off.

Results

In experiment A, although the experimental pond contained fewer fish of a smaller size in the beginning, in just under four months the experimental group went 224 to the pound, thirty-three per cent larger than the control group (Table 1 and Figure 1). Loss in the experimental pond totaled 8.2 per cent, 17,000 fish. Mainly, loss was caused by escapement, through a crack in the pond wall and a temporary break in an outlet screen. Mortality in the control pond was 24,860, or 9.7 per cent.

In experiment B, kokanee in the experimental group reached 440 per pound by July 20, while the control group went 830 per pound (Table 1 and Figure 2). In addition to being almost twice the size of the control pond fish, the experimental lot had 1,400 mortality in contrast to a 17,900 loss in the control pond.

In the final experiment, using equal numbers of eastern brook, the experimental fish were 272 per pound at termination of the tests, or 32 per cent larger than their control counterparts (Table 1 and Figure 3). Likewise, the loss of 2,505 in the test group was 81 per cent less than the 12,795 loss in the control.

It was found that manipulation of water flow increased the average daily temperature in the test ponds from two to five degrees. (Table 2 and Figure 4).

Water could be shut off for as long as twenty hours when the fish were small. Later, when the pond contained approximately a thousand pounds of fish (a pound of fish for seven cubic feet of water) flow could still be shut off for twelve hour periods when pond sprays were used.

Table 1. Growth rates ir experimental and control ponds, Fall River Hatchery expressed in numbers per pound.

	March 28	April 10	21	30	May 10	21	31	June 10	21	29	July 10	20
												20
Group A. Eastern brook												
Experimental Pond #4	6,464	5,136	3,760	2,088	1,872	1,216	1,024	672	400	320	266	224
Control Pond #5	6,200	5,136	4,608	3,776	2,464	1,728	1,376	1,008	704	628	496	340
Group B. Kokanee												
Experimental Pond #1	5,800		5,760	4,672	3,776	2.944	2,144	1,552	1,088	880	624	440
Control Pond #2			5,760	5,280	4,320	3,852	3,200	2,320	1,690	1,584	1,056	834
Group C. Eastern brook												
Experimental Pond #7			3,552	3,040	2,272	1,344	1,040	768	576	448	352	272
Control Pond #8			3,552	3,328	2,592	1,584	1,264	912	756	624	512	400

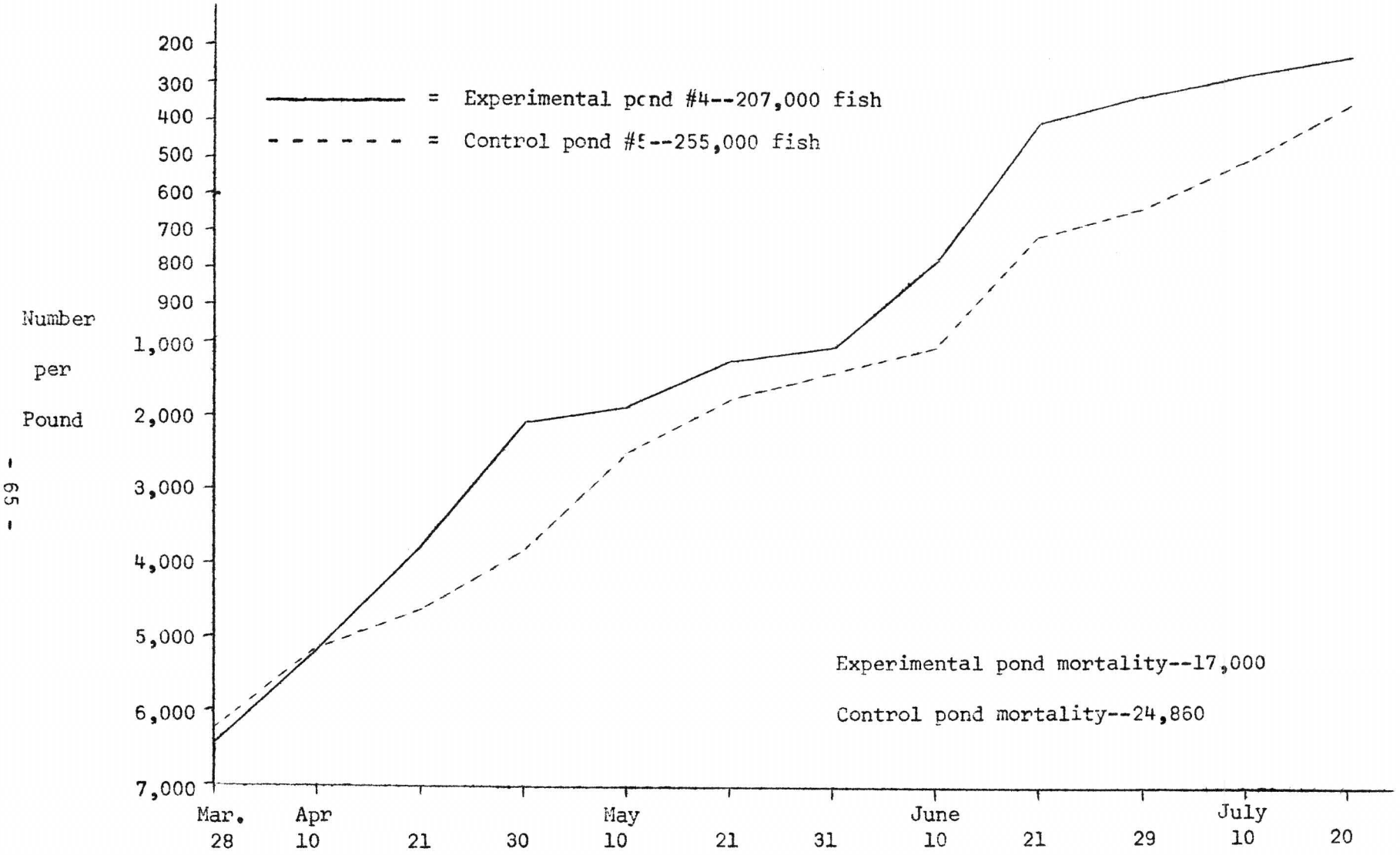


Figure 1.--Growth rates of eastern brook in experimental Group A, Fall River Hatchery

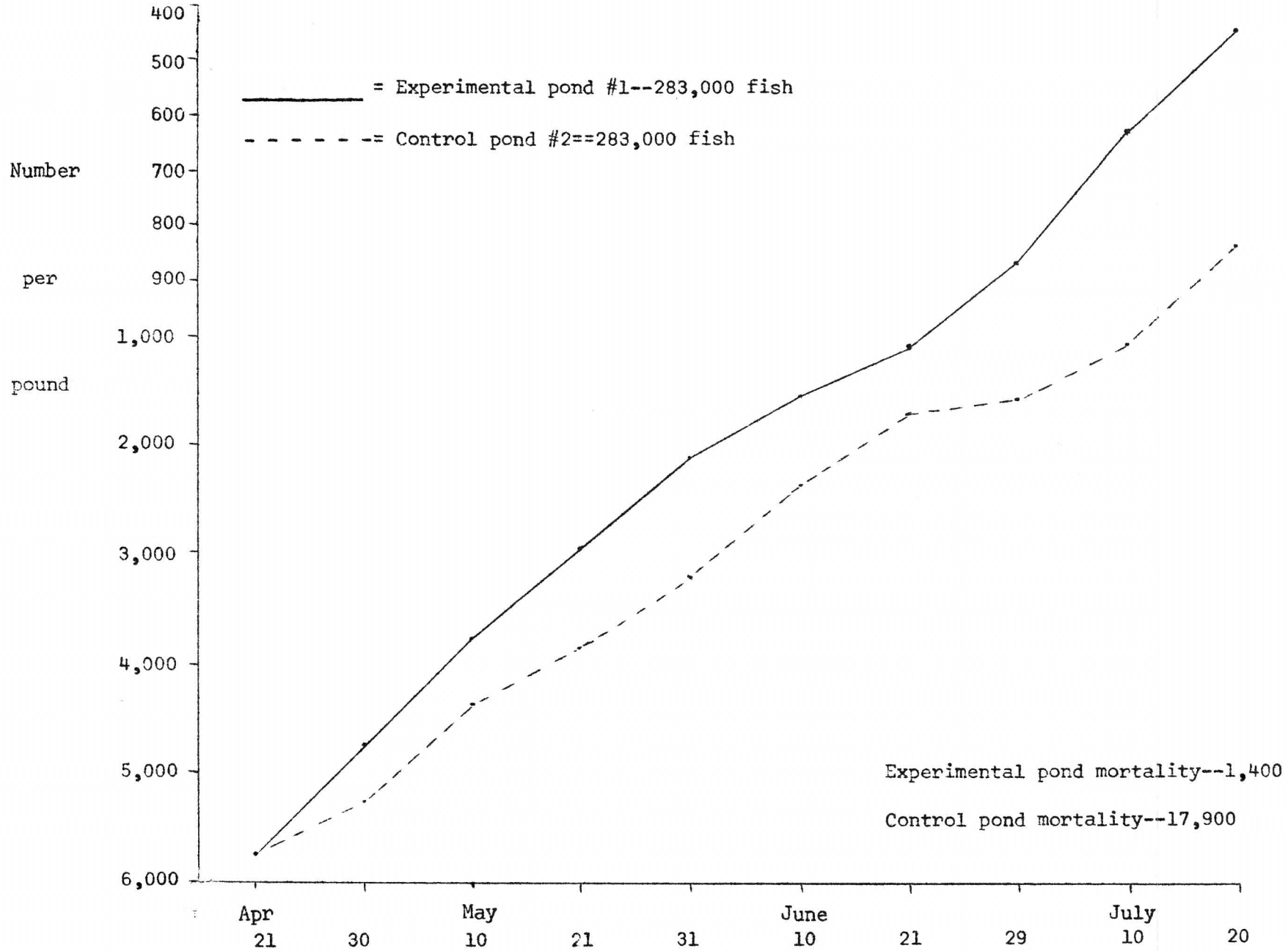


Figure 2.--Growth rates of kokanee in experimental Group B, Fall River Hatchery

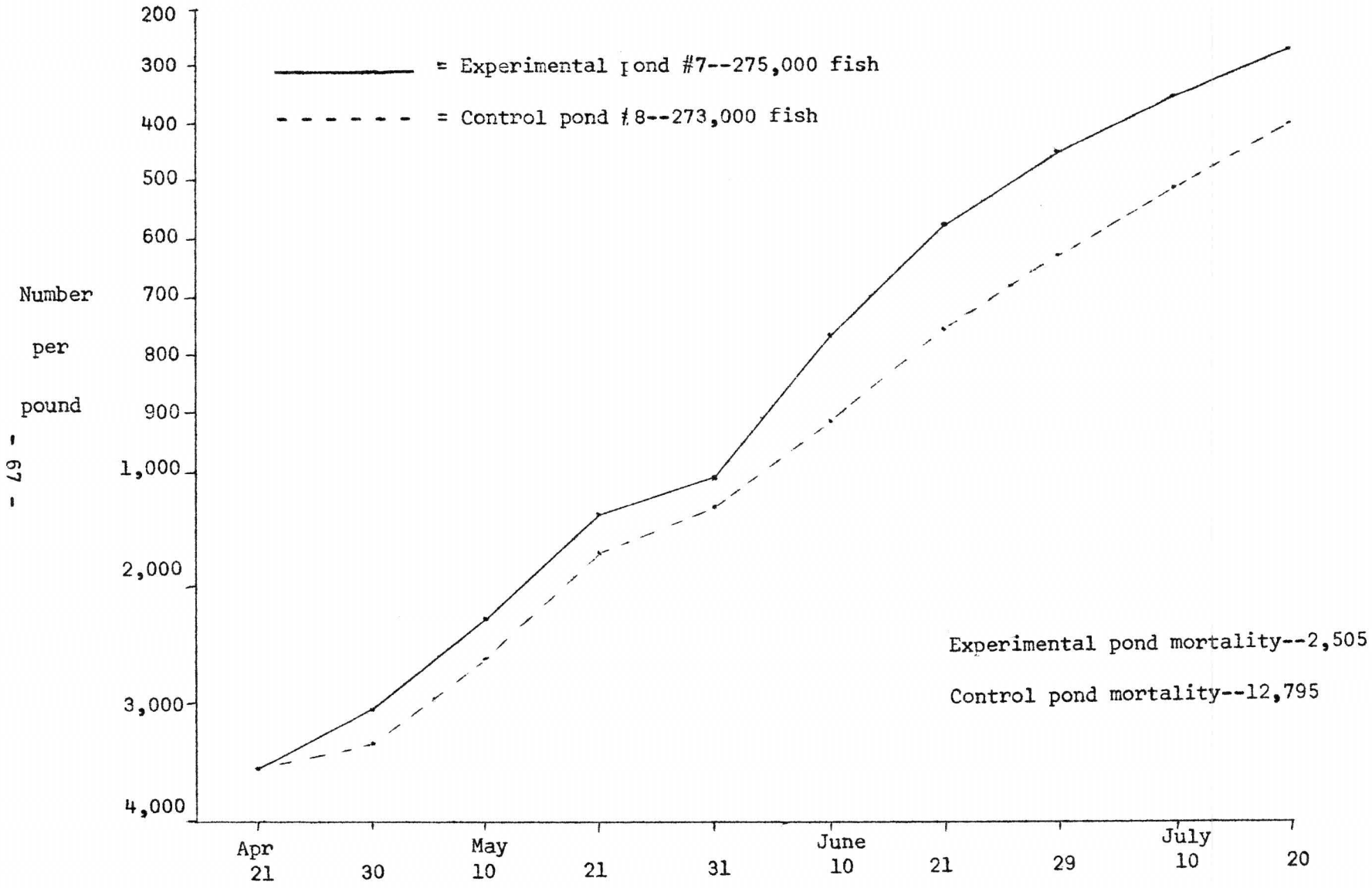
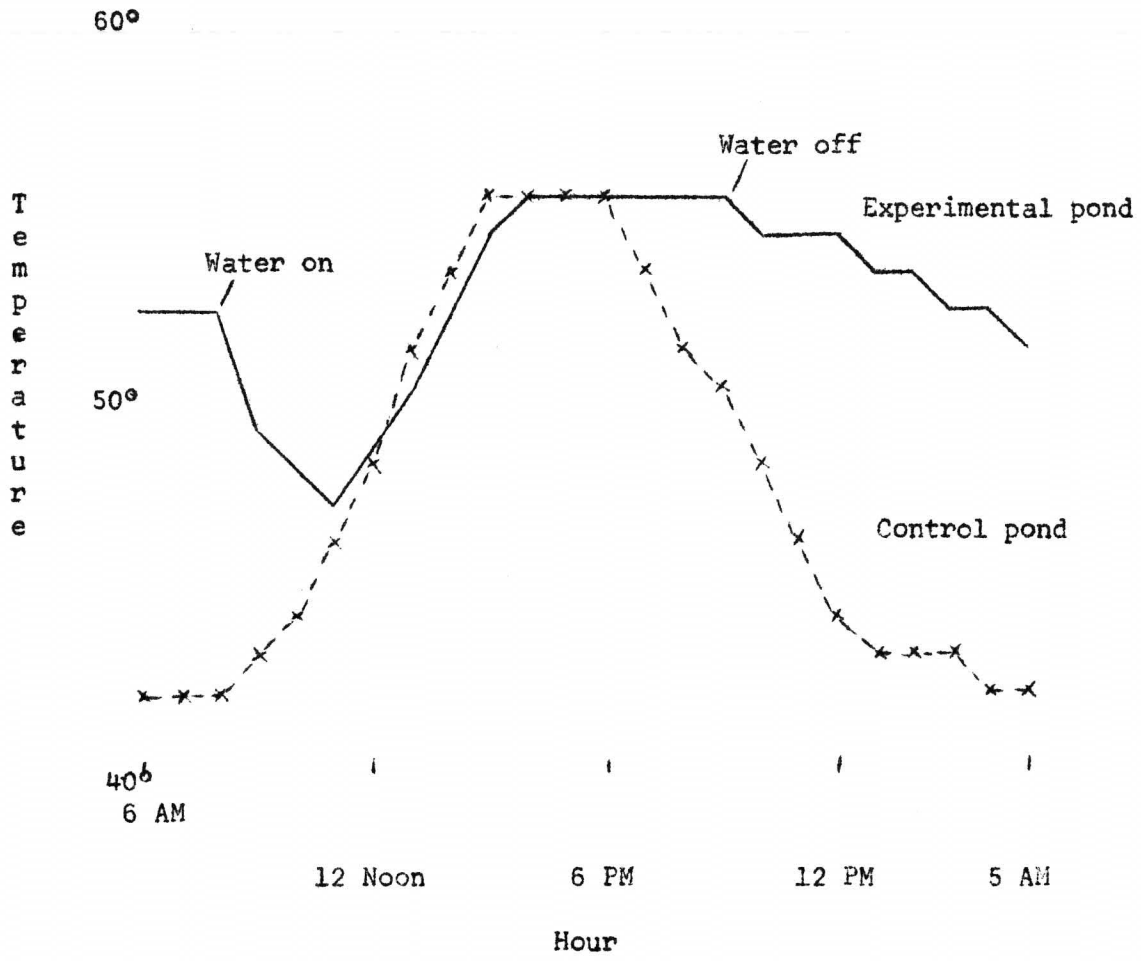


Figure 3.--Growth rates of eastern brook in experimental group C, Fall River Hatchery

Table 2.--Examples of temperature comparison between experimental and control ponds--Fall River Hatchery

	TIME	EXPERIMENTAL	CONTROL
	6 a.m.	52	42
	7 a.m.	52	42
Water on	8 a.m.	52	42
	9 a.m.	49	43
	10 a.m.	48	44
	11 a.m.	47	46
	12 p.m.	48	48
	1 p.m.	50	51
	2 p.m.	52	53
	3 p.m.	54	55
	4 p.m.	55	55
	5 p.m.	55	55
	6 p.m.	55	55
Water off	7 p.m.	55	53
	8 p.m.	55	51
	9 p.m.	55	50
	10 p.m.	54	48
	11 p.m.	54	46
	12 a.m.	54	44
	1 a.m.	53	43
	2 a.m.	53	43
	3 a.m.	52	43
	4 a.m.	52	42
	5 a.m.	51	42
	Averages	52.2	47.3

Figure 4.--Examples of temperature comparisons between experimental and control ponds, Fall River Hatchery



Conclusions

Although these experiments were rudimentary in nature and not thoroughly complete, the results show a careful and thorough series of tests should be made at Fall River Hatchery and possibly other stations faced with similar conditions. Such tests should include simultaneous thermograph recordings for the entire period in the experimental and control ponds and oxygen analysis more frequently and from different depths. Further, all tests should use lots equal in size and number of fish.

A variety of water flow manipulations should be tested carefully to determine the most satisfactory and efficient system from the standpoint of all factors, including growth rate, food conversion, mortality, and general condition of the fish.

ADJUSTABLE FRY GRADER

Paul Vroman
Oregon Game Commission
Philomath, Oregon

The separation of hatchery fish into individual size groups has long been used in Oregon as a management tool to arrive at a more uniform-sized fish for planting. We believe that grading decreases cannibalism, promotes a more uniform growth, and permits a more accurate estimate of weight by eliminating some of the variation in size often found in sample counts.

Because fingerling and yearling grading of fish had proven so beneficial, I started experimenting with methods by which smaller fingerling and fry could be graded at an early age. Among the main features which I tried to incorporate in the grader was: rapid adjustment for size, a means of preventing gilling, and a quick release of those fish that were gilled.

In our first attempt at grading fry, we built a wooden box with a slatted bottom and one end open to allow the larger fish to slide over the end. This model was first used in 1960 on Cutthroat fry, and results were so encouraging that we believed further work should be carried on. It was also through this first attempt at grading fry that we realized the importance of a means for making rapid adjustment for various sizes, and some means of releasing gilled fish.

In the first adjustable model I used 3/4" wood quarter-round for the grading bars. Wood on becoming wet, of course, tends to swell and warp and was used only because of the ease of construction with limited tools. However, this design was tested and showed enough promise to warrant the expenditure for construction of an all-metal model.

In search for available material, aluminum quarter-round base shoe 1/2" by 3/8" was chosen for the adjustable bars. On other parts, aluminum was used wherever possible.

In the model the bars are held in a closed position by means of a spring. A hand operated lever mounted at the head of the grader is used to open the bars to their maximum, thus releasing gilled fish. Upon release of this lever the spring returns the bars to their original setting. The size adjustment setting is by means of a thumb screw bearing against the hand lever, thus increasing or decreasing the openings between the bars. Two perforated pipes are used to supply a water spray for moving the fish along the grader bars. Legs at the head of the grader can be adjusted to change the slope and control the speed by which the fish slide down the grading bars. It was found desirable to have valves on each of the spray pipes in order to further control the movement of fish down the grader bars.

This grader is designed to fit a standard hatchery basket or trough. Weight is approximately 25 pounds. Baffles are placed underneath the grader at the end of the grading bars to aid in separating the two size groups into their respective containers.

At Alsea, fry are fed for about one week in the baskets and then transferred to concrete tanks 15 feet long and 3 feet wide by 30" deep where they are held for about three months. The transfer of fingerling from these tanks to outside ponds was chosen as the opportune time to grade with the fry grader. In past years the first grading came when the fish had been in the outside ponds for about two months and reached approximately 60 per pound. With this new fry grader however, fish can be graded at 300 per pound, a great saving in pounds of fish handled.

In the spring of 1962 we graded Steelhead averaging about 325 per pound into approximately two equal groups. The larger fish came off the end of the grader at 297 per pound, while the smaller ones graded out at 385 per pound. As is shown in figure 1, 78 percent of the larger group were over 50 mm long and 86 percent of the smaller group were under 50 mm in length.

At the end of one month, samples were taken and measured. Large fish averaged 143 per pound and smalls 182 per pound. Each group had doubled its weight (figure 2).

At six weeks from the original grading it was found that a large share of the fish in the smaller group had shown sufficient growth to warrant regrading (figure 3). In this grading we attempted to take off only about 20% of the smaller fish. No measurements were made of this group at this time.

On November 20th, 147 days after the original grading, we again measured the three groups. The two larger groups are still quite uniform as may be seen in figure 4, however at this time we regraded all of our fish through our larger grader.

In conclusion, I feel that this grader has an important place in hatchery management. It will satisfactorily divide fish into size groups at an early age with a minimum of labor, and no injury to the fish from gilling. Fish can be grouped by size for more uniform growth, reduction of cannibalism, and more efficient feeding with the new dry diets.

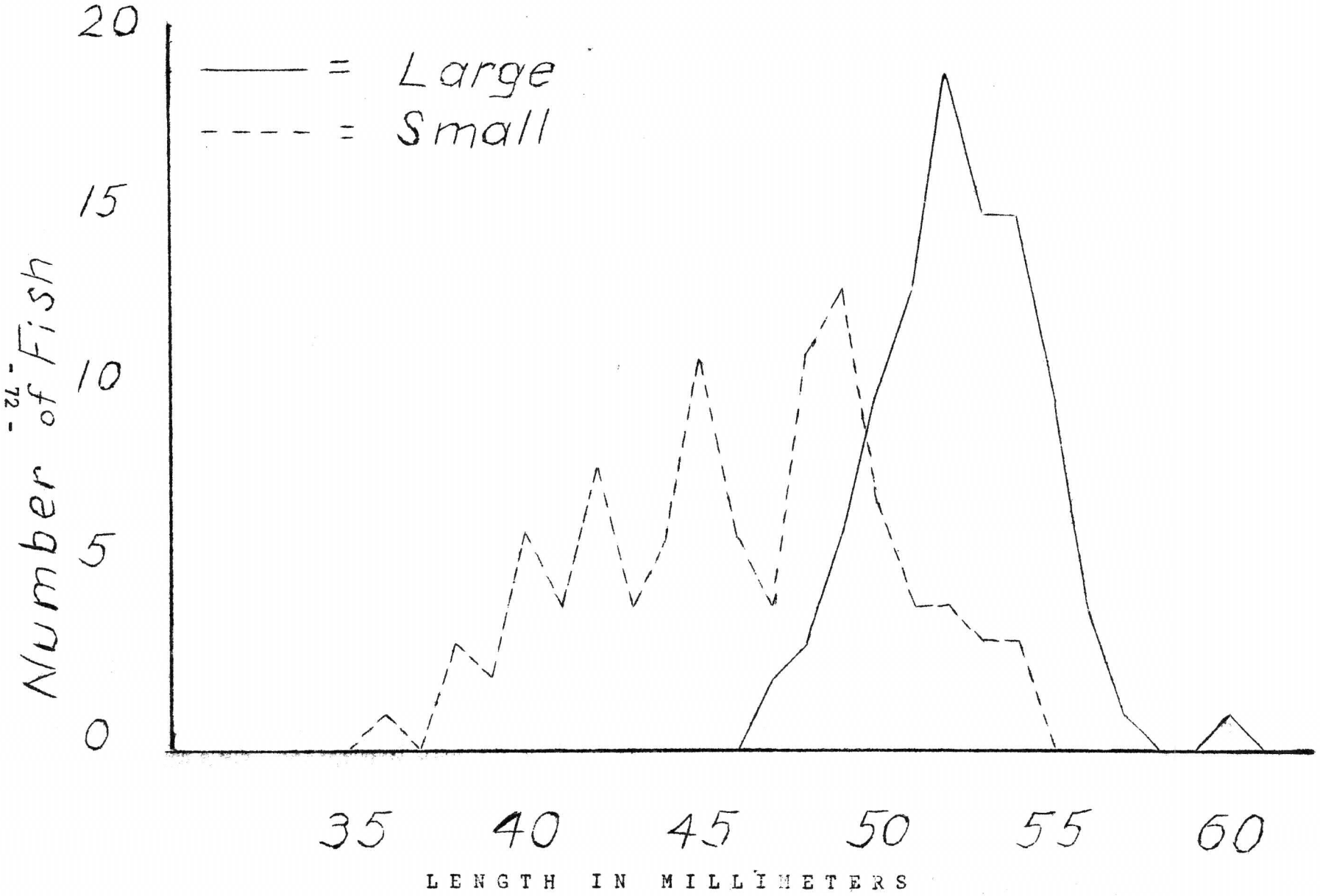


Figure 1.--Size frequency distribution of two groups of steelhead fingerling after grading.

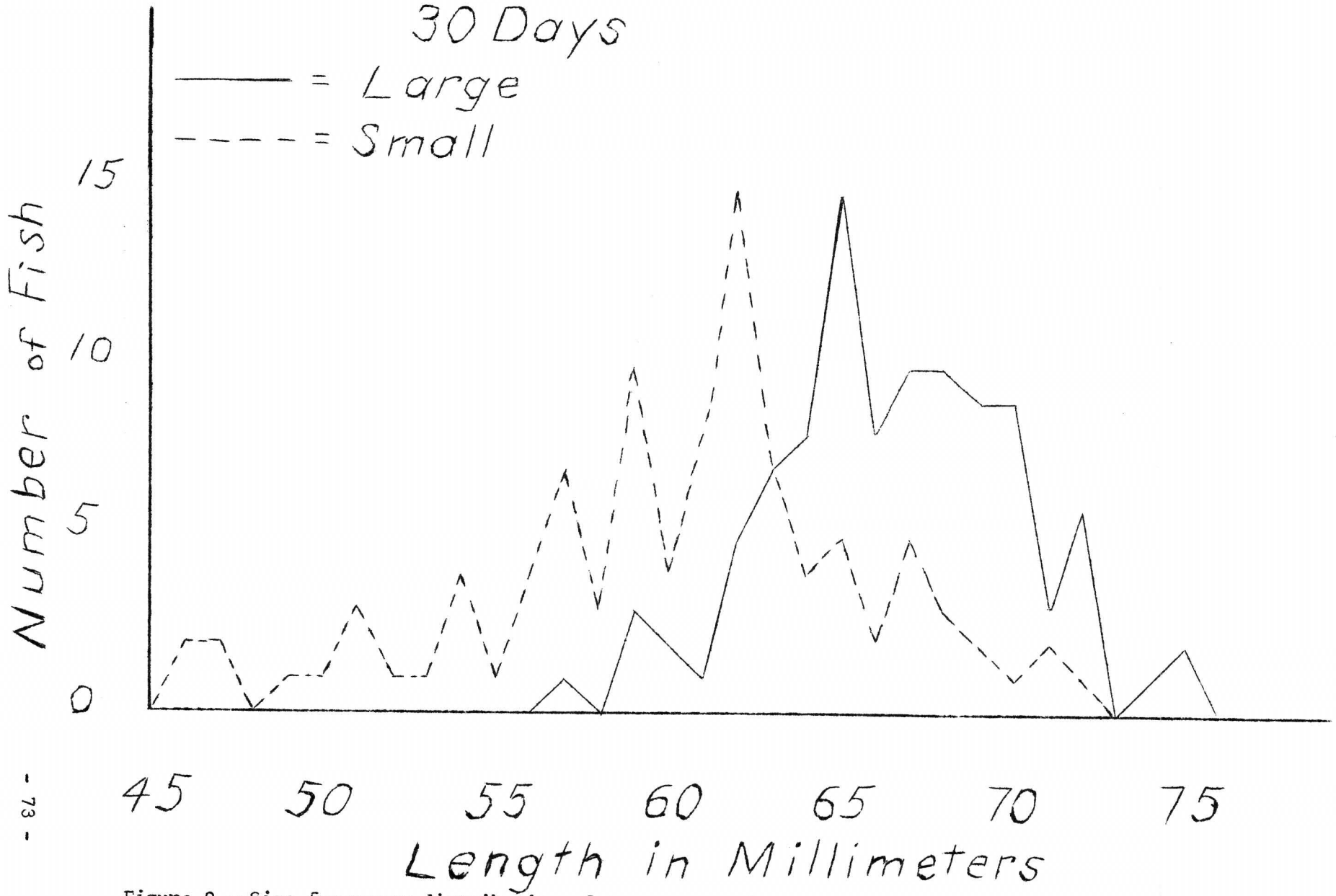


Figure 2.--Size frequency distribution of two groups of steelhead fingerling 30 days after grading.

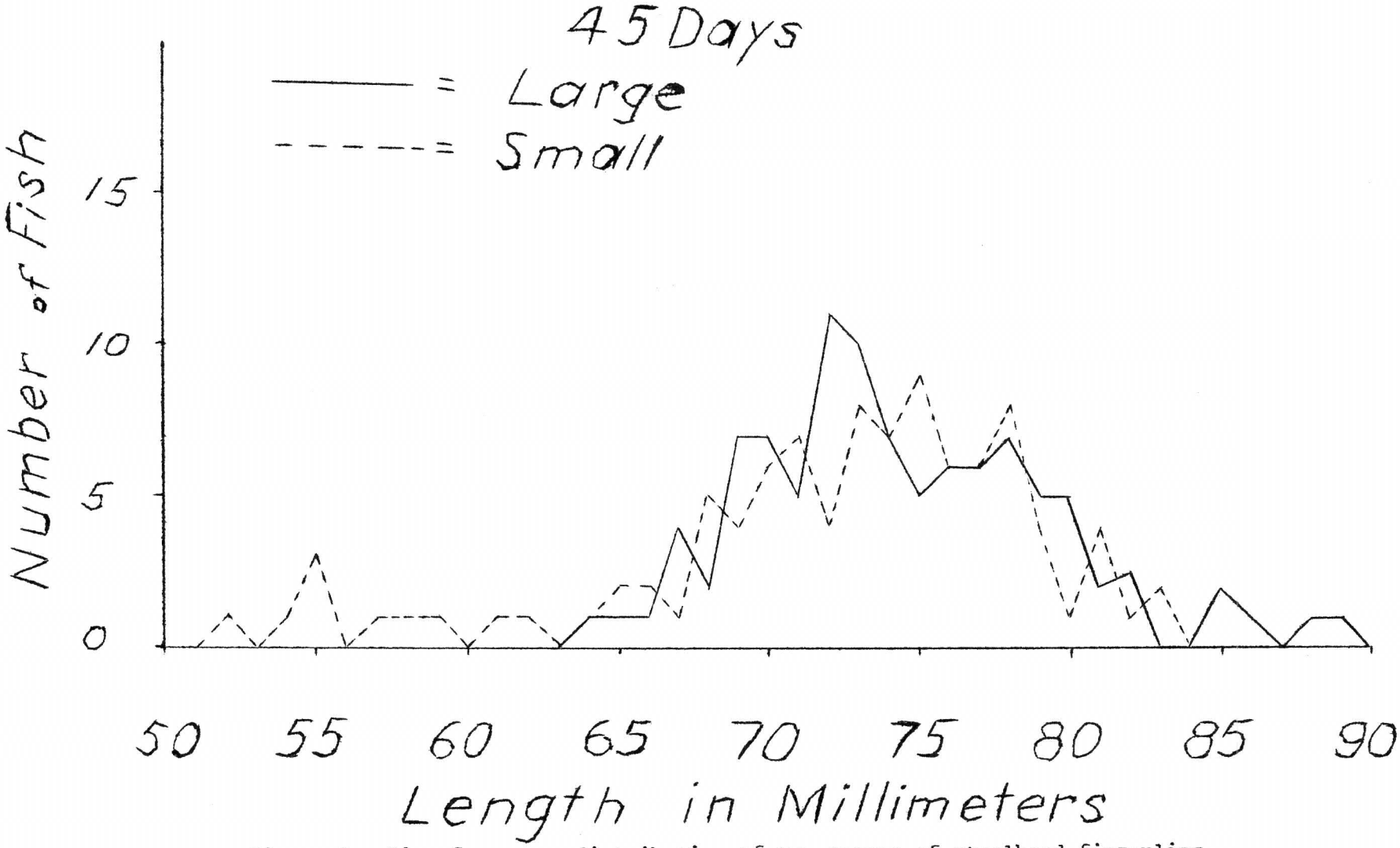


Figure 3.--Size frequency distributicn of two groups of steelhead fingerling 45 days after grading.

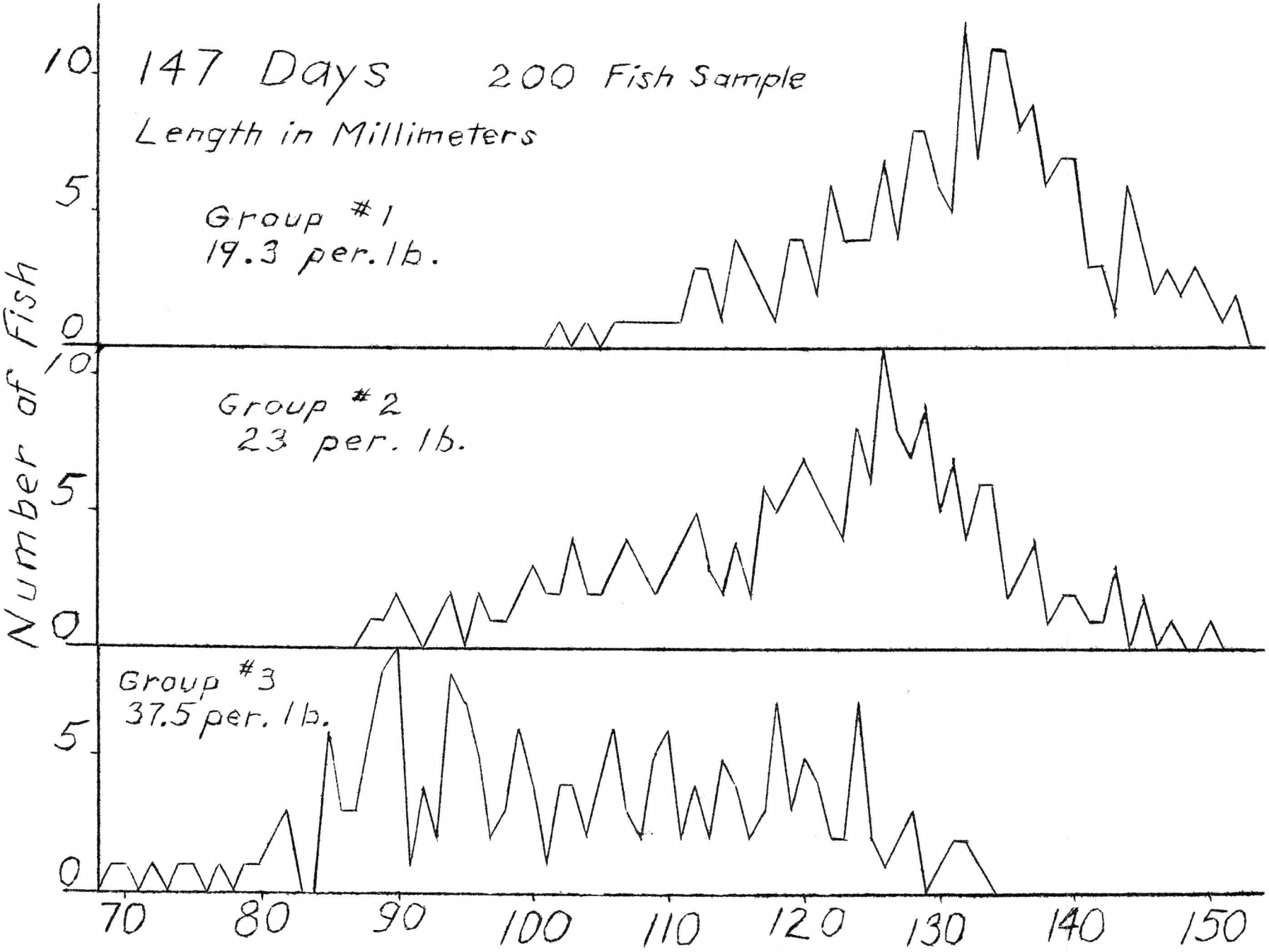


Figure 4.--Size frequency distribution of three groups of steelhead trout 147 days after original grading. Groups 2 and 3 were obtained by regrading the small group 45 days after the original grading.

USE OF TROXYMITE IN REPAIR OF CONCRETE

Fred W. Bitle
Bureau of Sport Fisheries and Wildlife
Winthrop, Washington

Troxymite multi-purpose resurfacer is designed for simple patch work and/or complete resurfacing. Worn, cracked and pitted old concrete is made better than new in one simple application of troxymite. A thin layer protects new concrete floors from elements which injure and destroy concrete. Non-toxic, it's safe to use anywhere! Non-sparking, it's ideal for hospitals and around chemicals and solvents. Troxymite is packaged in two components - one containing a special curing agent, the other a blend of aggregate, pigment and resin. Special instructions are to mix these components thoroughly, being careful to blend all curing agent furnished for each 'unit'.

If smaller quantities than full 'unit' are needed, use standard household measuring units. Be very careful to insure exact measuring as ration of mixing is most important: one (1) cup 'aggregate' mix and two (2) tablespoons 'curing agent'.

There are several warehouses throughout the United States and salesmen to cover all areas. By writing to the Texas Refinery Corp., 830-850 North Main, Fort Worth, Texas, additional information will be sent upon request and a salesman will be directed to call.

Our unit was sent from a warehouse in Tacoma, Washington. The freight charges were two-dollars and fifty-cents ($2.50) for a 1, 4-gallon 'unit'. The cost of one unit of 4 gallons, which is the smallest amount that one can purchase, was fifty-eight ($58.00) or $14.50 per gallon, The salesman stated that on a two (2)-unit order shipping charges would be paid. They also allowed 1% - 10 days discount.

The pictures that were shown show a fairly large patch made with Troxymite, normally this is much too large for the expense of the material is too great. This was merely done to show how it can be used. In this case the patch was too large to complete in one operation, the material 'sagged', so it was applied in two operations, by filling about one-half full and when that had set up was finished to the surface of the surrounding area.

Troxymite was used in the concrete troughs in the hatchery for the repair of the grooves where the trough screens set; some of the grooves have chipped and worn away over the years to where it was necessary to place caulking compound or sash putty under the screens to make them fish tight.

The operation to repair the slot was to fill a little above level with Troxymite, place some heavy cup-grease on the edge of the aluminum screen and set the screen solidly in the Troxymite and let the material set up firmly but not completely, then remove the screen. Should the screen be allowed to stay in place until the Troxymite set up solid, even though it had been greased, the material would have adhered to the screen making it extremely hard to remove.

Other areas where Troxymite has been used with success is on floors where the concrete has chipped or been chipped when some one has removed ice with a sharp instrument. Troxymite can be spread as thin as 1/16". It is most amazing how tight Troxymite adheres to almost any surface. We have used it to fill a crack with a piece of spruce wood as one surface and galvanized metal the other. Apparently water will not penetrate the material due to the resin base.

USE OF 'DARAWELD' IN REPAIR OF CONCRETE

Fred W. Bitle
Bureau of Sport Fisheries and Wildlife
Winthrop, Washington

"Daraweld" is a high polymir emulsion, chemical-engineered for you to mix with portland of Lumnite cement grouts. Daraweld gives these grouts resilience and lasting adhesion--adhesion that resists water and the action of heat, cold, oil, most acids and other corrosive materials. New mortar, concrete and plaster can be bonded to all concrete products, masonary, brick, tile, wood, steel and glass with the use of Daraweld. Grouts made with Daraweld are particularly effective in repairing old concrete floors where holes must be filled, worn spots smoothed over. Daraweld is secured from the W. R. Grace and Company, a Dewey and Almy Chemical Division, main offices in Cambridge 40, Mass., Chicago 38, Ill., and San Leandro, California. We secure ours through the Columbia Concrete and Pipe Company in Omak and Wenatchee, Washington.

In repairing a 14' section of walkway edge, the following procedure is recommended: Chip all loose cement and gravel from the area to be patched and carefully brush away all dust; make a mixture of a quarter (1/4) cup Daraweld into eight (8) cups of cement and add sufficient water to make a smooth lump-free mixture, of the consistency of thick cream; let set for a few minutes while you are preparing the grout. A good mixture for grout in a large batch is to use pea-gravel, 3 parts pea-gravel, two parts sand and one of cement. On smaller patches or thin patches, use one part cement to two parts sand and add one-eighth (1/8) cup of Daraweld to each complete mixture. One can tell if there has been sufficient Daraweld used in each batch, as when one is finishing the cement it will have a glassy appearance and small bubbles will appear on the surface as the trowel moves back and forth.

Estimate the size of the batch needed to fill this form and prepare as instructions given above. Take the mixture of Daraweld, cement and water previously mixed, and by the use of an old paint brush brush a thin layer over the area to be patched. While still moist, work the grout into the form, tamping the grout into the form good and solid. Finish, and let set for from 10 to 15 hours before removing the forms.

As soon as the forms are removed the green concrete should be stoned and finished by brushing with a mixture of one part fine sand and cement and sacked with straight cement to give a neat appearance.

Daraweld costs in our locality - $9.50 per gallon. It is also obtainable in quart size cans but cost runs higher when purchased in these amounts.