

SH 151 .N671 1967
Proceedings of the Northwest Fish
500255 1 0

DIVISION OF RESEARCH LIBRARY
OREGON FISH COMMISSION

APR 27 1968

Proceedings of the
**Northwest Fish Culture
Conference**



SH
151
.N67
1967

UNIVERSITY OF WASHINGTON
COLLEGE OF FISHERIES
Seattle, Washington

December 14-15
1967

SH
151
1967
1967

PROCEEDINGS OF THE
EIGHTEENTH ANNUAL NORTHWEST FISH CULTURE CONFERENCE

December 14 and 15, 1967

The Eighteenth Annual Northwest Fish Culture Conference was held on December 14 and 15, 1967 at the College of Fisheries, University of Washington, Seattle.

So many attended the conference that the auditorium at the College was inadequate to hold the group, making it necessary to use the much larger auditorium in Guggenheim Hall. The rapid increase in the number of those interested in fish culture presents very real problems. Some of them were brought into focus in the paper presented by Wally Hublou (pages 97 to 99). As an outgrowth of this discussion, the members voted to request that a committee be formed, composed of the immediate past presidents of each of the eight member-agencies, with Paul Cuplin, Idaho Fish and Game Department, as chairman. The committee is to investigate ways and means of expediting the business of the conference, and to work out a method of relieving the host institution of part of the burden of putting on the conference and preparing the proceedings.

Only minor changes have been made in the manuscripts included in these proceedings. In keeping with tradition, we ask that no portion of these reports be reproduced or quoted without written permission of the author(s) involved.

We are grateful for the assistance we received from the staff and graduate students of the College of Fisheries who helped with the conference. We are especially indebted to Mrs. Eileen Peterson who carried on the correspondence, organized the program, and prepared the proceedings. We also wish to thank the Bureau of Commercial Fisheries, Biological Laboratory, Seattle, for printing the proceedings, and to acknowledge the contribution from the Charles Bell Fund to pay the cost of the covers and binding.

1967 Committee:

Lauren R. Donaldson, Chairman
Ernest O. Salo
James W. Wood

TABLE OF CONTENTS

December 14

	Page
Development of a New Oregon Pellet Formulation John Westgate, Fish Commission of Oregon	1
Modifications of the Oregon Starter Mash Thonas B. McKee, Fish Commission of Oregon	6
Idaho Production Diet Tests, 1967. Paul Cuplin, Idaho Fish and Game Department	9
Ascorbic Acid Deficiency Syndrome in Coho Salmon L. M. Ashley, J. E. Halver, C. E. Smith and R. R. Smith, Bureau of Sport Fisheries and Wildlife	10
Folic Acid Anemia in Coho Salmon C. E. Smith and J. E. Halver, Bureau of Sport Fisheries and Wildlife	11
An Approach to Establishing a Loading Capacity Formula for Trout Hatcheries Robert G. Piper, Bureau of Sport Fisheries and Wildlife	12
Photoelectric Egg Sorter W. G. Taylor, Bureau of Sport Fisheries and Wildlife	14
Electrocuting and Tetracycline Mark Sampling of Big Creek Hatchery Coho Returns Charles O. Junge, A. Kenneth Johnson and Irving W. Jones, Fish Commission of Oregon	17
Results of the First Year's Operation of a New Hatchery Using Burrows Ponds Quentin Smith, Fish Commission of Oregon	20
Development and Use of an Egg Counter Denny McClary, Fish Commission of Oregon	22
Progress Report on Frozen Food Pellet Feeders Chris Jensen, Oregon Game Commission	23

PROPERTY OF THE LIBRARY
COLUMBIA RIVER INTER-TRIBAL
FISH COMMISSION
729 N.E. Oregon, Suite 200
Astoria, Oregon 97103
(503) 731-1004 • FAX (503) 238-3557

	Page
Water Temperature Controls in an Experimental Hatchery. . . James L. Fessler, Oregon Game Commission	25
Collapsing Membrane Disease of Brook Trout Eggs and Sac Fry Jamieson E. Holway, Bureau of Sport Fisheries and Wildlife	27
Soreback of Rainbow Trout: Results of 1967 Fish Quality Experiments James W. Warren, Bureau of Sport Fisheries and Wildlife	28
A Quantitative Comparison of Peritoneal Washes and Feces for Detecting Infectious Pancreatic Necrosis (IPN) Virus in Carrier Brook Trout James L. Billi, Bureau of Sport Fisheries and Wildlife	32
Control of <u>Ceratomyxa</u> in a Hatchery Water Supply David A. Leith and Keith D. Moore, Fish Commission of Oregon	35
A Viral Epizootic at Cultus Lake Robert W. Mead, International Pacific Salmon Fisheries Commission	38
A New Virus from Cultus Lake? Donald F. Amend, Bureau of Sport Fisheries and Wildlife	42
Oral Immunization of Coho Salmon Against Furunculosis at Quilcene National Fish Hatchery. William J. Walsdorf, Bureau of Sport Fisheries and Wildlife	44
Failure to Orally Immunize Juvenile Coho Salmon Under Hatchery Production Conditions. James W. Wood, Washington State Department of Fisheries	48
1967 Laboratory Trials and Tests of the Furunculosis Oral Vaccine Program. Doug Anderson, Bureau of Sport Fisheries and Wildlife	50

	Page
Columnaris Exposure and Antibody Production in Seaward and Upstream Migrant Sockeye Salmon	52
M. P. Fujihara, Battelle Memorial Institute, Pacific Northwest Laboratory	
Size and Sensitivity of Trout to Aflatoxin B ₁	53
J. E. Halver, G. N. Wogan, L. M. Ashley and R. R. Smith, Bureau of Sport Fisheries and Wildlife	
Diquat for Treatment of Columnaris in Coho Salmon	54
Reginald E. Morgan, Bureau of Sport Fisheries and Wildlife	
December 15	
<u>Ceratomyxa shasta</u> Infections in Salmonids.	55
J. E. Sanders and J. L. Fryer, Oregon State University	
Advances in Dry Salmon Feed.	58
R. E. Noble, Washington State Department of Fisheries	
Histopathology Associated with Possible Toxaphene and 2,4-D Toxicity in Rainbow Trout	60
William T. Yasutake, Bureau of Sport Fisheries and Wildlife	
Toxaphene Poisoning of Rainbow Trout	61
J. David Erickson, Snake River Trout Company, Inc.	
Chlorinated Hydrocarbon Insecticides and Fish.	65
Max Katz and H. E. Johnson, University of Washington	
FDA Clearance and Registration of Drugs for Use in Fish Culture	69
G. W. Klontz, Bureau of Sport Fisheries and Wildlife	
Uptake and Distribution of Zinc-65 in the Coho Salmon Egg (<u>Oncorhynchus kisutch</u>)	71
Gary Wedemeyer, Bureau of Sport Fisheries and Wildlife	

	Page
Delay in Spawning Caused by High Water Temperatures.	73
James W. Wood, Washington State Department of Fisheries	
Fish Can Smell Salt	75
C. L. Johnson, Bureau of Sports Fisheries and Wildlife	
K. Oshima and A. Gorbman, University of Washington	
Transplantation of Adult Coho Salmon	76
Roy Sams, Fish Commission of Oregon	
Rearing of Coho and Fall Chinook Salmon in Wahkeena Pond . .	80
Roy Sams, Fish Commission of Oregon	
A Progress Report on the Introduction of Coho Salmon in the Great Lakes	83
T. B. Durling, Michigan Department of Conservation	
Ground Water Toxic to Fish	87
Robert R. Rucker, Bureau of Sport Fisheries and Wildlife	
The University of Washington's Spawning Channel at Big Beef Creek	88
E. O. Salo and K. Victor Koski, University of Washington	
Relationship of Food Supply to Fish Production at Fern Lake	89
Paul R. Olson, University of Washington	
Some Effects of Marking Chinook Salmon by Fin Clipping . . .	92
Fred Cleaver, Bureau of Commercial Fisheries	
Food Energy and the Productive Value of Fish Feeds	95
Robert R. Smith, Bureau of Sport Fisheries and Wildlife	
Niagara Springs Hatchery	96
Charles R. Quidor, Idaho Fish and Game Department	
Suggestions Concerning "Proceedings of the NFCC"	97
Wallace F. Hublou, Fish Commission of Oregon	
Historical Record	100

DEVELOPMENT OF A NEW OREGON PELLET FORMULATION

John W. Westgate
Fish Commission of Oregon
Clackamas, Oregon

Wet fish in the Oregon Pellet, 40% of the diet, often creates a sticky product. The smaller pellets, particularly, tend to cling together and stick to machinery. Clumps of pellets must often be broken apart before feeding. For these reasons, it is difficult to completely automate Oregon Pellets.

We wouldn't have sticky pellets if wet fish was removed from the formula. However, we might not have a good diet either. Feeding trials with starter diets derived from the Oregon Pellet formula suggested wet fish is a very important ingredient, and probably no more than half could be deleted.

We don't know why wet fish is needed in our diets. It may be protein and fat quality. At any rate, our previous trials suggested if we delete part of the wet fish, the formula should be fortified with some additional ingredient, such as dried skim milk.

Fish diets often contain skim milk, even though it is expensive. We don't know if good results from skim milk are due to its protein or some other factor. MNC, a partially delactosed dried whey, could provide similar but less protein, virtually the same carbohydrate, and other factors found in skim milk. Lactose could provide the carbohydrate, but nothing else.

In addition to some dairy product, we thought a higher level of fat might enhance a low-fish Oregon Pellet formula. For that matter, more fat might help the regular Oregon Pellet.

In the first of two feeding trials, we evaluated a new Oregon Pellet formula containing only 30% wet fish; and dried skim milk, MNC, lactose, and a higher fat level in this low-fish formula. We also tested higher fat in the regular pellet. This trial lasted for 21 weeks, July 20 to December 14, 1966. Duplicate lots of 500 spring chinook salmon were reared in 6-foot circular tanks. The fish averaged 145 per pound at the start. We fed them on an equal dry per cent body weight basis, adjusting for differences in dietary moisture.

Figure 1 shows results in per cent average fish weight gain. Diet 1 is the regular Oregon Pellet with 40% fish, the maximum

control. Diet 2 also contains 40% fish, but here we increased total fat content to a calculated 15% dry weight by replacing some of the cottonseed meal with corn oil.

Diets 3-10 contain only 30% wet fish. Herring meal replaced the deleted dry weight of fish, while all other ingredients except those under test remained at the same dry weight level as in the regular Oregon Pellet (Diet 1). Diet 3 contains no additional fortification, and could be termed a minimum control. Diets 4-9 tested dried skim milk, MNC, and lactose; 10% in Diets 4-6 and 5% in Diets 7-9. Lactose level in Diets 6 and 9 is equivalent to the lactose in the skim milk of Diets 4 and 7, the difference made up with alpha cellulose flour. All dairy products were added at the expense of cottonseed meal. Diet 10 contains more fat, as in Diet 2.

Increasing the fat in the 40% fish formula (Diet 2) produced a little more weight, but the difference wasn't significant. Samples of these fish suggested they were fatter as well as a little longer than those fed the regular pellet. We found Diet 2 to be even more sticky than the regular Oregon Pellet.

The 30% fish formula with no fortification (Diet 3) produced significantly less weight than the regular formula (Diet 1). When the low-fish formula had skim milk at either 10% (Diet 4) or 5% (Diet 7), or MNC at 5% (Diet 8), weight gain was enhanced and became not significantly different from Diet 1. The difference in weight gain from skim milk and 5% MNC was not significant. Lactose (Diets 6 and 9) and 10% MNC (Diet 5) didn't improve the low-fish formula. High fat (Diet 10) enhanced weight gain, and this weight is not significantly different from the weight produced by the regular formula (Diet 1).

All lots suffered high mortality, with no apparent regard to diet. Food conversions were affected by this mortality. Hemoglobins and hematocrits were satisfactory.

These results suggested the low-fish formula should contain the higher fat level or be fortified with skim milk or MNC. A combination of higher fat and dairy product might be especially good. MNC is considerably cheaper than dried skim milk so it would be the logical choice. Substituting soybean oil for corn oil might further reduce costs, since it is about 5¢ a pound cheaper.

Objectives of our second trial were to determine if MNC would still help when the low-fish formula contained the higher

fat level; and to evaluate soybean oil in place of corn oil. In this trial, the fish averaged 501 per pound at the start and were reared in aquaria. Otherwise, methods were comparable with the first trial.

Figure 2 shows per cent average fish weight gains obtained from the second trial. Diet 1 is the regular Oregon Pellet with 40% fish. The other diets contain only 30% fish and the higher fat level. Diets 3 and 5 contain soybean oil, the others corn oil. Diets 4 and 5 contain 5% MNC.

Differences in weight gain during the second trial were not significant, nor were mortality, hematocrits or hemoglobins. Although differences in dry weight food conversion were not significant, all the low-fish formulations (Diets 2-5) produced significantly better food conversions than the regular Oregon Pellet (Diet 1) on an as fed basis and they were much less sticky.

We concluded: (1) MNC is probably not needed in the low-fish formula with high fat; (2) soybean oil can be used instead of corn oil; and (3) Diet 3 should be tested under production conditions.

Table 1 shows composition of low-fish and regular Oregon Pellet formulas. Calculated ingredient cost of the low-fish formula is about 12% more than the regular formula. However, during the second trial we obtained a food conversion of 2.02 with the regular formula (Diet 1) and 1.69 with the low-fish formula (Diet 3). Based on these conversions, ingredient cost to raise a pound of fish would be less with the low-fish formula.

Table 1. Composition (%) of regular and low-fish Oregon Pellet formulas

Ingredient	Regular formula	Low-fish (30%) formula
Herring meal	22.0	28.0
Cottonseed meal	22.0	20.0
Tuna viscera	20.0	15.0
Turbot	20.0	15.0
Soybean or corn oil	1.83	6.18
Crab meal	4.0	4.4
Distillers dried corn solubles	3.0	3.4
Wheat germ meal	3.0	3.4
Kelp meal (Algit)	2.0	2.2
Vitamin premix	1.5	1.7
Choline chloride (liquid, 70%)	0.65	0.7
Antioxidant (BHA-BHT)	0.02	0.02

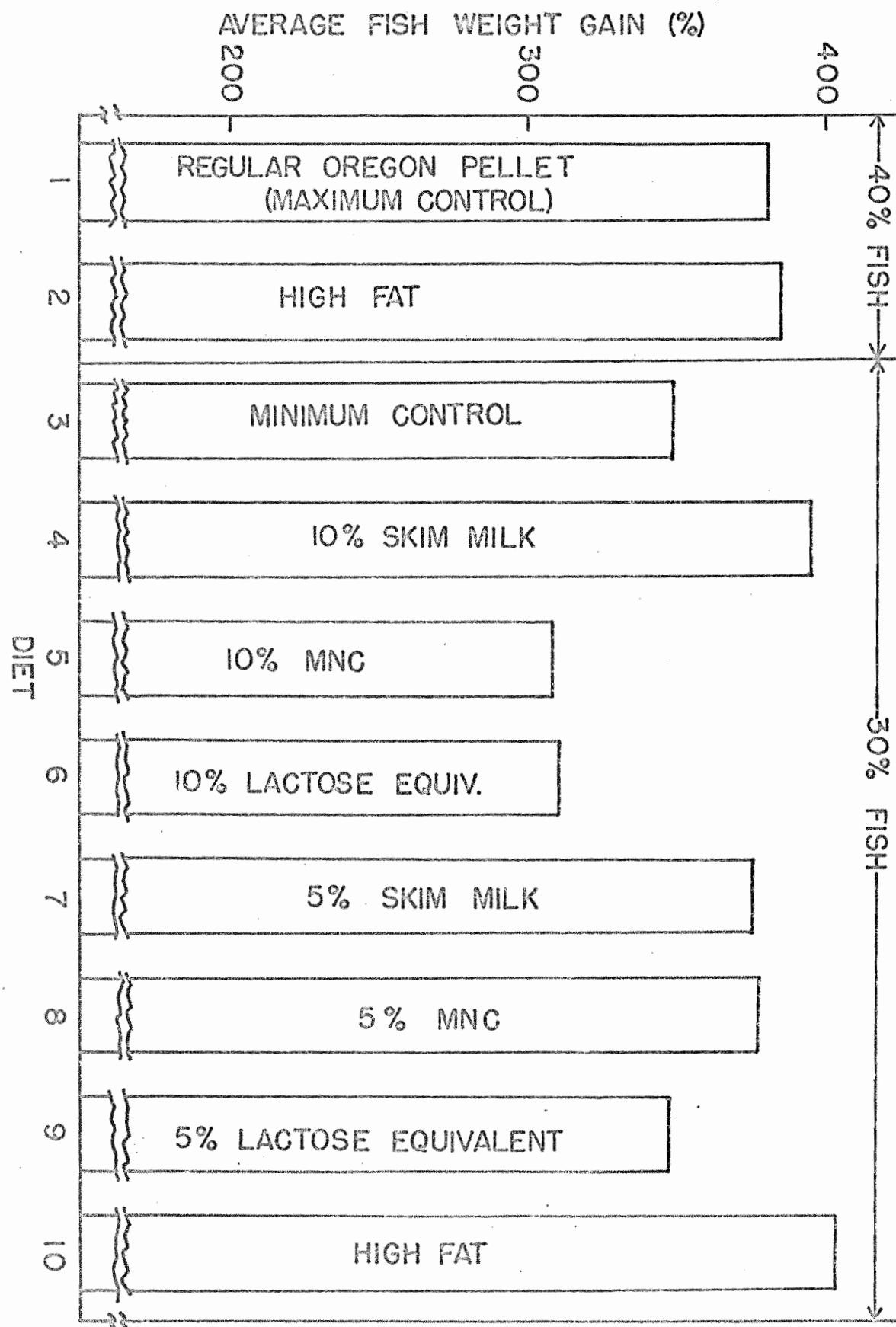
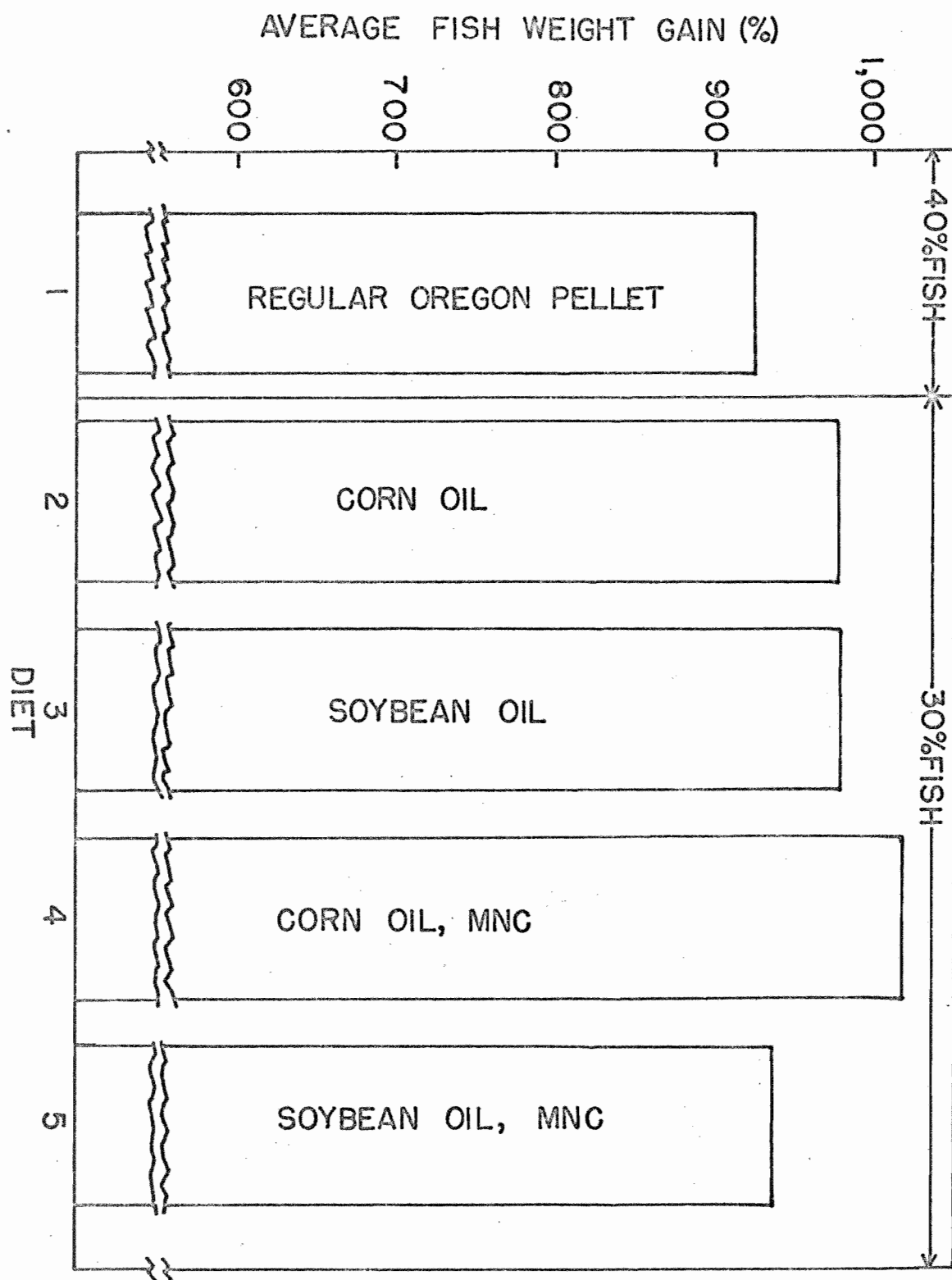


Fig. 1. AVERAGE FISH WEIGHT GAIN (%), FIRST TRIAL.

Fig. 2. AVERAGE FISH WEIGHT GAIN (%), SECOND TRIAL.



MODIFICATIONS OF THE OREGON STARTER MASH

Thomas B. McKee
Fish Commission of Oregon
Clackamas, Oregon

INTRODUCTION

Oregon Starter Mash, a modification of the Oregon Pellet formula containing 24% wet fish and 10% dried skim milk was developed through a series of experiments conducted at Clackamas in 1966. Dwain Mills of our group reported on these studies at last year's Northwest Fish Culture Conference. At that meeting, Howard Drago also reported on the production testing of the mash at Marion Forks Hatchery.

This year we tested modifications of the Oregon Starter Mash formulation. The objectives of the experimentation were to evaluate:

1. A higher level of skim milk
2. Salmon meal as a substitute for herring meal
3. Salmon meal and herring meal as replacements for wet fish, on a dry weight basis
4. Dried buttermilk as a substitute for dried skim milk

PROCEDURES

The diet studies were conducted at Blackamas Laboratory for 45 days, from February 8 through March 24, 1967. We established 20 lots of 500 fall chinook fry averaging 1,152 per pound and reared them in glass aquaria. The fish were fed for 35 days in water averaging 42°F and for 10 days in 54°F water. Oregon Starter Mash and nine modifications were fed. Each diet was fed to two lots of fish. For the first 35 days all lots received an equal quantity of food and for the last 10 days were fed on per cent body weight basis.

RESULTS

Results of the studies are shown in Table 1. This table also shows diet description, which will help in explaining the results.

The values shown are average for replicate lots, with one exception, where the replicate was lost.

The results were statistically analyzed by analysis of variance for a factorial experiment. The parameters included average fish weight gain in grams and per cent mortality. Because of experimental design, those diets with buttermilk are only compared with those containing skim milk, herring meal and wet fish. We concluded from the analyses that:

1. The higher level of skim milk produced significantly greater average fish weight gain only in the absence of wet fish. Differences in mortality between high and low skim milk levels were not significant.
2. Salmon meal was superior to herring meal in both parameters. There were significant interactions between meal types and wet fish, suggesting salmon meal especially enhanced those diets containing no wet fish.
3. Neither salmon meal nor herring meal satisfactorily replaced wet fish; those diets without wet fish produced unsatisfactory weight gain.
4. There was no statistical difference in those results between skim milk and buttermilk.

In summary, we found that salmon meal was superior to herring meal. The higher milk level is not needed when 24% wet fish is present. Wet fish is necessary in the present Oregon Starter Mash formulation. Further testing may be needed before buttermilk can be substituted for skim milk because the values obtained from buttermilk were slightly less. Salmon meal and a high skim milk level appear to show promise towards development of a dry Oregon Starter Mash.

Table 1. Summary of results, Clackamas fall chinook starter diet experiment, 1967

Description	Wet fish level (%)	24			None			24	
		Herring		Salmon	Herring		Salmon	Herring	
		Skim milk		Skim milk	Skim milk		Skim milk	Buttermilk	
		10	20	10	10	20	10	10	20
Results	Dairy product level (%)	685	682	636	655	1,126	1,056	694	710
	Final fish per pound	0.27	0.27	0.32	0.30	0.01	0.04	0.26	0.24
	Average wt. gain (grams)	0.6	0.8	1.2	0.9	3.9	4.0	1.3	1.4
	Mortality (%)								

IDAHO PRODUCTION DIET TESTS, 1967

Paul Cuplin
Idaho Fish and Game Department
Boise, Idaho

Abstract

Feeding trials this year tested the omission of three per cent kelp meal from the diet, the addition of ten per cent dried shrimp meal, and the production diet ground fine enough to pass through a 50-mesh-per-inch screen.

Results indicate the three per cent kelp meal was beneficial, the addition of ten per cent dried shrimp meal gave results equal to the production diet, and finely ground ingredients in the diet gave the same results as the regular grind.

Cutthroat diet tests indicated a need to increase calcium pantothenate as fish on all test diets displayed dietary gill disease. The high level of herring meal (69 per cent) in our number one fry diet was found to be unnecessary; 42 per cent herring meal was adequate for a fry starter feed.

ASCORBIC ACID DEFICIENCY SYNDROME IN COHO SALMON

L. M. Ashley, J. E. Halver, C. E. Smith and R. R. Smith
Bureau of Sport Fisheries and Wildlife
Western Fish Nutrition Laboratory
Cook, Washington

Spinal curvatures (lordosis and scoliosis), subcutaneous petichial plus intraocular hemorrhages and bloody ascites have been reported recently for vitamin C deficient salmonids by McLaren et al. (1947) and Kitamura et al. (1965) in rainbow trout and by Poston (1967) in brook trout. Spinal curvatures were also reported for sockeye salmon (Halver and Shanks, 1960) and for rainbow trout (Shanks et al., 1962) fed diets lacking the amino acid L-tryptophan which condition has been reported to increase the demand for vitamin C in the diet. These symptoms in the tryptophan deficient salmon and trout disappeared in 7-14 days after tryptophan was replaced in the diet but anomalies due to vitamin C deficiency were not reversed after restoration of ascorbic acid for long periods of time. The essential difference appears to be that the vitamin C deficiency may inhibit normal formation of collagen, cartilage, developing bone (osteoid) and dentine in the growing animals. Collagen, an albuminoid protein, is the most abundant protein in the body. Failure of normal development of this substance can impair the entire supporting system of the body including the walls of the vascular system. Thus the classical signs of scurvy include internal hemorrhages, bleeding gums, loose teeth, and sore joints especially in the lower extremities.

Recently completed experiments on coho salmon fed a diet lacking vitamin C showed fish with scoliosis and lordosis, together with subcutaneous hemorrhages in between 15 and 20 percent of the population. In addition, anomalies of gill and ocular cartilages, not previously reported for C deficient fish, were found in nearly all deficient fish histologically examined. A further anomaly in the form of adrenal cortical cell hyperplasia was also observed when vitamin C was absent from the diet of coho salmon fingerlings.

FOLIC ACID ANEMIA IN COHO SALMON

C. E. Smith and J. E. Halver
Bureau of Sport Fisheries and Wildlife
Western Fish Nutrition Laboratory
Cook, Washington

Juvenile coho salmon, Oncorhynchus kisutch, fed a folic acid deficient diet and sampled at 6, 9, 12 and 14 weeks developed macrocytic anemia. The anemia, first observed at six weeks, was characterized by a significant reduction in red blood cell (RBC) counts as well as macrocytosis and poikilocytosis of erythrocytes (Table 1). The abnormally shaped erythrocytes observed in peripheral blood smears, appear to be an important aid in the identification of folic acid deficiency in coho salmon. Fish recovered after eight weeks on a diet adequate in folic acid and exhibited a normal blood picture.

Gross manifestation of the deficient were as follows:
(1) extremely pale gills, (2) exophthalmia, often accompanied by ascites fluid, (3) dark coloration and (4) reduction in growth.

TABLE 1

Effects of Folic Acid Deficiency on Average Red Blood Cell (RBC) Counts and RBC Length of Juvenile Coho Salmon

No. Wks. on Diet	RBC's/mm ³ Blood		RBC Length (microns)	
	Controls	Deficient	Controls	Deficient
6	860,000 (680,000--1,120,000)	498,000 ¹ (310,000--650,000) ²	14.8 (13--16)	16.5 ¹ (12--22) ²
9	1,200,000 (950,000--1,380,000)	384,000 ¹ (200,000--530,000)	15.3 (13--17)	16.5 ³ (14--20)
12	950,000 (800,000--1,260,000)	190,000 ¹ (120,000--270,000)	15.1 (13--17)	16.9 ¹ (15--19)
14	990,000 (790,000--1,210,000)	146,000 ¹ (40,000--280,000)	15.1 (14--16)	16.9 ⁴ (15--20)

¹ p < .01 Control versus deficient

² Range

³ p < .02 Control versus deficient

⁴ p < .05 Control versus deficient

AN APPROACH TO ESTABLISHING A LOADING CAPACITY FORMULA FOR TROUT HATCHERIES

Robert G. Piper
Bureau of Sport Fisheries and Wildlife
Bozeman, Montana

A straight line relationship can be demonstrated between length of fish in inches and percent body weight to feed when plotted on logarithmic graph paper. Haskell, in the Progressive Fish Culturist, Vol. 17, No. 3, 1955, states, " . . . if the carrying capacity of a trough or pond is known for any particular size of fish, at a particular size of fish, at a particular temperature, then the safe carrying capacity for other sizes and temperatures is that quantity of fish which will require the same weight of feed daily." Since there is a straight line relationship between percent body weight to feed and fish length in inches, a formula can be derived whereby fish size in inches can be substituted for weight of food fed daily to calculate the safe carrying capacity of various sizes of fish. Haskell's safe carrying capacity was based on the permissible weight of fish in pounds per cubic foot, assuming a standard water flow. By substituting inflow of water for volume of the raceway in this standard condition, a formula is established in which loading capacity of a raceway is based on size of fish and gallons per minute inflow. Haskell's formula is:

$$\begin{array}{lcl} \text{Permissible weight} & = & \frac{\text{Weight of feed daily} \times 100}{\text{of fish in pounds} \quad \quad \quad \% \text{ of body weight}} \end{array}$$

After establishing the permissible weight of fish in pounds per cubic foot for a given size fish in inches following Haskell's method, the size of fish in inches can be substituted for food levels, thereby basing the loading capacity of a raceway on fish size instead of amount of food fed. This can be expressed by taking the length of the fish at which the feeding level was calculated and determining a "loading factor" by dividing the weight of fish permissible per cubic foot of water by the length of the fish.

$$\text{Loading Factor} = \frac{\text{Known permissible weight of fish (pounds)}}{\text{Given length of fish (inches)}}$$

This will then give you a "loading factor" that can be used with any size fish.

$$\begin{array}{lcl} \text{Permissible weight of fish} & = & \text{Length of fish} \times \text{"loading factor"} \\ \text{in pounds per cubic foot} & & \end{array}$$

Since we know the permissible carrying capacity, if the water inflow is substituted for the volume of the raceway, a formula can be established in which loading capacity of a raceway is based on gallons per minute flow instead of volume of raceway. This can be done in the following manner.

$$\begin{array}{lcl} \text{Permissible weight} & & \text{Permissible weight of} \\ \text{of fish per gallon} & = & \frac{\text{fish in raceway}}{\text{Gal. per min. inflow}} \\ \text{per minute} & & \end{array}$$

Then the loading factor is determined.

$$\begin{array}{lcl} \text{Loading factor} & = & \frac{\text{Permissible weight of fish} \\ & & \text{per gal. per min.}}{\text{Length of fish in inches}} \end{array}$$

We can now determine the permissible weight of fish per gallon per minute inflow for fish of other sizes.

$$\begin{array}{lcl} \text{Permissible weight of} & & \\ \text{fish in pounds per} & = & \text{Length of fish} \\ \text{gal. per min. inflow} & & \text{in inches} \quad \times \text{Loading factor} \end{array}$$

In this way, we can establish a consistent loading formula in an experimental set-up, taking into consideration differences in fish size. Furthermore, when weights of fish vary in different raceways, adjustments in water inflow can be calculated by using the loading factor.

$$\frac{\text{Pounds fish present}}{\text{Length of fish} \times \text{loading factor}} = \text{Gallons per minute required}$$

There are various other factors such as water chemistry which, undoubtedly, affect carrying capacities. If the assumption of Haskell is granted, however, that primarily oxygen and accumulated metabolic products limit carrying capacity, a working formula or rule is established which may help in the design and management of hatcheries.

For example, temperature change appears on the feeding charts to have a uniform effect on metabolism. Once the "loading factor" for a hatchery has been established, adjustments for variations in water temperature can be made. For each degree Fahrenheit increase in temperature, the "loading factor" would be decreased by approximately 6%. Likewise, an increase in water temperature would decrease the "loading factor."

PHOTOELECTRIC EGG SORTER

W. G. Taylor

Bureau of Sport Fisheries and Wildlife
Little White Salmon National Fish Hatchery
Cook, Washington

Last year, at Little White Salmon Laboratory, eyed coho salmon eggs were color-sorted with an electronic sorting machine. The portable unit was brought to the hatchery and demonstrated by Mandrel Industries, Houston, Texas. It sorted opaque and live eggs at an overall rate of about 250,000 eggs per hour. The unit operates basically on an optical principle which compares light reflectance from an egg with that of a pre-selected color standard. Eggs which exceed the reflectance limits of this standard are rejected.

Eggs to be sorted are fed by a vibratory feeder into a revolving conveyor bowl. Centrifugal force moves the eggs out and up on the concave bowl surface forming a single line of eggs at the outer edge of the bowl called the transfer edge. The eggs are picked up by a revolving conveyor disc which is tangent to the transfer edge and which contains a row of ferrules on the periphery. Vacuum on the ferrules lifts each egg individually from the transfer edge and carries it through a lamphousing. In the lamphousing two phototubes measure the difference between light reflected from the egg and that from a pre-selected background plate. The background colors are quickly interchangeable and are chosen to represent the reflectance value of eggs being sorted. A signal from the phototube is amplified and fed to a classifier which decides whether the egg is to be accepted or rejected. If rejected, a magnetic memory unit is energized and the egg is ejected by air into a reject discharge chute. Live eggs are carried to the accepted discharge chute where the vacuum is cut off and the egg is released.

The results of the test show that 94.4% of the bad eggs were sorted correctly into bad egg baskets and 5.6% were missorted into good egg baskets. The main source of this error was due to a build-up of fungused eggs in the reject chute, which caused the eggs to be deflected to the accept discharge chute. Trial and error adjustments of the background reference also caused variation in sorting efficiency. Live eggs in the dead egg baskets averaged .75% of the total live egg count.

To determine the effects of machine sorting on eyed eggs, the eggs were randomly selected in basket lots and separated into three groups. The eggs were sorted three days after shocking. A control group was sorted by the routine hatchery salting method. One group of machine sorted eggs was manually fed into the conveyor bowl. In order to determine the effect of vibration on eyed eggs, the third group was fed into the sorter using the vibratory feeder. The identity of all these groups was retained through incubation and rearing until the fish were 275 per pound.

Egg Mortality

Sorting Machine	Number Eggs Sorted	Mortality	
		1 1/2 days after Sorting	Cumulative to Swim-up
Vibratory feed	202,460	11.8%	14.0%
Hand feed	165,096	11.6	13.1
Salted control	<u>362,375</u>	10.7	11.8
Total	729,931		

After 1 1/2 days the two machine-sorted groups showed slightly higher losses than the salted control. At swim-up no long-term effects were apparent in the hand-fed group. Although the differences were minor, it appears that vibration increased the long-term mortalities slightly relative to the other groups. There was no significant mortality difference during the rearing period.

To determine the feasibility of sorting trout eggs, a trial quantity of rainbow trout eggs was sent to the Mandrel Industries laboratory in Houston. It was felt that rainbow and brook trout eggs could be readily sorted by fitting smaller ferrules on the pick-up disc. Time study data were submitted by several trout hatcheries. These data were based on hand-picking and were compared to the machine rate of 250,000 eggs per hour. Time advantage is based on man hours per million eggs picked.

Hatchery	Species	Egg Mortality	Time Advantage
A	Rainbow trout	35 %	9.1 x
B	Rainbow trout	2	3.4
C	Rainbow trout	13.8	6.9
D	Brook trout	42.2	14.8

In the first case, a 35% mortality in rainbow eggs was theoretically machine sorted 9.1 times faster than by hand-picking. At 2% mortality, the time advantage was 3.4. Since the machine sorts at a constant rate, the advantage of machine sorting over hand-picking would increase with an increase in egg mortality.

The use of a counter to enumerate eggs as they are sorted is being investigated. This would combine sorting and counting with no additional labor. Another device which could be integrated into the system is a checkweigher.

The sorter would feed live eggs to the scale weigh hopper, through the surge hopper. An electric-eye cut-off is placed on the scale dial at the desired net weight of an egg basket. When the scale indicates the set weight, the surge hopper gate closes and a horn or light tells the operator that the scale hopper is ready for discharge. At this point, the operator presses a discharge button to the egg basket. When the scale empties to zero, it closes the scale hopper gate and opens the surge hopper gate. This allows the accumulation that occurred during basket loading to go into the scale hopper, slowing or stopping the sorter. This system would provide an automatic count of weighed baskets from which the total weight of eggs in the hatchery could be figured. It could be used with or without the electronic counter.

The machine appears to have excellent potential, particularly when integrated with auxillary equipment. Since it was a demonstration model and had never been tested with eggs, minor adaptations should further improve the sorting efficiency and minimize possible egg damage. If no damage results, the sorting rate can be substantially increased with an increase in conveyor speed.

ELECTROCUTING AND TETRACYCLINE MARK SAMPLING OF
BIG CREEK HATCHERY COHO RETURNS

Charles O. Junge
A. Kenneth Johnson
Irving W. Jones
Fish Commission of Oregon
Clackamas, Oregon

INTRODUCTION

Recent success at our hatcheries is currently resulting in tremendous overescapements of coho salmon back to the hatcheries. These returns create problems which affect production records and hatchery research studies. Escapements based on numbers of fish handled tend to be underestimated. In addition the magnitude of handling problems frequently makes adequate sampling impossible. It must be stressed that representative sampling is necessary for all quantitative studies concerned with returning fish. The present study is directed at these problems.

At Big Creek Hatchery, a program was designed to allow enumeration of the entire 1967 coho escapement and meet the sampling requirements of various studies involving fin and tetracycline mark recoveries. Prompt handling was particularly important because of limited holding facilities which might induce straying if overcrowding were permitted for an extended time period. A method of electrocuting surplus fish was used to facilitate handling and insure continuous access to the holding facility during peak migration.

ELECTROCUTION

Limited time did not permit a technical study of electrocution. However, with a straightforward approach using standard 120 volt, 60 cycle AC current, we were able to accomplish our basic purposes. A 10' x 12' electrocution pen was installed adjacent to the sorting alley. Flow conditions attracted fish from the alley to the pen. Jacks had free access to this pen at all times. Adults could only enter when a gate between the alley and the pen was removed. This greatly reduced sorting effort since, for the most part, jacks and adults were killed independently.

A portable electrocution unit was designed to create electrical fields throughout the pen. The frame of the unit was made from 1 1/4" diameter plastic (PVC) pipe which acted as an insulator. This frame held three "hot" electrodes at the top (8' long) and four neutral electrodes at the bottom (9' long), all of which were parallel to each other and staggered on 3 foot centers. The electrodes were 1/2" diameter thin-walled metal conduit. The entire unit weighed approximately 35 lbs. A push-button contact operated the electrocution unit and a warning bell simultaneously. All units were securely grounded and thoroughly insulated for safety.

Applying the current continuously for 30 seconds killed or completely paralyzed the majority of the fish and sufficiently narcotized the remainder so that they could be handled easily. The above procedure was adequate for all quantities tried, which at times was as high as 500 adults.

Fish in the sorting alley, which were only separated from the electrocution pen by a metal pipe fence, were unaffected by the electrical field.

Some electrocuted fish experienced various degrees of tissue damage which ranged from small to massive hemorrhaged areas with occasional vertebral damage. This condition affected the quality of the meat and was of concern to a local fish processing company.

To alleviate the problem, low and high voltage DC current was tried with some improvement. A future study involving various power supplies and arrangements of electrodes is being considered.

SAMPLING

Electrocuted fish were netted into a hoist-operated platform which spilled the fish onto the sampling deck. All fish were checked for marks, aged (by length), sexed, and these data were recorded on sampling sheets. All marked fish were placed in a sampling box. One out of every 10 unmarked fish was selected by random numbers from the sampling sheet and also placed in a sampling box. Nonsampled fish were placed in large bins (one for jacks and one for adults) from which they were removed by the fish processing company.

The best tetracycline marks are usually found in the vertebrae. Vertebral bones are readily accessible by means of a core

cutter and are fairly small and easily handled. Cores were cut from the caudal peduncle area of the sampled fish.

To speed sampling, two core tables (one for jacks and one for adults) were built. Each had a recessed centimeter ruler and attached drill press to drive the core cutter. The drill press was operated by a treadle with attached microswitch. Difficulty was experienced with the Canadian type cutter, developed for pituitary sampling, so a simplified cutter was designed which worked more effectively.

Styrofoam boards 1 1/2" thick with multiples of 25 or more holes (1 1/4" dia. x 1 1/4") were used for core receptacles. Individual core boards were put in plastic bags and stored in a freezer in plastic 55 gallon drum liners to prevent dehydration.

It was necessary to file the core samples in an orderly manner for identification of specific data. For this purpose, the data were positioned on recording sheets to correspond with the position of the cores in the styrofoam boards. These data included age, sex, length (and fin mark when present).

TETRACYCLINE MARK ANALYSIS

To read the bones, we put the boards under hot tap water, strip the flesh from a sample, cut off a single vertebra and either scrape it or digest the tissue with 2% trypsin. We then wash the bones in hot water for a few seconds and read them under a microscope provided with ultraviolet light. Mark quality is graded according to mark intensity; 0 for no mark, 1 for a faint ring, 2 for a readily visible ring and 3 for a brilliant yellow mark.

From a total of 22,319 fish handled 3,809 core samples and length measurements were taken. Preliminary results of mark reading indicate that at least 97% of the returnees were hatchery produced.

ACKNOWLEDGMENTS

This study would not have been possible without assistance from Vernon Knowles, Superintendent of Big Creek Hatchery, and his staff.

RESULTS OF THE FIRST YEAR'S OPERATION OF A
NEW HATCHERY USING BURROWS PONDS

Quentin Smith
Fish Commission of Oregon
North Nehalem Hatchery

Hatchery production has been increased by better nutrition, pathology, and fish culture techniques. One of the areas holding great promise for further improvements in the quality of the fish produced (providing greater survival) is better designed ponds.

Roger Burrows and his staff began working on a new type pond at his Entiat laboratory. The work on this phase was continued when he moved to the Abernathy laboratory. The early results from use of this pond, as reported at previous Fish Culture Conferences, were very favorable, both from the standpoint of feeding, growth, and pond cleaning and maintenance.

We were impressed that the use of these ponds was a step forward in fish culture. Our first use of these recirculating ponds was at our Trask hatchery. We built four of the 15' x 50' ponds. We were well satisfied with the way the fish performed in these ponds.

In November 1966 our first hatchery was completed incorporating these new ponds. Initial construction provided 14 Burrows recirculating raceways. These ponds are 17' x 75' x 4' deep. The water is pumped to the ponds from the North Nehalem River. Hatchery capacity is 1 million yearling coho and 1 million or more 90- to 120-day reared fall chinook.

The hatchery is unique in that no fish diverting barrier in the stream has been included in the construction. A concrete sill about 1 foot high has been built across the stream to maintain the water level in the pump sump at low water period. At most flows you cannot see any evidence of this sill. If by any chance we need some kind of a barrier, this would be a good place to put an electrical one.

We have been operating this new hatchery for a full year. We are well pleased with the operation of these new ponds. An evidence of our endorsement of these ponds is the fact that we've recently completed six more at this hatchery, a 43 per cent increase in pond area. A new hatchery, under construction on the South Santiam River, will have 10 of these ponds. Another hatchery expected to be in operation next October on Elk River (southern Oregon) will have 14 of these ponds.

At present we have about 74,000 coho averaging 19 per pound in 13 of the ponds and 35,000 steelhead about 11.7 fish per pound in the 14th. The fish feed well, distribute themselves throughout the ponds and appear to like the environment.

Roger reported the ponds to be self-cleaning. We have been amazed at the extent to which this actually happens. We clean the screens one, two, or three times per day depending upon the need. As of this date the fish have been in the ponds 294 days and ponds still don't need cleaning. The North Fork Nehalem at times carries a heavy silt load. The silt and dirt wash through the ponds without settling out. No dirt remains on the bottom.

We had over 5,200 coho jacks return to the hatchery adult pond this year from a release of 270,000 smolts last March which were reared in the ponds for the last three months of their freshwater life history. This is more than three times the survival rate at Alsea, one of our better coastal hatcheries.

We are well pleased with the first year's use of these new ponds.

DEVELOPMENT AND USE OF AN EGG COUNTER

Denny McClary
Fish Commission of Oregon
Cascade Hatchery

In the Oregon Fish Commission we attempt to accurately enumerate our eyed salmon and steelhead eggs. When we have egg takes of less than 1 million, we count the eggs with our 100-egg counter. This was our most accurate method until the development of the Universal Egg Counter. When the egg takes are over 1 million, we recommend the eggs be weighed and sampled. At some hatcheries the 100-egg counter is used even with egg takes of up to 5 million or more.

Both of these methods are subject to error depending upon several factors. I wanted to develop an egg counter that was fast, accurate, required a minimum of manpower to operate and was relatively cheap to purchase or build.

The machine I have with me is the second one I've made and satisfies our needs.

The counter is made of a revolving 1/4-inch plexiglass disc having 200 holes. This disc revolves on a 1-inch solid plexiglass base. The disc and base are put together at an angle such that the eggs are added at the bottom and are carried to the top for discharge through a hole in the 1-inch plastic base. Each time the disc makes one revolution 200 eggs have been discharged from the counter. The revolutions are enumerated with a Veeder Root counter. The 1/4-inch disc is powered by a 1/100 h.p. motor. About 216,000 eggs can be counted per hour, or 1,650,000 eggs per day. These counts are based on actual performance the last couple of months. To date this machine has counted almost 30 million eggs.

The actual operation is simple. An operator starts up the counter and dips eggs into the lower side. The eggs are counted and slide down a chute into another basket or other container. By watching the counter dial, a given number can easily be put into the basket. The only manual work is dipping the eggs and changing the baskets.

The machine is 12 inches by 15 inches and weighs about 20 pounds. I have applied for a patent on the machine.

PROGRESS REPORT ON FROZEN FOOD PELLET FEEDERS

Chris Jensen
Oregon Game Commission
Portland, Oregon

An insulated pellet feeder commercially designed for automatic feeding of frozen fish pellets is presently being used in experimental feeding of chinook and steelhead trout at two Oregon Game Commission hatcheries.

The feeder, designed and constructed by Neilsen Metal Industries of Salem, Oregon, in cooperation with the U.S. Fish and Wildlife Service, is similar in operation to the regular dry pellet feeder. Pellets pass from a hopper through a gate and flow out upon a spinning disc-type flinger which distributes the feed over the surface of the pond. The new feeder, in addition to being insulated, has several other features. The pellet receptacle consists of two cannisters, one of which can be removed for filling with pellets inside of the freezer. The base cannister is equipped with an agitator designed to prevent the feed from bridging and/or adhering to the inner walls of the hopper. The capacity of the upper cannister is approximately 40 pounds of frozen fish pellets.

Preliminary findings indicate that the feeder under normal operating conditions (in outside air temperature reaching a maximum of 90° F) will maintain the larger sized pellets at a satisfactory temperature level throughout the day.

It was found that when a feeder was completely covered with a white paper protective coating, it was more effective in maintaining the cold temperature of the pellets (12° F colder) than was a plain galvanized metal cannister.

A cannister filled with frozen pellets and stored in the freezer until morning was more effective in maintaining temperatures (8° F colder) throughout the day than was the paper-covered feeder.

Frozen pellets placed in an outside feeder at 7:45 a.m. reached a temperature of 38° F at 4 p.m., as the feeder was exposed to 83° F outside air temperatures during the day.

Some difficulty was experienced with the smaller sized fingerling feeds sticking to the agitator near the end of the

feeding period. The doughing condition sometimes prevented the last 1 to 5 pounds of feed from leaving the cannister. The larger sized pellets (1/8" or larger) were fed effectively without sticking or doughing on the agitator.

Feed residues did not adhere to the inside cannister wall (polished stainless steel) under normal summer operating conditions. Apparently, the moisture which forms when the pellets reach the thawing stage (29-32° F) is absorbed rapidly and thus does not form on the inner wall of the food container.

The experimental work with the frozen food pellet feeder will continue for several years with the objective of perfecting a suitable feeder for use in our larger salmon and steelhead hatcheries.

WATER TEMPERATURE CONTROLS IN AN EXPERIMENTAL HATCHERY

James L. Fessler
Oregon Game Commission
Corvallis, Oregon

The Oregon State Game Commission's Research Laboratory located in Corvallis was built in 1964. The water supply for this facility is furnished by a 37-foot well and a 7 1/2-horse-power turbine pump. The water temperature is a constant 53°F. Under most hatchery conditions, this temperature would be considered ideal for rearing salmonids. Since the experiments conducted at the laboratory require variable water temperatures for varying hatching times on the same group of fish and to provide seasonal water temperatures for the experiments themselves, we looked into different types of water temperature controlling devices. We were interested in a temperature range of 40 to 65°F and about 30 gallons/minute. To chill the water down to the desired temperature between 40 and 53°F, a Dunham Bush package chiller was installed. The initial cost and installation of this unit was \$3,500 and the operating cost is approximately \$1/day. The accuracy to which the temperatures can be regulated is $\pm 0.5^\circ\text{F}$. To facilitate warming the well water to any desired temperature between 53 and 65°F, an A. O. Smith, permaglass, gas-fired water heater was installed with a Lawler Bam thermostatic water controller. The water heater itself is glass-lined and the Lawler mixing valve is made of bronze. The initial cost of the heating unit, mixing valve, and installation was \$1,000. The gas water heater has a capacity of 84 gallons and an input rating of 251,000 B.T.U. Operating costs for this unit run \$10/day when the heater is being used to full capacity or is heating 30 gallons of water/minute from 53 to 65°F. This commercial type heater along with the Lawler Bam mixing valve gives temperature controls to $\pm 0.5^\circ\text{F}$.

Under present experimental conditions in the laboratory, we are trying to approximate annual stream temperature patterns on a weekly basis and have found it necessary to run the water heater for a period of four to five months during the summer and during this period the heater was run at full capacity for about one month. During the rest of the period when the heater was being used, the burners would cycle off and on as controlled by the thermoswitch.

With the installation of the water chiller and the water heater, we have been quite flexible in controlling water temperature to our particular needs and we still have the added advantage of using well water which thus far has been free of fishery disease organisms and parasites.

COLLAPSING MEMBRANE DISEASE OF
BROOK TROUT EGGS AND SAC FRY

Jamieson E. Holway
Bureau of Sport Fisheries and Wildlife
Bozeman, Montana

Collapsing membrane disease has been a problem in the propagation of brook trout in several New England hatcheries, particularly where the water is very soft. It has occurred at the Berlin National Fish Hatchery in New Hampshire in epizootic proportions. However, the manager has been able to control the disease by artificially hardening the water with calcium chloride.

The work reported in this paper was an attempt to produce the disease under controlled laboratory conditions by simulating the water conditions at Berlin. This work has shown that the disease condition is reproducible and has cast additional light on its cause and control. The evidence indicates that physical and chemical properties of the environment are the causes of the disease. More important, perhaps, is the description of this distinct morphological condition.

This disease is characterized by the collapsing of two inner layers within the yolk sac. These layers collapse much the same as a balloon collapses when punctured. The fluid is retained within the yolk sac by a third outer layer. In the collapsed position, the membranes appear as a white spot.

This disease was formerly classed with white spot. A review of the literature reveals that white spot is an ill-defined descriptive term rather than a distinct disease and may actually be many different diseases. White spot is typically defined as a coagulation of the yolk protein. We were able to produce six different conditions of eggs and sac fry, including the collapsing membrane disease which could be called white spot.

Collapsing membrane disease can now be classed as a separate disease, because it has a distinct morphology and known causes and controls.

SOREBACK OF RAINBOW TROUT: RESULTS OF
1967 FISH QUALITY EXPERIMENTS

James W. Warren
Bureau of Sport Fisheries and Wildlife
Hagerman, Idaho

As fish culture advances, more and more attention is devoted to the quality of the product released. Studies have shown that fish of poor quality and appearance are not only handicapped and yield a poor return but may also be rejected by the sportsman. Experiments were recently completed at two National Fish Hatcheries that were aimed at testing some of the fish-cultural factors of fish appearance. These tests explored some of the possible causes of fin erosion and soreback of rainbow trout.

Description of Soreback

Definition: A primary focal lesion occurring as an ulceration at the anterior base of the dorsal fin. This lesion oftentimes involves the dorsal fin proper and frequently progresses into the underlying musculature and other tissues.

Etiology: Lesion itself is caused by the nipping of the specific site by cohorts. Why this nipping takes place is not fully understood.

Epizootiology: Primarily a problem of rainbow trout 3 to 9 inches in length which are reared under crowded conditions in concrete raceways. Seldom progresses into significant lesions at temperatures below 52 - 54°F. Lesions most frequently found on largest, most competitive fingerlings in population. Seldom observed on runts of population.

Symptomatology: Easily observed large dorsal lesions. Usually early stages identified as a small whitened patch at the insertion of the dorsal fin. At temperatures below 52°F disease seldom progresses beyond this stage. At warmer temperatures this whitened "target" is repeatedly nipped and after the teeth of the fish begin to harden the skin and underlying musculature will be chewed away.

Pathology: The lesion has characteristics of a simple traumatic injury of external cause. Affected fish are not particularly debilitated and secondary infection is seldom a problem.

Diagnosis: Soreback is readily recognized in an affected population of rainbow trout by the obvious large dorsal lesions.

Prognosis: Generally good. If victim fish are isolated, epithelium will cover lesion in 5 to 7 days and pigmentation will usually be complete in 30 days.

Treatment: No uniformly successful chemotherapy has been found.

Prophylaxis: Measurable success has been obtained for the control of soreback as well as other appearance problems by maintaining low population densities when the fish are in the 3 to 9 inch size range. Soreback is a juvenile disease.

Public relations: Sportsmen reject fish with soreback and other detracting appearance defects. Survival to the creel or to adulthood may be limited when other than intact fish are stocked.

Experimental

The effects of cover (shade), diet and population density upon the appearance, growth rate and conversion were tested on Ennis strain rainbow trout fingerlings at the National Fish Hatcheries at Hagerman, Idaho and Ennis, Montana during the spring and summer of 1967. With the exception of water temperature, fish cultural and environmental factors were as identical as possible at the two hatcheries. Growth and conversion data were collected at five complete monthly inventories. Appearance data were obtained by sampling each population three different times and enumerating the intact pectoral and/or dorsal fins and recording any lesions found. Growth and conversion data were statistically evaluated by Don Worland of the Bureau of Commercial Fisheries laboratory in Seattle who employed the IBM 704/7094 computer and the UCLA Health Services computer program for factorial designs.

Results

Treatment means: Growth rate, conversion and appearance

<u>Treatment</u>	<u>Growth Rate</u>	<u>Conversion</u>	<u>Appearance</u>
Hagerman, Idaho	1.052 ¹	1.538 ²	46.94 ³
Ennis, Montana	0.660	1.776	28.57
OMP diet	0.887	1.705	39.27
IOF diet	0.826	1.609	36.25
Low population	0.867	1.627	39.65
High population	0.845	1.688	35.87
Open raceways	0.864	1.666*	38.38*
Shaded raceways	0.848	1.648	37.14

¹ Increase in length in inches per month

² Pounds of food required to produce one pound of fish

³ Average percent of fish with intact dorsal and/or pectoral fins

* Not significant at the 95% confidence level ($F_{.95}$)

Discussion

The results at the Ennis station were likely due to the high stocking rate which was based upon their cooler water temperature (54°F vs. 59°F at Hagerman). At the end of the test all raceways had an average of 7% soreback and very poor fin condition. Fish at Hagerman were about normal, 0.1% soreback and average fin condition. This indicates that stocking levels greatly affect the condition of the fish and cannot be based simply on temperature alone.

Factors found to favor growth and conversion also favored fish condition as judged by their appearance. The inspection team repeatedly observed, however, that the runty fish maintained intact fins and brighter coloration than their larger cohorts. It is speculated that the runts are "non-combatants" and do not enter into competition and associated "nipping battles" in the mainstream of raceway life. Marking experiments have generally revealed that the runts do not contribute greatly to the catch or reach adulthood in the proportions that the larger fish do.

These tests revealed that shading of raceways may favor young fish up to about 3 inches, but may indeed be detrimental

to larger fish. Good nutrition is probably essential to good fish appearance but these trials did not indicate that diet alone would assure good fish appearance. In the final analysis we must hark back to the fact that, in these tests at least, the greatest single factor in the production of high quality fish as judged by their appearance is the population density levels at which they are reared during their juvenile life.

A QUANTITATIVE COMPARISON OF PERITONEAL WASHES AND FECES
FOR DETECTING INFECTIOUS PANCREATIC NECROSIS (IPN)
VIRUS IN CARRIER BROOK TROUT

James L. Billi
Bureau of Sport Fisheries and Wildlife
Coleman National Fish Hatchery
Anderson, California

Infectious pancreatic necrosis is an acute virus disease of trouts, and typically causes high mortality among the young. At present there is no treatment for the infection; the most effective control is achieved by prevention. In order to prevent the disease, sources of the virus must be determined. Depending on purpose, time, and economic factors, the quality of the examination may differ from absolute certainty such as is needed for IPN-free brood stock to "reasonable assurance" of freedom from the virus as is the case of large populations involved in shipments or transfers of fish. It has been the practice to combine feces or peritoneal washes from five fish and do the testing on such pools. If virus could still be dependably detected in larger pools, population samples could be increased and there would be a greater probability of detecting IPN virus carriers.

The objectives of this experiment were: 1) to compare fecal samples and peritoneal washes for efficiency and sensitivity of IPN virus detection; 2) to compare 5, 10, and 15-fish pools of material for efficiency in detecting virus; 3) to quantitate the IPN virus in carrier brook trout (Salvelinus fontinalis) and determine if fluctuations in virus level occurred, and 4) to determine whether non-carriers remained non-carriers.

MATERIALS AND METHODS

Three hundred yearling brook trout were obtained from the Leetown National Fish Hatchery which were survivors of an IPN epizootic. Virus was isolated from the sample and subsequently identified by serum neutralization tests to be IPN virus.

Initial survey. Initially every fish was individually examined, then portions of individual samples were combined into 5, 10, and 15-fish pools.

Repeat surveys. The individual fish which showed no virus in the initial survey were considered to be negative; they were

resurveyed but only fecal samples were taken. Those which were negative after the second test were examined a third time, and if necessary, a fourth time.

Confirmation. IPN virus-caused cytopathology (CPE) in rainbow trout gonad (RTG-2) cells is characteristic, but similar CPE can be caused by fecal toxicity. Therefore, a fish was considered a carrier only if medium from affected cultures successfully produced CPE upon transfer to fresh RTG-2 cultures.

Quantitation of positive fish. Virus was quantitated in both feces and peritoneal washes from carrier fish. New samples were taken, filtered, serially diluted to 10^{-6} , and inoculated into cell cultures. Five cultures were used for each dilution. The infectious doses necessary to cause 50% CPE (ID₅₀) was determined by the Kärber method.

Thirty-three of those fish whose fecal samples showed virus were periodically resampled and titrated four times. Seven of those showing virus in the peritoneal wash were titrated once.

RESULTS

Relative efficiency of feces and peritoneal washes. In the initial survey, 63.3% of the individual fecal samples and 4.7% of the peritoneal washes showed virus. These results showed the superiority of feces for viral detection and indicated which method was to be employed in the balance of the work.

Additional carriers were detected among those fish which had been negative on the first examination; 56.3% showed virus in the second fecal sampling. A third sampling showed 18 and a fourth screening detected 8 additional carriers. After 4 screenings, only 7.3% of the original 300 fish were considered non-carriers.

Five percent of the cultures showed CPE which did not transfer. Therefore, the CPE was attributed to toxicity or other non-viral factors.

Relative efficiency of pooled samples. Knowing from individual assay the actual incidence of carriers, 5, 10, and 15-fish fecal pools showed equal sensitivity; over 90% of the positives were detected by all 3. Since fewer filtrations, cultures, etc. are required, the 10 and 15-fish pools were judged more efficient.

In the peritoneal washes, one of the 5-fish pools and one of the 10-fish pools were positive. These contained one positive individual fish. The 15-fish pool containing the sample from this fish was not affected.

Quantitation of feces and peritoneal washes. The amount of virus present in feces from individual fish ranges from $10^{1.7}$ to $10^{7.5}$ ID₅₀ per ml. Successive titrations showed that most but not all carriers shed virus each time they were sampled. Those which were high tended to repeat at high titer, low titer fish tended to stay low. The time lapse between the initial survey and fecal titration was 9 to 167 days.

Those fish which were used in the peritoneal wash titrations were positive in the initial survey. Of the seven titrated, five were negative, one titered $10^{4.3}$ and one $10^{1.3}$ ID₅₀ per ml.

DISCUSSION

Gross virus detection in feces is easy. It becomes progressively more difficult as 100% certainty is approached.

Based upon information gained solely from the initial survey, it can be said that at least one fish per pool is positive. As more fish are combined, less can be said about the percentage of carriers in the population. To determine the exact level, individual fish would have to be sampled; but for determining only if carriers are present, pools should be used.

Some of the feces from fish which showed virus in the initial survey showed no virus when periodically titrated afterward. This indicates that carrier fish may shed virus some of the time, but not necessarily all of the time.

Since fish used in this work were together from hatching and since a few more carriers were detected at each testing, it is conceivable that additional testing might show all to be carriers. This possibility points out the need for utmost caution when attempting to certify brood stock as being IPN virus free. A history of freedom from IPN, as well as negative results when fecal samples from 10% or more of the population is tested in cell culture, should give a reliable "IPN-free brood stock" label.

CONTROL OF CERATOMYXA IN A HATCHERY WATER SUPPLY

David A. Leith and Keith D. Moore*
Fish Commission of Oregon
Sandy and Madras, Oregon

In November 1964, the Fish Commission of Oregon began conducting studies to determine the feasibility of artificially propagating spring chinook salmon and steelhead trout in the vicinity of Portland General Electric's Pelton-Round Butte hydroelectric development on the Deschutes River in Oregon.

From November 1964 to October 1966 we conducted several incubation and rearing tests to evaluate the potential of water obtained from the Deschutes River. We found that: (1) the river water could be used successfully to hold spring chinook and steelhead adults and incubate eggs and fry; and (2) water temperatures were adequate to insure good growth and production of smolts of both species in one year of rearing. However, we discovered that the water was infected with the myxosporidian fish parasite, Ceratomyxa shasta, which caused losses of steelhead fingerlings as high as 87% and up to 23% loss of chinook fingerlings. We attempted to control the disease without success with 0.2% sulfamethazine in the diet.

Since there was no known treatment for the disease and it was not practical to avoid the parasite by using ground water, in 1967 we tested three water treatment techniques in an attempt to control Ceratomyxa in the Deschutes River water. We used: (1) treatment with ultraviolet light; (2) filtration; and (3) filtration combined with chlorination.

To test ultraviolet light, we used a Model PVC-2 ultraviolet sterilizer manufactured by Steroline Systems Corporation, Santa Fe Springs, California. Since we did not know what dosage would be required to kill Ceratomyxa or how much interference to expect in Deschutes River water, we used the highest dosage practical under the conditions of the experiment (an estimated rate of 325,000 microwatt seconds/cm²).

To test the feasibility of filtering, we used a portable water treatment unit called a Water Boy manufactured by Neptune MicroFLOC, Inc., Corvallis, Oregon. This device uses a filtering process similar to the flocculation and sand bed filter system used for municipal water supplies.

*Presented by Wallace F. Hublou

MicroFLOC indicated their process was capable of removing particles the size of Ceratomyxa spores but they could not guarantee that it would remove 100% of the infectious agent. Therefore, we tried chlorinating a portion of the filtered water and then dechlorinating it in a bed of activated carbon. We tried a 1-hour exposure to a chlorine residual of about 3 ppm. Chlorine was added in the form of sodium hypochlorite. Residuals were difficult to control precisely and they varied from 2.2 to 5.3 ppm (mean 3.4 ppm) throughout the experiment.

We evaluated the three water treatments in a 70-day bioassay from July 17 to September 24 using 1967-brood Deschutes River steelhead fingerlings held in 6-foot diameter circular tanks (one lot of 500 fish per treatment). All fish were fed Oregon Pellets ad libitum. Prior to the experiment, the test stock had been reared to an average size of 263 fish/lb. in spring water at the Oregon Game Commission Wizard Falls Hatchery, and they presumably had not been exposed to Ceratomyxa. Results of the experiment are given in Table 1.

Treatment of the raw river water with ultraviolet light was effective in combating Ceratomyxa. The dosage we used was approximately 10 times the rate required to kill most bacteria. Recent evidence from a test conducted at the U. S. Fish and Wildlife Service, Little White Salmon National Fish Hatchery, indicates that a considerably lower dosage killed Ceratomyxa in prefiltered water (Roger Burrows, personal communication). We suspect that substantially lower dosages would also be effective in unfiltered Deschutes River water.

Table 1. Results of water treatment tests, Pelton Pilot Hatchery, July 17-September 24, 1967

Treatment ¹	Total mortality (%)	<u>Ceratomyxa</u>
Regular river water--CONTROL	20.2	Positive
Ultraviolet light (regular river water)	1.0	Negative
Filtered water	6.2	Positive
Chlorinated-dechlorinated (filtered water)	0.4	Negative

¹ One lot of 500 fish per treatment

MicroFLOC filtration did not prevent the disease, however it apparently reduced the incidence of the parasite as fish loss was reduced substantially.

A 1-hour exposure to chlorine apparently killed the infectious particles passing the filter, since the fish did not become infected with Ceratomyxa. Further work may show shorter exposure periods to be equally effective, since the organism is apparently sensitive to chlorine, residuals lower than 2-3 ppm would probably also be effective. A residual of 0.5-1.0 ppm might be adequate to indicate when enough chlorine has been added to oxidize both the organisms and the organic load in the water.

Both ultraviolet treatment and chlorination-dechlorination are feasible methods for treating fairly large volumes of water; however, both are quite expensive. For example, installation costs for a 2,000 gpm system, using either technique, would probably be in the vicinity of \$100,000. Of the two methods, we feel that ultraviolet sterilization is the most practical and fail-safe for application to hatchery water supplies.

A VIRAL EPIZOOTIC AT CULTUS LAKE

Robert W. Mead
International Pacific Salmon
Fisheries Commission
Cultus Lake, B.C., Canada

Introduction. An outbreak of disease in the spring of 1967 among groups of sockeye salmon fingerlings at the Salmon Commission research station at Cultus Lake, B.C., caused extensive loss and has been attributed to a virus. The present report will present the history, course of the disease, clinical description, and gross necropsy findings in the cases.

History and Course of the Disease. The Cultus Lake sockeye fry were largely obtained from an upwelling artificial spawning ground located on the premises of the research station. About 3 per cent were obtained from the natural spawning grounds at Lindell Beach. During the spawning season there had been a substantial pre-spawning mortality in the lake. Bacterial cultures from the adults revealed no significant pathogenic species, and virological examinations were not conducted.

Upon emergence the fry were placed in eight 300-gallon fiberglass rearing tanks and started on Abernathy starter mash fed eight times per day. The tanks were supplied with water from Cultus Lake as was the spawning ground. The experiment called for fry concentrations ranging from 36 to 54 per cubic foot of water; however, during the course of the fry collection, the concentration reached 500 per cubic foot in a few ponds for a few days. A total of 190,000 fry were collected during the course of the emergence. The water temperature began at 48°F and rose to about 50°F at the time of the disease outbreak, at which time it was reduced to 45°F. The average wet weight of a Cultus Lake sockeye fry at emergence is 3,000 per pound. The emergence began about April 18 with a peak from May 6 to 14, then dropped off.

A few mortalities were noted about the end of April but increased to about 1% of the total per day by May 15, and continued at 1% to 3% per day for the remainder of the epizootic. There were no significant lesions seen in the dead fish until May 29th when fish which behaved and appeared grossly in a characteristic way were noticed among the mortalities. These characteristics will be described under clinical signs.

Also, during the spring of 1967 a trout farm approximately 20 miles from the research station and on a different watershed

drainage was experiencing a similar epizootic among young rainbow trout. The trout farm had purchased eggs from a Washington State trout farm. These eggs hatched about Christmas, 1966. Eggs from their own stock hatched sometime later and at the middle of March mortalities began among both groups. On two occasions, trout were brought from this farm to the field station for examinations by one of the veterinarians. These rainbows were present on the field station on the 28th-30th of March and on April 13th and 14th. These fish were confined to one laboratory room and all equipment used in handling them was disinfected before reuse. After examination the carcasses were destroyed by burning. The mortality at the trout farm continued into July when the remaining fish were destroyed.

Clinical Signs

Sockeye salmon fry

1. Behavior. The affected fish became lethargic and occasionally darker in color than other fingerlings in the same pond. Many of the fry would drift with the current rather than maintaining their position in the water. Most of those affected seemed to still be feeding when the first external signs appeared. The most obvious signs were exophthalmia and a grossly distended abdomen. The behavior of the fish during the course of the disease can be broken into three progressive stages.
 - a. Stage one: Affected fish could maintain an upright position against the current but were oriented with the posterior body riding lower in the water than the anterior body.
 - b. Stage two: The fry would drift aimlessly with the current. Some lateral rotation was seen but not a predominant feature. Such drifting fish would occasionally develop very erratic and frantic swimming movements with considerable longitudinal rotation. They would descend to the bottom of the pond and continue the frenzied activity along the bottom for 10-15 seconds. Such fish would then become quiescent and continue drifting with the current.
 - c. Stage three: Fry would float at the surface of the water with rapid opercular movement the only sign of life. Occasional violent swimming activity as seen in stage two with return to drifting in 10-15 seconds was observed occasionally. Fry eventually drift against the outlet screen and remain until death.

Necropsy findings: Sockeye and rainbow

1. Gross external characteristics

- a. Largest fish in best condition most seriously affected.
- b. Exophthalmia - 100%.
- c. Ascites - 80%.
- d. Pale gills.
- e. Occasional petechiation in buccal cavity.
- f. Hemorrhagic areas at base of pectoral fins in 30% of moribund fry.
- g. Hemorrhagic areas seen in 80% of affected fry in body musculature at level of dorsal fin or slightly anterior to it. In some cases the area of the dorsal fin was swollen, quite hyperemic, and appeared necrotic.
- h. Scoliosis was observed only rarely early in the outbreak, but was seen more frequently after the 14th of June.

2. Gross internal characteristics

- a. All tissues edematous and very friable, including the skin.
- b. Petechiation and some larger hemorrhagic areas seen in the peritoneum along kidney area and in lateral body wall. Fat around spleen and pancreas contained petechial hemorrhages. The membranes covering the brain and heart contained areas of hemorrhage in some moribund fish.
- c. Body cavity filled with a clear fluid. Stomach and intestine contained some clear mucous but not grossly distended. Residual yolk sac also distended with fluid in case of youngest trout.
- d. Liver and spleen pale in most cases. All body organs were very friable and edematous.
- e. Some incidence of hemorrhagic enteritis.

Discussion

It was of concern to us to identify if we could the source of the infection in the sockeye. Rucker in 1954 isolated the old sockeye virus from sockeye adults in Cultus Lake, therefore a transovarian or lake water source of the virus would be plausible. The unexplained adult mortalities during the spawning season would seem to enforce this possibility. However, some other groups of sockeye fingerlings raised in another part of the laboratory but on the same water supply, diet and from the same basic spawning population were not involved in the epizootic, but were shown to be susceptible by inoculation and by contact with infected fish.

The other possible source of infection would be the rainbow trout and would involve a break in the isolation of the trout samples with contamination of the sockeye stocks in some way. Similarity of the characteristics of both outbreaks and the apparent identical viruses recovered from both the rainbow and sockeye point to this possibility.

During the course of the outbreak of the disease samples were sent to the Western Fish Disease Laboratory for virus isolation. Rainbow trout from both the trout farm experiencing the epizootic and from a trout farm located near the spawning grounds in Cultus Lake were submitted for virus determinations. The latter samples showed no virus while the former harbored a virus which appears identical to that one isolated from the sockeye.

A NEW VIRUS FROM CULTUS LAKE?

Donald F. Amend
Bureau of Sport Fisheries and Wildlife
Western Fish Disease Laboratory
Seattle, Washington

A virus was isolated from rainbow trout and sockeye salmon undergoing extensive losses in the Chilliwack area of British Columbia, Canada. The isolated viruses from each species of fish were indistinguishable from one another and were presumed to be the same virus.

This is the first report of an epizootic occurring among salmon and trout due to the same virus, and this is the first report of a virus epizootic in the Fraser River system. The virus has the following characteristics:

1. Size. The virus will pass a 100 m μ filter, but not a 50 m μ filter.
2. It contains essential lipids as it is inactivated by 10% chloroform in 24 hours at 4°C.
3. It is sensitive to pH 3.0 for 30 minutes.
4. It is sensitive to 34°C for 20 hours.
5. It grows best at about 18°C on FHM cells.

The virus can be easily separated from IPN because IPN will pass a 50 m μ filter, contains no essential lipids, and is not inactivated by heat at 34°C. However, these physical characteristics do not separate this virus from the sockeye virus or chinook virus. The following biological characteristics may possibly separate the virus from the other two:

1. Moribund fish typically have muscle necrosis and muscle hemorrhaging, particularly around the dorsal fin.
2. The hematological picture is distinct from the sockeye virus, but not necessarily different from the chinook virus.
3. Unlike the other two viruses the kidney does not appear to be the primary target organ.
4. Rainbow are naturally infected with this virus.

It is not known if the virus is distinctly a new virus. However, studies are still in progress and a positive identification will have to be determined by a histopathogenesis study, a serological comparison, and an electron microscope study. The virus will not be named until a definite decision is made as to its identity; but the virus probably belongs in the myxovirus group.

ORAL IMMUNIZATION OF COHO SALMON AGAINST FURUNCULOSIS
AT QUILCENE NATIONAL FISH HATCHERY

William J. Walsdorf
Bureau of Sport Fisheries and Wildlife
Quilcene, Washington

Two furunculosis vaccines were obtained from Dr. G. W. Klontz of the Western Fish Disease Laboratory and fed as directed. Furunculosis soluble antigen vaccine and heat killed vaccine were added to Oregon Moist Pellets and fed to two groups of production juvenile coho salmon. A control group was fed regular O.M.P. Mortality in the H.K. group was approximately one-third that of the control group during the four-month trial period. Mortality in the F.S.A. group was approximately one-half that of the control group despite their being held in reused water for part of the trial period.

The O.M.P. containing the vaccines was fed to the two immunization groups starting with a 10-day treatment beginning April 14, 1967. The 10-day initial treatment was followed by a once a week treatment for 10 weeks which ended on June 27. Both vaccines were fed at the rate of 150 micrograms per fish for the total treatment. All fish used in the tests were obtained from the run returning to the Quilcene hatchery and were approximately 500 per pound when the oral immunization began. The F.S.A. group started as 1,160,000 fish but was reduced to 720,000 in September when crowded conditions made it necessary to reduce pond loads. The H.K. group contained 500,000 fish and the control group 200,000 fish.

Average water temperatures throughout the summer were cooler than normal with the highest weekly average temperature reaching 54 degrees in August. Warmer water temperatures probably would have increased the severity of the infection and provided a better test of the efficiency of the vaccine. Furunculosis had caused greater losses at Quilcene in previous summers when July and August water temperatures averaged two to three degrees higher. Low water conditions in early July made it necessary to hold the F.S.A. group in reused water from the H.K. and control groups for six weeks. During the reuse period some of the F.S.A. ponds were receiving supplemental fresh water. Pond 10 which received the most fresh water was the F.S.A. pond with conditions most similar to the H.K. and control ponds. Pond 18 with the least fresh water had the least similar water conditions. In comparing the effectiveness of the H.K. versus the F.S.A. vaccines, pond 10

should give more comparable data than comparing the F.S.A. group over-all. The varied water conditions in the F.S.A. group produced considerable mortality differences. The ponds which received the most fresh water had the lowest mortalities.

Deaths caused by furunculosis were first identified from ponds 17 and 18 of the F.S.A. group on July 28. Holding fish in reused water appeared to be associated with the disease outbreak. Approximately three weeks after the F.S.A. group were placed on reuse water from the H.K. and control ponds the first incidence of furunculosis was recorded and the mortality in the F.S.A. group increased sharply. Furunculosis mortality was recorded from the control group in early August, but positive identification of furunculosis was never obtained from the H.K. group.

Total mortality, July 1 through October 31:

Control group	3.64%
F.S.A. group	2.00%
H.K. group	1.20%
Pond 10 (F.S.A.)	1.39%
Pond 18 (F.S.A.)	3.19%

Results indicate that the vaccine did provide protection against furunculosis. Mortality in the control group was more than three times greater than that of the H.K. group. The F.S.A. vaccine does not appear to be as effective as the H.K. when total mortality figures are considered (Figure 1). The effectiveness of the F.S.A. can be better evaluated in Figure 2 where individual ponds within the group are considered. Pond 10 which received the least reused water, provides information which has a greater comparative value as its water conditions are most similar to those present in the H.K. and control groups. The 1.39% mortality from pond 10 is only slightly higher than the 1.20% mortality of the H.K. group, and much lower than the 3.19% of the control group.

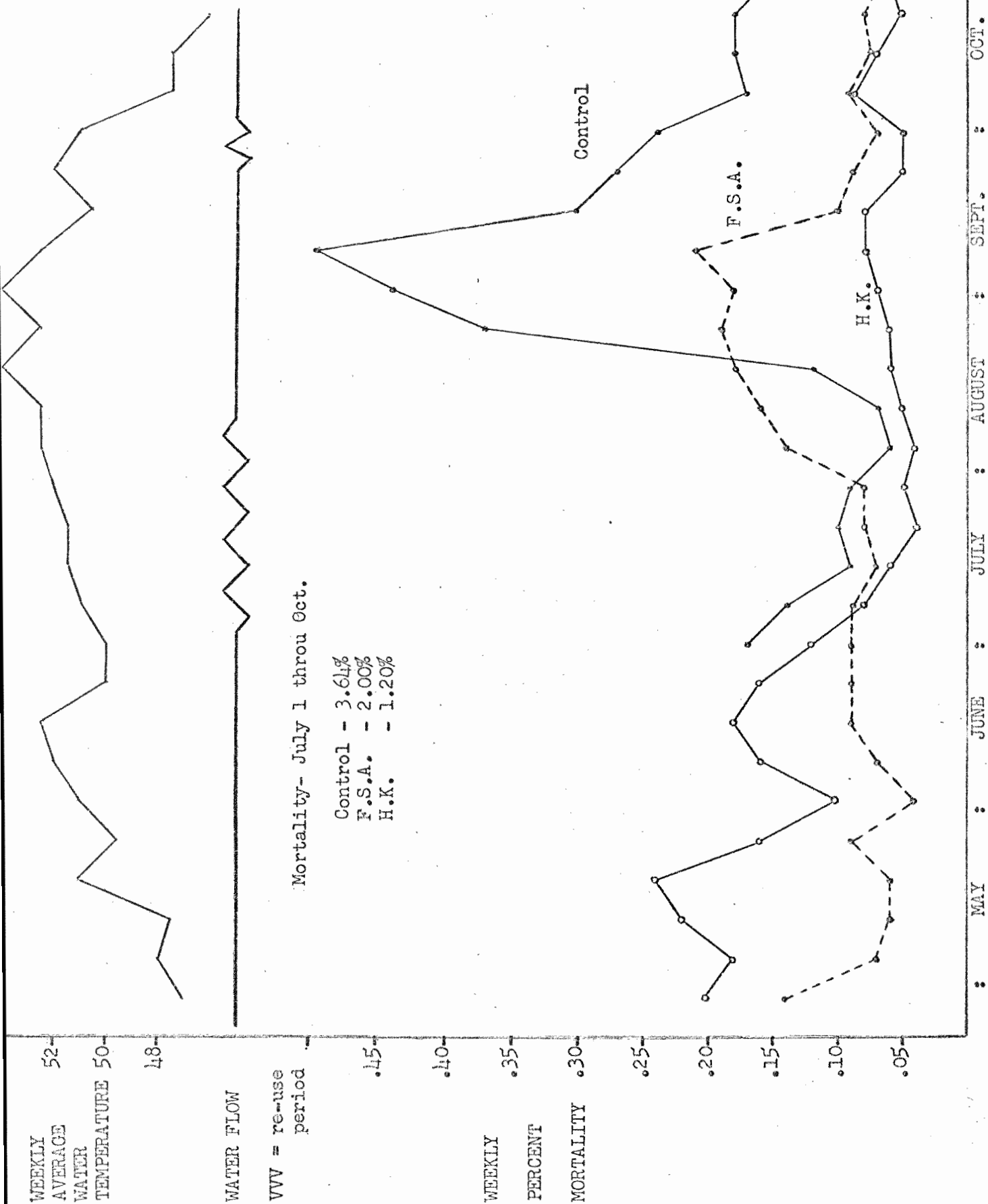


FIGURE 1. - Mortality of oral immunization (FSA & HK) groups and control group.

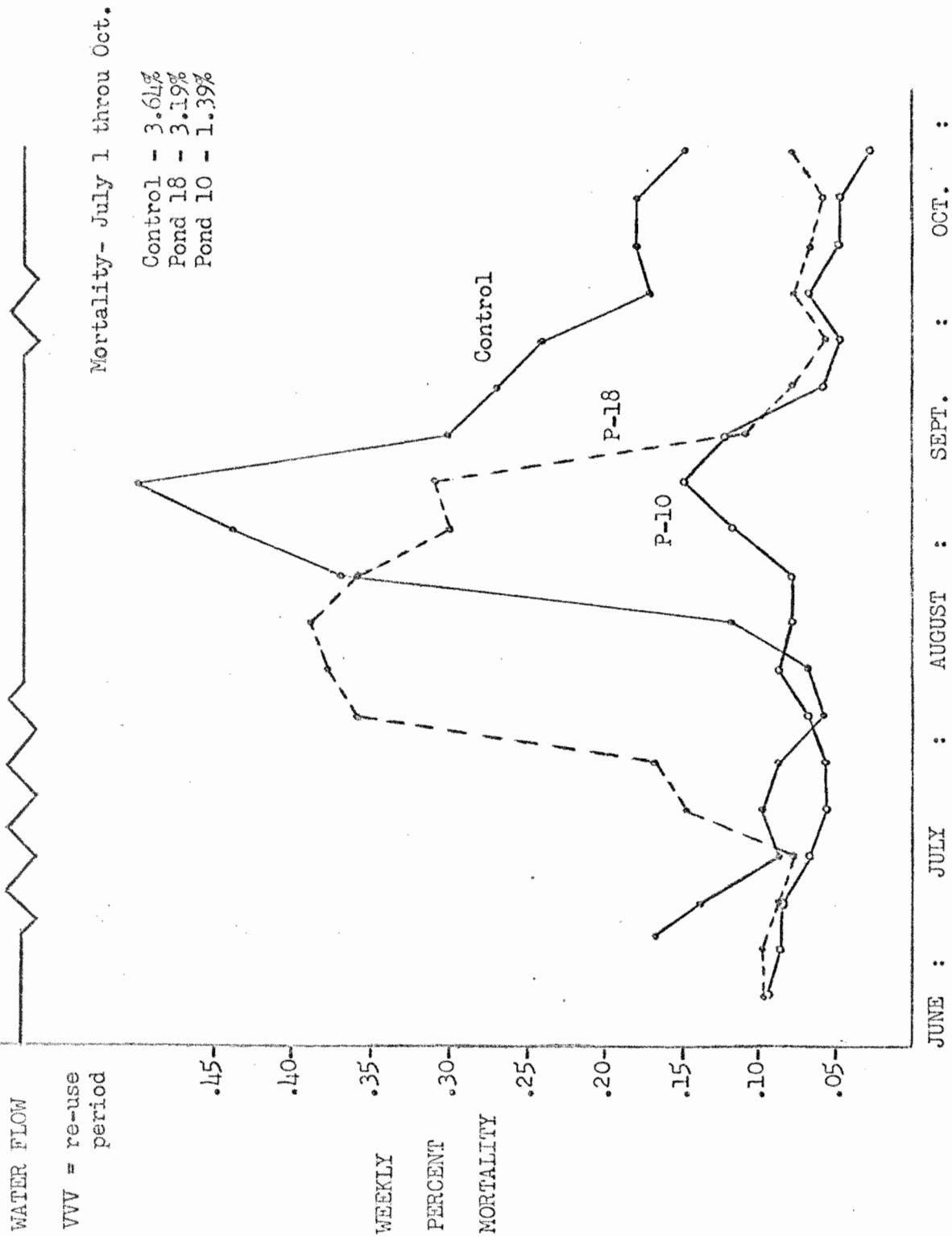


FIGURE 2. - Mortality of two FSA ponds and the control group. P-18 received the maximum of re-use water, P-10 the minimum of re-use water.

FAILURE TO ORALLY IMMUNIZE JUVENILE COHO SALMON
UNDER HATCHERY PRODUCTION CONDITIONS

James W. Wood
Washington Department of Fisheries
Seattle, Washington

Following apparent success in protecting juvenile coho at Issaquah Hatchery against furunculosis disease in 1966, it was decided to test the oral vaccine FSA (furunculosis sonicated antigen) at two hatcheries, Issaquah and Green River, in 1967. FSA for the 1967 tests was commercially prepared and purchased for the purpose of conducting these tests. In addition, a heat killed oral vaccine (HK) was supplied by G. W. Klontz of the Western Fish Disease Laboratory. Both oral vaccines were incorporated into the Oregon Moist Pellet diet.

Issaquah Hatchery

The following groups of 1966-brood coho were involved in the experiment at Issaquah:

<u>Lot Designation</u>	<u>mg Vaccine per Fish</u>	<u>Initial Number of Fish</u>	<u>Number of Ponds After Division</u>
FSA	0.150	320,000	5
HK	0.200	280,000	4
Control	0	320,000	4

Furunculosis disease first appeared during the second week of June 1967. Within a week the disease appeared in all coho ponds. It was allowed to run its course in all ponds without drug treatment. The loss reached a maximum in most ponds during the week ending July 8 and subsequently fell to a near normal level by the week of August 19. No disease, other than furunculosis, was involved from mid-June through the termination of the experiment in late August. Losses encountered by the various groups from June 3 through August 19 are as follows:

<u>Lot Designation</u>	<u>Loss Range of Ponds in Lot</u>	<u>Loss in Entire Lot</u>
FSA	1.7 - 5.4 %	4.2%
HK	2.4 - 10.8	5.6
Control	1.1 - 2.9	2.1

A statistical test (analysis of variance) indicated there is no significant difference in the loss rates of the various groups. The FSA and HK oral vaccines were neither beneficial nor detrimental this year at Issaquah.

Green River

FSA, at a level of 0.150 mg/fish, was given to one-third (500,000 fish) of the hatchery production of 1966-brood coho at Green River. Following pond division in late May and early June 1967, this resulted in 6 ponds in the FSA test group and 11 ponds in the control group. A sharp rise in the loss rate, due to furunculosis, occurred in all ponds shortly after the ponds were divided. Terramycin treatment was administered to one-third of the FSA test ponds and to approximately two-thirds of the control ponds at this time. The loss rate was reduced to normal within a few days in the treated ponds but continued to increase in the untreated ponds, both FSA and control. During the latter part of July the ponds formerly untreated in June were administered Terramycin, however, by this time the loss rate was already declining at a rapid rate. Compared with one control pond left untreated, this late season treatment was of little benefit. The FSA was without apparent benefit in either the Terramycin treated or untreated test ponds.

The following is a summary of the results of the Green River experiment:

<u>Lot Designation</u>	<u>Terramycin Treatment</u>	<u>Percent Loss</u>
FSA	No*	4.2
Control	No*	4.4
FSA	Yes	1.4
Control	Yes	1.4

*Terramycin not administered until late in season

1967 LABORATORY TRIALS AND TESTS OF THE
FURUNCULOSIS ORAL VACCINE PROGRAM

Doug Anderson
Bureau of Sport Fisheries and Wildlife
Western Fish Disease Laboratory
Seattle, Washington

When the first FSA--the name we use for the furunculosis vaccine--was made over three years ago, little did we realize the problems we would experience in making it available to widespread use. The majority of the problems stemmed from the myriad of variables that must be taken into account from the time the organisms are initially isolated, through the relatively complex production method, and finally in the fish. Each step must be carefully monitored with respect to temperature, time, pH, and introduction of stray organisms.

As we learned through repetitive trials the variables presented minimal difficulties if the procedure was followed exactly. However, as our oral immunization trials grew larger so did the FSA requirement. It followed that several potential commercial sources of FSA were sought which resulted in only one favorable reply--Norden Laboratories in Lincoln, Nebraska.

After several weeks, phone calls, and visitations, Norden said they could have one kilogram of FSA for our 1967 field trials. As it turned out they were able to provide us with 300 grams of FSA. The first trial lots were injected into albino rainbow trout at WFDL and the results indicated that, although their FSA resembled gunpowder, it was as effective antigenically as that we made. The chemical analyses were also very encouraging. Thus, we elected to put the Norden FSA into use--the results of which you have already heard.

In looking back on the 1966 hatchery trials we found it hard to believe that the 1967 trials had failed because of the FSA concept. We had tested it too many times to have it fail just because we did not prepare the vaccine. The only explanation that seemed logical was that the FSA did not get to the antibody forming sites in the fish--but why?

To test this theory we fed a group of 1966 brood coho from Dungeness hatchery some of the Norden FSA in the same fashion as it was fed at the hatcheries. For a comparison we fed another group of the Dungeness coho FSA we had prepared two years ago.

To test the efficacy of the two vaccines, lots of fish (75-100) were removed from the main group and exposed to furunculosis infected fish. The challenges were done in duplicate with isolates from Issaquah and from Quilcene. During the course of two months five challenges were done and in every case the WFDL-FSA demonstrated significant efficacy over the Norden FSA.

One interesting sidelight to come out of the challenges was the effect of the location of the challenged groups with respect to each other and the infected groups within the trough. The infected groups were always placed at the head-end of the trough and the WFDL-FSA fish were alternated with the Norden-FSA fish in either the middle or the downstream compartments. When the WFDL-FSA fish were in the middle compartment--upstream from the Norden-FSA fish--the percent deaths in the WFDL-FSA group was one-half that in the Norden-FSA group. However, only a few percent less deaths occurred in the WFDL-FSA fish when they were downstream to the Norden-FSA fish. Also, there was no significant difference in percent deaths in the Norden-FSA fish no matter where they were placed with respect to the WFDL-FSA fish.

In addition to the live-dead figures we determined by the fluorescent antibody technique that there was very little if any circulating antibody in the Norden-FSA fish while all the WFDL-FSA fish had easily detectible amounts of circulating antibody.

It is, therefore, our conclusion that the Norden-FSA, though highly antigenic, was unable to reach the antibody forming sites in the fish. This was probably due to the particle size being too large to pass through the gut wall; but we do not know this for sure at this point.

COLUMNARIS EXPOSURE AND ANTIBODY PRODUCTION IN
SEAWARD AND UPSTREAM MIGRANT SOCKEYE SALMON*

M. P. Fujihara
Battelle Memorial Institute
Pacific Northwest Laboratory
Richland, Washington

Chondrococcus columnaris population estimates made at various Columbia River dams suggest ladder facilities are probable sites for repetitive exposure of migrant salmon to the disease. Using antibody production and isolation of the pathogen as criterion of exposure, salmon passing through upper river ladders showed a higher incidence of antibody development and infection than at lower river ladder facilities.

Columnaris exposure during seaward migration was slight until the juvenile salmonids reached the Columbia River estuary. Duration of exposure at the estuary was probably insufficient to cause mortality. Although most of the adult sockeye salmon lost residual titers developed as juveniles, all the adults were exposed upon reentering fresh water and developed antibodies. Many developed high agglutinating titers by the end of upstream migration and were probably immune to the disease.

Control rainbow trout exposed to columnaris present in Bonneville ladder water, and to organisms released from stock rainbow trout in the laboratory, did not die from exposure but developed antibodies against the disease.

*This paper is based on work performed under United States Atomic Energy Commission Contract AT(45-1)-1830

SIZE AND SENSITIVITY OF TROUT TO AFLATOXIN B₁

J. E. Halver, G. N. Wogan,¹ L. M. Ashley and R. R. Smith
Bureau of Sport Fisheries and Wildlife
Western Fish Nutrition Laboratory
Cook, Washington

Rainbow trout fed 20 ppb aflatoxin B₁ in the test diet after 4, 8, 12, 16 or 20 weeks of CTD developed different degrees of hepatoma directly related to size before insult, whereas control fish fed CTD failed to exhibit even one hepatoma after 20 months on test. Response in numbers of gross hepatoma per group varied inversely with age before fish received the toxin. Total dose per fish was nearly constant at 10 mcgm during the 20 month test period.

Another study showed total dose at an early age spread over 2 to 20 weeks resulted in nearly the same total numbers of tumors at termination. More aflatoxin B₁ only increased tumor incidence slightly but when 0.4 mcgm was ingested over the first four weeks, growth was impaired and some acute liver damage appeared early in the experiment. Survivors developed classical hepatoma at 12 or 20 months of treatment.

Positive controls showed over 90% with hepatoma at 20 months of continuous aflatoxin B₁ insult and some fish had metastasis to other organs. Coho salmon fed the same dietary level of aflatoxin B₁ as the positive trout controls failed to develop a single gross hepatoma after 20 months on experiment.

¹Cooperator, Department of Nutrition and Food Science,
Massachusetts Institute of Technology, Cambridge, Massachusetts

DIQUAT FOR TREATMENT OF COLUMNARIS IN COHO SALMON

Reginald E. Morgan
Bureau of Sport Fisheries and Wildlife
Western Fish Disease Laboratory
Seattle, Washington

Diquat, an aquatic herbicide, was tested in vitro and in vivo for control of columnaris disease. The compound was non-toxic to rainbow trout and coho salmon under a variety of environmental conditions. In vitro testing of Diquat showed that a concentrate of over 10 ppm Diquat liquid was necessary to completely inhibit Chondrococcus columnaris in a one-hour treatment. Eight ppm Diquat failed to control the disease in experimentally infected coho salmon. Treatment at 16 ppm Diquat was effective when two or more consecutive treatments were given immediately following exposure of the fish to the pathogen. However, the degree of control was lessened when treatment was delayed a day or more.

A duplicate experiment confirmed these results, and the fish which received the most treatments experienced the least loss. Paraquat and PMA also showed some degree of control. However, none of these treatments completely controlled the disease because once treatment was discontinued, all fish died from the disease. It has not been determined how many treatments are required to adequately control the disease.

CERATOMYXA SHASTA INFECTIONS IN SALMONIDS

J. E. Sanders and J. L. Fryer
Oregon State University
Corvallis, Oregon

Ceratomyxa shasta occurs widely among adult salmonids returning to areas of the Columbia River basin located in Oregon. Epizootics in juvenile salmonids, caused by this parasite have been observed at Bonneville and Little White Salmon hatcheries and at several locations in the Deschutes River. The infections at Bonneville and Little White Salmon hatcheries occurred only after the use of mainstream Columbia River water for rearing purposes. Both of these hatcheries are located below the confluence of the Columbia and Deschutes Rivers. These observations suggested that the Columbia River above its confluence with the Deschutes River might not contain the infectious stage of Ceratomyxa shasta. Studies were carried out to examine this possibility and to determine the distribution of the parasite in the Deschutes River basin.

Liveboxes containing coho salmon or steelhead were installed at five locations in the Columbia River system. Four of these sites, John Day, McNary, Ice Harbor (on the Snake River) and Priest Rapids dams, were located upstream from the mouth of the Deschutes. The fifth site, The Dalles Dam, located downstream from the confluence of the Deschutes and Columbia Rivers served as a control group. An additional control lot was placed in the Deschutes River at the Oak Springs Hatchery. Seventy-five fish exposed from 22 to 30 days were recovered from the four locations above the confluence of the Deschutes and Columbia Rivers. No infections caused by Ceratomyxa shasta were found in any of these fish. Infections caused by this parasite were present in both control groups. These results suggest that in Oregon the infective stage of Ceratomyxa shasta is limited to the Deschutes River drainage area and the Columbia River below its confluence with the Deschutes River.

The three major tributaries of the Deschutes River, Metolius, Deschutes (mainstream) and Crooked Rivers, join at Lake Billy Chinook (formed by Round Butte Dam). Liveboxes were placed in each of these rivers just prior to their entrance into the lake. Infections caused by Ceratomyxa shasta were observed in only the mainstream Deschutes sample. Ceratomyxa shasta was found to be the agent responsible for a fish kill which occurred at Suttle Lake. Species primarily infected were rainbow and

brown trout, however several diseased Atlantic salmon were also observed. Suttle Lake is drained by Lake Creek which then enters into Metolius River. Results from these experiments indicate that the Deschutes (mainstream) and Metolius Rivers contain the infective stage of Ceratomyxa shasta. The disease is probably prevented or retarded in the Metolius River, due to low water temperature (high 40's maximum).

Infectivity experiments were also conducted at Bonneville Hatchery. Fall chinook and coho salmon were exposed to Columbia River water for a seven week period (February 7 to March 28), then removed and placed in Tanner Creek water (the normal hatchery supply) until August 11. No infections caused by Ceratomyxa shasta were observed. This experiment suggests that salmonids exposed to Columbia River water during this period do not become infected with this disease. Low water temperature and/or the possible absence of the infectious stage of Ceratomyxa is believed to be the principal factor involved in these results.

Groups of fall chinook and coho and later kokanee and coho were exposed to Columbia River water. Spores of Ceratomyxa shasta were detected in only 3% of the fall chinook and 5% of the kokanee. All of the coho examined in the first experimental group and 53% of the second group were infected with Ceratomyxa shasta. These experiments suggest that coho are more susceptible than fall chinook or kokanee to infections caused by Ceratomyxa shasta.

Filtration experiments using a 0.45 μ pore size Millipore filter were successful in removing the infectious stage of Ceratomyxa shasta from Columbia River water. Coho salmon exposed to this filtered water for 30 days failed to become infected while a second group exposed to unfiltered Columbia River water experienced a 53% loss due to Ceratomyxa.

Coho salmon, infected with Ceratomyxa shasta, as determined by sampling, were placed in filtered (0.45 μ pore size) Columbia River water. The effluent from this infected group was siphoned into an aquarium containing previously unexposed coho. Presumably the infectious stage of Ceratomyxa shasta released from the infected group would cause the disease to appear in the second group. No infections caused by Ceratomyxa shasta were observed in the previously unexposed fish. A duplicate experiment again failed to transmit the disease in filtered Columbia River water.

ACKNOWLEDGMENTS

This work was supported by the Fish Commission of Oregon, project 815. We are indebted to the Oregon Game Commission for their assistance and support of the work performed in the Deschutes River study. The authors are also indebted to Mr. John Conrad, State Fish Pathologist, Fish Commission of Oregon, for the data collected at the Oak Springs sampling site.

ADVANCES IN DRY SALMON FEED

R. E. Noble
Washington State Department of Fisheries
Olympia, Washington

The advantages inherent in being able to use a complete dry ration for feeding salmon or trout remain unchanged from the list presented on previous occasions.

Transportation savings
No freezers needed, less space required for storage
Better quality control, less chance of spoilage
Ability to use automatic feeders
Better size distribution and uniformity of pellets
and others

These represent the major advantages of dry feed over a moist product.

Based upon these factors and an obvious need for a suitable dry salmon feed, a project proposal, Evaluation of Dry Feed for Hatchery Salmon, was presented to the Commercial Fisheries Research and Development Act PL 88-309 by the Washington State Department of Fisheries. The Department of Fisheries, in turn subcontracted, by means of a Service Contract, with the University of Washington to provide facilities and administer the technical steps necessary to develop a dry fish feed suitable for salmon.

The program is presently in the second year of study and several points of interest have evolved.

The dry diet as prepared by Dr. Alexander Dollar in the first year of study was a complete failure when fed to fall chinook. The diet, incidentally, was a modification of the Idaho production diet. Dr. Dollar has left the University and his place was taken by Dr. George Pigott, who is presently in charge of the work being done at the University of Washington.

With the change of personnel the general direction of research was changed from compounding and evaluating a dry salmon diet to methods of preparing diet ingredients and of developing commercially feasible techniques of compounding or pelletizing dry feeds.

Techniques for preparing pellets that were less dense than those made by conventional methods were investigated.

A satisfactory technique of preparing a low specific gravity pellet was developed. This consists of atomizing fine droplets of water on a vibrating layer of meal. By controlling the water droplet size, a pellet of desired size can be obtained. This technique involves an increase of approximately 10% moisture, so pellets must be dried prior to storage. It was discovered that a commercial iron ore pelletizer was available, using the same principles developed for fish feed. This machine is made by the Dravo Company and consists of a small inclined, rotating disc above which spray nozzles are arranged.

Preliminary feeding tests indicate that this technique of preparing pellets does not significantly affect nutritional components.

The pellets were formed from the Abernathy diet and compared with moist Abernathy diet and the regular Oregon moist pellets in a feeding test using fall chinook as the test animal.

The test fish were fed 125 days and the percent gain for fish fed the dry diet was 2094 percent as compared to 2047 percent gain for the moist fed Abernathy diet. The fish receiving the Oregon moist pellets had a gain of 2459 percent over starting weight.

Survivals from start of feeding were 95%, 96% and 97% for fish fed Oregon moist, Abernathy moist, and Abernathy dry, respectively. These diets will be retested in 1968.

HISTOPATHOLOGY ASSOCIATED WITH POSSIBLE TOXAPHENE
AND 2,4-D TOXICITY IN RAINBOW TROUT

William T. Yasutake
Bureau of Sport Fisheries and Wildlife
Western Fish Disease Laboratory
Seattle, Washington

Dr. Rucker's paper, "Ground Water Toxic to Fish," discusses the possible association of toxaphene and esters of 2,4-D (chlorinated hydrocarbon compounds) with a chronic mortality at the Shelton hatchery. Unusually high rainbow trout mortalities have been experienced in a number of Washington State Game Department hatcheries for the past several years. No evidence of bacterial, viral, or parasitic organisms could be found to explain this condition. However, histological examination showed a consistent diffuse hepatic parenchymal cell degeneration. This type of histopathological picture suggested that the etiological agent could be an environmental toxin or toxins. Liver cell changes were not localized but appeared to occur throughout the whole organ. In an earlier stage of the disease, liver cells of the fish exhibited numerous degenerating cells. In the terminal stage of the disease necrotic areas were prevalent. Occasional kidney tubule involvement was also found but was not as consistent as the liver pathology. No significant changes were observed in other tissues.

TOXAPHENE POISONING OF RAINBOW TROUT

J. David Erickson
Snake River Trout Company, Inc.
and
Idaho Springs Trout Company
Buhl, Idaho

In 1965 the Idaho Springs Trout Company with facilities near Hagerman, Idaho, set about to increase production of market trout. Three large ponds with a total capacity of six acre-feet were constructed.

A concrete dam and adjustable headgate were constructed on Billingsley Creek for the purpose of diverting a part of this stream into the three ponds.

This spring-fed stream, running at a flow of 100 to 180 cubic feet per second at 55° to 65°F was considered ideal for trout rearing. However, the possibility existed that insecticides or herbicides could enter the stream from farm and ranch operations upstream. To prevent such an occurrence, the Idaho Springs Trout Company management set in motion an information program.

The information program was an attempt to inform all land and water users in the area of the dangers inherent in improper use of chemicals and of their liability in the event of fishery damage. There was no damage from agricultural chemicals during the period December, 1965, through February of 1967. Chlorinated insecticide levels in Billingsley Creek were less than 0.0001 PPM. Organic phosphate insecticides were not detectable at sensitivity of 0.001 PPM. The new trout rearing facilities performed exceptionally well during this period. On the morning of March 28, 1967, the ponds held approximately 200,000 pounds of trout.

At this time there was a sudden mortality and distress among trout in the ponds and in the creek. Company employees immediately removed 600 pounds of dead trout from Billingsley Creek. These were wild trout and fish which were previously planted by the Idaho Fish and Game Department. The distress and mortality indicated an obvious toxicant.

Within two hours, personnel of the Idaho Springs Trout Company and the Idaho Fish and Game Department located the sheep spraying operation responsible for the fish kill. The spray

equipment was set up approximately 40 feet from a tributary stream to Billingsley Creek at a distance of one mile upstream from the trout ponds. The equipment consisted of a compressor-mixing tank, high pressure hose, crowding chute (for holding individual sheep) and insecticide of 43% to 45% toxaphene.

The owners of the sheep admitted to using 13 gallons of the insecticide on March 26 and 27. Some of the material drained from the sheep into the tributary stream. The sheep also waded the stream after being sprayed. Photographs and water samples taken at this time for evidence established the responsibility for fish lost to both the public and private fisheries. However, the owners of the sheep operation remained skeptical. How could a small amount of insecticide kill fish in a large stream?

Herein lies the difficulty in obtaining public awareness. Many land and water users have no concept of the minute quantities of insecticides sometimes involved in water pollution. Information such as that provided in Table I should have adequate circulation among people involved in fisheries, conservation, recreation activities, and agriculture. Each case of pollution damage to a fishery needs to be thoroughly studied to obtain more information.

In this regard, events and data on the "Billingsley Creek case" have been recorded. The public lost more than 600 pounds of rainbow trout from a one mile stream section. Idaho Springs Trout Company lost 10,000 pounds of rainbow trout which died throughout a three week period. The company was also faced with a delay in marketing the survivors because of toxaphene residue.

To examine the extent of the residue problem, samples were taken periodically by both the trout company (note Table II) and the Federal Food and Drug Administration. In comparison with mutton and beef standards the toxaphene level in these trout was found to be minimal. FDA officially approved the trout as marketable on July 7. Having the residue problem solved, the company management turned to the question of compensation for the 10,000 pound loss.

Because of thorough documentation--including photos, witnesses, recording of events, and analyses--the collection was no problem. The sheep operator's insurance firm paid in full both to the state and to the trout company for losses incurred.

Although this was a very serious loss to the Idaho Springs Trout Company, there were certain benefits gained by the experience. Certainly those in the area of Billingsley Creek fisheries will use agricultural chemicals more judiciously. This experience should provide a point to those of us who rear fish and those who liberate fish. That is, you don't have to retreat from those who pollute our waters.

Table I. 24 hour LC50* values of insecticides for rainbow trout. Data provided in 1965 by Fish-Pesticide Research Laboratory. Water was 55°F. Fish were 43-58 mm.

<u>Insecticide</u>	<u>Parts per billion</u>
endrin	0.7
dieldrin	6.0
toxaphene	7.6
DDT	8.0
Perthane	9.0
Strobane	12.0
aldrin	14.0
heptachlor	15.0
methoxychlor	20.0
chlordane	22.0
TDE	30.0
Lindane	30.0
rotenone	32.0
pyrethrins	56.0
naled	70.0
malathion	100.0

*Lethal concentration which kills 50%

Table II. Toxaphene concentration in Billingsley Creek water and rainbow trout from ponds. All analyses done by Wisconsin Alumni Research Foundation.

Date of sampling	Toxaphene concentration in water (PPM)	Toxaphene concentration in trout (PPM)
March 29	.005	0.43 (trout dying)
March 29		0.28 (trout swimming normally)
April 21	No apparent toxaphene*	0.13 PPM (trout swimming normally)
May 22--#1		0.98 PPM (trout
#2		1.30 PPM swimming normally)

*Sensitivity of analysis--.0005 PPM

CHLORINATED HYDROCARBON INSECTICIDES AND FISH

Max Katz¹ and H. E. Johnson²
College of Fisheries
University of Washington
Seattle, Washington

Of all the compounds produced by man, there are none more toxic to fish than the chlorinated hydrocarbon insecticides. Although these insecticides are indispensable in maintaining our standard of living, they have been demonstrated to be an environmental poison and their harmful effects on fish and wildlife have been documented many times over. Surveillance programs conducted by the Federal Water Pollution Control Administration and by agencies of the State of California have indicated the presence of several of these substances in concentrations of a fraction of a part per billion in all of the major watersheds of our country.

Many major fish kills resulting from the chlorinated hydrocarbons have been observed in various parts of the United States but there has been no careful evaluation of the effects of sublethal concentrations of insecticides on fish.

A study was carried out, which is reported in detail by Johnson (1967) to determine the effects upon fish of continuous and prolonged exposure to sublethal concentrations of the chlorinated hydrocarbon insecticide, endrin. Endrin was selected because it is not only a widely used insecticide, but it is the most toxic to fish of any substance known. It is widely distributed throughout the major watersheds of the United States.

The experimental fish was the medaka, Oryzias latipes, the Japanese rice fish. This fish was selected because it is an excellent laboratory species, it becomes sexually mature within

¹ This study was supported by Training Grant (5-T01-ES00019) from the Office of Resource Development, Public Health Service.

² Now at Michigan State University, Fisheries and Wildlife Department, Lansing, Michigan.

three or four months after hatching; and a female, after it starts depositing eggs, will continue to deposit eggs daily for as long as eighty days.

The fish were tested in a specially designed apparatus which was supplied with a constant flow of Seattle tap water. In addition, a constant amount of endrin solution was continually added to the water to give concentrations which varied in the different containers from 1.2 ppb to 0.03 ppb. Several experiments with different concentrations of endrin and a control could be carried on at the same time. Four male and four female fish were placed in each of the tanks, and were allowed to acclimate before the endrin solution was introduced. The eggs that were produced by each fish were removed and were allowed to incubate in separate containers which contained water without any toxicant. Three series of experiments (which lasted from 39 to 99 days) were conducted. The complete details of the experiments are contained in the thesis of H. E. Johnson (1967). The higher concentrations used in the study, 0.3 ppb and above, were lethal to the fish; but at the lower concentrations, for 0.3 ppb to about 0.03 ppb there were serious effects on the developing and hatching fry. Over 75 percent of the fry at the lowest concentration tested, 0.03 ppb, exhibited abnormalities that would have prevented their survival for any length of time.

Observation in the field by several workers has indicated that trout populations have been severely affected by chlorinated hydrocarbon insecticides. George Burdick and his co-workers (1964) in a classic study, correlated the high mortality of lake trout fry in Lake George, New York, with the contents of DDT in their tissues.

Anderson and Everhart (1966) found strong evidence that the decline in the land-locked salmon (Salmo sebago) in Sebago Lake, Maine, was correlated to repeated applications of DDT in the watershed. Jean-Paul Cuerrier and associates (1967) found DDT and its metabolites in the eggs of wild rainbow trout in Jasper Park, Alberta; in the eggs of cutthroat and eastern brook trout brood stock held at the Maligne Trout Hatchery; in rainbow eggs from commercial egg supplies, and in eggs from provincial and Canadian trout hatcheries. There was up to 90% fry loss during the two-month period following the swim-up stage.

In addition, Cuerrier et al. (1967) analyzed 16 commercial dry trout feeds. All of these had DDT and its metabolites in concentrations ranging from 4 to 657 ppb, with most of the feeds having over 100 ppb chlorinated hydrocarbons. These latter data illustrate the complexity of the problems that the fish culturist must contend with, for not only will his fish absorb the pesticides directly from the water but they get substantial quantities of these substances in their food.

Literature Cited

- Anderson, R. B. and W. H. Everhart. 1966 "Concentrations of DDT in land-locked salmon (Salmo salar) at Sebago Lake, Maine." Trans. Amer. Fish Soc. 95, 160-164.
- Burdick, G. E., E. J. Harris, H. J. Dean, T. M. Walker, J. Skea and D. Colby. 1964 "The accumulation of DDT in lake trout and the effect on reproduction." Trans. Amer. Fish. Soc. 93, 127-136.
- Cuerrier, J. P., J. A. Keith and E. Stone. 1967 "Problems with DDT in fish culture operations." Naturaliste Can., 94, 315-320.
- Johnson, H. E. 1967 "The effects of endrin on the reproduction of a freshwater fish (Oryzias latipes)". Ph.D. Thesis, University of Washington, 136 pp.

FDA CLEARANCE AND REGISTRATION OF DRUGS
FOR USE IN FISH CULTURE

G. W. Klontz
Bureau of Sport Fisheries and Wildlife
Western Fish Disease Laboratory
Seattle, Washington

The legal basis for the Veterinary Investigational Drug Regulations is found in the Food Additive and New Drugs provisions of the Federal Food, Drug and Cosmetic Act of 1938. The section applying specifically to our field was amended last year to include food and edible game fish. Before a drug can be employed to prevent or to treat fish diseases the using agency and the producer must comply with the specific VID Regulations and submit the following to the FDA:

1. The chemical identity of the drug.
2. A sample label displaying the following information:

"Caution--Contains a new drug for use only in investigational animals in clinical trials. Not for use in humans. Edible products of investigational animals are not to be used for food unless authorization has been granted by the U. S. Food and Drug Administration or by the U. S. Department of Agriculture."
3. The name and address of each investigator to whom the drug is to be shipped, the quantity of drug, and the batch or code mark of each shipment. Such records are to be maintained by the producer for at least two years and be subject to inspection by a representative of the FDA.
4. The approximate number of animals treated.
5. Upon written request by the FDA, the sponsor shall be certain that the chemical is shipped only to investigators who:
 - a. are qualified by scientific training and/or experience to evaluate the safety and/or effectiveness of the chemical,
 - b. shall maintain complete records of the investigations, and

- c. shall furnish adequate and timely reports of the investigation to the sponsor. The sponsor shall monitor such reports and notify the FDA and other investigators of any significant hazards.
6. If the drug is given to food-producing animals (food and game fish), a supplemental statement shall contain the following information:
- a. A commitment that the edible products from such animals shall not be used as food without prior authorization from the FDA or the U. S. Department of Agriculture.
 - b. Approximate dates of the beginning and end of the experiment or series of experiments.
 - c. The maximum daily dose(s) to be administered to a given species, the size of the animals, maximum duration of administration, method of administration, and proposed withdrawal time, if any.
7. Authorization for use of edible products from treated food-producing animals (food and game fish) may be granted by the FDA or U. S. Department of Agriculture under regulations which include, in part, the following:
- a. Data to show that consumption of food derived from animals treated at maximum levels and with minimum withdrawal periods, if any, specified will not be inconsistent with public health, or
 - b. Data to show that the food does not contain drug residues or metabolites.

The VID Regulations also require that the sponsor shall not commercially distribute nor test-market a drug until a New Drug Application is approved by the FDA. As these VID regulations are new to most of us in fisheries, the FDA advises us to comply with them wherever applicable.

UPTAKE AND DISTRIBUTION OF ZINC-65 IN THE COHO
SALMON EGG (Oncorhynchus kisutch)

Gary Wedemeyer
Bureau of Sport Fisheries and Wildlife
Western Fish Disease Laboratory
Seattle, Washington

Zinc uptake and distribution in the developing coho salmon egg were measured using radioisotope tracer techniques. The uptake was affected by pH, temperature, Cu^{++} , a 2,4-fluorodinitrobenzene and the azo dye, malachite green; but not by azide ion or 2,4-dinitrophenol. About 70% of the total accumulated zinc was bound, rather firmly, to the chorion; about 26% was found in the perivitelline fluid, about 2% in the yolk, and about 1% in the embryo. Temperature, pH, inhibitor, and kinetic studies indicated that zinc uptake involved physicochemical sorption to the chorion together with passive diffusion into the yolk and embryo.

Table I. Zinc⁶⁵ distribution in the eyed coho salmon egg following incubation in 0.02 μC of radioactive zinc⁶⁵, 12°C, 120 min. pH7.

Sample	Uptake (cpm/gm)	Zinc Distribution (%)
Chorion	29,457	70.8
Perivitelline fluid	10,900	26.1
Embryo	973	2.3
Yolk	348	0.8
Incubation medium	1,232	-

Table II. Effect of malachite green on zinc uptake by the coho salmon egg. One hour treatment: 45 min. incubation with 2 μ C/ml zinc⁶⁵, 12°C.

Malachite green ppm	Yolk (cpm/gm)	Chorion (cpm/gm)
0	265	20,389
1	274	20,300
5	1,590	18,650
10	2,230	16,951
25	8,510	11,620

DELAY IN SPAWNING CAUSED BY HIGH WATER TEMPERATURES

James W. Wood
Washington Department of Fisheries
Seattle, Washington

A comparison was made of the spawning records for the last four years that eggs were artificially taken from Cowlitz River spring chinook. These were the years 1951 and 1952, when eggs were taken from fish held in the river at the Ohanapecosh Station; 1965, when fish were trapped at Mayfield Dam and hauled to the George Adams Hatchery on Hood Canal; and 1967, when fish were trapped at Mayfield Dam and hauled to the new Cowlitz Hatchery. The fish hauled to George Adams Hatchery in 1965 were held in a pond modified as a prototype of the holding ponds at Cowlitz which were utilized for the first time in 1967.

It was noted that the adults held at Cowlitz Hatchery in 1967 matured late compared with those held at George Adams Hatchery in 1965 or with those held at the Ohanapecosh Station in 1951 and 1952. In addition, adults released above Mayfield Dam in 1967 were observed, during stream surveys by Jack Thompson, to spawn at their normal time in the Ohanapecosh. In considering the various factors that might be responsible for the delay in maturation (type of holding facility, drug treatment, and light), the drastic difference in water temperatures between Cowlitz Hatchery and the other locales appeared to be the only factor responsible. Although water temperature records of the Ohanapecosh are incomplete for the entire spring chinook holding season, those available for the warmest part of the summer do not exceed a maximum of 58°F, nor do those for George Adams Hatchery in 1965. In contrast, Cowlitz River water temperatures at the hatchery reached a maximum of 70°F during each of four consecutive weeks in 1967 and exceeded a 65°F average for a six-week period in August and September.

Pertinent water temperature and spawning records for Cowlitz River spring chinook are summarized below:

<u>Location</u>	<u>Year</u>	<u>Week of Peak Spawning</u>	<u>Maximum Water Temperature During Holding</u>
Ohanapecosh Station	1951	September 1	57°F
" "	1952	" 6	58
George Adams Hatchery	1965	" 16	58
Cowlitz Hatchery	1967	October 21	70
Ohanapecosh River	1967	September 2	est. 58

Liver, kidney, and spleen samples taken on September 5, 1967 from the adults held at Cowlitz Hatchery revealed pathological changes identical to those described in the literature from spawned-out chinook and sockeye. Tissue degeneration in these adults had apparently taken place at a normal rate but sexual maturation was delayed for some six to seven weeks by the warm water temperatures.

FISH CAN SMELL SALT

C. L. Johnson, K. Oshima¹ and A. Gorbman¹
Bureau of Sport Fisheries and Wildlife
Western Fish Nutrition Laboratory
Cook, Washington

Studies dealing with the mode of action of thyroid hormone on the central nervous system and behavioral processes have been growing. The present experiments describe the electrical activity in the olfactory bulb of thyroidectomized and control rainbow trout evoked by stimulating the nasal cavity with graded concentrations (0.01M-0.12M) of sodium chloride solutions.

Control and thyroidectomized fish were divided into four test groups: (1) untreated (no injection); (2) saline (physiological saline injected); (3) Tx-10 (total dose of ten micrograms DL-thyroxine in physiological saline); and (4) Tx-100 (total dose of 100 micrograms DL-thyroxine in physiological saline).

Electrophysiological measurements were made on two control and two thyroidectomized fish each day. During the experiment the fish were secured in a plastic trough and the gills perfused with tap water. The brain was exposed from the olfactory bulb to the cerebellum by removing the skull cap with a dental saw. Electrical signals were detected with bipolar stainless steel electrodes placed on the surface of the brain and recorded by a polygraph through an electroencephalographic preamplifier. With this system the effect of midbrain separation, olfactory tract section and thyroxine administration were measured. The data have permitted some conclusions concerning: (1) the pattern of spontaneous and sodium chloride induced electrical activity in the olfactory bulb, (2) the centrifugal influences of the more posterior parts of the brain upon the olfactory bulb and (3) the differential sensitivity to thyroid hormone of isolated bulbar and midbrain elements in thyroidectomized and intact rainbow trout.

The internal and external environments are equally important interacting systems on which the survival of the fish depends. We believe that studies of this design will be an important tool in elucidating behavioral as well as physiological processes.

¹Cooperators, Department of Zoology, University of Washington, Seattle, Washington

TRANSPLANTATION OF ADULT COHO SALMON

Roy Sams
Fish Commission of Oregon
Clackamas, Oregon

Approximately 164,000 coho salmon returned to Fish Commission of Oregon hatcheries in 1964. Since this number of fish was considerably in excess of the capacity of the hatcheries, we decided to transplant part of the surplus to other streams to determine if they would spawn in their new environments.

The streams selected to receive adult coho transplants were ones which either had no known coho runs, had barriers preventing salmon migration, or had recently been opened by habitat improvement such as laddering or blasting obstructions. Preference was given to the first two of these situations since there was a possibility of contamination in the third category from remnant runs which could create problems when we tried to determine the success of spawning.

During the fall and winter of 1964, 38,185 coho adults were transplanted into Columbia River tributaries and Oregon coastal streams. Approximately 200 fish were hauled in each truck load, requiring 191 trips. Trucks from the Oregon Game Commission, Washington Department of Fisheries, and Bureau of Sport Fisheries and Wildlife assisted in hauling the fish.

Approximately 7,000 of the hatchery-surplus coho were released into Willamette River tributaries above Willamette Falls at Oregon City. We decided to use the Willamette system to evaluate the success of spawning by the newly transferred coho. Eleven tributaries, six where no coho runs existed and five where coho runs were believed to be well below their production potential due to partial migration barriers, were selected as evaluation streams.

Spawning ground surveys were conducted on 10 of the 11 streams originally selected. A total of 39 stream miles was surveyed. These 10 streams had received 6,185 coho. A total of 1,053 fish and 939 redds were counted during the surveys. On six of the streams, where essentially all of the stream above the release site was surveyed, the number of redds counted expressed as a per cent of the total females introduced ranged from 43 to 101% and averaged 64%.

The fish generally migrated from 2 to 5 miles upstream before spawning, if no barriers were present. In one instance, they migrated 19 miles above the liberation site. In six of the study streams, some fish migrated downstream (generally under 1 mile) before spawning. Where tributaries entered near a liberation site, they were also used for spawning. From 3.3 to 41.7% of the fish transplanted into a particular stream were observed in the tributaries of the receiver stream.

Seven streams were surveyed in March 1965 to determine the success of fry emergence. Fry were observed in six of the streams and were fairly numerous in two of them. Three of the study streams were checked in August and September and yearling coho were observed in all three, being quite abundant in two of them.

As a result of working with transplanted coho in 1964-65, the following determinations were made:

1. Transplanted adult coho spawned quite successfully.
2. The fish distributed themselves relatively well in the receiver stream and its tributaries.
3. Juvenile coho resulting from the transplants survived through the low summer flow in at least three of the study streams (the only ones investigated for this purpose).

Additional information regarding the 1964 adult coho transplant study is presented by Pearson, Conover, and Haas, unpublished.¹

As a result of the demonstrated success in transplanting adult coho in 1964, the practice has continued through the 1967 run. A total of 28,244 hatchery-surplus coho were transplanted in 1967 bringing the total number so handled for the four years to 119,188 fish. Of that number, 24,000 have been released in Willamette River tributaries where evaluation of this method for establishing runs is continuing.

The present study is to determine the merits of egg, fry, fingerling, and adult coho transplants. Efforts are being directed toward obtaining the distribution and population estimates of yearling fish resulting from each type of introduction. Most of the study streams are above impassable barriers, while the others are believed to not have coho runs.

¹Report in preparation for publication in Fish Commission Briefs

In 1966, three streams which received adult transplants and three which received unfed fry introductions were selected for juvenile population studies. An index unit 2,500 feet long was selected on each stream. The basis for selecting the index area was largely governed by accessibility. However, each was believed to be somewhat representative of the stream as a whole, with a good combination of riffles and pools for rearing.

With one exception, three surveys were made on each stream to determine the size of the yearling coho population. Two surveys were conducted on the other stream. All of the surveys were conducted in September and October after the low summer flow period. The fish were marked with a diagonal clip of the upper caudal lobe on the first survey and the lower caudal lobe on the second survey. The fish population could then be analyzed on the basis of three marks, that is, upper caudal, lower caudal, and both.

The calculated number of yearlings present in the index units on the three streams receiving adult transplants were 1,193, 1,431, and 505 fish, while on the three streams receiving fry plants the number of yearlings present were calculated to be 568, 354, and 1,259 fish.

The study was repeated in 1967 with eight Willamette River tributaries, four of which had adults introduced and four had unfed fry releases. Calculated populations of juveniles present in index units on streams stocked with adults ranged from 883 to 3,001 while those units receiving fry transplants had calculated juvenile populations of 543 to 2,740 yearlings. Either of these ranges compare favorably with calculated populations obtained from studies on five western Oregon streams having natural sustained runs of coho. In the latter case, the calculated populations ranged from 350 to 2,855 fish in a 2,500-foot section of stream.

Originally, we intended to survey in the fall and winter of 1967 for the presence of adults in those streams which had received adult coho transplants in 1964. However, we decided to forego the surveys due to the large number of hatchery-surplus coho available in 1967 and our desire to quickly bring the Willamette system to its fullest production for the species. We felt we were obligated to take this approach since a large share of the justification for building the new 3.5 million dollar Willamette Falls fishway was based on the potential for this species. Also, we believed the ultimate evaluation of our efforts will be in the trend of the annual counts of coho passing above Willamette Falls.

Because of our efforts in transplanting fry, fingerlings, and adult coho, we had expected a much larger escapement of coho above Willamette Falls in 1967 than those obtained in 1965 and 1966. The 1967 escapement was calculated to be 8,700 fish compared to escapements of 9,264 in 1965 and 6,318 in 1966.

The Willamette system received considerable damage from a devastating flood in the winter of 1964. Stream surveys showed that considerable changes were made in the stream bottoms, that gravel bars known to contain many redds were completely removed from some streams, or were left high and dry as a result of channel changes. The effects of the flood could have significant bearing on the size of the 1967 Willamette escapement. Actually, the coho escapement may have been considerably smaller had it not been for our efforts with the 1964 brood. This is borne out by observations this year of adult coho where they had not been observed before. Most of these fish observed were returns from fry plants.

In summary, the conclusions derived from our studies are:

1. Coho adults spawned successfully in all of the streams examined.
2. Good numbers of fry resulted from the transplants.
3. Populations of coho yearlings resulting from adult transplants compared favorably with those produced by natural sustained coho runs.
4. Because of the tendency of the transplanted adults to disperse upstream from the liberation site we felt they were especially useful in situations where access was available to only the lower end of the watershed. This is a common situation in the Willamette River system.

REARING OF COHO AND FALL CHINOOK SALMON IN WAHKEENA POND

Roy Sams
Fish Commission of Oregon
Clackamas, Oregon

Wahkeena Pond was constructed in the spring of 1961 using funds supplied by the Bureau of Commercial Fisheries. Research studies have been conducted continuously since that time using BCF funds. From 1961-65 the studies were to determine the time and rate of stocking that would provide the best survival and growth of coho salmon reared on natural food. In FY 1966, the effect of fertilizing the pond was studied. Four applications of 3,600 pounds each of ammonia sulfate (20% N) and single super-phosphate (20% P) were made between July 21 and September 15.

Fish of the brood years 1961 and 1962 were planted as unfed fry, while those of broods 1960, 1963, and 1964 were fed for 60 days and weighed from 267 to 360 to the pound when stocked in the pond. The number of fry planted was approximately 101,000 each year except for the 1961 brood when about 400,000 were stocked.

Survival to time of release into the Columbia River ranged from 2.4 to 13.5% for those broods stocked as unfed fry and from 8.4 to 51.0% for those stocked after 60 days of feeding.

The size at time of release ranged from 67.5 to 109.0 to the pound for those broods stocked as unfed fry and from 27.5 to 63.0 to the pound for those stocked after 60 days of feeding. Other data concerning these studies are shown in Table 1.

Based on the results of studies of natural rearing of five brood years, it appeared that the best production we could expect was about 50,000 yearling coho salmon. Also, that the fish would be somewhat inferior in size, being between 27.5 and 109.0 to the pound. The next logical step was to determine the results to be obtained from artificial feeding.

In FY 1967 (1966-67) the Fish Culture and Research divisions cooperatively conducted a study to determine the growth and survival of coho and fall chinook salmon reared on Oregon Pellets. Approximately 1 million coho of the 1965 brood were stocked in the pond on May 31-June 1, 1966. The fish weighed 257 to the pound after being fed 60 days at the Sandy Hatchery. Thirty per cent of the fish were marked dorsal-left maxillary before being introduced into the pond. The fish were fed 113,050 pounds of

Oregon Pellets while in the pond. The pond was drained and 571,466 fingerlings, weighing 17.5 to the pound, were released into the Columbia River on February 20-24, 1967. In addition to those fish released alive, an estimated 150,000 died in the silt-laden pond on the second day of count out.

As of December 11, 1967, some 3,448 coho jacks from the 1967 release have either been taken in the Wahkeena trap, recovered at Columbia River hatcheries, or known to be caught in the sport fishery conducted near the pond outlet. This is a very minimal figure since only limited creel censuses have been made. Approximately 22.3% of the returning jacks have been marked.

From March through June 1967, the rearing of artificially fed fall chinook was studied. One million 1966 brood fish were stocked in the pond on March 22-23, 1967. They weighed 430 to the pound after being reared at Oxbow Hatchery for 50 days. A total of 12,750 pounds of Oregon Pellets was fed from March 23 to May 31, 1967. By May 19, the fish weighed 90 to the pound.

In view of the rapid growth of the fall chinook and a predicted prolonged high spring freshet of the Columbia River, we decided to see if the fish would leave the pond of their own volition. The Columbia River was already too high to allow drainage of the pond. We diverted all Wahkeena Creek water into the pond and pulled the screens and stop logs, allowing the overflow to return to the creek through the secondary outlet. A 100-foot seine, 8 feet deep with 1/4-inch stretch mesh was attached to the center of the overflow culvert and extended out into the pond in hopes it would lead the young fish to the outlet. A wire mesh trap was constructed at the downstream end of the culvert to capture the outmigrants.

The trap was operated approximately 16 hours a day from May 25 through June 5. A total of 671,638 fall chinook averaging 86 per pound migrated from the pond. The rate of migration ranged from 126,137 fish on the second day to 1,989 fish on the last day the trap was operated. The pond was drawn down on October 13, 1967, and 81,040 fall chinook, weighing 42.4 to the pound, were released into the Columbia River. This brings the total reared to 75.2% of the number stocked.

Wahkeena Pond will be stocked with 3 million fall chinook in March 1968.

Table 1. Yield and survival data for coho salmon reared in Wahkeena Pond, 1961-67.

	Brood Year					
	1960	1961	1962	1963	1964	1965
Date introduced	5/23/61	3/30/62	4/5/63	5/19/64	5/17/65	6/1/66
Number introduced	101,150	400,500	101,000	101,000	101,000	1,000,052
Size of fish introduced per pound	360	1,300	1,220	360	267	257
Length of rearing period (days)	273	336	318	281	267	265
Per cent survival for rearing period	39.5	2.4	13.5	51.0	8.4	72.1 ¹
Number of fish released	38,960	7,857	12,939	50,154	7,034	571,466
Fish per pound	44.0	109.0	67.5	63.0	27.5	17.5
Total pounds of coho reared	894.0	86.3	202.0	822.3	511.9	41,274

¹ Includes an estimated 150,000 killed by silt when the pond was drawn down.

A PROGRESS REPORT ON THE INTRODUCTION OF COHO SALMON
IN THE GREAT LAKES

T. B. Durling
Michigan Department of Conservation
Lansing, Michigan

In the spring of 1966, the Michigan Department of Conservation planted approximately 850,000 coho salmon smolts in three streams tributary to lakes Superior and Michigan. These smolts were reared from eggs generously donated by Oregon.

Marked differences in the growth, distribution, and return patterns were exhibited by the fish in the two lakes.

The Lake Michigan fish were stocked in Bear Creek, a small tributary of the Manistee River at a point approximately 40 miles from the lake, and in the Platte River approximately 19 miles from the lake. The Bear Creek smolts averaged 16 per pound while the Platte River fish weighed 20 per pound. The plantings were made between March 22 and March 25, 1966.

It was estimated that about 10,000 of these fish returned as "jacks" to the streams in which they were released in October and November, 1966. The fish ranged in weight from one to seven pounds and averaged two pounds. Thirty two precocious females were spawned and produced apparently normal fingerlings which have been reared to a present weight of 36 per pound.

During May, 1967 reports were received that commercial fishermen were harvesting substantial numbers of coho salmon in Indiana waters in the extreme southern portion of Lake Michigan. Before required administrative procedures to restrict such fishing could run their course, about 30 days elapsed and about 22,000 coho were taken.

Michigan commercial fishermen's reports of small numbers of coho being taken incidental to fishing for other species indicated that the fish began to move northward during the late spring. A minor mortality from kidney disease occurred during the summer. Geographical distribution of the dead fish indicated the fish continued their northward migration through the summer.

Sport fishermen, with the aid of exploratory fishing effort conducted by the Department of Conservation, finally located

concentrations of cohos near the mouth of the Manistee River and in Platte Bay, near the mouth of the Platte River in late August, 1967.

A statistically based creel census yielded an estimate that 26,000 cohos ranging up to 22 pounds and averaging 11.5 pounds in weight were taken in about 70,000 angler days of fishing effort between early August and mid-October. As many as 900 boats were observed from a single observation point off Manistee on a particular weekend day.

An excellent fishery developed in the Manistee River itself. A creel census yielded an estimate that 5,000 fish were taken between mid-September and mid-October. Stream fisheries in Bear Creek and Platte River were closed early in the run because it was believed fishing activity was obstructing the runs to the spawning stations, and because the fishermen persisted in attempting to take the concentrated fish by snagging, netting and by hand.

Approximately 8,000 adult salmon were stripped for spawn at the newly constructed Platte River spawn taking station and 7.8 million eggs were taken.

Fifteen thousand green salmon in the ratio of 5 males to 4 females were transferred to selected streams in the Lake Michigan and Lake Huron watersheds to assess natural spawning potential.

An additional 77,000 fish were captured in the Platte River and Bear Creek weirs. These were donated to state and charitable institutions or were sold through commercial marketing channels.

Thus, a total of about 163,000 mature salmon have been accounted for in returns from the initial release of 659,400 in Lake Michigan tributaries. This represents an accountable survival of approximately 25%.

Very limited straying was observed in the Lake Michigan fish, with confirmed reports of salmon observations being received from only 5 streams other than the two which were planted.

The Lake Superior fish were released in Huron River only 7 miles above the mouth on May 16 and 17, 1967. The fish migrated into Lake Superior in less than 24 hours.

A small number of jacks were caught in a trap installed in the Huron River during the fall of 1966. These fish were considerably smaller than the Lake Michigan fish, ranging from 10 to 16 inches in length and averaging 14 inches and 1 pound in weight.

Beginning in April of 1967 and continuing throughout the summer and early fall, reports were received of excellent coho fishing in most of the protected bays along the Wisconsin and Michigan shores of Lake Superior. Cohos commonly appeared in the catch of a traditional lake trout troll fishery along the exposed shore of Lake Superior west of the Keweenaw Peninsula.

By October, it became apparent that the Lake Superior cohos were straying to a considerable degree, as reports were received of concentrations off stream mouths and in streams in Minnesota and all along the Wisconsin and Michigan shoreline.

A concentration of adult fish averaging 4 pounds in weight occurred off the mouth of the Huron River in October and provided an excellent fishery. The stream fishery was closed in the Huron River because of obstruction of the run and because of illegal fishing activity. Although only a small percentage of the Lake Superior fish were ripe when captured in the Huron River trap, around 100,000 eggs were taken.

Some effort was made to statistically assess the angler harvest, but the wide distribution of the fishery made it difficult to obtain an acceptable estimate. However, we believe that an angler catch for Michigan waters alone of 15,000 fish is reasonable. Many more were caught by sportsmen in Minnesota and Wisconsin and incidental to the commercial fishery in all three states.

It was observed that the eggs taken from Lake Michigan fish were extremely delicate and required careful handling to avoid rupturing the yolk. Yolk sac rupture at the time of fry emergence was noted in one small lot of eggs left to hatch undisturbed on egg trays. The balance of Michigan production eggs, which were routinely subjected to physical shock to separate the "blanks," hatched normally and normal fry development is occurring.

Approximately one million eyed eggs were donated to other Great Lake states and to Ontario on the understanding that the resulting fish would be reared to smolt size and released in Great Lakes tributaries.

A tremendous amount of enthusiasm among mid-Western sportsmen has been generated by the early success of the coho salmon. Proposals for financing an expanded anadromous fish hatchery and fish passage program have been well received by Michigan legislators and we are optimistic about the continuing success and expansion of the program.

GROUND WATER TOXIC TO FISH

Robert R. Rucker
Bureau of Sport Fisheries and Wildlife
Western Fish Disease Laboratory
Seattle, Washington

The Washington State Department of Game hatchery at Shelton was built in 1947. When the first fish hatched the water from the west spring was found to contain excess nitrogen gas. The problem was solved by changing the water supply to the east spring which contained normal amounts of gas. Commercial dry feeds were started in 1956 without incident. About 1960 a higher than normal annual mortality started to develop. Some of the fingerlings became listless, were dark in color, and oriented themselves at the sides of the troughs near the surface of the water. Death ensued within 7 to 10 days. Mortalities of 20% to 50% in some groups of fish were attributed to this syndrome.

No parasites of consequence were found. A few bacteria on occasion have been found but none of significance. We were unable to transmit the disease and all attempts to demonstrate the presence of a virus have failed. A type of pathological change in the liver, diffuse hepatic parenchymal cell degeneration, indicated the presence of a toxic agent in the food or in the water.

Chemical analysis of the spring waters showed that the west spring (excess nitrogen) had 0.03 ppb toxaphene and 0.2 ppm esters of 2,4-D, the east spring about half these levels, while the central spring was clean. An analysis of whole fish from one group revealed concentrations of 4 ppb toxaphene and 9 ppm esters of 2,4-D.

From this "spade work" at the Shelton hatchery, I believe we should plan future work on the premise that the ground water is toxic and a cause of the chronic mortalities.

THE UNIVERSITY OF WASHINGTON'S SPAWNING CHANNEL
AT BIG BEEF CREEK

E. O. Salo
K. Victor Koski
College of Fisheries
University of Washington
Seattle, Washington

Included in the facilities at the University's research station at Big Beef Creek near Seabeck on Hood Canal is a 600-foot spawning channel for chum salmon. The channel contains four different grades (as determined by the percentages of fines) of spawning gravel, and each grade is replicated three times. The 10-foot wide channel is partitioned lengthwise to accommodate varying concentrations of spawners. Ninety-six plastic stand-pipes were built in to sample the intragravel water. Although the channel was not completed until December, 50 pairs of salmon spawned in selected spawning pens.

Below the channel three ponds were developed in the intertidal area for (1) the retention of freshwater run-off from the channel during periods of high tides (this is necessary to keep the channel from becoming inundated), (2) the capturing of adults prior to disposition in the channel and (3) the rearing of the fry in freshwater and, if desirable, in salt water. Two of the ponds are equipped with tidal gates and one has a trap for adult fish.

The long-range research and instructional programs for Big Beef include experimental salt-water rearing of chum and coho salmon, of steelhead and cutthroat trout, shrimp culture and oyster farming.

RELATIONSHIP OF FOOD SUPPLY TO FISH PRODUCTION AT FERN LAKE

Paul R. Olson
College of Fisheries
University of Washington
Seattle, Washington

Fern Lake is a 23.9-acre, mineral-deficient lake located in the Puget Sound lowland of western Washington. The Washington State Department of Game, who owns the lake, has made it available to the University of Washington* for studies to improve the productivity. The lake, as well as the watershed, has been the site of ten years' study, relating the production of steelhead trout to that of zooplankton and insect emergence; other limnological observations also have been made.

A summary of the data on the contents of trout stomach indicated that during the winter period, November through January, the trout were unable to find sufficient food, often resulting in a weight loss in the fish. The trout fingerlings planted in June fed mainly on zooplankton and a few emerging bottom insects. During August and September terrestrial insects became important as food. In the winter, with very little else available, the fish fed on zooplankton. In the spring and early summer, as midge emergence occurred, the fish actually moved about the lake seeking bottom areas where emergence was peaking. The fish that did not migrate from the lake as one-year-olds had grown large enough to eat amphipods and young crayfish. In late summer they again relied almost completely on terrestrial insects. As the fish grew larger they preferred larger, bite-size organisms, but if these were not available they fed on zooplankton.

Because the preferred food appeared to be the emergent midge, an intensive bottom study was made in which five bottom types were defined and estimates were made of the standing crop. The estimates of the insect population of the profundal and sublittoral areas were 400 pounds and 65 pounds wet weight per acre, respectively. The overall average for the lake was 230 pounds per acre. However, because of a two-year life cycle and mortality, only 20 percent of the organisms in the profundal zone emerge per year. In contrast, close to 100 percent of the organisms in the sublittoral zone emerge per year. The annual average of insect emergence at Fern Lake is approximately 80 to

*Work performed under Contract AT(45-1)1385 with the United States Atomic Energy Commission.

100 pounds of insects dry weight. It appears, tentatively, from four years' data, that the relationship between total annual dry weight of emergent insects and total pounds of fish produced per year is good.

The zooplankton standing crop in the lake for a nine-year period has varied between 300 and 900 pounds wet weight, or 12 to 46 pounds dry weight. The average increased to 70 pounds following fertilization. The relationship of average annual zooplankton production to pounds of fish produced was not a simple relationship, being influenced by species composition and availability of other preferred foods. There was an indication, however, that when there was a high survival rate of fingerlings, the standing crop of zooplankton (dry weight) was reduced.

Steelhead trout production in Fern Lake for an eight-year period averaged 450 pounds of fish wet weight (19 pounds per surface acre). Regardless of the number of planted fish, varying between 7,000 and 20,000, the survival eleven months after planting was approximately 2,600. One year, as a result of fertilization, the number and poundage of yearling steelhead trout produced were approximately double those of the control years.

Steelhead Trout Data, Fern Lake, Washington

Year	No. planted	One-year survival	Average weight (gms)	Per cent survival	Holdovers at time of plant	Total yield yearlings (lbs)	Total yield holdovers (lbs)	Total annual yield (lbs)
1959-60	20,000	2,400	75	12		370	?	?
1960-61	20,000	3,200	92	16		520	None	520
1961-62	11,000	1,600	75	15	1,000	185	170	355
1962-63	11,000	2,900	70	27		445	None	445
1963-64	11,000	2,200	80	20	1,200	260	170	430
1964-65	7,000	3,200	64	47	600	440	132	572
1965-66*	11,000	5,700	92	52	600	813	158	971
1966-67	11,000	2,700	48	24	1,000	180	178	358
Average one-year survival, except 1965-66: 2,600								
Average yield per surface acre, except 1965-66: 19 lbs								
Yield per surface acre, 1965-66: 41 lbs								

*Application of a ton of mineral nutrients was started August 19, 1965

SOME EFFECTS OF MARKING CHINOOK SALMON BY FIN CLIPPING

Fred Cleaver
Bureau of Commercial Fisheries
Biological Laboratory
Seattle, Washington

A recent study of ocean mortality and maturity rates for fall chinook salmon from Columbia River hatcheries was based on the recovery of marked fish in the fisheries and at the hatcheries. Because marking is known to influence growth and survival of salmon, it was necessary to examine the differences between marked and unmarked fish before applying the results of the marking studies to unmarked hatchery fish.

The data for study were kindly made available to me by the organizations conducting the Columbia River hatchery evaluation program. These include the fishery agencies of California, Oregon, Washington, and Alaska, the Fisheries Research Board of Canada, and the U. S. Fish and Wildlife Service. The hatchery evaluation study was based on release of large numbers of marked fish at 12 hatcheries. The marked fish were roughly 2.5 inches long and about 200 to the pound when released, although the size varied appreciably. Fish bearing a common mark (adipose fin - right or left maxillary bone) were released from all 12 hatcheries. The Kalama hatcheries and Spring Creek were also allotted unique marks (adipose fin - a ventral fin - a maxillary). Differences between marked and unmarked fish in growth, maturity and survival were evident.

Marking was shown to cause decreased growth in chinook salmon among the fish from the 1961-64 broods which had returned to the hatcheries by the fall of 1966. The average marked 2 year old fish was 1.06 inches, 3 year old 0.75 inch, and 4 year old 0.39 inch shorter than unmarked fish. Because maturity is associated with growth, the full effect of marking is probably masked by delayed return among marked fish. Fish which lacked only the adipose fin at return were least affected, but no comparison was made of the damage due to different marks; the effects did not appear consistent from station to station. Removal of part of a maxillary bone proved to be more harmful than I had expected.

The average effects of marking on age at maturity were striking. For all marks at all hatcheries, the average percent of the returning fish that were marked increased from 2.7% for 2 year olds to

3.8% for 3's and 5.0% for 4's. There were few 5's. The degree of delay also varied between hatcheries. It was high at Kalama and hardly apparent at Spring Creek. The causes for the differences are not clear. Perhaps it is related to variation in size or time at release or to differences in ocean feeding ground and maturation schedules.

The relative numbers of marked and unmarked fish that returned to a hatchery were used to estimate the change in survival caused by marking the 1961 brood. The actual returns are of course biased by straying, mark regeneration and delayed maturity; thus it was necessary to adjust the returns for these biases in order to estimate mortality caused by marking. Because only Kalama (AD-RV-RM) and Spring Creek (AD-LV-RM) had large numbers of marked fish for which the hatchery of origin was certain, estimates of loss were limited to these. About 5% of the total spawners for the two marks were recovered from streams or hatcheries other than that from which they came. Because it is unlikely that all strays were found, 5% must be a minimal figure.

Estimates were made of the reduction in hatchery returns due to regenerated fins. At Spring Creek, 17% of the marked fish that returned had a mark that could have resulted from fin regeneration. Accidental fin loss was considered negligible. At Kalama, about 1% of the marked fish were judged to have originated by regeneration of an experimental mark.

The effect of delayed maturity was calculated by substituting maturation rates of unmarked fish for those of marked fish and estimating the return of marked fish that would have been observed if no delay existed. From the return of marked fish with and without delay due to marking, loss due to delay was derived. It was 16% for Kalama and 4% for Spring Creek.

The observed return of AD-LV-RM fish to Spring Creek was only 0.275 as good as that for unmarked; at Kalama the return of AD-RV-RM marks was 0.388 as good as that for unmarked fish. From the above numbers, it was estimated that the Kalama fish were reduced 21% by straying, regeneration and delayed maturity. $(1-.95 \times .99 \times .84)$; Spring Creek returns were lowered 24% by these factors $(1-.95 \times .83 \times .96)$. To account for the remaining differences between marked and unmarked fish other causes of disappearance must have removed fish at the rates of 51% for Kalama and 64% for Spring Creek; 51 and 64% are the fractions of marked fish that were lost for reasons other than straying, regeneration,

and delayed maturity. This represents the post-release death of marked fish which the unmarked fish did not experience.

This analysis by no means exhausts the knowledge that the evaluation program data can yield. It is hardly a beginning. Beyond providing much better estimates of straying, regeneration and marking loss than are provided here, the prospect is rich in knowledge.

For example, even the marked adult fall chinook in the Columbia River fishery were substantially larger at the same age than were the wild fish. The 1961 brood Spring Creek fish (AD-DV-RM) at 3 years averaged 18.2 pounds. The unmarked fish which were a mixture of wild fish and hatchery fish averaged only 14.1 pounds. Not only are there real differences between wild and hatchery fall chinook, but even the hatchery chinook vary substantially between hatcheries--few Kalama fish return in their third year, and they are abundant as 4's and 5's. Most Spring Creek fish that enter the river are in their third year, and few remain to spawn as 5's. In 1965, the young Spring Creek fish that were marked were nearly gone from the river by July. July was the month of greatest abundance for Kalama fish in the Columbia River estuary, and they were common until September.¹ At sea, the Kalama River fish were found farther north than were the Spring Creek fish. Their ranges overlap to a large extent, but the differences are obvious.

From Sims' studies mentioned above, it appeared that much less than 30% of the 1966 plants of hatchery fall chinook reached the sea. Study of mark recoveries at sea indicated that about 1% of the hatchery plants survived to begin their third year of life, the age at which they begin to be caught in large numbers.

In spite of these staggering losses, the data for the 1961 brood suggest that the yield to all fisheries from the Kalama hatcheries was about 500,000 pounds and the yield from Spring Creek was about 1,500,000 pounds. If survival of fingerlings is very low and yield is still great, the prospect of greatly improving yield by small increases in fingerling survival is good.

The hatchery returns were also calculated assuming that no fish were taken at sea. The numbers of hatchery fish returning to the Columbia River would more than double if there were no ocean fishing.

¹From Carl Sims' data, U.S. Bur. Comm. Fish.

FOOD ENERGY AND THE PRODUCTIVE VALUE OF FISH FEEDS

Robert R. Smith
Bureau of Sport Fisheries and Wildlife
Western Fish Nutrition Laboratory
Hagerman, Idaho

A method of feed evaluation is needed which is relatively inexpensive, easily performed and rapid, and which evaluates whole diets or diet ingredients on the basis of their availability to fish. Proximate analysis gives the crude composition of feeds but does not indicate the availability of nutrients. Long-term feeding trials are slow and expensive and therefore are of little use in evaluating any particular batch of feed.

At the Hagerman station methods are being adapted to fish which have been used successfully in other fields of animal nutrition. Feeds are evaluated on the basis of protein quantity and quality and on the availability of the energy producing portion.

The gross energy and nitrogen content of the test material are determined. The test feed is then fed to individual fish in metabolism chambers. Feces, urine, and gill excretions are collected during a suitable test period, usually five days. The excretions are then analyzed for nitrogen and gross energy. From the data are then calculated:

1. The apparent digestibility of the protein and the gross energy of the diet.
2. The biological value of the protein.
3. The metabolizable energy content of the diet.

Recent tests indicate a very close correlation between results of long-term feeding trials and results predicted from metabolizable energy determinations. With the system used metabolizable energy can be determined in about two weeks using five to ten fish.

NIAGARA SPRINGS HATCHERY

Charles R. Quidor
Idaho Fish and Game Department

The Niagara Springs Steelhead Hatchery, constructed by Idaho Power Company, is located on Snake River, eight miles due south of Wendell, Idaho. The base cost of this station was \$750,000. The Idaho Fish and Game Department is contracted to operate the station.

The water source for the station is Niagara Springs, which flows an average of 328 cfs of water at a constant temperature of 58° F.

The hatchery consists of 14 concrete raceways, 300 feet long, 10 feet wide and 4 feet deep. A movable bridge spans the 14 raceways, from which all cleaning, grading, loading and feeding of the fish are done. The feed is stored in two 11-ton bins which are connected to an automatically operated conveyer which fills the 14 feeders connected to the bridge. The hatchery building is an all-steel building, which houses the office, incubator room, storage room, fry feed room, garage, workshop and restrooms. The incubator room is equipped with 20 stacks of Heath vertical incubators, and two Heath 8-foot troughs. A water chiller is located at the lower end of the raceways and is used to drop the water temperature for transporting the steelhead. There are three, three-bedroom, frame residences at the station.

At the present time steelhead eggs are shipped in from Oxbow Hatchery in Hells Canyon. These eggs are hatched and the fingerlings are reared until they reach 7 1/2 to 8 inches in length, at which time they are moved to the Pahsimeroi River where they are released into two one-acre acclimation ponds. These fish are free to migrate out of these ponds at will.

This hatchery, as well as the release ponds and the trapping station that will be built on the Pahsimeroi River, is owned and will be financed by Idaho Power Company. It is operated by three full-time Fish and Game Department personnel, and has an annual production capacity of 3,500,000 eggs, and approximately 1,600,000 smolt.

The purpose of the hatchery is to attempt to relocate the steelhead run that normally ran Snake River. This run will be maintained in Pahsimeroi River, a tributary of Salmon River.

SUGGESTIONS CONCERNING "PROCEEDINGS OF THE NFCC"

Wallace F. Hublou
Fish Commission of Oregon
Clackamas, Oregon

Present organization of the conference is very simple and is as follows: First, next year's chairman is selected at the end of the current conference. Then, about 1-2 months before the next meeting the chairman announces the time and place of the conference and requests submission of titles for reports so he can prepare an agenda. The agenda is distributed, the conference is held, and written summaries of each talk are submitted to the chairman. As soon as possible after the conference, the "Proceedings," consisting of all of the written summaries, are distributed to all persons that attended the conference and to others who did not attend but have requested to be on the mailing list.

There is nothing basically wrong with this procedure. We have been using it for many years and it works fine. However we have problems now that have been sneaking up on us in recent years and that, in my opinion, need to be recognized and given attention.

All of the problems I refer to are the result of growth. It is obvious that there are many times the number of attendees now than there were several years ago. Last year we had a registered attendance of 236. The mailing list, which I pared down unmercifully last year and have been paying for ever since, contains about 400 names.

This growth has naturally refined and changed some of our techniques. In the beginning this conference was intended to be more or less a workshop--very informal with spirited discussion. Almost everyone attending gave progress reports of their endeavors for the year. We had little difficulty covering everything we chose to bring up in about a day and a half.

Now, for contrast, look at this year's program. The chairman was swamped with 41 papers--all to be delivered in 2 days. I think Doc and his associates did a wonderful job of keeping speakers on schedule and I think it has been a very good meeting. But I think you will all agree that this is no workshop atmosphere and that discussion had to be held to a very minimum to permit covering the whole program in the allotted time. Whether

good or bad this is a change due to growth. Next year could be more of a problem yet if more papers are submitted. I think we need to arrive at some method of controlling the amount of material presented. Or, we need to lengthen the conference to 3 days. Maybe we need to run more than one session at the same time. Perhaps we should assign special topics to talk about. Each agency could submit only so many papers. The chairman could be given more authority so he can produce an agenda to his liking by selecting or rejecting papers. This is what I call Problem #1-- what to do about or how to handle the growth of the group in terms of more material offered for presentation at the conference.

Another problem caused by a larger group is increased cost. At present the host agency must stand the expenses of holding the conference, which isn't too expensive, and compiling, printing, and distributing the Proceedings, which is costly. Total cost cannot be predicted accurately ahead of time because the number and length of written summaries are not known nor is the mailing list determined until after the conference is held. Thus, the host agency is most likely faced with an unbudgeted, open-ended bill that can be a very real problem.

Last year's conference cost the Oregon Fish Commission \$548 in supplies and services plus about 6 weeks of clerical help. We don't pay our clerks much but the total bill still came to about \$1,000. This does not include my time or the time of any of our biologists or librarian who helped a lot in one way or another. Last year I was able to budget the necessary funds to do the job-- this year I wouldn't have been able to do it. We simply don't have that kind of money surplus to our regular needs this year. I assume cost to hold the conference causes problems in other agencies also. How should we solve it? We could have a registration fee (this has been done on two occasions that I can remember). However, I don't think this is necessarily the right answer as the most costly item is connected with putting out the Proceedings. There are over 160 names on the mailing list of persons that do not attend. A registration fee at the conference would not be an equitable way of paying for all the copies. We could charge for each copy of the Proceedings, or we could have annual dues. I don't have the solution but we must come up with one if we are to solve the problem of cost to hold the conference.

Problem #3 concerns the handling of the Proceedings, the written record of the conference. A brief glance at past reports will show that the quality of the written material is much higher than it used to be. This is a result of a lot of time spent

preparing good copy by every speaker, the administrators that review the reports, and the chairman and his assistants. Is it all worth it? Why go to so much trouble preparing something of such high caliber when we stipulate that the material can't even be referred to without the author's written permission. Nor can the authors count the reports as published material. Did you know that many libraries all over the country are on the mailing list? You'd be surprised to know of all the places and persons receiving the reports of this conference. As far as I am concerned, once you have printed 400 copies and distributed information all over the country you have in effect "published" the information whether you want to call it that or not. I believe we should either consider the Proceedings as the published record of the meetings--or reduce very drastically the amount of time, effort, and expense involved. If we cut back I recommend limiting the mailing list to only those who attend--this is the way it was in the beginning. Or, if we decided to charge for each copy and consider it a publication then 500 or more copies should be printed each year.

The last problem I want to talk about is really more of a suggestion to permit advance planning and budgeting of expenses. I think the host agency, chairman, and location of future conferences should be decided 2 years in advance instead of the customary 1 year. This would be of definite help to the future hosts and shouldn't be too hard to decide on.

There are other problems I could cite, however, I am content to bring up only these four.

I suggest that we select a committee to consider these problems during the coming year. This committee should report their recommendations at next year's conference. At that time we can vote on the issues and hopefully come up with improved organization and procedure. I recommend that the eight formal participating Northwest agencies and institutions, that is, FCO, OGC, BSF&W, WDF, WDG, IDF&G, UofW, and OSU, each select a representative for the committee. Each committeeman should be a past chairman so we can get the most experienced crew together than we can. The chairman of the committee should be next year's conference chairman.

ANNUAL NORTHWEST FISH CULTURE CONFERENCES
HISTORICAL RECORD

<u>Year</u>	<u>Location</u>	<u>Host Agency</u>	<u>Chairman</u>
1950	Portland	U.S. Fish & Wildlife Service	Perry
1951	Wenatchee	U.S. Fish & Wildlife Service	Burrows
1952	Seattle	Washington Dept. of Fisheries	Ellis
1953	Portland	Oregon Fish Commission	Cleaver
1954	Seattle	U.S. Fish & Wildlife Service	Rucker
1955	Portland	Oregon Game Commission	Rayner
1956	Seattle	Washington Dept. of Game	Millenbach
1957	Portland	U.S. Fish & Wildlife Service	Johnson, Earlan
1958	Seattle	Washington Dept. of Fisheries	Ellis
1959	Portland	Oregon Fish Commission	Jeffries
1960	Olympia	Washington Dept. of Game	Johansen
1961	Portland	Oregon Game Commission	Jensen
1962	Longview	U.S. Fish & Wildlife Service	Burrows
1963	Olympia	Washington Dept. of Fisheries	Ellis
1964	Corvallis	Oregon State University	Fryer
1965	Portland	U.S. Fish & Wildlife Service	Halver
1966	Portland	Oregon Fish Commission	Hublou
1967	Seattle	University of Washington	Donaldson
1968	Boise	Idaho Fish & Game Dept.	Cuplin