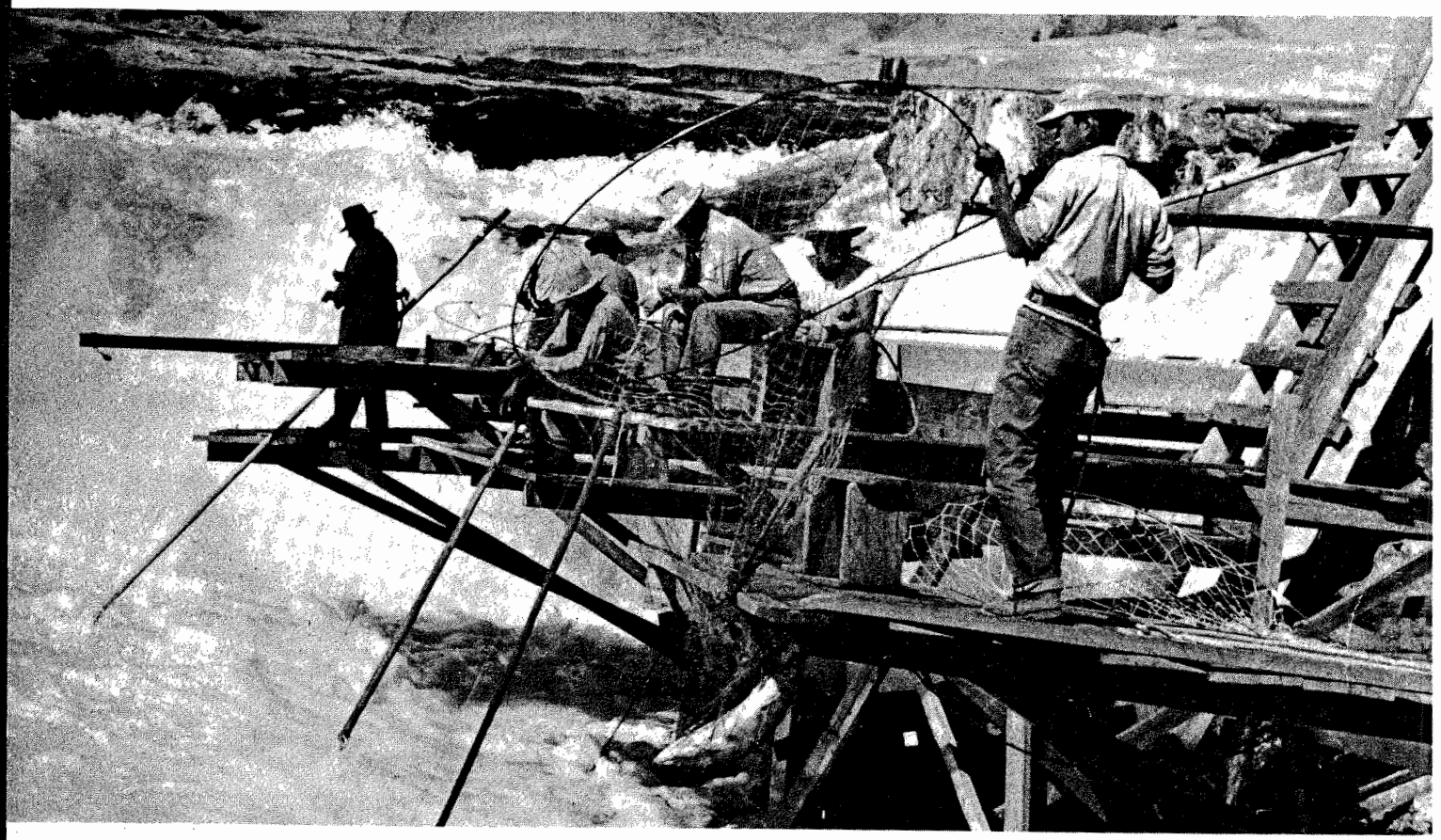
Wagner

PROCEEDINGS of the

Northwest Fish Culture Conference



EGON STATE UNIVERSITY
VALLIS, OREGON

Dec. 2-3
1964

Proceedings of the
Fifteenth Annual
NORTHWEST FISH CULTURE CONFERENCE
Corvallis, Oregon
December 2 and 3, 1964

Included herein are the abstracts of presentations made at the Fifteenth Annual Northwest Fish Culture Conference held at Oregon State University, Corvallis, Oregon, December 2 and 3, 1964. This report was published by the Oregon Agricultural Experiment Station.

As Chairman, I wish to express my appreciation for the excellent effort made by those participating in the program. The presentations displayed, in an interesting manner, the kinds and quality of work underway concerning the artificial propagation of fishes.

Attendance, for the second year in a row, exceeded two-hundred persons. I feel this is a direct indication of the increased interest and importance of fish culture.

As in previous years, abstracts of reports are presented in the proceedings with only minor editing. In accordance with the policy established by this organization, none of the enclosed abstracts or any portion thereof may be reproduced without express permission from the author involved.

Finally, I wish to thank the Agricultural Experiment Station and the Department of Microbiology, Oregon State University, for their assistance in presenting this program. Oregon State University will continue to take its regular turn as a host agency for the conference.

Dr. John E. Halver, U. S. Fish and Wildlife Service, Western Fish Nutrition Laboratory, Cook, Washington, is Chairman for the 1965 Conference.

J. L. Fryer
Chairman 1964

Department of Microbiology
Oregon State University
Corvallis, Oregon

FIFTEENTH ANNUAL NORTHWEST FISH CULTURE CONFERENCE

Table of Contents

THE TEAM

George W. Klontz
Western Fish Disease Laboratory
Seattle, Washington

A team is defined in Webster's Dictionary as "a number of persons associated together in any work". The process by which a team functions is defined as teamwork. Webster defines teamwork as "work done by a number of associates, usually each doing a clearly defined portion but all subordinating personal prominence to the efficiency of the whole."

How does this apply to our situation? Our team is composed of three groups of people: (1) Administrators; (2) Fish culturists or fish husbandmen; and (3) Fish biologists. We are all working toward the common goal of raising as many large, healthy fish as we can so as to keep the commercial and sports fishermen happy and still have enough fish left over to return to our hatcheries. In this respect we are quite different than farmers in that we do not reap the crop we sow. In a sense, then, this work of ours is definitely a labor of love, and it is understandable why at times we get discouraged. It is at this point we may begin to wonder if another of Webster's definitions of a team might not be more applicable: "A team is two or more asses harnassed to the same wagon."

What all this is leading up to is a suggestion that we as a team should take a "seventh inning stretch" to re-evaluate the progress we have made the past fifty to sixty years. We should also look very seriously at where we want to go. I am proposing that we are now ready to start applying the principles of the practice of medicine to achieve our goal of more, larger, and healthier fish.

Just what are these principles of the practice of medicine? In essence they are just what we have been doing all along. They are the maintenance of a healthy stock of animals through a rational program of disease treatment, prevention and control. However, we have not called our work this because we are not Doctors of Medicine. But, we, for the most part, know just as much about our "patients" as the M.D.'s know about theirs, and as such are we not qualified to practice medicine on our animals the same as they are on theirs?

This means that all of us - administrators, fish culturists, and fish biologists - are scientists doing research on how to raise fish. And I think we all could do our job better by "believing what we see" and not "seeing what we believe".

CELLULAR REGENERATION IN THE FISH *Onocorhynchus kisutch*

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ABSTRACT

Cellular regeneration can be defined as the replacement of cells in tissues for those which have been destroyed by the normal life processes. Every cell that is engaged in performing certain specialized functions, such as a red blood cell that is involved in carrying oxygen; a white blood cell involved in combating disease or a cell in the intestine that is responsible for absorption of food; all will be eventually destroyed in carrying out these activities and will have to be replaced. Therefore, a fundamental question arises; "How and where do the cells arise to replace the lost cells in the adult animal?"

Recent developments in analytical techniques have provided us with a tool, namely, autoradiography, which enables us to obtain answers to this question.

The present study is involved in determining the cellular replacement which occurs in various parts of the gastro-intestinal tract with special emphasis on the mucosal lining of the intestine. Results obtained show that the embryonic cells which give rise to the formation of a new column of epithelial cells of the intestine come from the "valley" region of the mucosal lining. Also, the hindgut is the area which has the fastest rate of replacement followed by caeca, stomach and esophaegus, respectively. Fifty per cent of the mucosal lining appears to be replaced within eight days which is called the "turnover" time. The active site of cell proliferation appears to be in the basal portion of the "valley" and in a nest of granule cells in the sub-mucosa.

FURUNCULOSIS IN THE CHESTNUT LAMPREY

Dr. James D. Hall
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Corvallis, Oregon

My studies in Michigan on the biology of the chestnut lamprey, Ichthyomyzon castaneus, accidentally revealed what appears to be the first record of a bacterial disease from this group of fishes. I was holding adult lampreys together with brook trout in aquariums at the State Fish Hatchery in Grayling to determine the destructive effect of the lamprey. In October and November of 1960 a complete mortality occurred among the lampreys in one aquarium. The trout, taken from hatchery stock, were apparently infected with furunculosis, and several died with the disease at the same time. The coincidence of the mortalities led me to consider furunculosis as a possible cause of the lamprey mortality.

I was able to isolate the causative agent of the disease, Aeromonas salmonicida, from the first group of dead lampreys. Further study, including microscopic examination and inoculation with the bacterium, confirmed furunculosis as a cause of death. This paper reports on the diagnosis and experimental confirmation of furunculosis in the chestnut lamprey.

The lampreys and trout were being held together in two 50-gallon aquariums supplied by well water of constant year-round temperature of 10^{o}C. The density of trout and lampreys in each aquarium varied as a result of mortality, but averaged about 10 adult lampreys (5 to 8 inches) and 15 brook trout (6 to 10 inches).

Diagnosis of the disease in the first group of lampreys that died was made by bacteriological culture on Trypticase Soy Agar. Seven of eight dead lampreys tested provided positive coloration. One of these positive cultures was identified as A. salmonicida by S. F. Sniesko (personal communication).

The kidney of two of these lampreys was sectioned, stained, and examined microscopically. Bacteria resembling A. salmonicida were observed, but I could not positively identify the species.

In September 1961 an experiment was performed to confirm the cause of death. A 24-hour subculture of a positive culture from lamprey kidney was washed with 2 ml of sterile saline; 1 ml of this solution was used to make up dilutions of 10^{-1}, 10^{-3}, and 10^{-5}. The design and results after two weeks are shown below:

Contribution from the Institute for Fisheries Research, Michigan Department of Conservation; and Department of Fisheries, University of Michigan.

		Aq. #1		Aq. #2		
Number Lampreys	10	10	10	10	10	10
Treatment 10/8/61	autopsy TSA	0.1 ml saline	none-	0.1 ml 10^{-1}	0.1 ml 10^{-3}	0.1 ml 10^{-5}
No. dead 10/21/61	all neg.	0	0	6	0	0

The failure of lampreys given the more dilute injection to die may have been the result of several factors, including the low titre of the inoculum and possible attenuation due to storage of the culture for about three months prior to use. Bacteria were recovered by culture from the dead lampreys and identified as A. salmonicida by Griffin's test. Thus, furunculosis is thought to be the cause of death in these lampreys.

Although furunculosis has been recovered from many fishes, there is no record of its occurrence in any lamprey, nor has any other bacterial disease been reported for the lampreys. The fact that no bacterial disease has been reported as a result of the extensive studies on the sea lamprey, Petromyzon marinus, suggests that my result may be due to a fortuitous combination of the disease organism and many lampreys held in close proximity to it.

The significance of this disease to the natural population of the lamprey remains unknown. Its presence in the lamprey population in the Manistee River near Grayling is suggested by recovery of a positive culture from a lamprey that died in a sterilized aquarium within 24 hours of being brought from the river. None of 20 normal appearing, live lampreys produced a positive culture upon autopsy.

There was evidence that the bacterium was relatively more virulent to the lamprey than to trout, at least under the experimental conditions. Although spread of the disease was not rapid (about 5 weeks elapsed between the first and last deaths of the 10 lampreys in the first 50-gallon aquarium to be infected) the temperature was below optimum for transmission of the disease. Several brook trout remained alive in the aquarium after all the lampreys had died, suggesting the relative virulence of the bacteria to the chestnut lamprey. Further experiments on the virulence and pathology of the disease in other lampreys are planned at Oregon State University.

FISH DIAGNOSTICS LABORATORY

Dr. Robert R. Rucker
Western Fish Disease Laboratory
Seattle, Washington

The need has long been recognized for a fish diagnostics laboratory where state and private fish-rearing groups can obtain help for disease problems, seek advice on the general health of their fish, and obtain certificates for the interstate shipment of eggs and fish. Such a laboratory could be supported by federal, state, or private funds or operated on a self-sustaining basis. Personnel might include a director familiar with fish diseases, their diagnostics and treatments. He could be supported by a laboratory technician, a hatchery aid, and others as the need indicated. Facilities should include about ten troughs or tanks for holding small groups of stock fish and about 100, one-gallon containers for holding experimental fish. A pure, disease-free, temperature-controlled water supply should be available. There must be facilities with equipment and supplies for the examination of living and preserved fish for parasites, bacteria, viruses, and pathology. A small laboratory should be included for water chemistry determinations and perhaps for pesticide evaluation.

A diagnosis might consider the following procedure. First, the fish would be examined for gross evidence of disease, then the body surface, lesions, and gills for parasites and bacteria in wet mounts. The muscle and viscera would be examined for parasites, and for bacteria in stained smear preparations and by culturing on different media.

The presence of an infectious agent could be confirmed and demonstrated by injecting young fish with a homogenate of suspect, small fish or organs from large fish or body fluids from the larger fish. A bacteria-free filtrate could be used to demonstrate the presence of virus by injecting fish and inoculating tissue cultures.

The presence of an infectious agent might also be demonstrated by immunological methods. Tissue extracts from diseased fish will react with test serums containing specific anitbodies. Some of the techniques would be gel-diffusion, neutralization reaction or immunofluorescence.

No diagnosis would be complete without a histopathological examination and report. Whole small fish or samples from large fish would be fixed, sectioned and stained (Hematoxylin-eosin and Giemsa). Special methods would be employed only when indicated.

This examination would require about two weeks. However, more information could be supplied within this time. Some patrons might desire a red blood cell count, hemoglobin and hematocrit evaluations, a differential count, or definite chemical determinations.

The above information would be helpful as an aid for the successful rearing of fish. Certainly there should be laboratories where fish diagnostics are available to all, where diseases could be identified and treatments determined, and where certificates of "good health" could be obtained.

TREATMENT OF SALMON AND STEELHEAD WITH SULFONIMIDES

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Clackamas, Oregon

INTRODUCTION

Sulfonimides are used to control bacterial diseases of juvenile trout and salmon by incorporating the sulfa into the diet as required. The feasibility of administering sulfonimides to adult salmon and steelhead to control bacterial diseases such as furunculosis is undergoing investigation. However, as the adult salmon and steelhead are presumably non-feeding when returning to the parent stream, appropriate application techniques were needed. Of those tested, two were utilized: oral force-feeding and intramuscular injections. The objectives were to: (1) determine the ability of the sulfonimides, Sulmet (sulfamethazine), Gantrisin (sulfisoxazole), Bactrovet sulfadimethoxine, S.E.Z. (sulfaethoxypyradiazine) and S-4 (a quadrasulfa containing sodium, sulfamethazine, sulfamerazine, sulfaquinoxaline, and sulfathiazole) to produce blood levels in adult salmon and steelhead greater than 5 mg per 100 ml. of blood (mg. %); (2) develop a suitable method for administering the sulfas.

METHODS AND MATERIALS

Adult Experiments

The oral force-feeding method was investigated first. The intramuscular injection was the alternate technique.

Bactrovet was administered in two forms to spring chinook, coho and steelhead: tablets and two injectable solutions. One solution was the commercial 10 per cent preparation and the other a 25 per cent laboratory compound.

Other sulfas fed orally were injectable S.E.Z. 25 per cent solution and Sulmet, S-4, Grantrisin, and S.E.Z. tablets. Spring chinook received the S.E.Z. solution, Sulmet tablets, and Gantrisin tablets while Sulmet, S.E.Z., Gantrisin, Bactrovet and S-4 tablets were fed to coho. Intramuscular Bactrovet injections were given to spring chinook, coho and steelhead.

Juvenile Experiments

A test was conducted on juvenile coho to determine if sulfa levels could be analyzed from some part of the fish other than blood with no sacrifice of accuracy. The fish were fed Oregon pellets containing Sulmet. Blood was taken from the fish, the blood analyzed for Sulmet content and the remaining carcasses of each sample homogenized in a blender. The homogenized fish were centrifuged and the supernatant body fluids analyzed.

The newly acquired quadrasulfa, S-4, was fed in Oregon pellets to juvenile coho at three rates: 2, 2.5 and 10 grams of S-4 per 100 pounds of fish.

RESULTS AND CONCLUSIONS

Adult Experiments

The force-fed Bactrovet tablets produced slight blood levels in the steelhead (Figure 1) but none in the chinook or coho. The injectable Bactrovet solutions given orally yielded short-term low blood levels (Figure 1) in all species.

The highest and most persistent blood sulfa levels from a single feeding were achieved by feeding S-4, Gantrisin and Sulmet tablets to chinook and coho (Figures 2 and 3).

Intramuscular Bactrovet injections given to chinook, coho, and steelhead produced satisfactory blood levels (Figure 4) but caused hemorrhagic abcesses at the injection site. However, this technique might be employed when no other course is effective.

Juvenile Experiments

The resulting blood and tissue levels obtained were comparable (Table 1) indicating that this technique might be employed on fish too small to furnish adequate blood for analysis. The juvenile coho receiving the S-4 produced blood levels proportional to the amount fed (Table 2). No toxic effects were observed in any lot. However, toxicity tests should be conducted prior to application of this sulfa on other species.

Table 1. Comparison in Mg-Per Cent Between Blood Sulfa Levels and Tissue Sulfa Levels of Juvenile Coho Fed Oregon Pellets Containing 5 Gms and 2 Gms of Sulmet per 100 Pounds of Fish.

Fish Size	Replication	Treatment	Water Temp.	Hours After Start of Feeding 24	48	72	96
Yearling 1/	A	Blood only	54°F.	3.5	2.0	-	-
		Carcass only		5.2	2.4	-	-
		Whole Fish		6.5	2.0	-	-
	B	Blood only		6.5	6.5	8.5	-
		Carcass only		7.2	6.8	9.3	-
	C	Blood only		1.5	6.5	4.8	-
		Carcass only		1.5	7.3	4.8	-
Yearling 2/	A	Blood only	44°F.	-	-	-	0.7
		Carcass only		-	-	-	0.7
		Whole Fish		-	-	-	0.7
Fry 2/	A	Whole Fish		-	-	-	0.8

1/ Feeding Rate - 5 gm sulmet per 100 pounds fish.
2/ Feeding Rate - 2 gm sulmet per 100 pounds fish.

Table 2. Tissue Levels in Mg-Per Cent of Juvenile Coho Fed Quadrasulfa (S-4) at a Water Temperature of 54°F.

Lot	Feeding Rate in Mg Sulfa per Lb. Fish	Hours After Start of Feeding 48	96
1	2.5	1.8	1.9
2	5.0	4.4	4.4
3	10.0	8.1	-

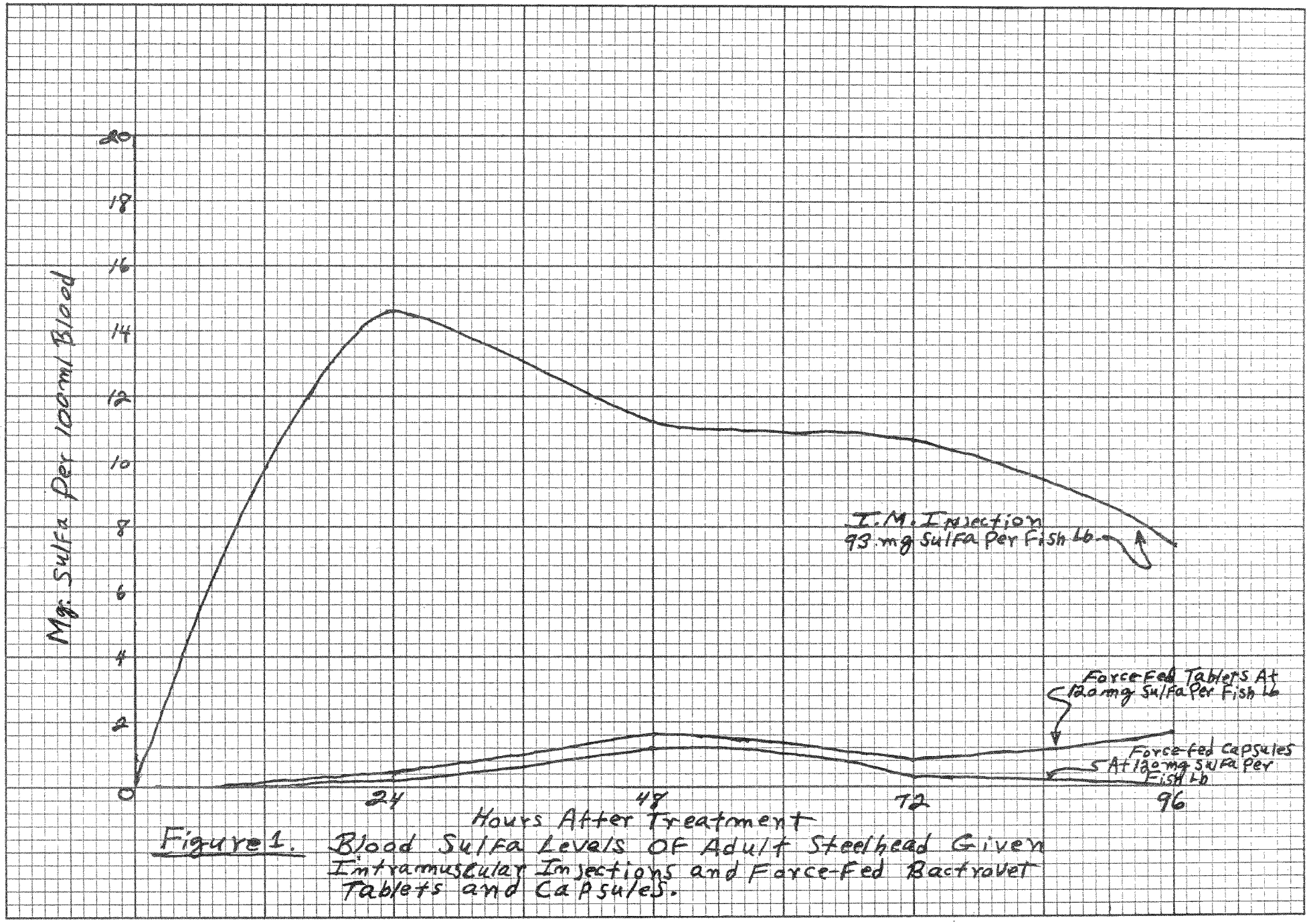

Figure 1. Blood Sulfa Levels Of Adult Steelhead Given Intramuscular Injections and Force-Fed Bactrovet Tablets and Capsules.

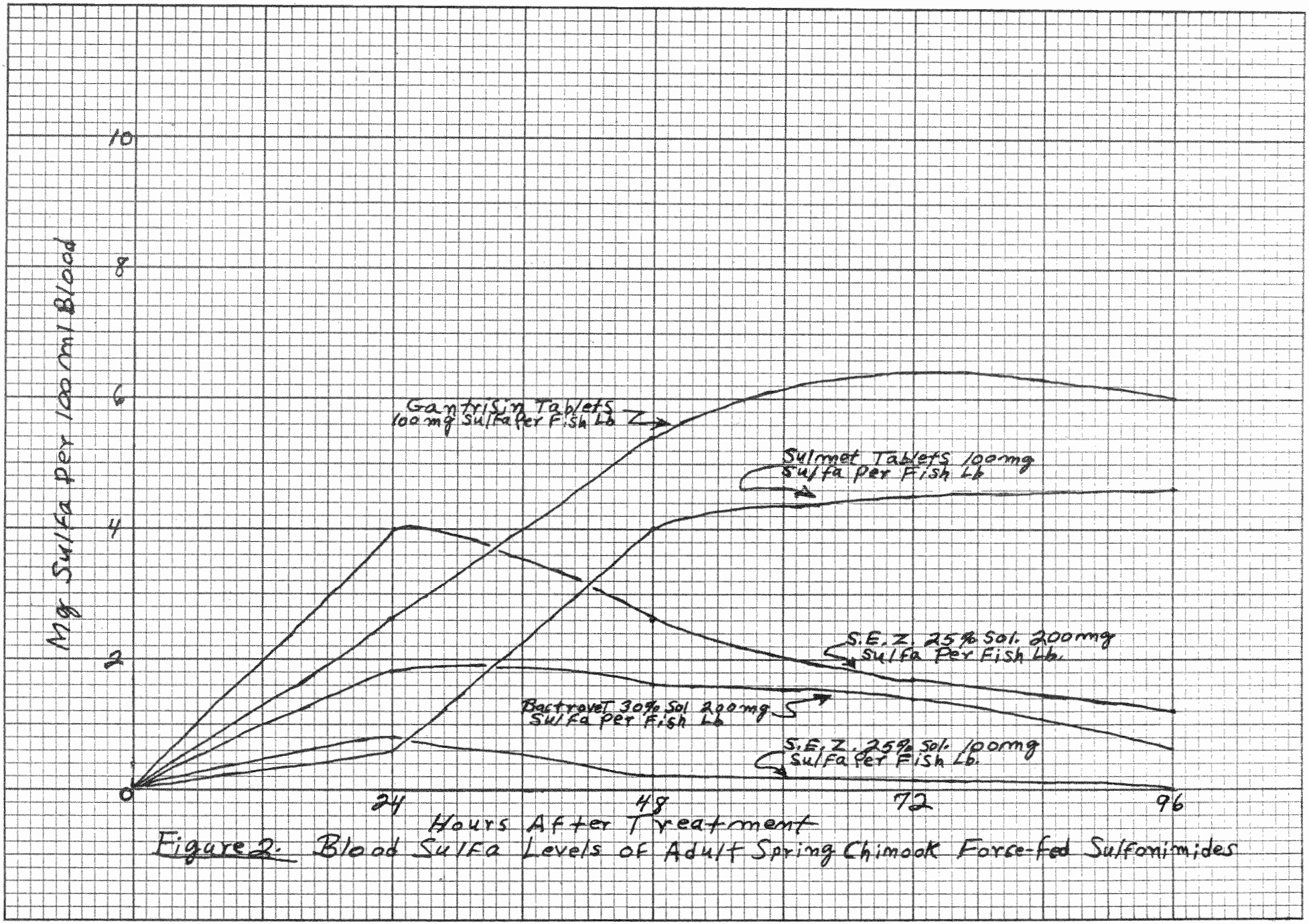

Figure 2. Blood Sulfa Levels of Adult Spring Chinook Force-fed Sulfonimides

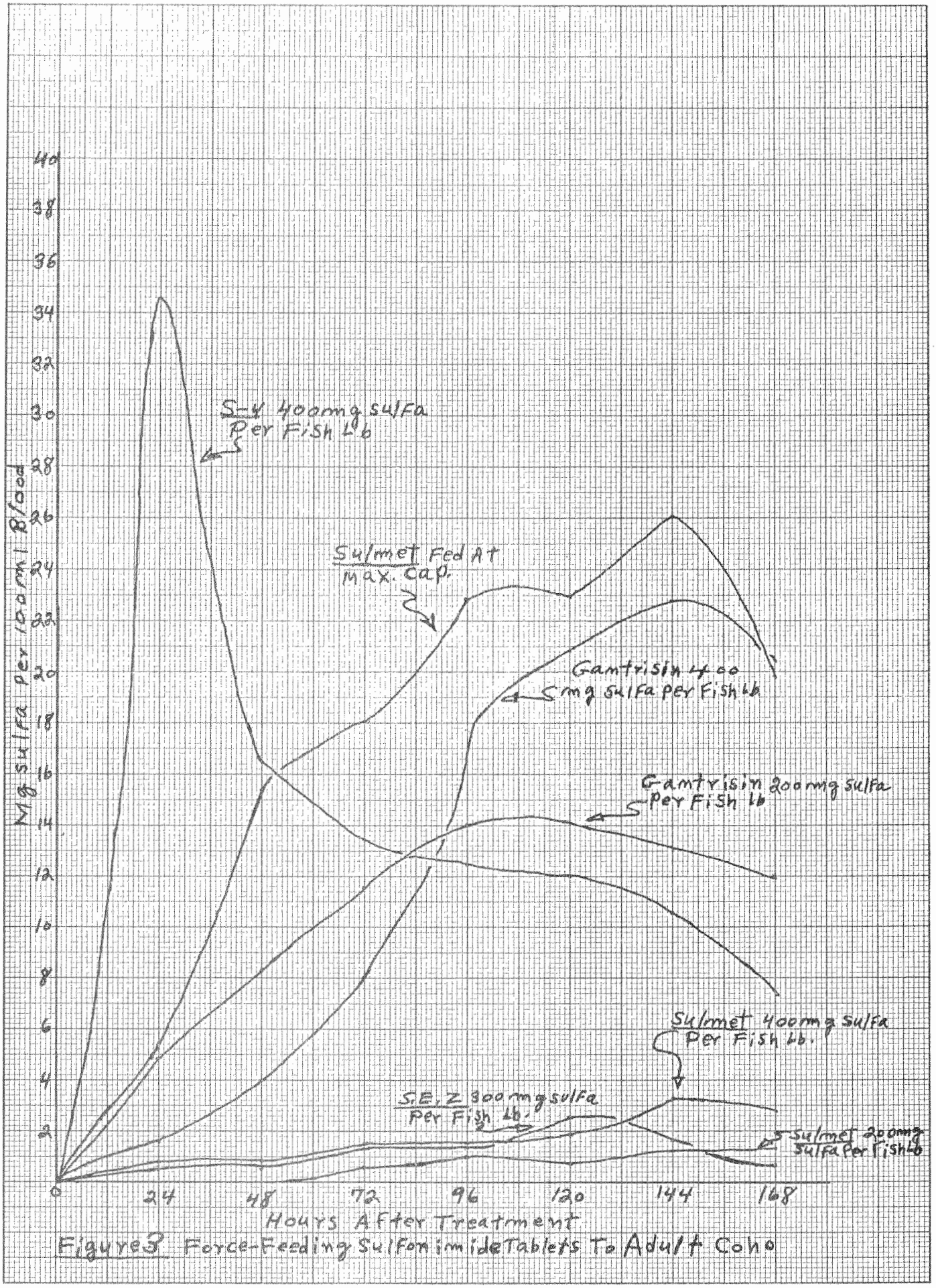

Figure 3 Force-Feeding Sulfonimide Tablets To Adult Coho

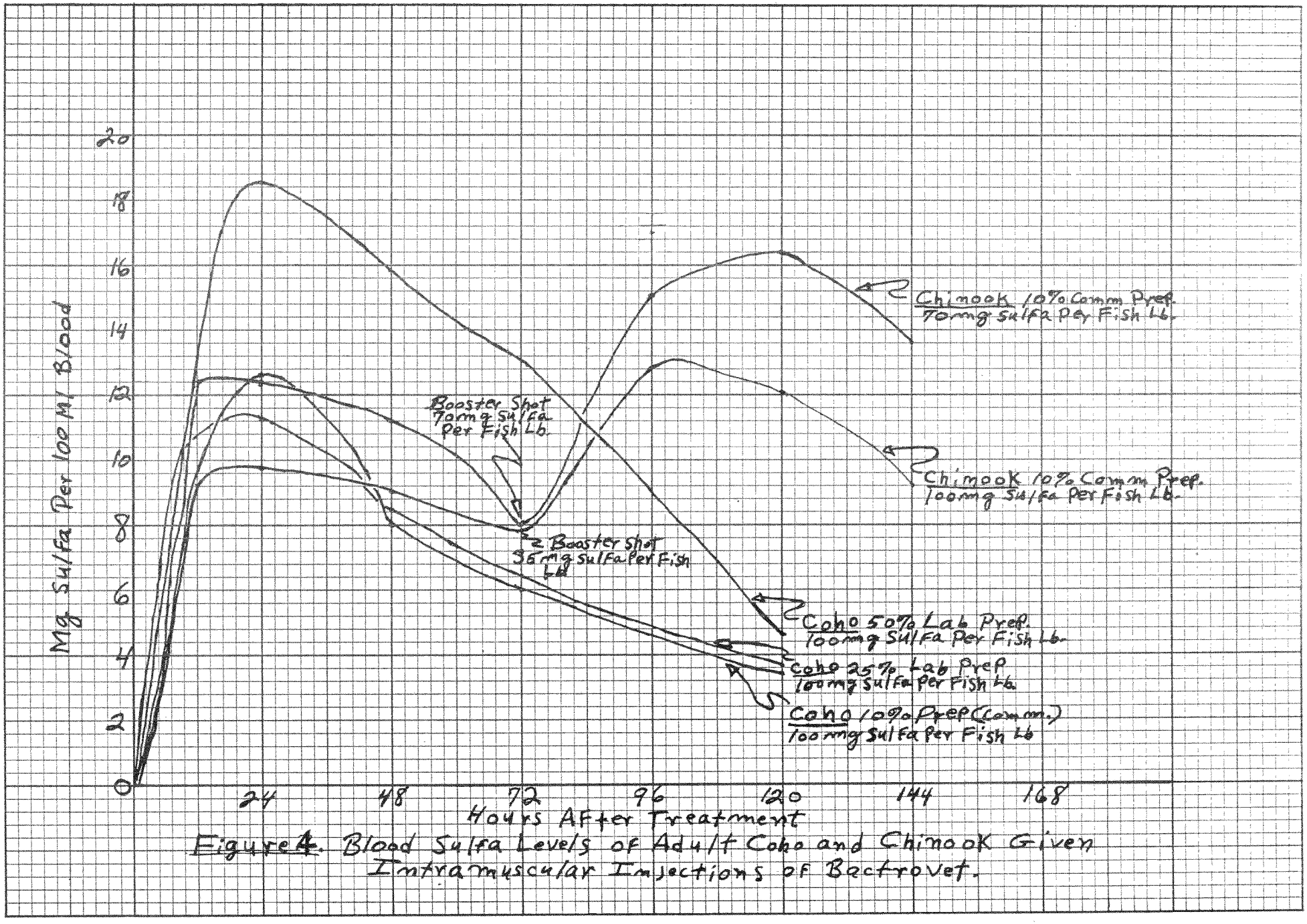

Figure 4. Blood Sulfa Levels of Adult Coho and Chinook Given Intramuscular Injections of Bactrovet.

DISEASES OF ADULT SALMON

James W. Wood
Washington State Department of Fisheries
Seattle, Washington

Interest in adult salmon diseases has increased during recent years. High prespawning losses in various runs of salmon which were at critically low levels are responsible for precipitating this interest. A number of various diseases have been described from adult salmon. A summary of these diseases, listed without regard to possible importance, follows:

Columnaris disease caused by Chondrococcus columnaris - responsible for fish deaths in natural as well as artificial holding areas (described from adult salmon by Ordal and Rucker in 1944).

Ceratomyxa shasta - a protozoan of the Order Myxosporidia responsible for prespawning loss in natural and artificial holding areas.

Aeromonas liquefaciens - causes a bacteremia, associated with Ceratomyxa infections. First found in adult salmon by Oregon Fish Commission personnel.

Ichthyophthirius multifiliis - a protozoan causing "Ich". Observed on adults in both artificial and natural holding areas, generally not considered as being involved in prespawning losses. In one instance, however, it was considered the cause of prespawning deaths in an artificial spawning channel.

Piscine tuberculosis - caused by one or more species of Mycobacterium. This disease is not thought to be a direct cause of prespawning loss but infected adults are less capable of surviving until spawning than are non-infected adults.

Bacterial kidney disease - caused by a species of Corynebacterium. Like tuberculosis, this disease is probably not involved directly in prespawning losses, but infected fish are generally less capable of surviving until spawning.

Furunculosis disease - caused by Aeromonas salmonicida. This disease has been observed in a number of salmon runs under both natural and artificial holding conditions. No information on its importance as a major cause of prespawning mortalities. Definitely needs more study.

Bacterial gill diseases - caused by a variety of species of myxobacteria and perhaps others. Such infections are thought

to be involved in postspawning death of Pacific salmon. Although the bacterial gill diseases are probably precipitated by the physiological degeneration of postspawning salmon, it is possible that the gill diseases kill many fish before the degenerative changes *per se* do. With high fish density, some evidence exists that the bacterial gill diseases may kill adults before spawning.

Trichodina sp. - this common hatchery parasite has been observed in considerable numbers on gills and body surfaces of adult salmon. Its importance is unknown.

Costia necatrix - this common parasite of young salmon has also been observed in large numbers on the gills of prespawning adults. Its importance is also unknown.

This list is certainly not complete but it does illustrate the number of diseases that are known from adult salmon and that have been implicated in losses. There is every indication that the susceptibility of adult salmon increases as the fish approach maturity. This point is worthy of consideration in any study conducted on the diseases of adult Pacific salmon.

PROGRESS REPORT
COLEMAN VIRUS DISEASE AND AUTOMATIC FISH FEEDER

John Pelnar, Manager
Coleman National Fish Hatchery
U. S. Fish and Wildlife Service
Anderson, California

Studies and experimentation with control measures continued at Coleman during the past season. Considerable knowledge of the virus, its characteristics and patterns have been obtained. Laboratory studies by the Western Fish Disease Laboratory have continued.

The operational program in utilizing 50°-60°F. water in hatching has been followed, but unsatisfactory results indicate that this method is not the requirement. Many other problems with the resulting fish were disclosed and losses indicated that the mortality from yolk sac trouble, white spot and constant parasitic infestation caused as much loss as the virus itself. It is not believed prudent to pursue this course further.

We held 125 female's individual egg groups through the incubation, hatching, fry and fingerling stages, which also passed through the normal virus stages.

All individual groups eyed up normally with an entire group eye of better than 90 per cent.

57 females eyed and hatched over 95%
30 females eyed and hatched over 90%
12 females eyed and hatched over 85%
2 females eyed and hatched over 80%
7 females eyed and hatched over 70-80%
4 females eyed and hatched over 60-70%
13 females eyed and hatched over 13-50%

Well over 80 per cent of the groups developed normally. There was no evidence of anything unusual. One female produced severely bloodied eggs; these eyed at 96.8 per cent, with fingerling survival of 79.5 per cent; bloody eggs are not necessarily to be discarded. These were judged to be worthless by our hatcherymen.

The resulting fingerling groups from each female were reared separately with no interchange of contacts except through hatchery operations, feeding and cleaning.

Of the entire 125 groups -

21 groups survived with less than 10% loss
32 groups survived with 10-20% loss
24 groups survived with 20-30% loss
13 groups survived with 30-40% loss
12 groups survived with 40-50% loss
12 groups survived with 50-60% loss
3 groups survived with 60-70% loss
3 groups survived with 70-80% loss
3 groups survived with 80-88% loss
1 group survived with 10 fish survived

None of the group showed 100 per cent loss.

Of this group 25 were from Keswick stock -

10 of these had less than 3.4 loss
1 of these had 6.8 loss
5 of these had 10-20% loss
2 of these had 20-30% loss
none of these had 30-40% loss
2 of these had 40-50% loss
2 of these had 50-60% loss
2 of these had 80-85% loss
1 of these had 10 fish survived

The one with ten fish surviving is felt to be the result of overlooking fertilization in spawning.

There seems to be very little difference in the virus incidence between the Battle Creek and Keswick stock.

The virus has failed to appear upon steelhead, kamloops, Kokanee or brown trout reared at the same period.

Operational treatment of first swimming fish with well water of 64°F., for periods of two hours, seems to have some beneficial effect. We propose to follow this further.

Progress in control of the virus is slow and there does not seem to be a solution now or in the near future.

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AUTOMATIC FISH FEEDER

The automatic fish feeder reported on last year has had several improvements added. The feeder has been used in feeding frozen pellet food of all sizes. Slides of the feeder were shown.

We are in the process of enlarging this feeder to cover the complete pond area.

THE MONSTER IMPROVED - DOES GRADING PAY

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

Continuing developments of our new fish-moving machine, although at a snail's pace, have been most encouraging. Most of the problems reported on last year have been overcome. The lack of funds for this sort of work is the acme of frustration.

We were able, however, this past summer to install a set of "Quick-Connect" couplers in the 5-inch aluminum suction line and demonstrated that small fish, 95 per pound, some even smaller, can be removed from 8 (25-foot diameter) circular ponds in one setting of the machine.

We now have an actual operating range of 40 feet, and we are very confident it can be increased to 60 feet, in place of the former 16, from one location.

It has also been demonstrated that fish can be removed from 4 large holding ponds (measuring 20 by 100 by 6 feet deep) from one setting of the device.

The problem of getting an even distribution of fish over the head of the grader was corrected by building a fish-diffusion chamber that has worked very well. The diffusion chamber is a triangular-shaped, enclosed wood box, attached to the head of the grader. All fish emerging from the snorkel tube must first pass through the chamber before dropping on the grader apron. The result is a uniform distribution of fish to each grading bar.

For grading purposes only, the problem of regulating the quantity of fish coming out of the machine at one time was corrected by installing an adjustable mirror on the grader. The mirror is so positioned the operator can always see the rate of discharge from the diffusion chamber and regulate the flow of fish accordingly. Good grading cannot be achieved unless a very uniform flow of fish, properly spaced, is maintained over the grading bars.

The rate fish are discharged from the machine is no problem insofar as loading tank trucks is concerned.

The ease with which grading can now be carried out was demonstrated to a group of interested hatcherymen on April 2, 1964. In this demonstration Len Mathisen, Regional Supervisor for the Game Commission, was the official timekeeper.

Starting at 10:30 a.m., the machine, grader and aluminum irrigation pipe were all moved into place and rigged for grading. Ponds 34 and 37, containing a total of 4,624 pounds of fish, averaging 5.6 fish per pound, were graded into 3 size groups and distributed into 3 pounds in 1 hour and 40 minutes. This grading was accomplished by only two men, with only slight assistance from a third man in moving pond crowders from one pond to the other. In larger holding ponds, containing 6-10,000 pounds of fish, I am confident the grading rate could be increased to 4,000 pounds per hour.

A new self-supporting crowder was developed this year for use between the two main pond crowders. This new crowder proved to be very helpful in speeding up the grading process and in reducing man-hours of labor required. With this device, and once all crowders have been installed, nearly all fish can be removed without a man being in the pond.

With the new crowder, the entire pond of fish being held between the two main pond crowders can be forced to one side of the pond from which the suction end of the snorkel tube is siphoning fish.

By maintaining a uniform density of fish at the suction end of the snorkel tube and by careful timing of the actual siphoning period, it is possible to estimate, within reasonable limits, the total poundage of fish being placed in the vacuum tank.

While we have actually had 1,100 pounds of fish in this small tank without apparent injury or mortality, we try to not exceed 1,000 pounds. From operating experience, we feel that 8-900 pounds is a reasonable load.

It requires about 3 minutes of actual siphoning time to place 1,000 pounds of large rainbow trout in the tank. Fingerling rainbow flow through the suction tube with great ease and require less siphoning time than large rainbow. Loads of 6-700 pounds are the rule for fingerling.

Now that grading has been made easy, the answer to the question, "Does grading pay?" is a very emphatic, "YES, IT DOES." (Pyle-Phillips, et al, 1961-1964 notwithstanding). These investigators, using small numbers of fish kept well-fed and thinned in hatchery troughs for only 20 weeks, and apparently evaluating survival within the hatchery only, concluded that grading was hardly worth the effort.

I submit that such small scale laboratory experiments are not applicable to the mass production techniques used here in the West. Had these investigators carried their work further to check on the survival of individual size groups in the wild environment, I am sure they would have found that grading does pay. These investigators did concede that small fish grew faster when segregated.

It is interesting to note that David C. Haskell, et al, 1958, states:

"Samples of sufficient size to insure one per cent accuracy are not feasible from a labor cost standpoint, though samples of sufficient size to insure five per cent accuracy appear to be feasible. The sample size to secure at least this degree of accuracy can be reduced by reducing in turn the size variation.

"Sorting and other practical methods for keeping trout uniform in size is justified on this basis, for in this way more accurate sampling can be obtained."

Haskell also states:

"An experiment in cannibalism under hatchery pond conditions but without feeding of the trout showed that larger trout apparently accounted for 100 per cent loss of fingerling in five days in a clear spring water pond and for 90 per cent loss in eight days in a cloudy lake water pound."

All factors being equal - size is the key to survival. This fact has been well-documented down through the years. In 1954, ten years ago, The Game Commission's Rock Creek Hatchery enjoyed a 4.7 per cent return of spring chinook reared 18 months. These fish were reared under the most adverse disease and nutritional conditions, and the per cent return did not include jack salmon or those fish taken in the off-shore troll fishery. Grading played an important role in their uniformity of size, which was between seven and eight inches at release.

The finest reference I have seen on the specific size of fish in relation to returns as adults was Wagner, et al, 1963, Transactions A. F. S. The following quotes are from this publication:

"An average difference of 2.6 centimeters between groups that migrated and those that remained in fresh water was found, the non-migrating fish always being the smaller."

"Evidence suggests that size of fish had a greater influence upon the total survival than time of release, within the time limits tested."

"No adult fish from the release of 2,730 steelhead at 26.5 per pound have been recovered."

"The present information points to the increase in survival of fish which were planted at a larger size in 1958. An estimated 10.0 per cent survival was obtained from the 7.7 per pound group, whereas the survival of the 13.0 per pound group was 0.8 per cent."

I would say the number of fish per pound alone should no longer be used as a criterion for planting size. Let's be specific and say steelhead must be a minimum of 19.0 centimeters, fork length, at one year of age.

In all those species where large surpluses of eggs exist, and perhaps even in those species that are not too plentiful, it would pay to grade out the slower-growing fish and concentrate production on the cream of the crop that will assure high returns. These grade-outs of small fish would make fine stock for the natural rearing ponds where Mother Nature operates an excellent grading system which might be called the "survival of the fittest".

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LABORATORY TESTS OF THE BACTERICIDAL ACTION AND FISH TOXICITY OF SIX QUATERNARY DISINFECTANTS

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ABSTRACT

Six antimicrobial quaternary ammonium compounds were tested for their toxicity to Rainbow trout fingerlings and for their activity against Myxobacteria suspected of causing Bacterial Gill disease in hatchery-reared Salmonids. Both of these determinations were conducted in deionized (soft) water and in hard spring water to evaluate the possible effects of different water supplies upon the efficacy of these agents.

The compounds tested were Hyamine 2389, Hyamine 1622, Hyamine 10X, Hyamine 3500, Cyncal and Roccal. Bactericidal tests of these compounds were conducted in the laboratory on artificial media and toxicity tests were carried out in aquaria under simulated therapeutic conditions.

Specific experimental results were as follows:

T E S T R E S U L T S (P P M)

Agent	99.999% Bacteria Killed	Fish Toxicity All Dead	Fish Toxicity All Live	Safety Index
		SOFT WATER SERIES		
2389	10.0	20.0	5.0	0.5
1622	2.0	20.0	5.0	2.5
10X	1.3	7.5	3.3	2.5
3500	1.0	7.5	5.0	5.0
Cyncal	1.0	7.5	2.8	2.8
Roccal	1.3	7.5	3.3	2.5

T E S T R E S U L T S (P P M)

Agent	99.999% Bacteria Killed	Fish Toxicity All Dead	Fish Toxicity All Live	Safety Index
		HARD WATER SERIES		
2389	20.0	100.0	10.0	0.5
1622	10.0	20.0	10.0	1.0
10X	10.0	10.0	5.0	0.5
3500	2.0	10.0	7.5	3.7
Cyncal	2.0	10.0	5.0	2.5
Roccal	4.0	10.0	7.5	1.9

Other applications of these compounds were discussed including the antiviral disinfectant activity of Roccal, Cyncal and Hyamine 3500 as well as the potential value of Hyamine 10X for the control of the protozoan Costia.

SUSCEPTIBILITY OF TWO SPECIES OF SALMON TO BOTULISM INTOXIFICATION

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While there is an extensive literature pertinent to botulism intoxification of mammals, primarly from a public health point of view, there is little information on the susceptibility of fish to the toxins produced by the various serotypes of the microorganism, Clostridium botulinum.

With the obvious advantages inherent in a hatchery feeding regime employing centrally-prepared diets has come the disadvantage of introducing conditions favorable for the growth in the diet constituents of C. botulinum. It was, therefore, of interest to determine the susceptibility of those species of fish reared in Oregon Fish Commission hatcheries to the various botulism toxins.

The two species tested thus far, chinook (Onocorhynchus tshawytscha), and coho (O. kisutch) salmon, showed a quantitative and qualitative similarity to intoxification. Both species are refractory to type A toxin, highly susceptible to type E, and to a lesser extent to types B, C, and D. Death occurred within 24-48 hours of feeding in the case of type E, as opposed to 4-6 days after feeding types B, C, and D. All the toxins were fed by soaking them into Oregon pellets just prior to feeding juveniles ranging between 40 and 60 per pound. Water temperature was maintained at 15°C. Various dilutions of the stock toxins were tested; however, no end-point or minimum lethal dose was determined.

CONTROL OF COLUMNARIS ON JUVENILE SPRING CHINOOK

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Idleyld Park, Oregon

There are usually four months in which columnaris becomes a serious problem at the Rock Creek Hatchery. During this time, July through September, it is quite common for the mid-day water temperatures to reach 66 to 68 degrees F. and the evening temperatures to exceed 70 degrees F. Columnaris is usually detected in June, when the water temperatures exceed 55 degrees F. on consecutive days. The disease progresses rapidly at these water temperatures, and once the typical symptoms and lesions appear, a heavy mortality follows. The infection can, however, be controlled in a short period of time. Our purpose, therefore, has been to find a method of preventing the occurrence of columnaris in the juvenile spring chinook.

In 1961 and 1962 during the four summer months, the salmon were fed Oregon moist pellets with sulfamerazine in an attempt to prevent columnaris. The salmon daily received 2 grams of sulfamerazine per 100 pounds of fish. When mortality was not controlled with 2 grams, sulfamerazine was increased to as much as 8 grams per 100 pounds of fish for a period of 5 to 10 days, or until mortality decreased. The high level of sulfamerazine was fed in a meat mixture one or two times a day. In addition, PMA (piridylmercuric acetate) was used during 1961 and 1962 as a prophylactic pond treatment for one hour, at concentrations of 1 to 750,000 and 1 to 500,000. Pond treating with PMA was carried out by shutting off the inflow, drawing the pond down to a predetermined level, and spraying the PMA solution over the water surface with a pump. The pump was operated for one hour after the PMA solution had been introduced into the water. The inflow was then turned on and the pond was allowed to fill normally but was not flushed out. All treatments were conducted with 75 to 100 pounds of water per pound of fish in the pond.

The above described treatments with PMA and sulfamerazine were partially effective as a control of columnaris, but did not prevent its recurrence. Because PMA by itself had shown some promise of controlling the disease, and because of the possibility of building up a sulfa resistant strain of bacteria with prolonged use of sulfa, it was decided to discontinue the feeding of sulfamerazine.

Pond treating with PMA at concentrations of 1 to 500,000 for one hour was used through the summer of 1963 in an attempt to prevent the occurrence of columnaris. The treatments were given every other day or for 2 or 3 consecutive days when columnaris was detected. When the lesions were no longer apparent, the number of treatments per week was reduced to one per week. In

approximately 2 weeks or less, lesions would again appear under reduced treatment. Again, the disease was being controlled but not prevented, and it appeared that treatments on consecutive days were more effective than on alternate days. It is believed that treating on alternate days partially controlled the bacteria on the day of treatment, but that the next day when not treated, the bacteria were allowed to multiply in such numbers that a slight infection was always present. With this in mind, a treatment schedule of 4 days each week was planned for the 1964 season.

Columnaris first appeared this past summer in July, a month later than usual because of lower water temperatures. At that time, a weekly treatment schedule was begun, using PMA for one hour at a concentration of 1 to 500,000 on Monday, Tuesday, Thursday and Friday. This schedule was continued through the summer until early October when water temperatures decreased to a safe level. Pond treating 4 days per week in 1964 with PMA was highly successful in preventing the occurrence of columnaris on the spring chinook at Rock Creek Hatchery. The columnaris, which appeared for only a short time in July of the 1964 summer season, was soon controlled.

TRYPTOPHAN REQUIREMENTS OF CHINOOK, SOCKEYE AND SILVER SALMON

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ABSTRACT

Diets containing casein and gelatin supplemented with crystalline L-amino acids to simulate whole egg protein were fed to triplicate lots of chinook salmon Oncorhynchus tshawytscha fingerlings for 10 weeks. Tryptophan content of the diet varied between lots at 0.15, 0.25, 0.35, 0.45, 0.65 and 1.05 per cent of the ration and was maintained isonitrogenous by ratio with proline. Almquist type plots of growth responses showed typical inflection points between 0.15 and 0.25 per cent of tryptophan in the diet treatments. In subsequent studies the same technique applied to sockeye salmon and silver salmon groups fed in duplicate for 10 weeks showed apparent requirements for tryptophan to be consistent at between 0.20 and 0.25 per cent of the ration when 40 per cent whole egg protein was fed.

NUTRITIONAL STUDIES AT ABERNATHY SALMON-CULTURAL LABORATORY

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The 1964 feeding trials were designed to test the protein quality of various fish meals with the objective of finding possible substitutes for salmon carcass meal. Salmon carcass meal has been used successfully as a diet component at the Salmon-Cultural Laboratory, Longview, Washington, for several years. The supply of this meal, sufficient for experimental work, would be inadequate for large scale production involving multiple hatcheries. The following is a list of fish meals which were tested: chinook salmon carcass (prepared in 1960), chinook salmon carcass (prepared in 1963), herring, turbot, dogfish (A), dogfish (B), rockfish, sole, and tuna. The three latter meals were fillet-scrap meals, the remainder were whole meals prepared from the entire carcass.

To briefly explain, these fish meals were added to the basal ration which consisted of dried skim milk, cottonseed meal, wheat germ, a vitamin supplement, peanut oil, CMC, and water. The fish meals were added in varying amounts dependent upon their protein content, and the amount of protein in the diet was held constant by adjustment of the water content to compensate for changes in the amount of fish meal. All experimental diets were maintained isocaloric by adjusting the level of peanut oil in the diet. The diets were fed at a protein level of 25 per cent and a caloric level of 2350 per kilogram of diet. The experiments were continued for a 24-week period.

Figure 1 shows the growth rates of the various diets and may be summarized as follows:

1. The salmon carcass meal prepared in 1960 produced better growth rates than did the salmon carcass meal prepared in 1963. The 1960 meal was a dry-rendered meal and had a low rancidity value. The 1963 meal was a precooked, pressed meal in which the solubles were defatted, dried, and returned to the meal. This process resulted in a meal with a lower fat content than the 1960 meal but with a higher rancidity value. It is assumed that the 1963 meal did not receive adequate handling after preparation which resulted in the high rancidity and consequent poorer utilization of the protein. Histopathological examination of the livers from fish fed this rancid salmon meal showed definite evidence of liver toxicity.

2. A dogfish meal (B) containing a high urea content produced inferior growth. The inferior quality of this meal was probably due to the method of preparation of the meal.

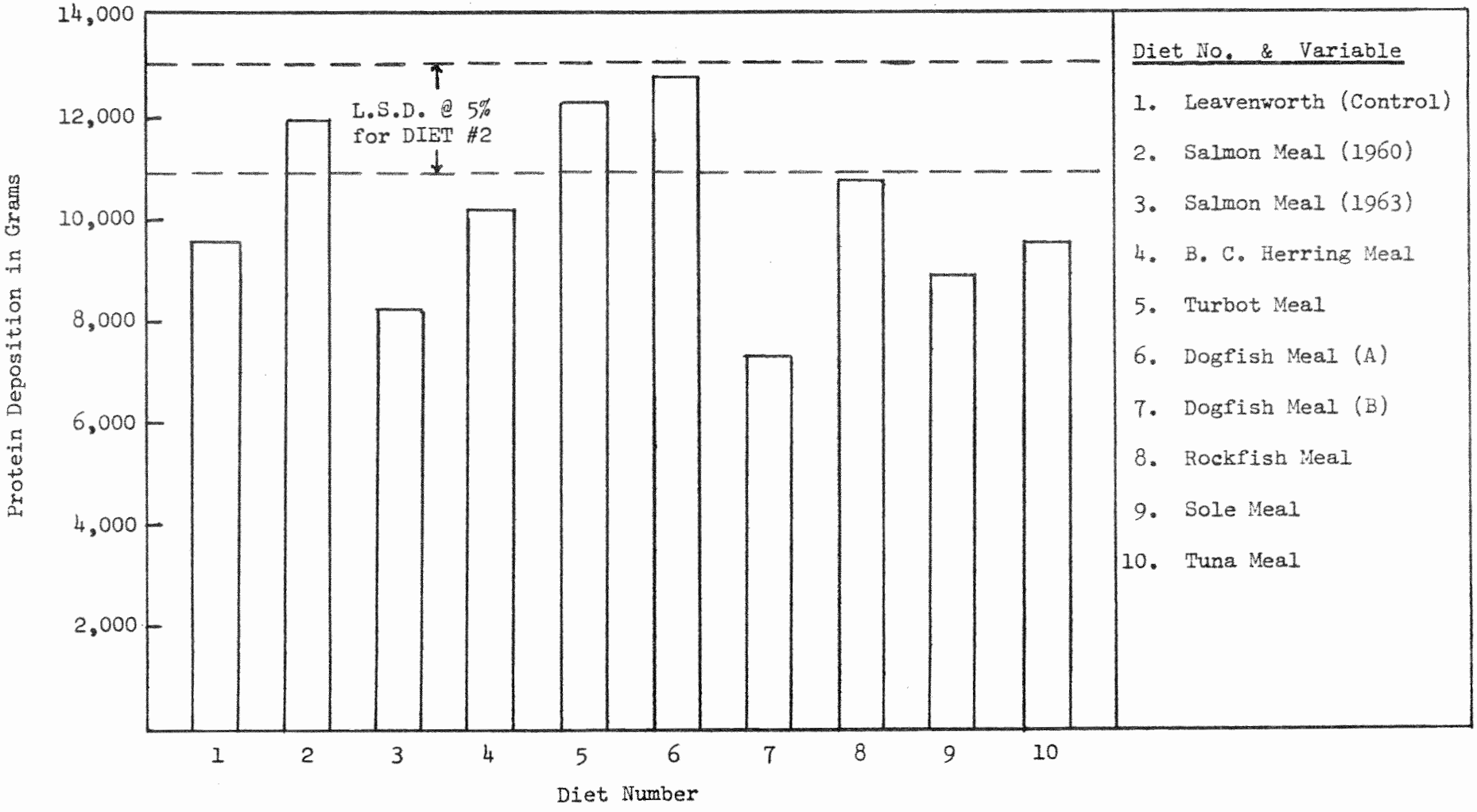

Figure 1:--Deposited protein levels of diet fish after 24 weeks of feeding, Abernathy Salmon-Cultural Laboratory - 1964.

3. Diets containing the turbot and the low-urea dogfish meals produced fish with growth rates equaling those fed the 1960 salmon carcass meal. Herring meal produced poorer growth than the 1960 salmon meal.

4. In general, the protein quality of the fillet-scrap meals were inferior to the quality of fish meals prepared from whole carcasses.

5. In other diets not shown in Figure 1, fish meals fed in combination did not produce fish with growth rates any better than did diets containing a single fish meal.

We have concluded that other fish meals may be substituted for salmon carcass meal in our composite meal diet. The ability to make such substitutions makes this ration a practical production diet as all portions of the mixture are in adequate supply. In addition, this diet compares in cost very favorably with other production diets. The cost per pound as fed is between $0.08 and $0.10, depending upon the size of the fish and the source of meals used. Our future plans are to feed this diet in 1965 on a large scale experiment to production stock.

A second feeding experiment was designed to determine the effect on the fish of different types of supplemental fat in a diet. Three diets were fed in this experiment. The first diet was our standard all-meal with the peanut oil supplement and was called the soft-fat diet. The second diet was the same all-meal diet, but instead of the peanut oil, it was supplemented with a hard fat which was collected from frozen beef spleen. The third diet was an all-meat diet consisting of hog liver and beef spleen.

At the end of 14 weeks of feeding, histological examinations of preserved livers from these fish showed marked differences between groups. The hard-fat group had concentrated a massive amount of lipo-protein within the hepatic cells while the soft-fat group had none of this material. The soft-fat group was characterized, however, by a fair amount of both neutral fat and glycogen accumulated between the liver cells. The livers from fish fed the meat diet also showed neutral fat and glycogen and in addition exhibited early stages of an intra-cellular deposition of lipoprotein.

At the end of 18 weeks of feeding, the group of fish being fed the hard fat was randomly split into 2 lots. The one lot remaining on the hard-fat diet and the other being put on the soft-fat diet to determine if the changes in the livers were irreversible. After 4 additional weeks of feeding the per cent hematocrit values of the fish were:

22.7% hard-fat group
22.2% recovery group
40.8% soft-fat group
33.4% meat group

The feeding was continued for an additional 8 weeks or a total of 26 weeks and terminated. At this time per cent hematocrits were:

16.0% hard-fat group
25.4% recovery group
36.0% soft-fat group
30.3% meat group

Histopathological evaluation of these fish at this time showed that the hard-fat group had continued to deteriorate and examination of the spleens and hematopoietic portion of the kidneys showed deteriorations resulting in the low hematocrit values. There was little or no recovery in the recovery group of fish, and the meat-fed group of fish continued to show liver lesions as well as the spleen lesions. No abnormalities were detected in the group fed the standard meal and peanut oil diet.

We have interpreted these results as evidence that fish metabolize hard fats very poorly. As a consequence, these fats infiltrate and accumulate within the liver cells as lipoproteins. In addition, prolonged feeding of hard fats severely inhibit the hematopoietic capabilities of the fish. These changes in the livers appear to be irreversible after they have once become established. The feeding of diets containing high percentages of meats and hard fats for considerable periods may be one of the reasons for poor survival and return of fish to hatcheries.

EFFECT OF AGE, GROWTH, AND DIET ON FINGERLING CHARACTERISTICS

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Contributing factors affecting hatchery success are sometimes difficult to determine. The quality of fish produced will depend on these factors. If differences between fish are measured and assessed, and quality standards defined, then criterion of normalcy can be established. Differences between hatchery-reared fish may not be due entirely to hatchery environment. In this experiment we measured the variation in the blood and body composition of chinook fingerling due to growth and the divergence created by diet. Age, growth, and diet effects were measured by analysis of the blood and body components of chinook fingerling at four-week intervals.

It was not our intent to produce comparable diets, but rather to determine if the diet fed would produce differences, and to determine if these differences varied with age and growth. The fish were fed exclusively on a meal or meat diet. As the fry were hatched from a single female and were reared at a constant temperature of 53 degrees F., there were no genetic differences, and the environment was optimum. The meal-fed fish received a diet containing 25 per cent protein and 2350 calories per kilogram and the meat-fed fish one containing 17.5 per cent protein and 1150 calories per kilogram.

Initially, the meat-fed lot weighed more than those fed meal. Those fed meal gained 81 per cent in weight during the second month of feeding while those fed meat gained only 66 per cent. Throughout the experiment, the meal-fed group continued to gain more rapidly than those fed meat and at the conclusion of the experiment had an average weight of 31.2 grams versus the 23.4 grams for the meat-fed group. The meal-fed fish had a higher fat deposition. The meat-fed fish had a higher body water content. Those fed meat showed lipoprotein deposition in the liver at 18 weeks and pathological examination of the body organs also revealed marked abnormalities in the spleen and hematopoietic portions of the kidney.

The hematology, as measured by per cent small cells, hematocrit, and total corpuscular count, showed that these values were affected by growth and diet. When the fish were 3 months old and had received the meal diet for 2 months, the levels of the small blood corpuscles increased to 51 per cent. This is appreciably higher than the average level of 21.2 per cent as determined for hatchery fish of comparable size. This increase in small cells was due, probably, to the rapid growth of these fish. In the group fed a meat diet, the heamtocrit levels fell sharply at

the 7th month, reflecting damage caused by the hard fat in the diet. Corpuscular counts in the meat-fed fish dropped from 1.1 million to 980 thousand during the same period. Hematocrits and corpuscular counts for meal-fed fish increased throughout the feeding period.

Physiological measurements showed that the composition of the blood plasma varied due to age and diet. Plasma glucose levels of both groups increased from 50 mg per cent to about 90 mg per cent in the 8-month period. The plasma cholesterol content of the meat-fed fish increased steadily for three months, then dropped sharply, indicating a pathological condition. The plasma protein and protein components were very similar regardless of diet. The pathological abnormalities were not shown by these measurements. Careful study of the electrophoretic patterns of the plasma proteins did show that there was a protein fraction in the meat-fed fish which was not present in the meal-fed group.

The chemical content of the blood plasma was relatively constant; however, measurable changes occurred in glucose levels and protein composition with age and growth, and cholesterol levels were dependent on the diet fed. Hematocrit levels and corpuscular counts increased due to age and growth.

This study as conducted shows us that in order to compare the inherent properties of fish, standards must be established for each age and size group.

A PROGRESS REPORT ON THE RESULTS OF RELEASING MIGRANT SIZE ZERO AGE COHO

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It has been demonstrated that in addition to time, the size of juvenile coho influences the seaward migrational tendencies. In waters where growth is found to be extremely slow, coho may reside for over 2 years in fresh water before they obtain a migratory size. Where growth is extremely fast, coho are known to migrate to sea during their first spring of life. (Noble, 1958). The latter pattern of life history has raised the question as to what is expected from the marine suvival and growth.

It is the intent of this paper to show the life history of three separate study groups of coho which migrated to sea at a normal migratory size, but all of which migrated as 6 month-old coho. The term "normal migratory size" shall be defined as fish between 20-80 fish per pound, as experienced in Puget Sound streams.

Studies were conducted on two groups of hatchery- reared coho which were induced to migrate to sea after spending six months in fresh water. A third group of the same age migrated as zeros from a freshwater lake. All three groups were subjected to conditions where the normal migratory size could be obtained in six months. Through marking experiments it was found that all three groups which were released at this age returned after a normal marine life history of approximately 18 months. Near-normal size and survival rates were obtained by the returning adults.

The eggs for two of the studies were from the coho salmon entering Chambers Creek near Tacoma, Washington. The progeny of the adults returning in 1957 and 1961 were incubated and reared in the warm (56°F.) spring waters of the Washington State Department of Game Hatchery on Chambers Creek. By the middle of May, after approximately six months of feeding, both groups obtained a migratory size of 29-47 fish per pound and were released into Chambers Creek after an identifying fin clip.

All fish returning from the 1957 brood returned as two year old mature adults in the fall of 1959. No jacks and only one three year old fish was observed. Approximately 60 per cent of the run was sampled for three year olds in the fall of 1960. The returns from 1961 brood followed a pattern identical to the 1957 brood, with the exception of one fish which returned as a three year old mature adult in the fall of 1964.

The third study was conducted on Cranberry Lake, a 170 acre spring-fed lake, on the very southern portion of Puget Sound (Johnson 1963). The 500,000 marked and 500,000 unmarked 1958 brood coho fingerlings were from the Green River stock and ranged in size from 321 to 348 fish per pound when planted into Cranberry Lake on May 11 and 12, 1959. During the month of June 1959, 4,531 of the unmarked group were marked as they were captured in downstream trapping facilities one mile below the lake. Their size ranged from 50 to 100 fish per pound and the silvery characteristics typical of yearling coho were present.

Returning two year old adults indicated that the size is several inches smaller than the parent stock. The mean length of the 1958 brood returning to Cranberry Lake as two year old fish was 22.9 inches for females and 23.9 inches for males. This would be some two to three inches shorter than the parent stock. One dorsal-marked three year old returned.

The returns from the 1957 brood marked and released into Chambers Creek can be compared with the returning unmarked fish from the 1956 brood Chambers Creek stock. The mean length of two year female adults measured during the fall of 1959 (1957 brood) was 20.9 inches while the unmarked three year fish (1956 brood) equaled 21.8 inches.

The females returning in 1963 from the marked 1961 brood were compared with the 1960 brood females returning in the fall of 1963. The 1961 brood females as two year old fish averaged 21.25 inches, and the 1960 brood averaged 22.68 inches.

The reason for the relative low mean length of the stream-reared returning adults at Chambers Creek is not fully understood. It has been theorized the September run, which was used for the study purposes, may have a different marine history than other stocks of coho. The adults returning in November averaged 7 - 8 pounds, or some 3 - 4 pounds larger than the September run. Beside the additional growth from a longer period at sea, the later run is theorized to spend a part of its marine life in ocean waters.

The per cent survival from downstream migrating coho to adult return was determined on the zero age lake migrants and on the zero age 1957 brood. From the 4,531 migrants leaving Cranberry Lake, 23 or 0.51 per cent returned at two years of age; from the 41,530 1957 brood released into Chambers Creek in the spring of 1958 at 47 fish per pound, a minimum of 0.352 per cent returned as mature adults. The rate of return of these two study groups can be said to be comparable to hatchery operations during the years where the unpastuerized diet was used.

Eggs taken from the Chambers Creek fish were fertilized and developed equally as well as eggs from regular three year fish.

Charts I and II summarize the three studies.

TABLE I

The Return from Releasing Migrant Size Zero Age Coho

Brood Year	Type Rearing	No. Released As Marked Fish	Age at Release	Size at Release Fish/lb.	Age Upon Return 1	2	3
1957	Accelerated	5,220	6 months	29	0	*0.352%	?
	Hatchery	34,310	6 months				
1961	Accelerated	19,738	6 months	32	0	0.40%	0.002
	Hatchery	32,954	6 months	47			
1958	Accelerated Lake	4,531	6 months	50-100	0	0.51%	0.02

* An estimated per cent return for the 1957 brood would be 0.5% as a portion of the run was not sampled.

TABLE II

Mean Length of Returning Coho as 2_1 Compared to the Normal Run or 3_2 (Fork length in Inches)

Area	2_1 Returns Male	2_1 Returns Female	3_2 Returns Male	3_2 Returns Female	Year of Return
Chambers Creek	20.0	20.9	23.3	21.8	1959
Chambers Creek		20.9		21.8	1963
Cranberry Lake	23.9	22.9		26.0	1960 for 2_1 1961 for 3_2

Statement:

The findings from releasing migrant size coho nearly one year prior to the normal release time could have management applications at either warm water hatcheries or at hatcheries using temperature controls; however, the smaller size of the returning adults may rule out the desirability of this practice.

Knowing that lake- or stream-reared coho may often obtain a migrant size in their first year, and return as 2_1 may explain some of the variables in research projects.

Conclusions:

1. Zero age coho of a migrant size did migrate to sea in the spring months.

2. These fish lived a normal marine life-history of 18 months, returning as mature two-year old coho.

3. The size upon return appears to be one to three inches smaller for a given stock; however, it is suggested this be pursued further.

4. Male and female ratio and fertility was normal.

5. Marine suvival rates were within the range experienced by hatchery-reared fish.

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PROGRESS REPORT

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This talk will serve as a progress report for the Hatchery Division, Washington State Department of Fisheries. I am pleased to report that we are making progress in Washington, and it is gratifying for me to see the excellent returns of salmon at all stations these past few years.

The year 1963 set a record for fall chinook handled at Washington hatchery facilities. The count for 1963 was 90,678 fall chinook; 83,400 from the Puget Sound and Coastal areas and 7,078 from the Columbia River.

The fall of 1964 resulted in a new record with a count of 103,146 fall chinook: 88,358 from the Puget Sound and Coastal region and 14,788 from the Columbia River. The actual count of fall chinook would have been much greater except for the necessity of blocking several hatchery trap sites because egg quotas were reached, and areas above these sites were saturated with adult salmon. For example, at the Skykomish hatchery in excess of 6,000 fall chinook were blocked out after the egg potential of the hatchery had been reached, and an estimated 25-35,000 fish were held below the hatchery racks at Green River.

Production of coho salmon is also showing well, with 67,000 adults counted by mid-November.

We, in Washington, are well aware that we do not stand alone in the general success of artificially propagating salmon. Actually, it is my feeling that this phase of fish culture has just scratched the surface of potential production, and I am confident that in the succeeding years the overall momentum and total production will be greatly increased. It may be that in the next decade some of the most dogmatic disbelievers in artificial propagation will be at least held at a neutral stand.

In spite of the excellent returns, we will eventually be faced with an economic evaluation of individual stations. To make this type of program feasible it will be necessary to identify numerous groups or lots of salmon by means other than fin clipping. Several new inovations in marking techniques such as tetracycline, coded wire and branding are presently under study, and some are in the stage of showing promising results.

Most of you have probably heard about the coded wire technique but may not have had the opportunity to see the mechanical apparatus involved or to determine for yourselves the problems involved.

Through the cooperation of Pete Bergman, Biologist for the Washington Department of Fisheries, who incidentally is fathering this project, and Pete's assistant, Bob Hager, Biologist, it is possible to present this group with a short film, a few slides, and a view of the actual tagging machine along with the detector.

The basic principle is to implant in each individual juvenile salmon a minute piece of color-coded wire; actual size is 1/25 of an inch in length by 0.010 of an inch in diameter; then later detect this wire by means of an electronic detector. The wire is a type 302 cold-worked stainless steel. The codes are placed on the wire by adding longitudinal color stripes, up to six different colors per wire, making it possible to obtain 10^6 different code combinations. If this number of codes is insufficient, simply change the length or diameter of the wire, or for that matter, the width of the color stripe and an entire new array of codes are possible. In effect, we are saying that the problem of making a sufficient number of different marks available has been solved.

Problems in the mechanics of tag application still exist, but experience and refinement of equipment will eventually solve these to the point of practical use. The problems that confront the recovery program will come into focus when the presently tagged coho return.

Experiments to date indicate that there is no tag loss when fish are properly tagged, there is no initial mortality, and for that matter no differential mortality up to one year for salmon held in rearing ponds, and the presence of the tag does not create tissue reaction in the snout area. The tagging speed is potentially comparable to fin clipping; for example, in the first semi-production run utilizing in excess of 170,000 yearling coho, the speed of tagging averaged 2,240 fish per day per operator, with a peak day of 3,960 per day per operator. This is based on a 6.5 hour working day.

On the second production run, with fish averaging 300-150 per pound, the tagging speed was 2,843 per day per operator with a peak day of 5,244 per day per operator. It is anticipated that by the fall of 1966 the potential of this marking technique will be sufficiently tested to determine ultimate success or failure.

A quick review of some of the slides shown gave an overall view of the marking tools and procedures.

IDAHO PRODUCTION DIET TESTS, 1964

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INTRODUCTION

Fish feed purchased for Idaho Fish Hatcheries has been open-formula dry diet since January, 1963. The diets reported upon were developed in cooperation with Dr. A. M. Dollar, University of Washington, College of Fisheries.

Continued testing of alternate ingredients and vitamins is necessary to develop the best diet for the least cost. The production and experimental diet tests reported were carried on by production hatchery personnel.

The diet test period reported is for the seven months from April through October, 1964.

Approximately 15,000 fish were used in each trial. Fish were not graded. A pound count was made at two week intervals and the ration adjusted accordingly.

A density of one pound of fish per cubic foot of water was maintained with the exception of Mackay where the density was .5 pound per cubic foot of water. Fish were reduced in number by random selection and the poundage of fish on each trial at a particular hatchery was equalized.

DIETS TESTED

The diets tested were the production diet (Table I) with the vitamin concentrate (Table 2) added; the production diet excluding choline chloride and ascorbic acid in the vitamin concentrate; a meat scrap meal diet and the production diet substituting corn gluten feed for soybean flour meal and adding one per cent A and D feeding oil.

COST OF DIETS

The production diet was purchased for $7.98 per CWT including a cost of $.79 per point five pounds of vitamin concentrate. Experimental diets were all priced at $15.00 CWT. Since the latter is not a realistic production cost figure, cost data was not computed.

Table I

DIET INGREDIENTS, IDAHO PRODUCTION DIET TEST, 1964

Diet	Fish Meal	Meat Scrap Meal	Liver Meal	Soybean Meal Flour	Whey	Dried Skim Milk	Yeast	Kelp Milk	A & D Oil	Salt	Vit. Conc.	Cond. Fish Solubles	Wheat Middlings
Production[1]	31	10	5	9	5	4	5	3	3	3	.5	.5	21
Production (Less Choline and ascorbic acid)	31	10	5	9	5	4	5	3	3	3	.5	.5	21
Meat Scrap Meal		83.5				5	5		3	3	.5		
Corn Gluten Feed	31	10	5	8	5	4	5	3	4	3	.5	.5	21

1. All fish were started with a fry diet containing 10 per cent delactosed whey, and 20 per cent wheat middlings; diet was then modified to contain 5 per cent whey, 4 per cent dried skim milk, and 21 per cent wheat middlings.

Table II Vitamin specifications, Idaho open formula diet, for 200 pounds of fish feed.

Guaranteed analysis per pound of vitamin concentrate.

Vitamin E	6,000 IU
Riboflavin	9,000 Milligrams
D Calcium Pantothenate	5,000 Milligrams
Niacin	10,000 Milligrams
Vitamin B_{12}	2 Milligrams
D Biotin	60 Milligrams
Thiamine Hydrochloride	12,000 Milligrams
Pyridoxine Hydrochloride	2,000 Milligrams
Folic Acid	300 Milligrams
Choline Chloride[1]	25,000 Milligrams
Ascorbic Acid[1]	20,000 Milligrams

1. All fish were started with choline and ascorbic acid added to the diet. Meat scrap, corn gluten feed diet and the special production diet had choline chloride and ascorbic acid added throughout the experiment.

RESULTS

Results of the feeding tests are presented in Table III.

The production diet, excluding choline and ascorbic acid, gave the best conversion of pounds of fish feed to pound of fish. The production diet with the substitution of corn gluten feed at a level of eight per cent and the addition of one per cent A & D feeding oil for nine per cent soybean flour meal was slightly less efficient than the production diet.

Table III

Results of 1964 feeding test at Idaho Fish and Game Department Hatcheries

Diet	Station	Lbs. of Feed per lb. of Fish	Mortality Per Cent	Hematocrit Readings
Production (no Choline or Ascorbic acid)	Ashton	1.81	2.3	36
	Hagerman	1.48	1.6	36
	Hayspur	1.43	.8	36
	Mackay	1.47	.2	40
Production (with Choline and Ascorbic acid)	American Falls	1.80	17.0	34
	Mackay	1.53	.4	40
Meat Scrap Meal	Hagerman	1.97	1.9	39
	Twin Falls	1.78	1.0	
Corn Gluten Meal	American Falls	2.0	16.0	36
	Ashton	2.2	13.5	43
	Hagerman	1.59	1.8	34
	Hayspur	1.50	.8	36
	Mackay	1.62	.3	41
	Twin Falls	1.52	.3	

The meat scrap meal diet was considerably less efficient than the other diets. This diet is the least expensive per CWT. It would compare favorably in cost per pound of fish produced with the other diets; however, the increased volume of food required to produce a pound of fish would be magnified in handling and transportation costs.

The hematocrit readings were reduced on the average of five points on all diets during these tests as compared to the 1963 feeding tests. It is believed this was caused by the addition of Santoquin at the rate of one pound per three tons of fish feed. Santoquin was added without the consent or knowledge of the Dept.

ACKNOWLEDGMENT

Special thanks to all of those who carried on the experiments, Hatchery Superintendents, B. D. Ainsworth, Norman Floyd, Harvey Albrethsen, Frank Gaver, L. W. Gaver, Walt Bethke, E. O. Bailey, Calvin Coziah, and Hark Misseldine and their respective hatchery crews.

OREGON PELLETS AS A STARTER DIET FOR SALMON AND STEELHEAD

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Fish Commission of Oregon
Clackamas, Oregon

Oregon pellets (1/32-inch diameter or 20-mesh crumbles) were fed as a starting diet to two ponds each of coho at Klaskanine and Sandy hatcheries; fall chinook at Bonneville and Oxbow hatcheries; and spring chinook at Willamette and Marion Forks hatcheries. The standard starter diet composed of equal parts beef liver, hog liver and pasteurized salmon viscera with salt to bind served as the control.

A summary of the results are shown in Table 1. Results of fork length measurements from fish samples taken at termination of the trials showed:

1. Very little difference in size distribution in coho fed by the two methods and no significant difference in mean fish lengths.

2. Very little size difference was noted in mean lengths of fall chinook at Oxbow whereas the pellet diet resulted in significantly greater mean length and larger size variation at Bonneville.

3. It appears that the pellet produced a greater size variance with spring chinook and observations at the hatcheries suggest that more pinheads resulted. However, a significant difference in mean length could not be demonstrated.

We concluded that coho, spring and fall chinook could be successfully started on Oregon pellets. However, we believe we need additional information on optimum particle size.

Also, in exploratory studies, steelhead trout were successfully started on 20- to 30-mesh moist and 30- to 40-mesh dried Oregon pellets. Both did better than the meat-fish control. The moist pellet did somewhat better than the dried.

Table 1. Summary of Results at Termination of Starter Diet Trials, 1964.

Species	Hatchery	Diet	Lot Wt. Gain %	Conv. (Dry)	Hematocrit (%)	Mortality (%)
Coho	Klaskanine	Control	278	1.50	31.0	2.6
		Pellet	277	1.65	34.4	3.9
	Sandy	Control	231	1.30	33.0	2.4
		Pellet	216	1.20	33.9	4.7
	Willamette	Control	254	1.52	37.8	1.1
		Pellet	258	1.67 1/	36.3	1.7
Spring Chinook	Marion Fks.	Control	340	1.35	39.6	2.4
		Pellet	378 1/	1.33	40.3	2.7
Fall Chinook	Oxbow	Control	246	1.40	39.4	2.5
		Pellet	281 1/	1.40	40.3	1.5 1/
	Bonneville	Control	199	1.80	31.6	5.3
		Pellet	256 1/	1.35 1/	37.8 1/	3.5

1/ Difference is statistically significant.

STUDIES ON OREGON PELLET FEEDING TECHNIQUES

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Fish Commission of Oregon
Sandy, Oregon

The Fish Commission of Oregon must provide feeding techniques to fit varying hatchery conditions. Environments range from those with predominately low water temperatures to situations where water warms early in the year and good growing conditions prevail through most of the rearing season. In order to produce fish of optimum size at liberation, it is necessary to restrict feeding at some stations. To gain insight into the effects of restricted feeding with Oregon pellets, a series of small scale, short term experiments were conducted with coho fingerlings under controlled temperature conditions.

Tests were conducted with three fish sizes (approximately 50-75, 90-105, and larger than 120 mm fork length) at three water temperatures (40°, 50°, and 60°F.).

Small groups of fish (300-400 grams per lot) were reared for 2-3 weeks in 7 gallon capacity aquaria. Duplicate lots were fed on a demand basis twice per feeding day and the total intake was restricted by reducing the number of days fed per week. Feeding frequencies investigated were 7, 6, 5, 4, 3, and 2 feeding days per week.

Data collected included (1) weekly measurements of growth in weight and fork length, (2) hematocrit samples as indices of blood condition at the beginning and end of each test, and (3) proximate analyses of fish carcasses and diet for each experiment.

Food consumption and growth in fork length are depicted in Figures 1 & 2 respectively. Indices of protein efficiency and fat deposition rate are presented in Figure 3.

The average amount of food consumed on a daily basis was restricted in a regular manner by reduction of feeding days per week, and growth was proportional to the amount of food fed. No feeding rate was so low that it completely restricted growth in length.

Comparable protein efficiency ratios were noted with most all of the feeding levels at 50° and 60° F. There is an indication that efficiency dropped for 2 feedings per week with the two larger fish sizes at 60° F. and for the same frequency with the largest fish at 50° F. Rate of fat deposition per unit of food fed seemed to increase with increasing feeding rates at 60° F. but this relationship was not as obvious at 50°F. Notable reduction in fat deposition occurred for feeding frequencies associated with declines in protein efficiency ratios. Data on diet efficiency and fat deposition at 40°F. were too variable to permit recognition of relationships.

Mean hematocrit values observed at the termination of the various treatments usually were in the range from 30 per cent to 40 per cent and considered satisfactory. One exception was the series with fish 90-105 mm fork length fed 7, 5, and 3 days per week. Mean values of about 20 per cent at termination reflected a low initial value of 28 per cent. Initial mean hematocrits for all other experiments ranged from 36 per cent to 44 per cent.

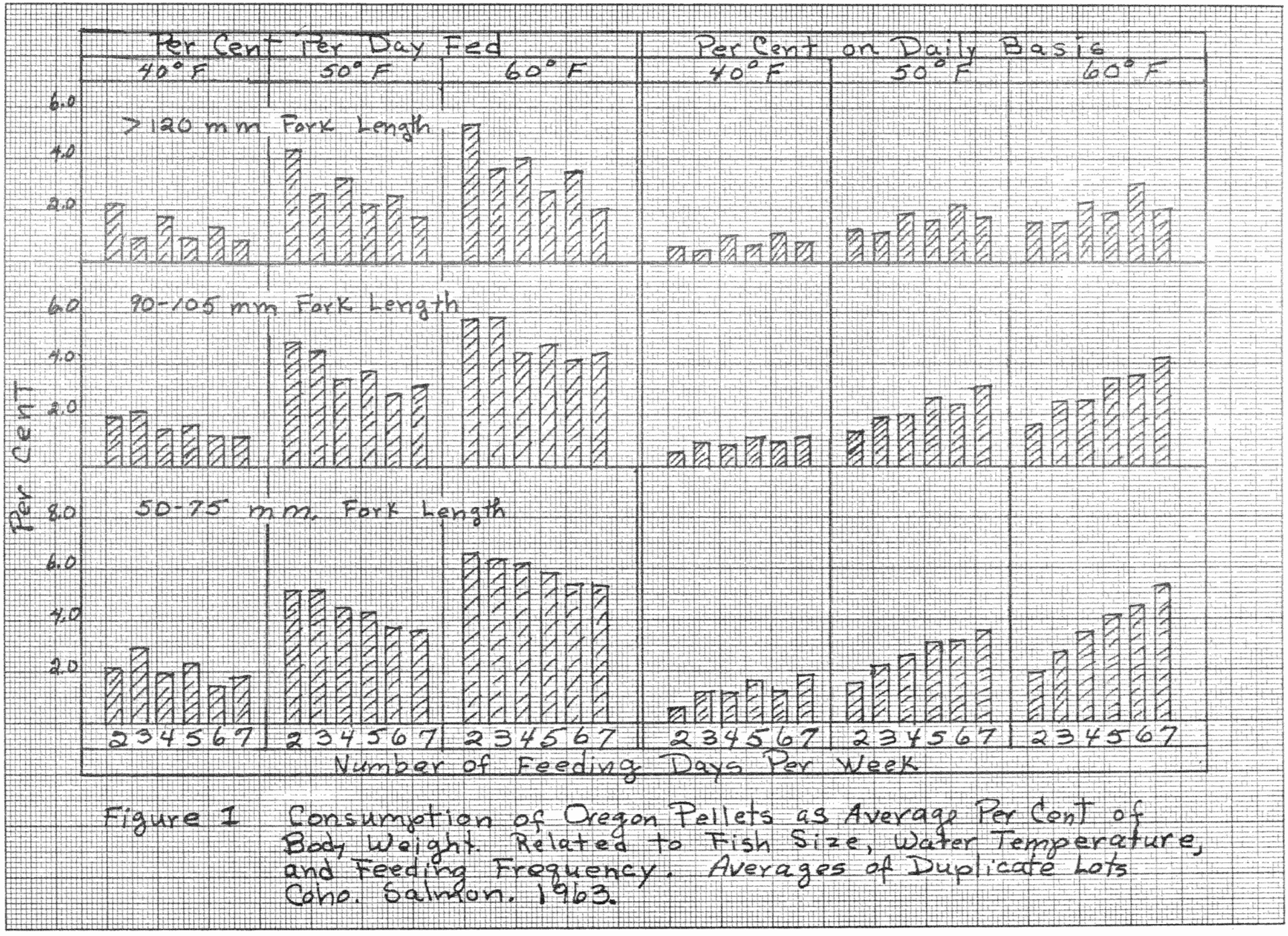

Figure 1 Consumption of Oregon Pellets as Average Per Cent of Body Weight Related to Fish Size, Water Temperature, and Feeding Frequency. Averages of Duplicate Lots Coho. Salmon. 1963.

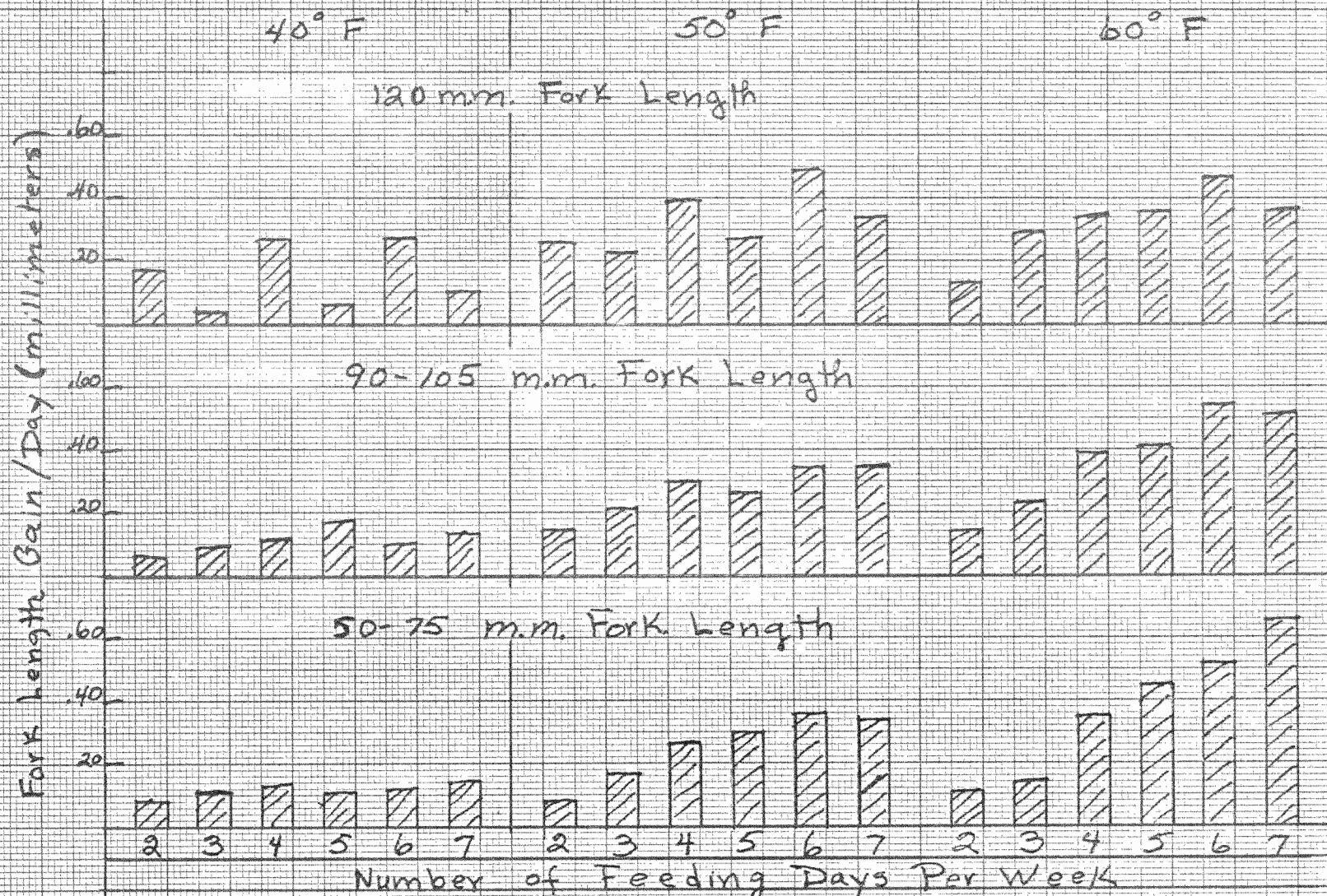

Figure 2. Gain in Average Fork Length Per Day Related To Fish Size, Water Temperature, and Feeding Frequency. Averages of Duplicate lots Coho Salmon, 1963.

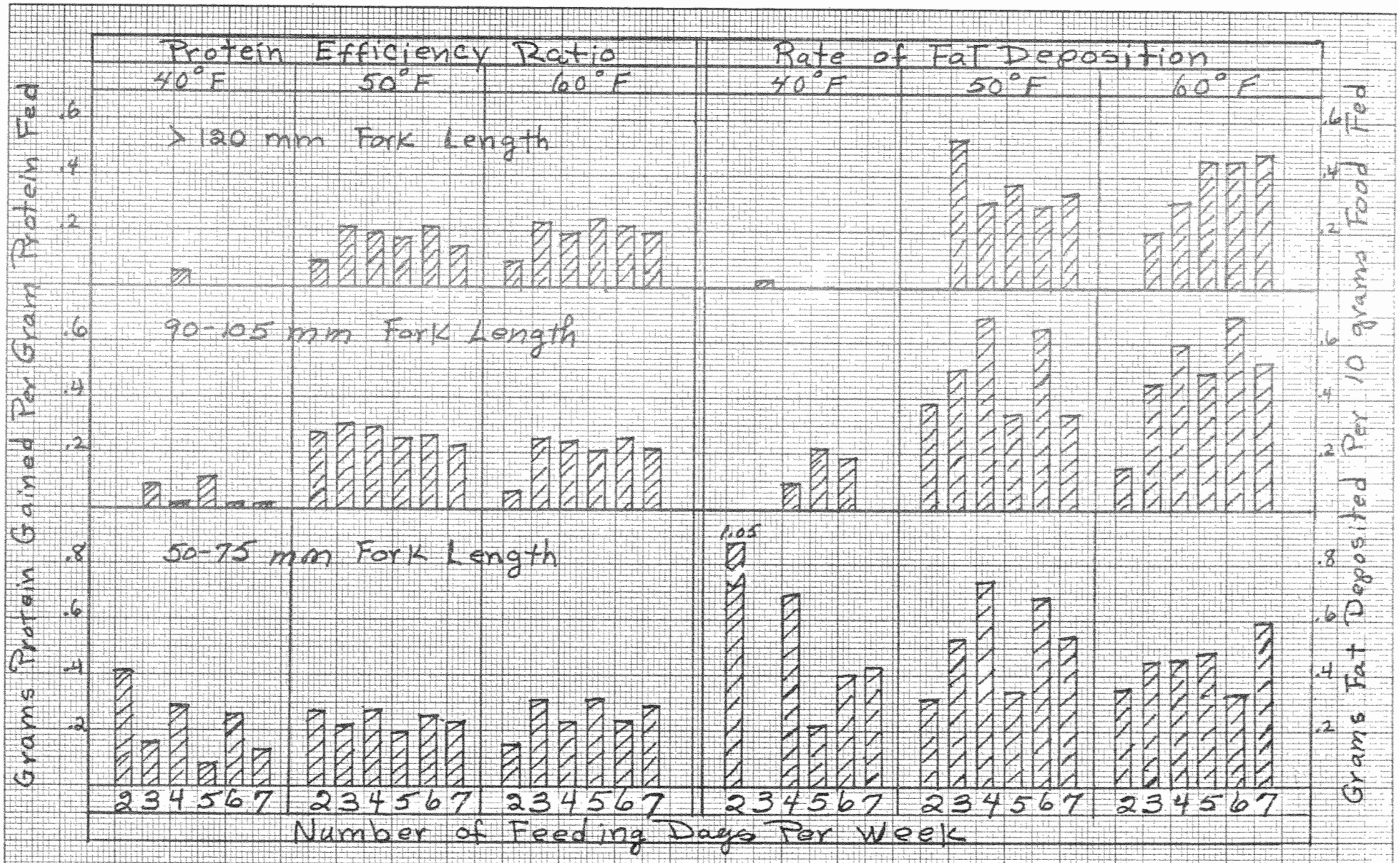

Figure 3. Protein Efficiency Ratios and Fat Deposition Observed for Oregon Pellets. Related to Fish Size, Water Temperature and Feeding Frequency. Averages of Duplicate Lots. Coho Salmon. 1963.

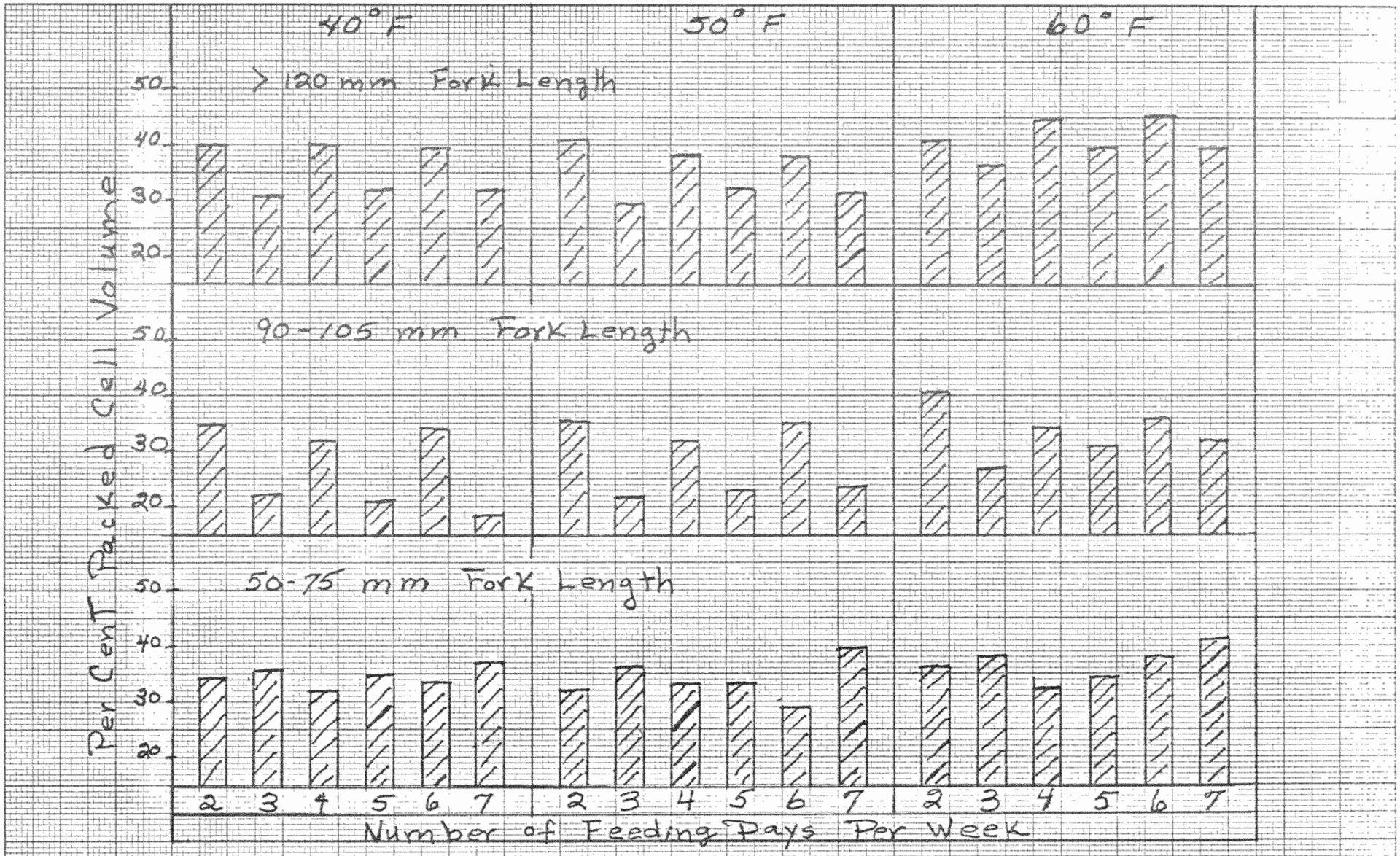

Figure 4. Hematocrit Values Related To Fish Size, Water Temperature, and Feeding Frequency. Averages of Duplicate Lots. Coho Salmon, 1963.

EVALUATION OF COTTONSEED OIL MEAL SUBSTITUTES AND BOTTOMFISH FILLET SCRAP IN THE OREGON PELLET
A PRELIMINARY REPORT

John Westgate
Fish Commission of Oregon
Clackamas, Oregon

Cottonseed oil meal, corn gluten meal, ground beet pulp, dried skim milk, and Alphacel (a non-nutritious filler) are being compared as ingredients at 22 per cent of the Oregon pellet. Results to date suggest that cottonseed meal, corn gluten meal, and dried skim milk contribute considerable nutritive value when used for this portion of the pellet. Beet pulp appeared to be unpalatable at this high level.

Fillet scrap from Pacific Ocean perch, English sole, and Dover sole are each being tested as the entire wet component (40%) of the Oregon pellet. Their use at this level is producing relatively poor growth and is associated with increased mortality.

Results to date are summarized in Table 1. The experiment will continue until December 30, 1964.

Table 1. Summary of Results After 16 Weeks Testing, Oregon Pellet Trials, Clackamas, 1964.

Ingredient Tested	Total Weight Gain(gms)	Mortality (%)	Food Conv. (Wet)	Calories Fed/100 gms Wt. Gain	Gms Protein Fed/100 gms Wt. Gain
Alphacel	2,813	0.3	2.35	494	66
Cottonseed Meal	4,194	0.5	1.56	357	60
Corn Gluten Meal	3,917	0.0	1.42	339	55
Dried Skim Milk	4,379	0.5	1.58	350	57
Beet Pulp Meal	-255	54.3	Discontinued after 12 Weeks		
Ocean Perch	3,269	4.1	1.46	367	53
English Sole	3,596	1.6	1.48	334	53
Dover Sole	2,891	3.3	1.65	360	57

EFFECT OF FINGERLING STAMINA ON ADULT SURVIVAL

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The ability of fingerling salmonids to swim as measured by the stamina tunnel has been assumed to be a valid determination of condition. In 1962, an experiment was initiated to determine if differences in the stamina of the fingerlings at time of release affected the survival to the adult stage.

In May of 1962, approximately 400,000 fall chinook fingerlings were randomly divided into two lots of 200,000 fish each. One of these lots was reared in 6 rectangular-recirculating ponds and the other in 4, 8x80 raceways. Water was introduced at the rate of 250 gpm in the rectangulars and 375 gpm in the raceways. At time of release, the loading rate was approximately 6 pounds per gpm of inflow. In July, the two groups were marked, the raceway fish by the excision of the right pectoral and the fish reared in the rectangular ponds by the excision of the left pectoral fins.

On September 19 and 20, the two lots of fish were released into Abernathy Creek. At time of release, the raceway fish averaged 20.5 grams or 22 per pound and the fish from the rectangular-recirculating ponds averaged 23.9 grams or 19 per pound. Random samples from each group showed performance ratings of 64 for raceway fish and 99 for the samples from the rectangular ponds. These are highly significant differences in stamina between the two groups.

The differences in stamina were imposed only by the pond type. All other rearing procedures including the diets fed were identical.

In 1964, the fish returned as 3-year-olds. The results of this return are shown in the following table:

	No. released	No. returned	% return
High Stamina (LP)	181,859	255	0.140
Low Stamina (RP)	198,715	170	0.086

Chi Square: 23.3, highly significant difference

In addition, 3 fish marked right pectoral and 2 fish marked left pectoral returned as 2-year-olds in 1963, all as males. The 3-year-old run from both groups approximated a 50:50 ratio of males to females. Actually, there were 121 females producing 605,000 eggs in the high-stamina lot and 81 females producing 405,000 eggs in the low-stamina lot. Both groups have successfully maintained themselves as 3-year-olds.

These data indicate that a 50 per cent increase in stamina results in a 60 per cent increase in adult survival. The conclusion is obvious. At present we are in the process of converting the 12, 8x80 raceways at Abernathy into 6, 17x75 rectangular-recirculating ponds. This conversion is costing about $1,500 per raceway. We can see no justification for further construction of raceway ponds and recommend the conversion of present low-velocity pond types into high-velocity ponds as rapidly as economically feasible.

ACUTE COPPER AND ZINC TOXICITY IN CHINOOK SALMON

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Western Fish Nutrition Laboratory
Willard, Washington

ABSTRACT

In the soft water conditions of the Western Fish Nutrition Laboratory, acute copper toxicity toward chinook salmon (Oncorhynchus tshawytscha) resulted from 13 to 14 ppb Cu regardless of age. The presence of 100 ppb Zn roughly doubled the sensitivity to Cu.

The critical age period for zinc toxicity was from hatch to 2800 heat units. Low concentrations of Cu exhibited a protective mechanism to zinc toxicity until 5000 total heat units, whereupon the Cu-Zn synergism appeared and comparative mortality was doubled.

TOXICITY STUDIES OF DIMETHYLSULFOXIDE ON SILVER SALMON

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Western Fish Nutrition Laboratory
Cook, Washington

ABSTRACT

Dimethylsulfoxide (DMSO) is noted for its penetration of biological membranes with a low order of toxicity to the cells. Immersion studies to determine the effect on fish and establish its lethal exposure time for 50 per cent of the fish population revealed a logarithmic response and indicated Haber's Law, which governs air-borne contaminants, may also apply to fish and water-borne contaminants. Mortality rate varied directly with temperature. Critical environmental concentration was observed between 2 per cent and 3 per cent. Fish exposed to 3 per cent expired in 1 month whereas fish at 1 per cent and below were still actively feeding and growing after 3 months. The oral studies showed no effect in the growth rate at the 0.5 per cent and 2 per cent levels; above this, there was a decrease in per cent weight gained. An 18 per cent level was fed for 6 weeks with no mortalities. Intraperitoneal injections of silvers and sockeyes produced an approximate LD-50 of 16gm DMSO/rg body weight and 13gm DMSO/rg body weight, respectively.

BLOOD CHANGES IN SILVER SALMON FROM DIMETHYLSULFOXIDE

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Western Fish Nutrition Laboratory
Willard, Washington

ABSTRACT

Hematological changes in fingerling silver salmon Oncorhynchus kisutch, were studied after immersion, injection and feeding of dimethylsulfoxide (DMSO). Fish immersed in 16 per cent, 12 per cent, and 8 per cent DMSO in hatchery water at 2^{0}, 12^{0} and 22^{0} centigrade showed evidence of toxic degeneration of blood leukocytes. Hematocrits were increased at all three percentages. Exposure at all concentrations indicated that the death rate varied directly with temperature while hematocrits varied inversely. Increased red blood cell fragility occurred in fish immersed in 16 per cent and 12 per cent DMSO as determined by the Modified Sanford Method. Intraperitoneal injection of 27.5, 13.75, 11.0 and 8.25 g/kgbw 100 per cent, 50 per cent, 40 per cent and 30 per cent DMSO produced changes similar to those resulting from immersion, i.e. evidence of toxic degeneration of blood leukocytes. Mild nuclear degeneration was also noted in some erythrocytes. Hematocrits, however, decreased with repeated injections. No hematological changes were observed when DMSO was fed at 0.5 per cent, 2.9 per cent and 11.7 per cent of diet for six weeks.

MARKING SALMONIDS WITH TETRACYCLINE ANTIBIOTICS

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Fish Commission of Oregon
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Feeding tetracycline antibiotics to fingerling salmon produces a compound fixed in growing bone tissue which can be later detected with fluorescent microscopy. Pacific salmon were first marked in this fashion by Douglas Weber and George Ridgway in 1961. The Fish Commission is carrying out research to gather information which may eventually permit Food and Drug Administration clearance and full-scale use as a marking method. Preliminary tests were conducted at Clackamas Laboratory. Oxytetracycline, chlortetracycline, and tetracycline were incorporated into standard Oregon pellets and special low-calcium moist pellets in concentrations of 1.1, 2.3, and 6.8 per cent active ingredient. Consumption of all drugs decreased with increasing level in the food. Chlortetracycline was poorly accepted and produced the weakest marks. Oxytetracycline was second in mark quality and produced fair marks. Tetracycline was eaten best, and produced the best marks.

In a second test for marking efficiency treatment levels of 0.5, 1.0, and 2.0 grams per kilogram of oxytetracycline and tetracycline were compared. A treatment level of 1.0 gram of tetracycline per kilogram of fish weight gave the best mark consistent with acceptable palatability in the low-calcium pellet.

One of the prerequisites of FDA approval is low levels of drug residue in fish tissue after withdrawal of drug treatment. Flesh, skin, viscera and bone were dissected from fingerling salmon which had been fed a 1 gram per kilogram level of oxytetracycline and then sampled at 3-day intervals following withdrawal of treatment. Results of the assay showed that the antibiotic level was below the sensitivity of the assay method in flesh and skin within 7 days and in viscera and bone in 16 days.

Coho fingerlings were held in temperatures of 40°, 50° and 60°F. and fed 1 gram per kilogram doses of tetracycline. Although results of the assay have not been received, examination of bones shows good marks at 50° and 60° and almost no mark at 40°F.

A test of multiple marking was conducted with fingerling coho, after the third feeding of 1 gram per kilogram doses of tetracycline, a sample of 100 fish was examined for marks. In the order of their application, 63, 95, and 100 per cent of the marks were identified.

In three instances, fingerlings were marked when fed therapeutic doses of antibiotic for treatment of furunculosis.

GROWTH RATES AND CONDITION INDEX FOR RAINBOW TROUT FED DRY DIETS

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Utah Fish and Game Department
Logan, Utah

During 1963-64, the Utah Fish and Game Department conducted tests whereby several commercial trout diets were fed to rainbow trout at the Experimental Hatchery in Logan, Utah.

The length-weight information obtained from these trout (Figure 1) was subjected to statistical tests to determine expected weights from given lengths and also to provide expected condition factors. An example of these expected values is given in Table 1.

Table 1 and its statistical derivation will be published in complete form later this year. Accompanying this information will be a second table giving feed amounts fed by ten-day intervals which should act as a useful guide for projecting feed orders.

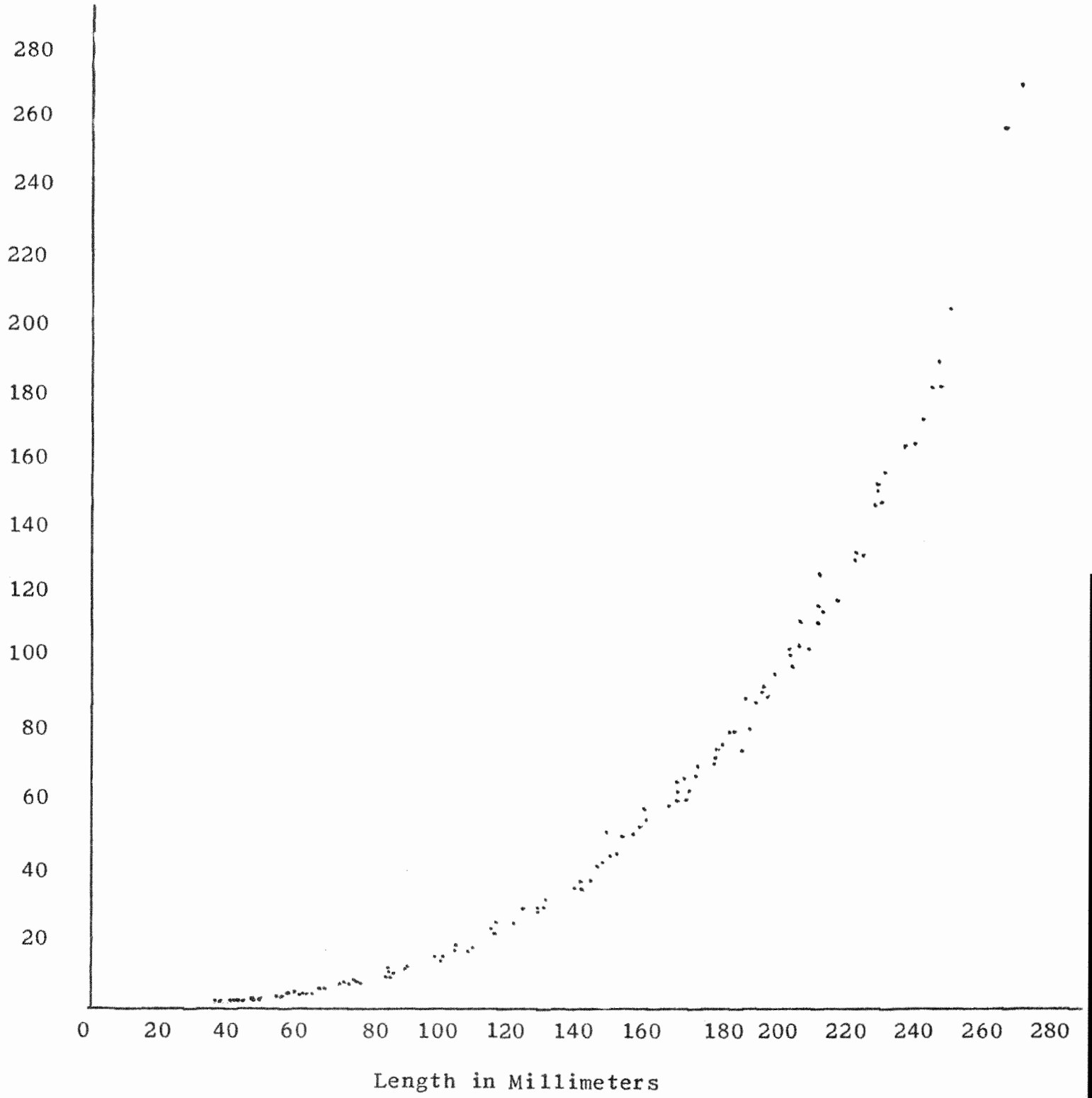

Figure 1. Length-weight relationship obtained from 12,800 fish. Each point represents an average from 100 fish.

Table 1.

Expected weights, condition factors (grams per centimeter) and fish per pound computed from measurements taken from 12,800 rainbow trout fed dry diets at the Experimental Hatchery in Logan, Utah (1963-1964).

Length in inches	Length in millimeters	Weight in grams	Grams/ centimeter	fish/ pound
1.37	35	.62	.17	722.80
1.41	36	.68	.18	666.12
1.45	37	.73	.19	615.26
1.49	38	.79	.20	569.48
etc	etc	etc	etc	etc
11.96	304	330.84	10.88	1.37
12.00	305	334.01	10.95	1.35

USE OF SIMAZINE AND ATRAZINE FOR CONTROL OF PONDWEEDS AND FILAMENTOUS ALGAE

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U. S. Fish and Wildlife Service
Miles City, Montana

At the Miles City National Fish Hatchery we are primarily concerned with the production of large numbers of fingerling predator sport fish such as largemouth bass, smallmouth bass, northern pike and walleye pike. Since none of these species can be readily fed in the same manner as trout or catfish, it is necessary to depend entirely upon the production of zooplankton for food for these predatory species.

Over the years many ways have been devised to increase zooplankton production, varying from the use of inorganic fertilizers to the use of high protein plant and animal meals. The use of these inorganic and organic materials will increase zooplankton production to a great extent but often has resulted in some troublesome side effects. The primary problem encountered is the stimulation growth of filamentous and blue-green algae. This growth of algae makes removal of the small fish difficult and expensive and often can result in a total loss of fish at draining time due to the high oxygen demand caused by the dying algae. This high oxygen demand caused by a rapid die-off of algae can result in the loss of an entire pond of fish prior to draining time.

Production of fingerling predator fish in ponds is therefore limited by the amount of zooplankton that can be produced and also is limited by the amount of algae produced in the process of increasing the zooplankton production. This is an oversimplification but basicly states the problem.

Many different chemicals and commercial preparations have been used in an effort to control algae and pondweeds and many of these are effective. Among the most effective are copper sulphate, sodium arsenite, Del-rad and Phygon. While these chemicals control pondweeds and algae they are all more or less toxic to fish and invertebrate life. Recent experiments at the Bureau of Sport Fisheries and Wildlife Warm Water Training School at Marion, Alabama, have discovered some chemicals that are very promising from the standpoint of controlling pondweeds and algae without materially affecting the zooplankton food supply needed for high production in ponds.

Until we began using simazine at Miles City (at rates of .5 to 2 ppm) we considered maximum production of the predatory fingerlings to be 100 to 150 pounds per acre. However, with the use of simazine or atrazine, it appears that these production figures can be at least doubled. This means that instead of producing

100 pounds of bass or pike fingerlings per acre it is now possible to produce 200 pounds per acre or even more with proper use of organic fertilizers. When simazine or atrazine is used, more frequent applications of organic fertilizer are required to maintain an adequate zooplankton food supply. We have tried various organic fertilizers and have found alfalfa hay plus tankage and/or torula yeast to be effective.

The use of simazine or atrazine for control of weeds or algae also seems to prevent wide fluctuations in the pH of the pond water. These wide fluctuations can be very detrimental to fish production if the pH goes over 10 as will sometimes occur when heavy pond weed or algae growths are present in production ponds. We have found it best to delay use of simazine in bass production ponds until small amounts of algae are present. This is usually about two weeks after the ponds have been filled and the brood fish placed in the pond. This delay seems to result in greater fry production. If pondweeds are a serious problem the application of 1 to 2 ppm of simazine or atrazine should be made simultaneously with the filling of the pond.

These chemicals also would seem to offer great promise for the control of pondweeds in farm and ranch ponds especially if the bass and bluegill combination is being used. They could not be used, however, where the pond water is subsequently used for irrigation purposes.

CONSTRUCTION AND OPERATION OF RAPID RIVER HATCHERY

Charles R. Quidor
Superintendent
Rapid River Hatchery
Idaho Fish and Game Department
Riggens, Idaho

INTRODUCTION

The Rapid River Hatchery is owned and was constructed by Idaho Power Co. at a cost of $279,000. The Idaho Fish and Game Department is contracted to operate this station on Idaho Power funds; it will be operated by two full-time employees.

PURPOSE

The purpose of this station is to relocate the spring chinook salmon run that normally migrated up the Snake River above the Idaho Power Dams. An attempt will be made to maintain spring chinook salmon in the Rapid River in the hope that a successful way may be found to restore them in the Snake River.

OPERATION

Spring chinook salmon and steelhead brought from Oxbow fish trap on the Snake River will be held at Rapid River Hatchery until they are mature. The fish will be spawned, the eggs hatched, and the fingerlings raised to release size. The salmon will be released at approximately 22 per pound. A small number of steelhead are being experimentally raised at the station. It is hoped the salmon will reach release size the fall following hatching.

HOLDING POND, RACEWAYS AND HATCHERY BUILDINGS

The holding pond is of steel and concrete construction, 80 feet long, 25 feet wide and 6 feet deep with a 6 inch grade to the bottom. It is equipped with a sprinkling system and two water intakes - one 24-inch line coming into the head-end and another 24-inch line coming into a defuser in the bottom. There is a perforated plastic hose along the bottom that is used in the daily treatment of the water with a one ppm solution of malachite green.

The twelve raceways are also of steel and concrete construction, 100 feet long, 6 feet wide, and 4 feet deep with a 6 inch grade to the bottom.

The hatchery building, residence and public restrooms are of redwood construction.

EGGS AND TEMPERATURE UNITS

Heath vertical incubator trays will be used for hatching eggs. Swim-up fry will remain in incubator trays until they are transferred to outside raceways.

The spring chinook salmon eggs developed to the eyed stage at 400 temperature units. Eggs started to hatch at 850 temperature units and hatching was complete at 1,000 temperature units.

FEED

The fingerlings will be fed a diet of Oregon Moist Pellets. This food is stored in a 15 ton freezer room located in the hatchery building.

WATER INTAKE AND SCREENING

The main water intake is the straight diversion from Rapid River, equipped with electrically-driven screens. The screens are stainless steel and are controlled by a float mechanism. If water below the screen drops one inch or more, a switch is engaged and water is pumped through sprayers, which washes debris off the screens. The screens revolve 10 seconds later and continue to revolve for at least two complete revolutions. The debris is washed into a trough and returned to the river below the intake.

WATER TEMPERATURES

The water temperatures at the hatchery during 1964 reached a high of 59 degrees Fahrenheit in the summer, and dropped to a low of 35 degrees Fahrenheit during November 1964.

FISH ON HAND

At the present time there are 11,000 steelhead fingerlings and over 800,000 spring chinook fry at the station.

PRODUCTION CAPACITY

This station is equipped to hatch approximately 1,000,000 eggs and is designed to raise 600,000 fingerlings.

Facilities can be expanded if the success of this hatchery indicates that it is desirable.

THE RECIRCULATING HATCHING UNITS AT THE COLLEGE OF FISHERIES, UNIVERSITY OF WASHINGTON

James N. Ellis, Hatchery Supervisor
College of Fisheries
University of Washington
Seattle, Washington

The College of Fisheries hatchery at the University of Washington, which is used largely for experimental research, is located on the shores of Portage Bay - a body of water situated between, but connected with, Lake Washington and Lake Union. The waters from Lake Washington flow into Portage Bay, through Lake Union and the Lake Washington Ship Canal into Puget Sound. A great many vessels of all sizes pass through Portage Bay on their way to and from Lake Washington.

The water supply for the hatchery comes from Portage Bay at a point near the east end of the bay where the waters from Lake Washington flow into the bay. Two pumps of 750 gallons per minute capacity provide water for the hatchery. One pumps water to a large tank located on the top floor of the College of Fisheries building and the other provides water to a holding pond and the brood-stock raceway. Water for the hatchery flows by gravity from the large tank to the troughs, tanks, and incubators in the hatchery.

For various reasons, however, this water is not suitable for hatching eggs: (1) Water temperatures vary from 40°F to 74°F. (At the beginning of the hatching season temperatures may go as high as 64°F and as low as 40°F later on). (2) Ships passing through the canal sometimes stir up the bottom mud or silt, which is pumped into the water system where it is deposited on the eggs. (3) Algae in great numbers are present in the waters at the beginning of the hatching period. While algae present no real threat they contribute to the over-all problem. (4) Hydrogen sulphide and other products of decomposition may be present in these waters. (5) Low oxygen content may obtain at certain times. (6) Raw sewage is pumped into Lake Washington.

To overcome the hazards to the eggs from the above conditions a semi-closed system of recirculation was designed and installed for the incubation unit. The operation of the unit will be described first. The construction will be outlined at the end of the paper.

The recirculating unit consists of six sets of Heath hatchery trays, 16 trays to each set. The sets of trays are placed side by side in a space about 12 feet long by 24 inches wide. They rest in a large pan which extends several inches beyond the over-all dimension of the trays, thus providing space to catch drippings and for lateral and depth adjustment of the units. The pan sets on top of a bottom reservoir. Water from the trays falls into the pan where it flows to the lower end of the pan and then falls through a six-inch hole into the lower reservoir. This water, the temperature of which has risen as it passes

through the trays, is chilled and then pumped into the top reservoir from whence it flows into the top tray of each unit and down through the trays into the pan, into the lower reservoir again.

Since the water is exposed to warm air, a refrigeration system designed specifically for this purpose was installed and is connected to the system. The water from the lower reservoir is pumped into the refrigerating unit and chilled, then is pumped back into the lower reservoir, where it is dispersed by means of a perforated pipe. The pipe is placed near the bottom of the reservoir and is held in position by means of short pieces of polyvinyl chloride pipe. The PVC pieces were cut into equal lengths of approximately one inch and the top end of each was shaped to fit the radius of the dispersion pipe. They then were fastened to the dispersion pipe and the bottom simultaneously by means of a polyester resin called Plylox. The Plylox was used with glass cloth to coat the inside of both reservoirs and by itself to coat the outside. Used as a glue and as a protective coating it is non-toxic, as far as we can determine.

The water from the lower reservoir is pumped by means of a plastic pump up to the top reservoir through a one-inch PVC pipe. This pipe extends up one end of the unit and then across the top of the top reservoir. Water is jetted out of this pipe through slots in the under side of the pipe, where it strikes the surface of the water in the upper reservoir, providing the aeration needed. The water flows out of the top reservoir through a one-inch PVC pipe and into the top trays. Smaller pipe can be used in place of the one-inch to reduce and thus regulate the flow. Two two-inch PVC overflow pipes also are provided to handle the excess water which flows back into the lower reservoir near the lift pump.

This pump is a one-inch plastic Deming Finite pump driven by a 3/4 hp single-phase motor turning at 3500 rpm. The only metal in the pump is the drive shaft of stainless steel.

The refrigerator is a 3 hp, single-phase, 220-volt, self-contained, water-cooled, water chiller. The chiller section is made from No. 316 stainless steel piping which can be cleaned inside if necessary. It has a 3-ton 30,000 BTU-capacity and is capable of maintaining a 50°F temperature at a flow of 60 gallons per minute in the recirculating unit. The chiller unit has a capacity of 10 gallons per minute. Because we operate this facility at partial capacity at the beginning of the season, a capacity control unit was installed, to prevent short cycling of the compressor. The refrigerator unit was built by the Puget Sound Refrigeration Company. By specifying our requirements and having the unit custom built we saved two-thirds of the cost of factory made refrigerators.

We try to maintain a temperature similar to that of the water in the holding pond in order to avoid having to temper the new eggs.

If for any reason the pumps should fail, the water would run out of the top reservoir in a few minutes and the trays would soon empty. To insure a water supply in this eventuality, a one-inch No. 124 Cla valve was installed in a waterline over the top reservoir. A plastic float valve opens and closes the Cla valve as the level of the water in the reservoir fluctuates. This valve provides a heavy flow of water, which the average valve cannot do. It is designed specifically for this type of installation and, while expensive, it is well worth having.

Another source of intermittent water-flow which is also regulated by a float valve was adapted from a refill valve found in toilet tanks. It is attached to the lower reservoir and adds a small amount of water as needed. The float is coated with a plastic paint.

PVC pipe is used throughout the installation. The water is not exposed to metal of any kind, except the stainless steel pipes of the chiller unit. PVC pipe is easy to work, although the elbows and other fittings are usually cemented to the pipe and are not always removable as is the case with metal pipe. PVC pipe comes in different thicknesses called schedules, 80schedule being much thicker than 60- or 40-schedule. The thicker schedules can be threaded in a machine shop if needed.

The reservoirs are made of 2 by 4 studs placed between the top and bottom plates. The bottom plates rest on 3/4-inch outside or marine plywood. The sides and ends which are placed inside the studs are of 1/2-inch marine plywood. The bottom reservoir is 14 feet 1 inch long by 26 inches wide by 12-1/2 inches high, which gives enough length to permit access to each end of the reservoir. Drain plugs are placed in the bottom of each reservoir, and a stand pipe screwed into a floor drain also provides a means of drainage. This pipe is under the pan drain-hole and is capped with a rubber stopper to prevent water loss from the system.

The upper reservoir is smaller, measuring 12 feet 2-1/2 inches long by 22 inches wide by 12 inches high. Its width is established in that the pipes coming out of the side can be only so long if they are to carry water into the end of the top hatchery tray. The top reservoir rests on 3/4-inch wooden crosspieces milled to fit the protrusions on top of the units. The pan which collects the water from the trays is fabricated from fiberglass cloth and polyester resin.

The overflow pipes should be placed low enough in the reservoir wall to prevent water from spilling over the top when the unit is partially shut down, but high enough not to

rob water from the tray outlets. To prevent splashing from the top reservoir, a flexible ribbed vinyl counter top (loose) was placed over the top distribution pipe and inside the walls of the reservoir.

Treatment of eggs is accomplished by adding the specified chemical or drug in proper amounts to the water and then flushing it down the drain when the treatment is complete.

The unit has worked very satisfactorily. Mud and silt are no longer a problem. Temperature can be controlled by setting a dial.

THE RECLAMATION OF WATER

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The reclamation of water has great potential for hatchery operations. Production at many hatcheries could be increased if the water was reclaimed and reused, and new hatcheries could be constructed in areas with little available water. A recirculating water reuse system would be, in effect, a controlled environment.

Experiments were initiated to determine the problems associated with water reuse and methods of overcoming these problems. A small recirculating system was set up which consisted of a fish-holding trough, a settling tank to collect the heavier debris and excrement, an aeration tank, and a recirculating pump. The pump forced the water through an aspirator into the aeration tank maintaining an oxygen level of near saturation at all times.

Tests were conducted with chinook fingerlings in creek water at pH 7.3 and in well water at pH 8. In both water supplies, ammonia levels increased rapidly during the first 10 to 14 days. Both the ammonia level and the pH then began to decrease, and before the end of 3 weeks only small amounts of ammonia were measured in the system. The pH had decreased below 6 and fish began dying. Investigation showed that a nitrifying bacterial culture which utilized the ammonia excreted by the fish had developed in the reuse system. In the nitrification process, the bacteria oxidize ammonia to nitrous and nitric acid which combine with basic salts to form nitrates. Since neither water nor solids containing basic salts were added to the reuse system, these salts were gradually depleted thereby decreasing the pH to a level toxic to chinook fingerlings.

An experiment was conducted with the addition of oyster shell in the aeration tank. The shell served effectively both as a filter and as a source of calcium carbonate to maintain the pH at 7.4 or higher. A group of fish held in the reuse system for 5 weeks doubled their weight and incurred no mortality. Only trace amounts of ammonia nitrogen were measured in the system.

A larger model reuse system utilizing a 6-foot circular tank for fish holding was set up to simulate conditions thought to be applicable to large scale operations. Reconditioning facilities were the same as used in previous experiments. Supplemental fresh water in an amount sufficient to provide a theoretical complete interchange once in 12 hours was added to the system.

Chinook fingerlings were held in the system for as long as 12 weeks with excellent results. The only difficulty encountered was an outbreak of bacterial gill disease in one group of fish which was successfully controlled by treatment with Lignasan. At the conclusion of the test lasting 12 weeks, the reuse system was carrying 104 pounds of fish per gallon per minute of fresh water; 10 pounds per gpm is considered maximum without water reconditioning. The only maintenance required was a weekly cleaning of the filter and settling tank.

Large scale tests were conducted utilizing two rectangular-recirculating ponds for fish holding. Water reconditioning facilities were constructed on essentially the same scale as was used in the model reuse system except that two 12-foot by 12-foot filter tanks were used instead of a filter in the aeration tank. The ponds were stocked with 1,200 pounds of chinook fingerlings each and the experiment carried on for 4 weeks. Although weight gains in the reuse ponds were comparable to those obtained in ponds not on reuse, the reconditioning facilities did not remove the metabolic waste products as effectively as desired. It was assumed that a large part of the bacterial culture was lost each time a filter was cleaned, and the heavy pond loading necessitated frequent filter cleaning. This assumption was confirmed by tests in the small reuse system. The filter was cleaned 5 times in 11 days and after more than 3 weeks of normal operation, the ammonia nitrogen level in the system had not decreased to the low levels measured prior to the frequent cleaning. These results indicated that an additional filter to increase the time interval between cleaning was needed in the large reuse system, and that the initial pond loading should be low to allow the nitrifying bacterial culture to become well established in the reconditioning facilities.

Adult salmon were held successfully in a recirculating water reuse system. Spring chinook salmon trapped at Detroit Dam and transported to the Salmon-Cultural Laboratory were held over 5 weeks before any mortality occurred. Although the time of death of these fish corresponded to peak spawning of comparable fish in the Willamette River tributaries, the fish held in the reuse system did not fully ripen. It appeared that some triggering mechanism that caused final development of the gonads in these fish was missing in the reuse system. Silver salmon from the Little White Salmon River and both silvers and fall chinooks from Abernathy Creek were held to full maturity. Outbreaks of fungus disease on the fish were successfully controlled by treatment with malachite green.

These experiments indicate that water reclamation and reuse is practical for both rearing fingerlings and holding adult salmon. Tests directed toward methods of increasing the efficiency of water reconditioning facilities will be continued.

A COMPARISON BETWEEN THE USE OF PLASTIC HATCHING JARS AND STANDARD TROUGHS FOR INCUBATING COHO EGGS

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Among the various types of egg-incubating and fry-rearing equipment used by fishery organizations, the use of hatching jars is noted. Jim Wharton, Australia, stated that with his particular siltation problem he could not successfully use hatchery troughs but could use hatching jars. The use of jars for incubating eggs has been described in the Progressive Fish Culturist in several articles (Vol. 15 No. 4, Vol. 16 No. 3, Vol. 21 No. 1, Vol. 22 No. 1, and Vol 23 Nos. 2 and 3). Since a silt problem exists at some of our hatcheries, we decided to run a series of tests comparing the jar method with the conventional hatchery trough.

Jars formerly used as shad batteries with spherical bottoms were acquired by the Sandy Hatchery and modified at a cost of $2.37 each for twenty-four jars. This cost included perforated plastic plates, surgical hose, hose clamps, and plastic tees to complete a hookup to a wooden headbox. Three plates with evenly spaced 7/64-inch perforations were constructed for each jar. A small plate with a short piece of 1/2-inch tubing cemented through the center was located to remain 1/2-inch from bottom and act as a base for gravel. A second but larger plate was made to fit over gravel and rest near the top of the spherical bottom to keep eggs or fry from going down into the gravel. The third perforated plate was placed near the top of the jar to rest on a cemented internal flange and act as the outlet screen. Acrylic tubing (3/8-inch) was provided to run through the two larger plates down into the 1/2-inch tubing of the bottom plate.

Cost of completely equipped new jars quoted in September 1963 by the Pam Company, 1951 N. W. Wilson St., Portland, Oregon, was $12.00 each for three jars or $8.00 each for thirty jars.

Most written references advised the use of gravel in the bottom of jars to aid in diffusing water through eggs or fry. Our studies were designed to compare these jars with and without gravel as well as to the normal hatchery troughs.

The Fish Commission statistical section proposed a three factor, completely radomized experiment using 1963 brood coho to study the different methods. This plan included the taking of eggs, loading the jars and the control troughs. Twelve of the twenty-four jars had gravel in the bottom - the remainder no gravel. Four lots of eggs were taken and were distributed in the two control troughs and the two types of jars. Stocking of jars with and without gravel was randomized as were the stocking

rates of five, six, eight, and ten thousand eggs per jar. The two shallow control troughs contained five baskets each stocked at sixteen thousand eggs per basket.

Each lot was mixed in a wash tub prior to measuring into jars or baskets. Eggs were not washed prior to stocking in the jar lots or hatchery baskets.

Water flow was turned on and adjusted to 1.0 gallons per minute in each jar and 12 gallons per minute to each shallow trough. Bi-weekly malachite treatments of 1:400,000 were administered starting on November 15, 1963 and continued through January 1, 1964, one week prior to shocking cleanup of egg mortality, and counting of each individual jar or basket. Malachite was added to headbox for treatment of jars.

All twenty-four jars were placed in a single row beneath a 16-foot shallow hatchery trough. Total eggs carried with the varying numbers per jar were 178,594. Assuming that ten thousand to each jar is not overstocked, 240,000 eggs could have been carried in this space. A double row of jars in a single trough space could carry 480,000 eggs or fry at this stocking rate.

Much more time was consumed working with the jars than with control troughs - mainly because they were new to us. Mud did collect in the jars and all through the eggs, but was easily eliminated after eggs were eyed by increasing water flow for a few minutes. A 14 x 18 mesh screen in the headbox supplying water to the jars kept anything larger than silt from entering the jars. Jars were not difficult to unload, pick mortality and reload, but it was time-consuming to count each individual jar. It required four hours to count the eggs in the ten control lot baskets compared to six hours to count the eggs in the twenty-four jars. Partially offsetting this was the one hour it required to tray down the ten baskets in the control lots.

Dissolved oxygen determinations were taken at intervals from jars at all stocking rates and from the control troughs. There was a significant decrease in measurable DO in the jars associated with increased numbers stocked in the jar. The decrease in DO did not, however, measurably affect survival.

Hatching started earlier in the jars than in the control troughs. All jars started hatching January 23, 1964 at 863 T.U. at which time there was no hatching noted in the control troughs. Hatching started in the troughs two days later.

The experiment terminated March 9, 1964 and all fry mortality was cleaned up and counted.

Conclusions:

1. Eggs had a significantly higher mortality than did fry.

2. There was no difference in survival between the jars with gravel (6.34%)and those without gravel (6.10%).

3. There was no significant difference in survival between the four general rates at which the jars were stocked: 5,000 - 6.04%; 6,000 - 6.67%; 8,000 - 5.30%; 10,000 - 6.28%.

4. There was no significant difference between survival in the troughs which were used as controls and the hatching jars:

   Lot 1 trough 2 - 6.34%
   Lot 4 trough 1 - 8.34%
   Total both troughs - 7.3%

A COMPARISON OF TECHNIQUES FOR SPAWNING STEELHEAD TROUT

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Because steelhead do not die after spawning, it was formerly the common practice to spawn steelhead by hand stripping, just as for other trout.

More recently, most agencies adopted the practice of killing females and cutting open the body cavity to take eggs, as is done with Pacific Salmon. The primary reasons for this method are: large number of eggs are frequently left in females when stripped, stripping involves a great deal of physical labor, and it was felt that there would be less physical damage to the eggs, therefore, they were of better quality.

A method using air pressure within the body cavity to expel eggs was reported by Wharton in 1957. This method has been adopted by some hatcheries of the Washington Department of Game and the U. S. Fish and Wildlife Service, and was tested in 1964 at Big Creek and Marion Forks Hatcheries of the Fish Commission of Oregon.

Big Creek is located approximately 20 miles above Astoria, and Marion Forks is on the North Santiam River with their egg taking station immediately below Detroit and Big Cliff Dam.

This experiment was designed to compare certain aspects of different spawning techniques such as: number of eggs left in females by different methods, quality of eggs taken as reflected in egg losses, and value of draining off ovarian fluid before fertilization.

Marv Hull of the Skamania Hatchery spent a day at Big Creek demonstrating these techniques. Chuck Hiltz also aided in assembling equipment. This consisted mainly of anesthetic, pressure gauges, air hose and needles. Air supply used at Big Creek was a truck tire because a small compressor was not available. Three types of anesthetic were used; MS-222, Methyl Pentynol, and Chloratone. None of these knocked the fish out as quickly as desired; however, Cloratone was the least satisfactory. From 3 to 5 lbs. air pressure in body cavity seemed the most satisfactory.

Four methods were tested: kill and cut open, anesthetize and strip into pan, anesthetize and spawn with air pressure into pan, anesthetize and spawn with air pressure into collander to drain ovarian fluid before fertilizing. The eggs from each female were kept separate until hatching. The eggs remaining in each female were mainly counted, with some at Big Creek being measured. The eggs in each lot were hand counted at time of eyeing. The chart indicates the numbers of fish at each hatchery used and number fish used in each method and final analysis.

There was no demonstratable consistent difference in loss of eggs by any of the methods. Broken eggs were observed in eggs taken by all methods, but it was not a major problem. Small numbers of eggs were left in the females by the incision method; more were left by the air method; and still more when stripping. There were differences between hatcheries in numbers of eggs left in females by stripping and by the air method. More eggs were left in the females at Big Creek by the air method than at Marion Forks. More eggs were left in females at Marion Forks by stripping method. At Big Creek the loss through eyeing was slightly higher in the groups where ovarian fluid was drained, although the difference was not significant. At Marion Forks the loss in drained eggs was lower but appeared to be only a variation between individual fish.

At Big Creek, approximately .5 million (1/2 million) Steelhead eggs were taken by air method. One hundred fifty-eight of those adults spawned were tagged, and if they survive and return, will provide additional information. This chance of surviving to return a second time no doubt varies greatly in different watersheds.

Based on our work, if it is important to take as many eggs as possible, then it is obvious that the incision method will provide the maximum number. If it is important to release adults to provide repeat spawners, then the air method provides an improvement over hand stripping, both in per cent of eggs obtained and ease in taking. With increased survival of hatchery-reared steelhead in the last several years, it is now common for hatcheries to have a substantial surplus available over what they can rear. Hatchery-reared fish are no longer as severely diseased as in earlier years and stand a better chance of surviving to spawn a second time. Therefore, since it is not essential that they be utilized for most possible eggs, it is felt it is worthwhile to release adult fish alive after spawning, on certain watersheds.

SUMMARY OF RESULTS, COMPARISON OF SPAWNING TECHNIQUES ON STEELHEAD TROUT, BIG CREEK AND MARION FORKS HATCHERIES 1964

Hatchery	Method	Number of Fish	Loss in Per Cent of Total Eggs		
			Left in Females	Through Eyeing	Total Loss
Big Creek	Incision	5	0.95 (0.22-2.19)	3.18 (0.64-11.30)	4.10
	Strip	5	15.26 (5.30-23.81)	2.64 (0.78-5.44)	17.50
	Air	5	12.19 (4.10-18.51)	4.39 (1.69-13.67)	16.04
	Air-Drain	5	10.93 (3.12-18.95)	6.53 (1.82-12.34)	16.76
Marion Forks	Incision	4	1.10 (0.03-0.24)	4.92 (2.83-1196)	5.02
	Strip	3*	28.10 (16.91-33.66)	7.38 (2.61-11.79)	33.41
	Air	4	1.54 (0.31-5.40)	10.97 (7.71-14.43)	12.35
	Air-Drain	4	6.96 (4.09-10.42)	5.96 (2.99-12.95)	12.51

*One additional fish stripped by an inexperienced spawntaker was omitted from the experiment.

INCUBATION OF PINK SALMON EGGS IN A SIMULATED INTERTIDAL ENVIRONMENT

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INTRODUCTION

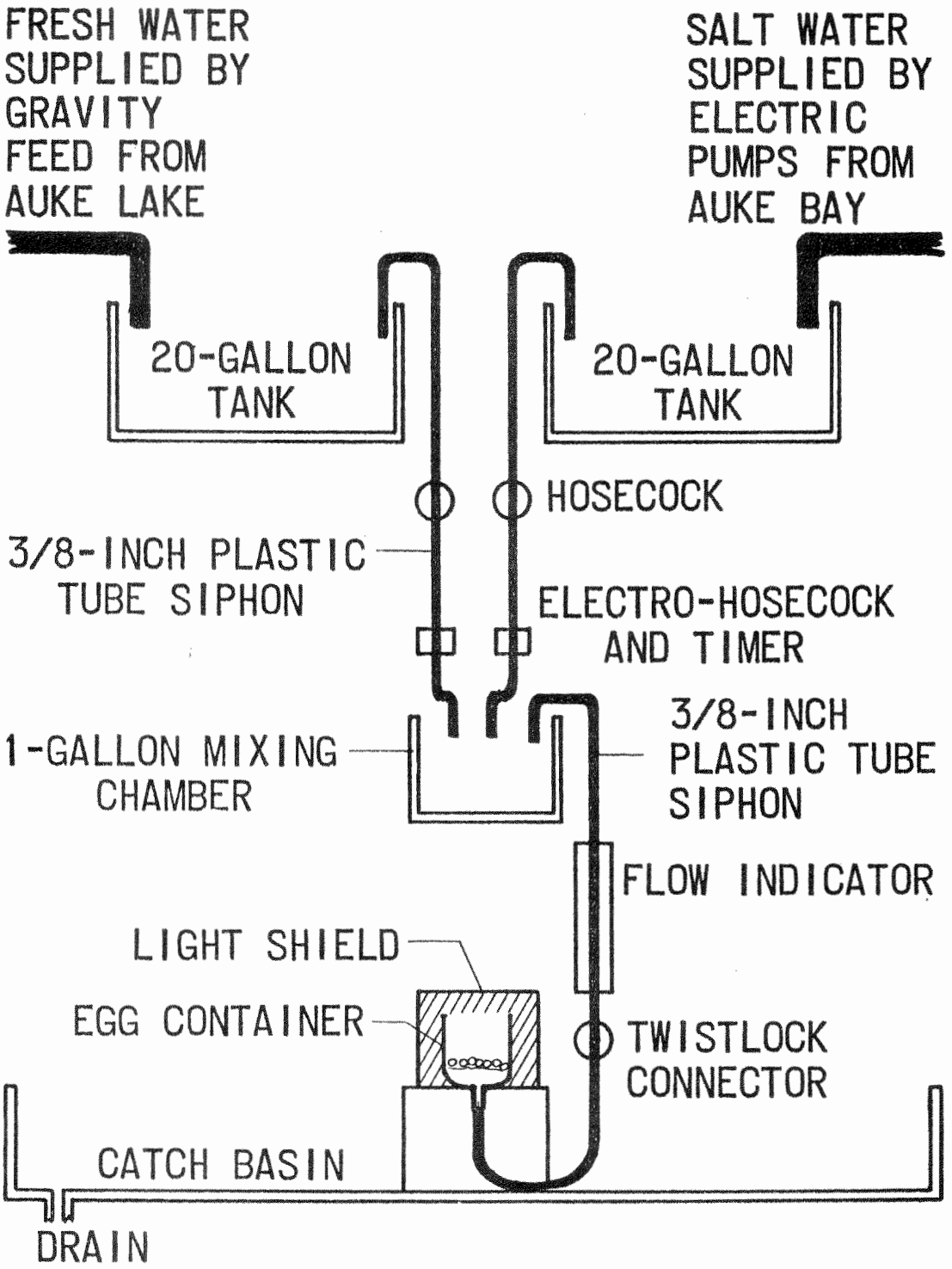
Over 50 per cent of pink salmon spawning in Prince William Sound, Alaska, occurs in intertidal streambeds (Noerenberg, 1963). The U. S. Bureau of Commercial Fisheries in Alaska is investigating the underlying causes of variations in survival in this environment (Helle et al., 1964). The intertidal environment is quite complex with constantly changing salinity, dissolved oxygen, and temperature, all of which are influenced by periodic tidal inundations. Because it is difficult to adequately assess the effects of these physical factors on salmon eggs in the natural environment, laboratory studies were initiated in August 1964 to simulate certain environmental conditions of the intertidal spawning grounds. Continuously flowing sea water from Auke Bay and fresh water from Auke Lake are both available at the Auke Bay Biological Laboratory. Flow-control systems were set up in which incubating salmon eggs could be alternately exposed to controlled dilutions of sea water and fresh water.

Rockwell (1956) found that chum salmon eggs could survive 3 to 4 days exposure to salinity of 26°/00 before dying from dehydration and that they could survive 8°/00 indefinitely at 52° F. or 13°/00 near the freezing point. He did not test their ability to survive periodic exposure to sea water with alternating periods of exposure to fresh water or diluted sea water. Hanavan and Skud (1954) observed poor production of pink salmon fry in pens at the 4-foot tide level of Sashin Creek, while production at higher levels was equal or superior to freshwater production. Eggs at the 4-foot tide level were exposed to salinities somewhat less than 30.7°/00 for approximately 16 hours each day. Eggs at higher levels were also subjected to high salinities but for much shorter intervals. Field studies at our Olsen Bay Field Research Station in Prince William Sound indicated that pink and chum salmon eggs can survive 28°/00 sea water for at least 4 hours twice daily. This report describes laboratory experiments which duplicate this feature of the intertidal environment. Size measurements of embryos and mortality rates of eggs were monitored to assess possible adverse effects of sea water.

METHODS

Fresh water and sea water were supplied to egg containers through a system of mixing tanks, siphons, and electro-hosecocks

controlled by electric timers (figure 1). Flow to each egg container was delivered at 240 ml. per minute. Buchner funnels with 55 mm. diameter porous perforated plates were used as egg containers. About 60 pink salmon eggs were placed in one layer on this porous surface. Light was excluded from the eggs by removable opaque covers above and a wooden support stand below. Water temperatures were monitored continuously by a recording thermograph which was readable to the nearest 0.1°C. Mean water temperatures were not always identical in all egg containers primarily because Auke Bay sea water was slightly warmer than Auke Lake fresh water. Dissolved oxygen levels were assumed to be nearly identical in all egg containers at any given time. Dissolved oxygen of the fresh-water supply was 7.33 mg./l. September 17, 1964, and 8.34 mg./l. October 19, 1964. Dissolved oxygen of the seawater supply was 8.04 mg./l. September 17, 1964, and 5.90 mg./l. October 19, 1964.



SCHEMATIC DIAGRAM OF ENVIRONMENTAL CONTROL EQUIPMENT USED TO SIMULATE INTERTIDAL CONDITIONS FOR INCUBATION OF SALMON EGGS.

For each of the four experiments, pink salmon eggs were taken from two females and fertilized with sperm from two males. Eggs for experiment 1 were obtained from Fish Creek spawners; eggs for experiments 2 and 3 were from Auke Creek fish. Both streams were near the laboratory so that the eggs were easily transported to the laboratory without danger of thermal or mechanical shock. The eggs for experiment 4 were from Lovers Cove Creek, Baranof Island, and required more handling plus a 1-1/2 hour trip by plane, but again no adverse thermal or mechanical problems were encountered.

After each experiment was completed, eggs were preserved in Stockard's solution. Head widths of twenty 2-week old embryos from each lot (experiments 1, 2, and 3) and eye diameters of ten 39-day old embryos from each lot (experiment 4) were measured with an ocular micrometer to the nearest 0.01 mm.

OBSERVATIONS

Experiment 1

Two egg containers were used in experiment 1. Each received 61 eggs which were incubated for 14 days. There were no mortalities of embryos and only six unfertilized eggs were found. Lot 1 received 1-1/2 hours of exposure twice daily to dilute sea water at salinity of about 12°/00. Lot 2 received only fresh water. Mean water temperature was 10.1°C. for lot 1 and 9.9°C. for lot 2. During the 7th day of the experiment, flow to the lot 1 container was accidentally stopped for about 10 hours, and water temperature of 13.5°C. was recorded for 3 hours.

Mean head widths of the embryos exposed to sea water was 0.56 mm. and was significantly greater than the 0.53-mm. head width of the freshwater controls (figure 2). This could have been due to the 0.2°C. difference in mean temperature.

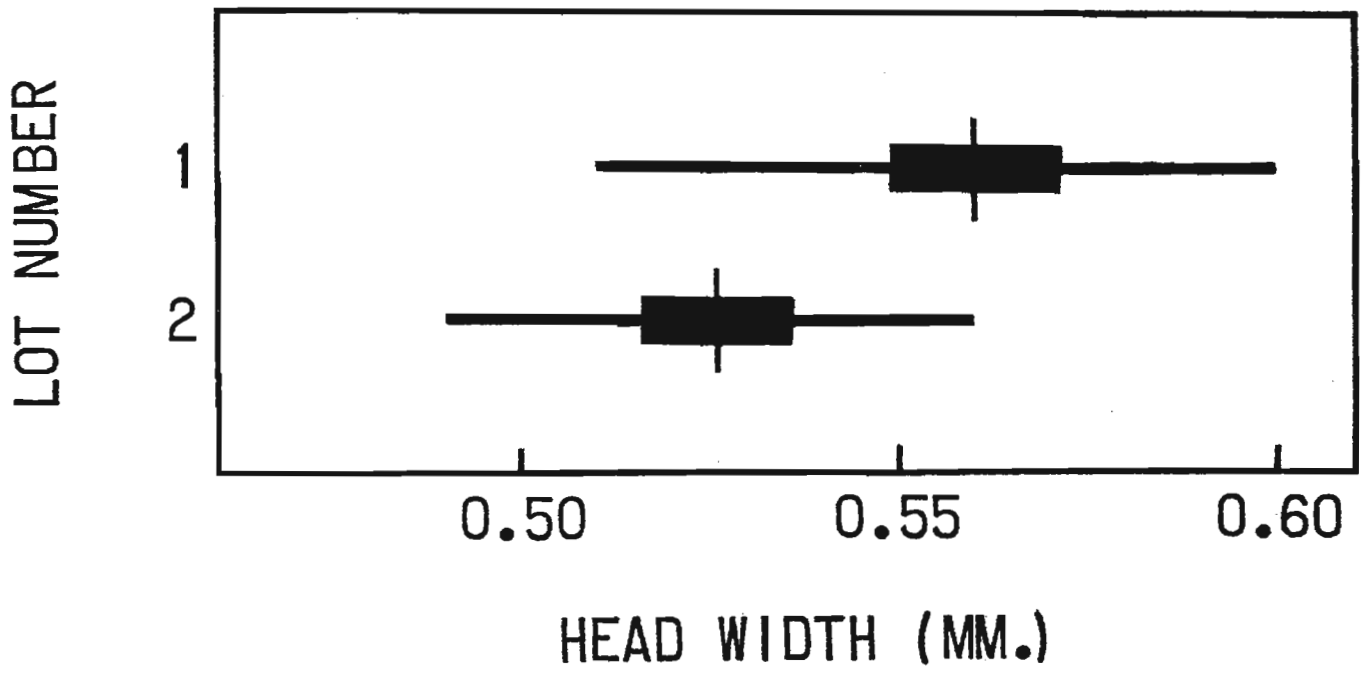


Figure 2.--Experiment 1-1964: Mean, range, and 95-percent confidence limits of head width measurements from 14-day-old pink salmon embryos.

Experiment 2

Five egg containers were used in experiment 2. Each container received 55 eggs which were incubated for 12 days. There were no mortalities of embryos and no unfertilized eggs. Lot 2 received a 4-hour exposure twice daily to sea water of salinity of about 12°/00. All other lots received only fresh water. Mean water temperature was 9.8°C. in all containers but lot 4 which had a mean temperature of 9.7°C.

Mean head widths ranged from 0.41 to 0.42 mm., and there were no significant differences between lots (figure 3).

Experiment 3

Eight egg containers were used in experiment 3. Each container received 60 eggs which were incubated for 12 days. There were no mortalities of embryos, and only three unfertilized eggs were found. Lots 1 and 3 each received a 4-hour exposure to sea water of about 28°/00 twice daily, while lots 6 and 8 each received about 18°/00. Mean water temperatures for the eight lots in numerical order were: 10.3°, 10.1°, 10.5°, 10.1°, 10.1°, 10.3°, 10.2°, and 10.2°C.

Mean head widths ranged from 0.39 to 0.41 mm. (figure 4). Embryos in two lots (3 and 6) showed definite indication of accelerated growth, but the replicate pairs (lots 1 and 8) of these lots showed no acceleration. Again, temperature differences probably influenced growth.

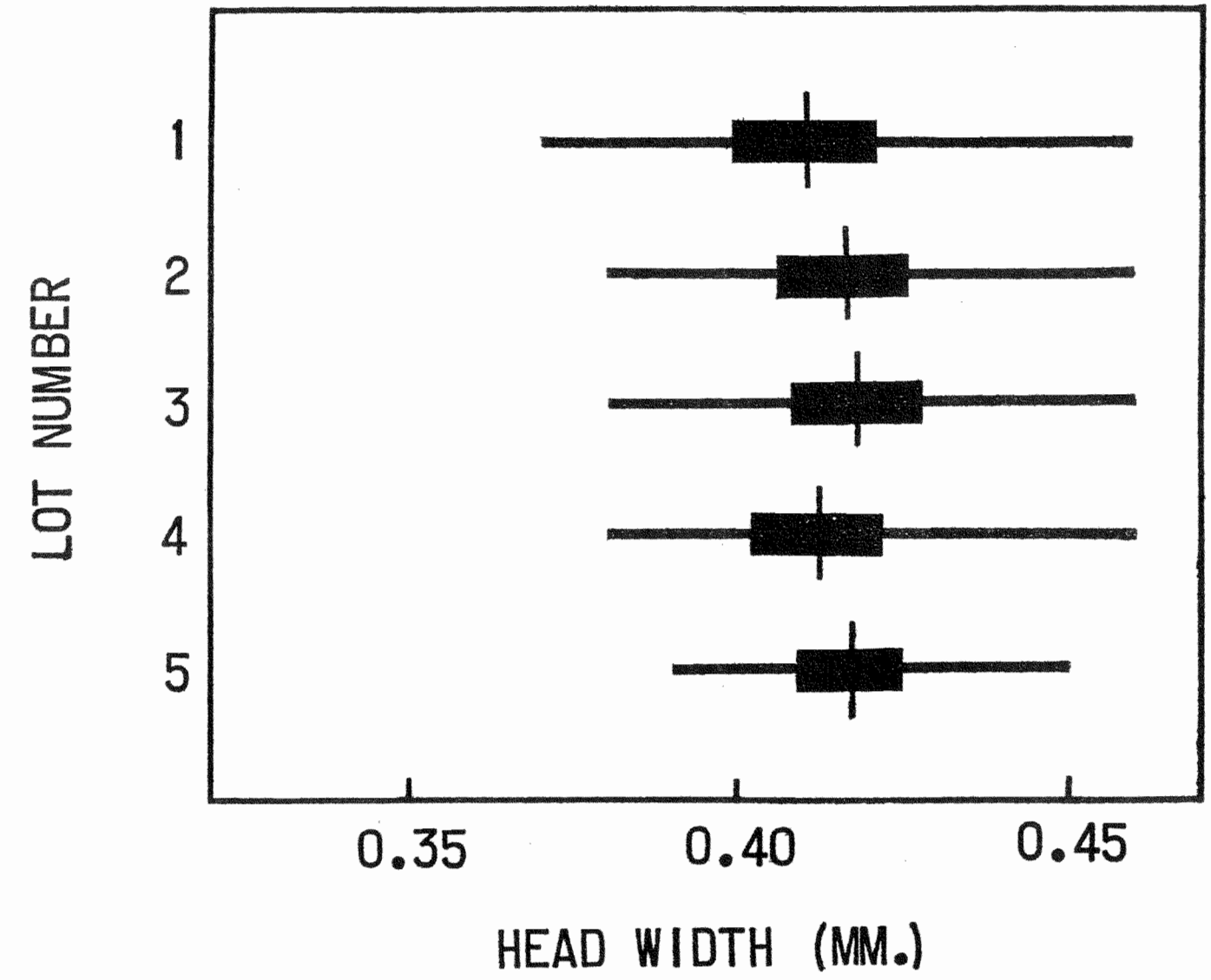


Figure 3.-- Experiment 2--1964: Mean, range, and 95-percent confidence limits of head width measurements from 12-day-old pink salmon embryos.

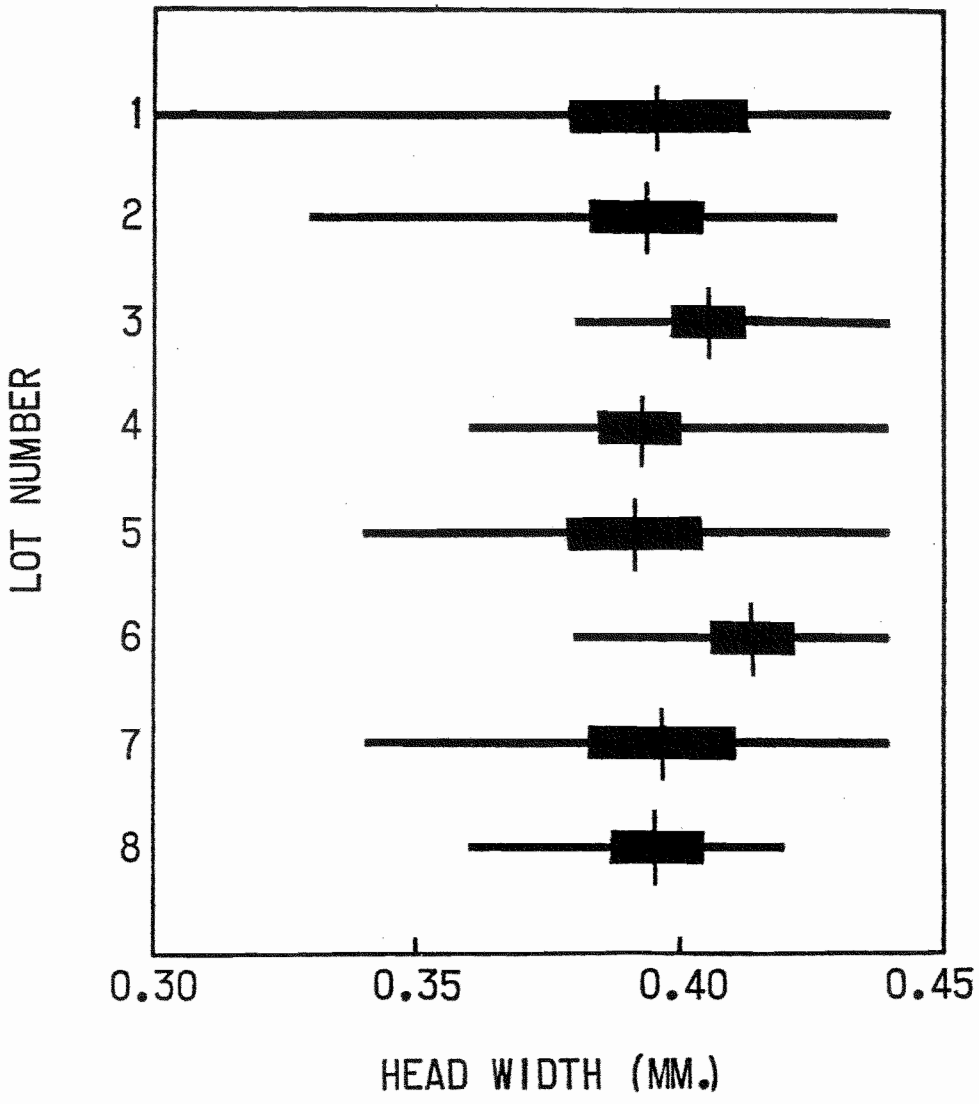


Figure 4.--Experiment 3-1964: Mean, range, and 95-percent confidence limits of head width measurements from 12-day-old pink salmon embryos.

Experiment 4

This experiment, comprising eight egg containers with approximately 65 eggs each is still in progress. At the time of this report the eggs had incubated for 39 days. There were eight dead embryos, five in seawater lots and three in freshwater lots. About 6 per cent of the eggs used in this experiment were unfertilized. Experimental treatments were the same as for experiment 3. There were several occasions when flow was briefly stopped because of mechanical problems with the seawater system. However, flow through the egg containers never stopped long enough to cause an appreciable rise in temperature such as occurred in experiment 1.

Mean eye diameters ranged from 1.16 to 1.21 mm. (figure 5). Embryos in lot 3 showed a slight indication of accelerated growth, but embryos in lot 1, which received the same seawater exposure, showed a slight indication of decelerated growth. Temperature records for experiment 4 were not summarized at the time of this report.

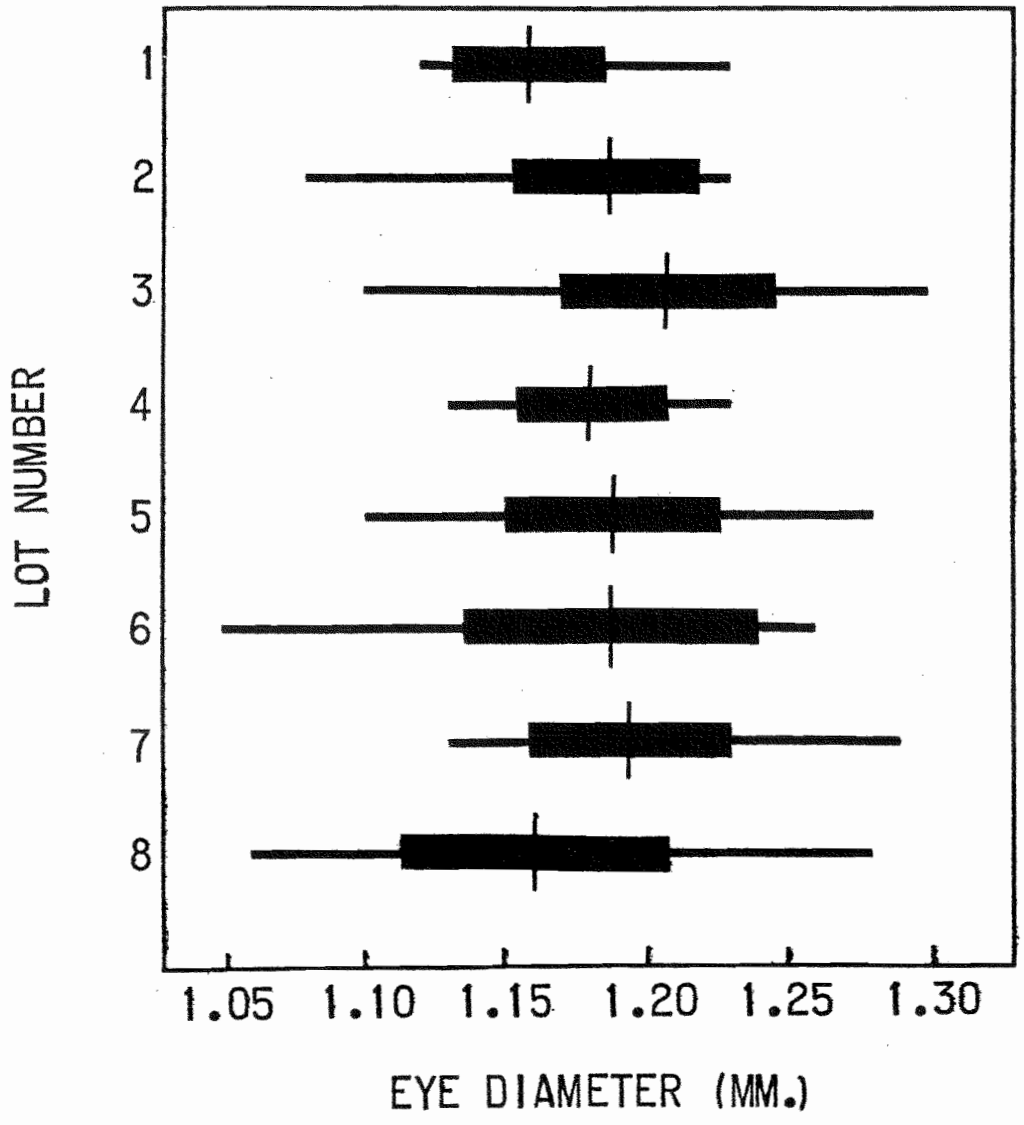


Figure 5.--Experiment 4-1964: Mean, range, and 95-percent confidence limits of eye diameter measurements from 39-day-old pink salmon embryos.

CONCLUSIONS

The results were inconclusive in that a definite tolerance threshold to seawater exposure was not attained and no startling differences in growth rates were noted. However, periodic exposure to sea water simulating an intertidal environment had no apparent deleterious effect on incubating pink salmon eggs. If the eggs in experiment 4 hatch successfully, longer daily periods of exposure to sea water will be tested.

Future experiments should incorporate adequate equipment to hold water temperatures within 0.1°C. and oxygen levels within 1.0 mg./l. of desired levels in all egg containers. Accidental stoppages of flow in the seawater pumping system were all due to mechanical problems. The freshwater supply is by gravity, and no problems were encountered with it. A saltwater storage reservoir on the laboratory roof will be incorporated in the seawater system so that terminal flow to the laboratory will be by gravity, thereby reducing the seriousness of temporary breakdowns in the pumping system.

ACKNOWLEDGMENTS

William McNeil gave advice during the planning stage of the project and collected the eggs from Lovers Cove Creek. Fred Thorsteinson and Rodney Kiesel tended the egg incubation apparatus when I was absent.

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THE INFLUENCE OF GRAVEL SIZE ON SURVIVAL TO EMERGENCE OF COHO SALMON AND STEELHEAD TROUT

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ABSTRACT

Experiments testing four sizes of gravel (1/4 to 1/2 inch, 1/2 to 3/4 inch, 3/4 to 1 inch and 1 to 1-1/4 inches) in troughs demonstrated the importance of gravel size in the survival to emergence of coho and steelhead. Emergence was restricted at gravel sizes smaller than 1/2 to 3/4 inch for steelhead and at sizes smaller than 3/4 to 1 inch for coho. Gravel size influenced the weight of steelhead. Only the smaller individuals emerged in 1/4 to 1/2 inch gravel. A similar pattern did not exist for coho, because the gravel sizes tested either prevented emergence entirely or permitted relatively high survival. None were intermediate. Time of emergence was not influenced by gravel size. Pilot experiments on Cottus perplexus migration into the gravel indicate that the minimum size adequate for emergence (1/2 to 3/4 inch for steelhead and 3/4 to 1 inch for coho) should be used in spawning channels and incubation boxes where cottid predation is a factor.

OPERATION OF THE ABERNATHY INCUBATION CHANNEL

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The Abernathy Incubation Channel is adjacent to the Salmon-Cultural Laboratory and has been operated previously by the Seattle Biological Laboratory of the Bureau of Commercial Fisheries. The experimental operation of the channel was transferred to the Salmon-Cultural Laboratory in July 1963.

The channel is 1,820 feet long and is divided by drop structures into 32 sections. The upper 11 sections are 70 feet long and the remaining 21 sections are 50 feet long. The bottom of the channel is 10 feet wide. It is lined first with an impervious clay layer and then with 1 foot of 1-2-inch graded gravel.

There are presently two main investigations being carried out using the incubation channel. The first of these is to determine the efficiency of the channel in establishing an experimental chum salmon run in Abernathy Creek. In this study we are seeking to determine the size of an exotic eyed-egg plant necessary to establish a significant, self-supporting chum salmon run. Eyed chum eggs from other areas have been imported to supplement the existing native run and the resulting fry liberated into Abernathy Creek. During past operations by the Bureau of Commercial Fisheries, the following numbers of exotic chum salmon fry were released from the channel: 1960 brood year 228,900, 1961 - 484,800, and 1962 - 825,800. Approximately 133,800 fry were liberated from 1962 native stock. Per cent survivals have varied from 75.0 to 95.5 for eyed eggs.

Over 647,000 fry from exotic chum eggs and 146,100 fry from native stock were released during our operations in 1963-64. Stocking rates for both groups were 400 eggs per square foot of channel used as compared to a maximum density of 140 eggs per square foot under previous operations. Survivals from eyed eggs were 81.7 per cent for the exotic eggs and 91.7 per cent for the native eggs.

Adult chum salmon are due this fall from the 1960 brood year release. Although a few chum have been seen at the mouth of Abernathy Creek, a distance of about 3 miles below the Laboratory, no adult chum salmon have moved into the laboratory holding pond. Any eggs which are taken will be planted in the channel as eyed eggs. No exotic chum eggs will be used this year.

The second main investigation is the evaluation of the incubation channel as an auxiliary incubation system for hatchery operations. The objectives of the present studies are (1) to determine the capacity of the channel in the incubation of fall chinook eggs and (2) to determine the significance of the contribution from channel-incubated fall chinook eggs to the adult return.

Approximately 4,000,000 surplus fall chinook eggs from Abernathy Creek were planted in the channel as eyed eggs in October 1964. In addition, 2,000,000 eyed fall chinook eggs were received from the Little White Salmon National Fish Hatchery and planted in the channel in mid-November. These eggs were planted at the rate of 435 eggs per square foot of channel used. This rate is 8.75 per cent higher than the 400 eggs per square foot stocking rate used with good success on chum salmon eggs during the 1963-64 season. Actually, the stocking rate per trough was not any heavier, it was found possible to increase the number of troughs per section and thereby increase the overall stocking rate. The fish produced by the channel will be compared with 5-month and 7-month-reared hatchery fish on the basis of adult returns. Fry from the channel will be unmarked. All hatchery releases will either be marked by fin clipping or with tetracycline.

The evaluation of the capacity of the channel for fall chinook eggs will be determined first from the fry survival and second from the size of the adult return. Future tests will include various stocking rates using both green egg and eyed egg plants and comparisons with first-feeding and 90-day hatchery-reared fingerlings.

THE GROWTH OF JUVENILE SALMONIDS IN REARING PONDS

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The growth and yield of juvenile salmonids in natural rearing ponds is influenced by the survival of fry and the available food supply. Primary production on which the food chain in a natural rearing pond is based is influenced by the fertility of the environment. The yield is composed of smolts and their competitors that are harvested following the rearing period.

The total yield from the Medco pond for 1961, 1962, and 1963 remained relatively constant at 109, 96 and 98 pounds-per-acre respectively. The yield of smolts varied from 1 to 77 pounds-per-acre over the same period depending on the size of the competitor population present.

The size and per cent survival of a group of juvenile salmonids appears to be influenced by the rearing density, quality of the environment, competition, and the length of the rearing cycle.

A favorable growth rate has been obtained in controlled rearing impoundments utilizing three methods:

(1) Low density. Summer steelhead fry stocked at a density of 800 per acre in freshwater at Hemlock Meadows grew from 0.7 grams to 24.9 grams in 133 days of rearing.

(2) Saltwater. Coho fry stocked at a density of 2,600 per acre in Lint Slough and reared in gradually increasing salinities grew from 0.4 grams to 19.5 grams in 90 days of rearing.

(3) Fertilization. One section of Medco pond stocked at a density of 5,000 summer steelhead fry per acre and fertilized produced a growth rate comparable to previous years without fertilization at densities of 2,200 to 3,800 per acre. The second section of the impoundment was fertilized and stocked at 3,000 fry per acre. This group of summer steelhead grew from 0.4 grams to 26.0 grams in 150 days of rearing.

The slope of the growth curves for the three populations of salmonids were comparable and much improved over rearing cycles of previous years.

THE EFFECTS OF STOCKING YEARLING, HATCHERY-REARED, WINTER STEELHEAD ON WILD POPULATIONS

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The effects of stocking large numbers of yearling, hatchery-reared, winter steelhead on wild stocks has long been a question of concern and speculation and has largely been ignored in hatchery evaluation programs. In general, an investigation of the matter would be hampered by the lack of historical records concerning the abundance of the wild population prior to and after the introduction of hatchery fish. An assumption would have to be made that population changes brought about by other environmental factors could be distinguished. There is little information presently available as to whether or not hatchery stockings are harmful or beneficial to wild populations, much less how, when, and where wild fish are influenced by such introductions.

Over the years that steelhead have been reared in hatcheries, indigenous stocks have been used for the obtainment and widespread distribution of hatchery fish and, thus, in any particular Oregon coastal stream, the pre-aquicultural stock has probably undergone dilution of the genetic pool to an extent that no truly wild strain of winter fish exists. For our purposes we shall consider a "wild" population as being made up of fish of unknown origin which are the result of natural propagation. It is such fish on which the angler would be totally dependent in the absence of fish of hatchery origin.

The Research Division of the Oregon State Game Commission, in the winter of 1959-1960, initiated a program to assess the role of artificial propagation of winter steelhead as a means of supplementing natural production and determine means of increasing the effectiveness of hatchery operations. Emphasis has been placed upon (1) assessing the contribution of hatchery-reared steelhead to the sport fishery in relation to fishing intensity and numbers of fish stocked, (2) determining the survival of hatchery-reared steelhead in connection with the conditions under which the fish were reared and released and (3) determining the cost of producing hatchery-reared steelhead for the creel.

Much associated data have become available concerning the catch and fishing success for wild steelhead, thus providing some insight as to how well the wild population has maintained itself under increased angling effort and hatchery releases.

The data are from two coastal streams, the Alsea and Wilson rivers. The Alsea is the larger with a six-year mean annual discharge of approximately 1,600 c.f.s., whereas the Wilson River has a six-year mean of 1,400 c.f.s.

The estimates of catch and angling effort as well as calculated fish-per-hour are shown graphically in Figure 1 for the Alsea River and in Figure 2 for the Wilson River.

During four migratory seasons on the Alsea River the catch of wild and hatchery fish has increased sharply. Fishing success in the form of fish-per-hour for hatchery fish has increased rapidly while fishing success for native stock has been strikingly constant. Angling pressure increased over four-fold from a low of 4,200 angler-days to 18,000 angler-days. The greater number of hatchery fish being caught is related not only to increased effort but increased numbers of fish in the river. The population increase is the result of larger numbers of fish being stocked and greater survival. The estimated populations (catch plus escapement) of hatchery fish which are not included in the graph are for 1960-61, 944 fish; 1961-62, 3,271 fish; 1962-63, 4,003 fish; and 1963-64, 7,646 fish. There has been a four-fold increase in the catch of wild steelhead. No population estimates are available for the wild fish.

Data collected over five migratory seasons on the Wilson River show a two-fold increase in angling effort from approximately 10,000 angler-days to 22,000 angler-days. The catch of wild steelhead has paralleled effort and has risen from 1,000 to approximately 2,100 fish. The fish-per-hour figure consequently has been quite constant for wild steelhead. The catch of hatchery fish has been variable and consequently so has fishing success. The numbers of fish stocked in the Wilson River has been relatively constant each year at approximately 100,000 yearlings. The variable catch is primarily the result of survival.

Fishing success for wild steelhead has varied only a little and is similar for both rivers in the face of increasing angling effort. It is not possible to say definitely whether the wild fish populations are increasing, decreasing, or remaining static. It is probable that the efficiency of fishing methods has remained constant during the period, and that the present levels of exploitation have not been sufficient to show any population decline or natural cycle of abundance, if it existed. What effects this rate of exploitation will have on future abundance of wild fish is not presently known.

The inspection of catch and population estimates for hatchery fish on the Alsea River reveals that the two variables are closely related. If the same degree of correlation exist between catch and population for wild stocks, then an increase in abundance can be assumed. Whether the increase is related in some manner

to the presence of hatchery fish in the stream or is just a fluctuation of the runs from natural causes is a matter of speculation.

If the wild populations have not been constantly increasing during the period of study, the runs in previous years were under-harvested.

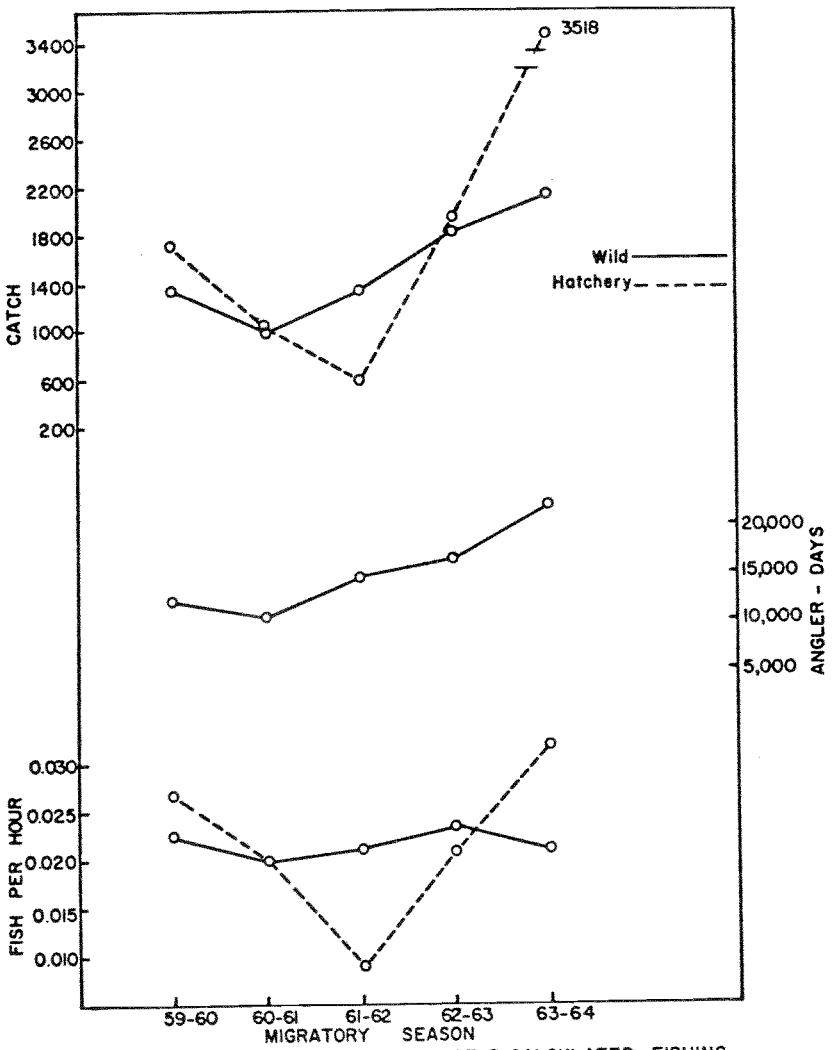

Figure 2 — ESTIMATED CATCH, EFFORT, & CALCULATED FISHING SUCCESS FOR THE WINTER STEELHEAD FISHERY ON THE WILSON RIVER

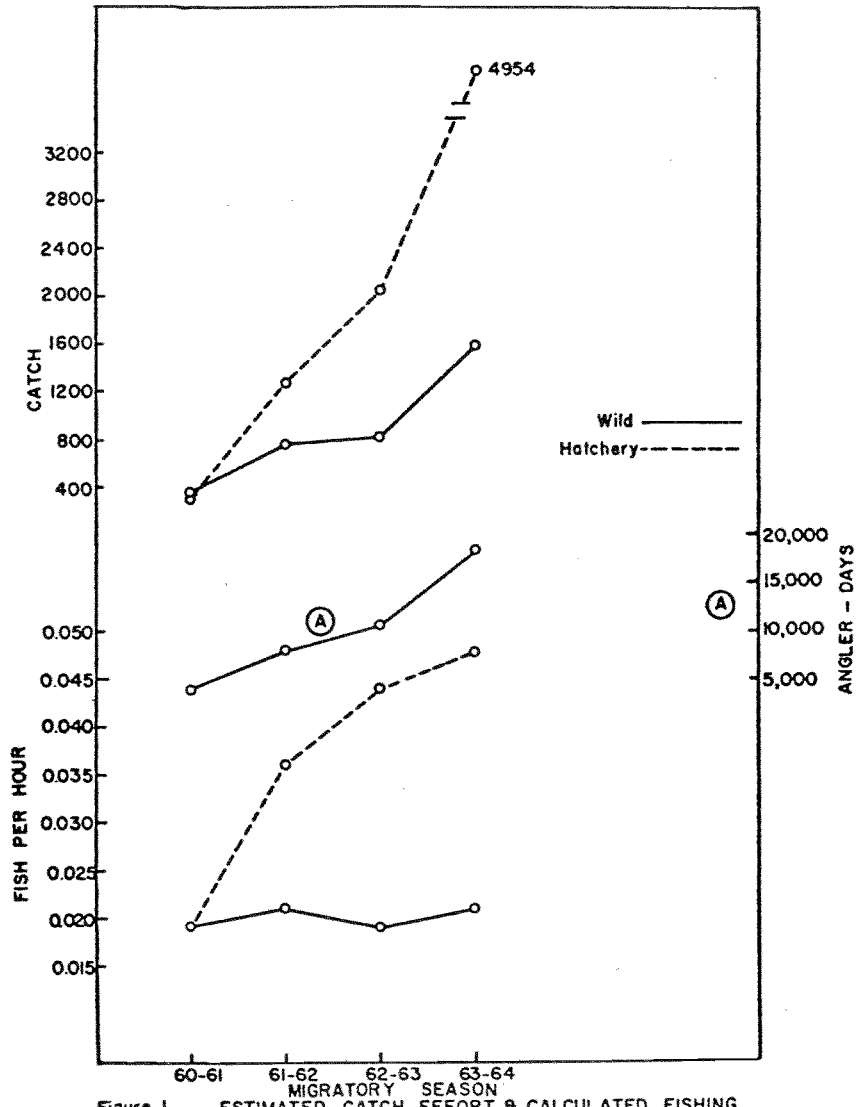

Figure 1 — ESTIMATED CATCH, EFFORT, & CALCULATED FISHING SUCCESS FOR THE WINTER STEELHEAD FISHERY ON THE ALSEA RIVER.

THE EFFECTS OF TIME, DENSITY, MOTION AND STARVATION ON THE SURVIVAL OF TRANSPORTED TROUT

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INTRODUCTION

The results of experiments conducted in 1958 to assess the effects of pre-transportation starvation, in-transit motion, density of fish transported and elapsed time of transportation on the survival of hatchery-reared rainbow trout, Salmo gairdneri Richardson, are reported. Significant post-transportation mortalities of fish have been reported from British Columbia (Black and Barrett, 1957), Ontario (Miller, 1957), Massachusetts (Stroud and Bitzer, 1955), California (Wales, 1954), Japan (Suehiro, 1951) and Oregon (Saltzman, 1953, Newcomb, 1955, Horton, 1956). I decided to test the influence of starvation, motion, density and time on the survival of transported trout after Black and Barrett (1957), Horton (1956) and Suehiro (1951) presented data which suggested that these factors might be implicated in delayed transportation mortalities.

METHODS

Experimental animals were fall-spawning rainbow trout which had been reared at the Roaring River Trout Hatchery, near Scio, Oregon. The fish were graded to an average weight of 60 grams, acclimated to laboratory conditions for seven days and normally starved for 48 hours before each experiment. In no case was the same specimen used twice for experimental purposes.

Tests were performed in five-gallon, widemouth glass jars containing 10 liters of Alsea River water. The test aquaria were suspended in a constant temperature tank by means of a motion device. Temperature of the water bath was thermostatically controlled to 15 ^{o}C. Water in the aquaria was aerated by means of compressed air and carborundum stones. At no time did the dissolved oxygen concentration in any test vessel drop below 6.0 ppm. The experimental apparatus was illuminated by two 40-watt fluorescent lamps.

In order to simulate the effect of in-transit motion, a device was constructed to oscillate eight aquaria within the constant temperature tank (Horton, 1963). Following a simulated transportation experience, fish were transferred for observation to hatchery troughs where they were retained for five days in individual test lots. All experiments were conducted at the Oregon State Game Commission's Alsea Trout Hatchery.

RESULTS

Effects of Motion and Time

An experimental series was designed to test simultaneously (1) whether post-transportation mortalities could be produced in the laboratory, and (2) if so, would the mortalities increase due to simulated transportation, and (3) would the mortalities increase with prolonged periods of sham hauling.

Into each of eight aquaria held by the previously described motion device were placed ten liters of water and ten trout. A control lot of ten fish was placed in a covered egg basket partially submerged in water in the observation trough. The motion apparatus was adjusted to 18 revolutions, or 36 oscillations, per minute and started.

At the end of one hour, fish from two aquaria were placed in baskets in the observation trough. Fish from two more aquaria were removed at the end of two, four and six hours, and were placed under observation. Four replications of this procedure were conducted. Mortality data accumulated over the five days of observation after experimental exposure are presented in Table 1.

Delayed mortalities were produced which began the first day after testing, peaked on the second day and declined thereafter. The mortality pattern was similar to that encountered following live transportation of trout during earlier experiments in Oregon (Horton, 1956)

Table 1. Mortality following sham transportation for four time periods.

Days after test	Mortality following motion at 36 oscillations per minute for: 1 hour	2 hours	4 hours	6 hours	Total daily mortality	Control mortality
1st day	1	1	2	10	14	0
2nd day	3	1	1	11	16	0
3rd day	1	3	4	4	12	0
4th day	1	0	0	1	2	0
5th day	0	1	0	4	5	0
Total mortality	6	6	7	30	49	0
Number Tested	80	80	80	80	320	40
Per cent mortality	7.5	7.5	8.8	37.5	15.3	0

More fish died after six hours in an oscillating test container than after four, two or one hours confinement under like conditions. That losses increased with duration of the experiment was obvious, but whether higher mortalities were due to water turbulence, stress of confinement, the concomitant accumulation of metabolic products, or other factors was not clear. None of the control fish died.

Effects of Motion, Time and Density

A second series of tests was designed to explore further the effects of motion. Additional treatments were incorporated into the series to probe the influence of fish density and length of experimental exposure on delayed trout mortalities.

Two levels of each factor (motion, density and duration) were arranged into a randomized block design. Four replications of the eight possible treatment combinations were tested. Each replication required the use of 160 animals in addition to 30 fish placed directly into the observation trough as controls. Per cent mortality data for the 32 individual tests are presented in Table 2.

Motion, as produced by oscillating aquaria, apparently had little influence on the magnitude of the experimentally induced mortality. Mean mortality among oscillated fish was 10.00

Table 2. Per cent mortality following motion, duration, and density treatments.

Motion (oscillations per minute)	2-hr. test, 1 fish/liter	2-hr. test, 3 fish/liter	8-hr. test, 1 fish/liter	8-hr. test, 3 fish/liter	Mean treatment mortality	Control mortality
0	0.00	0.00	0.00	6.67	9.17	0.00
	0.00	3.33	10.00	23.33		
	0.00	0.00	10.00	26.67		0.00
	20.00	3.33	20.00	23.33		
56	10.00	0.00	30.00	20.00	10.00	0.00
	0.00	3.33	10.00	10.00		
	0.00	3.33	10.00	3.33		0.00
	0.00	6.67	40.00	13.33		
Mean treatment mortality	3.75	2.50	16.25	15.83	9.58	0.00

per cent, while those in stationary aquaria suffered a 9.17 per cent delayed loss. The difference between these two treatment means was not significant.

There was no significant difference in survival between fish confined at a density of one fish per liter and those tested at three fish per liter. Other investigators have increased fish concentrations to the extent that their metabolites limited carrying capacity of liberating units (Haskell and Davis, 1958). Evidence that the fish densities tested influenced post-release deaths in the current study was lacking.

Length of experimental exposure had the greatest influence on subsequent trout losses. Delayed mortality averaged 16.04 per cent following eight hours of confinement in the test aquaria, while only 3.13 per cent of the rainbow trout died after the two-hour exposure periods. Difference between the two mortality rates was significant, indicating mortality rate to be related to exposure time. All control fish survived.

Effects of Duration of Experimental Exposure and Starvation of Fish

While analysis of the data presented in Table 2 did not suggest that density of fish or metabolic or other waste products were in any way related to the mortality observed, other workers had suggested causal relationships between such products and delayed mortality. Other than by varying density of fish, various levels of metabolic and other waste products could be experimentally obtained either by increasing exposure time or by holding fish for longer periods of time after feeding before introducing them into the experimental aquaria. To determine whether mortalities might be related to various levels of metabolic and other wastes, an experiment was designed in which every possible combination of five levels of pre-testing starvation period (0, 24, 48, 72 and 96 hours) and four levels of experimental exposure time (2, 8, 16 and 24 hours) were arranged into a statistical design with three replications. In this experiment, the aquaria were not rocked and the density was two fish per liter.

Analysis of data obtained from these tests (Table 3) revealed no significant correlation between the subsequent delayed mortality and any level of starvation period, experiment duration, or interaction between the two factors. The post-experimental loss averaged 31.08 per cent. It was concluded that neither length of exposure (2 to 24 hours) nor starvation period (0 to 96 hours), taken as individual or collective factors, had any pronounced influence on the delayed mortalities.

Table 3. Effects of duration of experiment and pre-test starvation period on per cent delayed loss[1]

Duration of experiment (hours)	Starvation period (hours)					Mean
	0	24	48	72	96	
2	65	55	50	20	35	30.7
	20	30	20	35	40	
	30	20	10	25	5	
8	70	75	35	40	30	37.3
	35	30	25	10	50	
	30	20	40	35	35	
16	35	10	20	35	45	33.7
	20	50	50	30	70	
	25	20	40	30	25	
24	15	15	35	20	20	22.7
	25	20	25	20	30	
	25	25	15	20	30	
Mean	32.9	30.8	30.4	26.7	34.6	31.1

[1] Nine of 60 control fish died in the observation trough.

CONCLUSIONS

Based on the research reported, it was concluded that simulated in-transit motion, density (concentration) of fish transported, and pre-hauling starvation period had no apparent influence on the magnitude of post-transportation trout deaths. In most instances the longer experimental time periods were associated with the higher percentage mortalities.

Subsequent research (Horton, 1963) related the varying experimental time periods with the presence of zinc (from galvanized iron source) as the factors responsible for post-transportation trout deaths in Oregon. The toxic effect of zinc increased with increasing zinc concentration and with increasing length of exposure to the metal. The result of this latter research is a subject in itself and is reported in detail by Horton (ibid).

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