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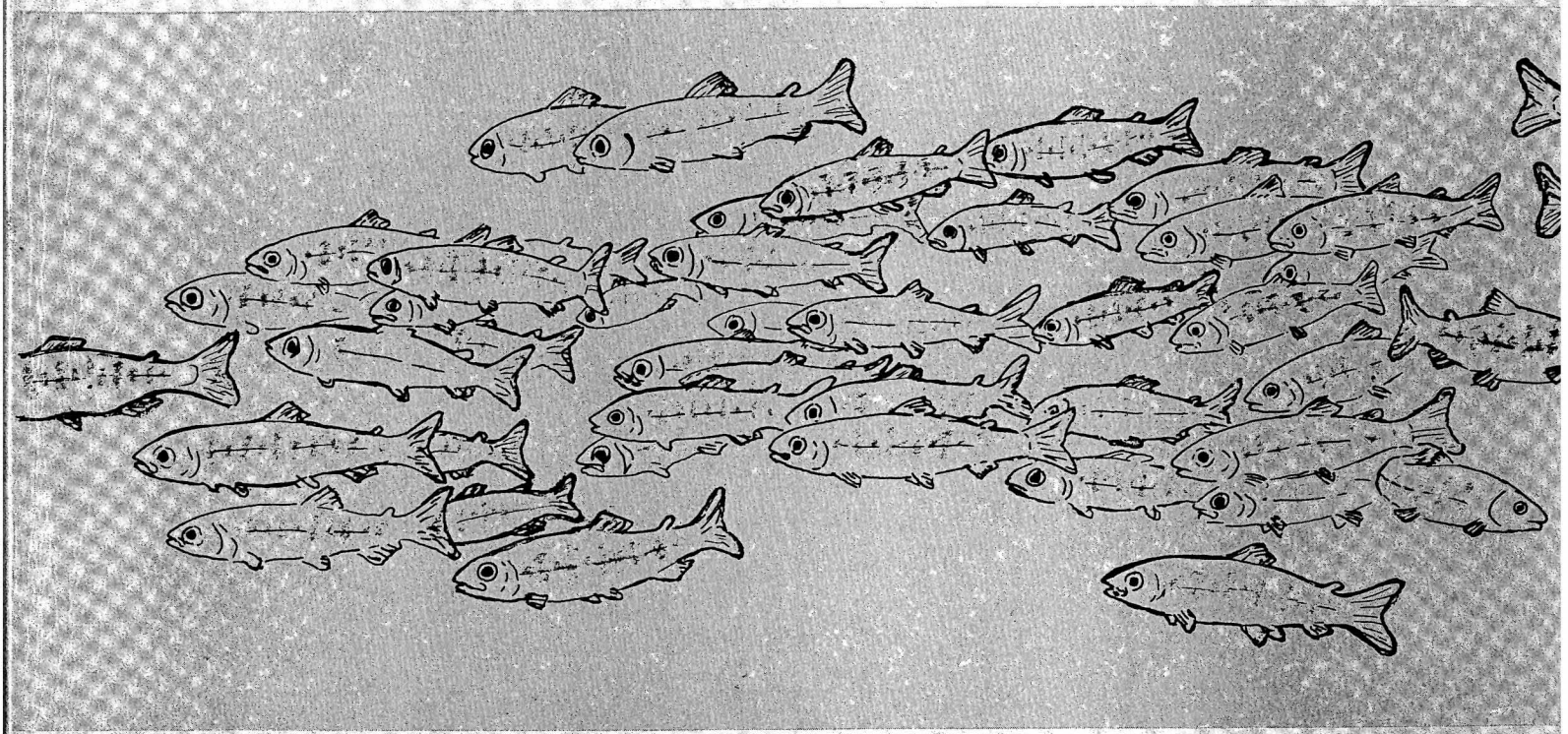
NORTHWEST FISH CULTURE CONFERENCE



TUMWATER, WASHINGTON

DEC. 5-6
1963

Fourteenth Annual
NORTHWEST FISH CULTURAL CONFERENCE
Tumwater, Washington
December 5 & 6, 1963

By the number of topics covered and the array of data presented, the Fourteenth Fish Cultural Conference can probably be classed as one of the most fruitful in the long array of these valuable conferences.

Forty-two topics were presented in the two-day period; and of this total, forty are herewith presented as copy. Two topics, Dr. L. R. Donaldson's illustrated talk on "Fish Culture in Denmark and Norway" and Dr. A. M. Dollar's illustrated narrative on "European Hatcheries, Fish Food and Fish Diseases" were of travelogue nature and could not readily be presented in publication; consequently these two topics are not included.

As Chairman, I wish to express my great appreciation for the large and spontaneous response of material presentations by fish culture technicians and their associates. Diligent participation is a necessary ingredient for a successful conference; it was apparent such an ingredient was present in the fourteenth conference.

As Chairman, I also wish to thank all the participants and those in attendance for bearing with me on holding to the Spartan schedule that was necessary to cover all the material.

This conference is fast becoming one of major proportions. Over two-hundred persons signed the attendance roster for this session.

As in previous years, summaries of reports are presented here without editing or correction except for a few minor obvious errors in dates or spelling.

Also, as has been previously stated, <u>none of the attached abstracts or any portion thereof may be reproduced without express permission from the author involved</u>.

Dr. John Fryer of Oregon State College was selected as Chairman of the 1964 Conference.

C. H. Ellis
Chairman
STATE OF WASHINGTON
DEPARTMENT OF FISHERIES

FOURTEENTH ANNUAL NORTHWEST FISH CULTURAL CONFERENCE

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PROGRESS REPORT ON THE MARKING OF PACIFIC SALMON WITH TETRACYCLINE ANTIBIOTICS

Douglas Weber and George Ridgway
Bureau of Commercial Fisheries
Seattle, Washington

Studies on the marking of salmonoid fishes with tetracycline antibiotics were initiated in 1961 at the Seattle Biological Laboratory. During the following year we determined through laboratory experiments, the dose required for marking, palatability of various forms of antibiotic, and methods of detection. By the spring of 1962 we felt that further experimentation required pilot studies, especially in regard to information on two additional factors.

First, we wanted to determine if the deposited tetracycline is permanent, and distinguishable, throughout the life of the fish. To meet this end, we marked 17 percent of the 1960 brood of silver salmon reared at Klaskanine hatchery. These fish were released in March of 1962; some returned as jacks in the fall of 1962, and others as adults in the fall of 1963. Results of the 1962 jack returns showed 21 percent marked; four percent greater than expected.

Commencing in mid-September 1963, the commercial fishery for silver salmon in Youngs Bay was sampled. It was assumed that most of the fish passing through the Youngs Bay fishery were reared in, and were returning to, the Klaskanine hatchery. Personnel of the Oregon Fish Commission sampled the entopterygoid bone from 22 percent of the commercial catch for us. Of the 822 individuals sampled, 17 percent were marked. The percent marked by sampling week is presented graphically in figure 1.

At the Klaskanine hatchery we sampled 10 percent of the returning adults. In a sample of 437 spawning fish, 21 percent were marked, the same percentage found in jack returns of 1962. Figure 2 protrays the number of marked adults per thousand adult returns. The number marked per thousand is based on our sample size as indicated at the base of figure 2, and expanded to the expected value if all adult returns were sampled.

Deposited tetracycline was readily detected in most skeletal structures after return as jacks or adults, but certain exceptions were noted: the gill rakers of returning jacks were well marked, however, as adults the gill rakers appear to be modified with the tetracycline having been removed by reabsorption of the bone; the maxillary, mandible and clavical became progressively heavier ossified, and by time of return as adults the overlaid bone often tends to obscure the mark. In other skeletal components, ribs, vertebrae, entopterygoid, the deposited tetracycline was easily detected after removal of flesh with hot tap water.

A comparison of length frequencies for both jack and adult returns is given in figure 3. The similarity in size of both marked and unmarked fish indicates no effect on growth due to tetracycline administration.

Our second objective was to determine if feeding of tetracycline antibiotics effected survival. In August of 1962 we marked one-third of the sockeye salmon production at Leavenworth hatchery. Oxytetracycline (OTC) was administered in a dry diet after one-third of the sockeye fingerlings were previously marked with an adipose-maxillary clip. Thus, upon release, approximately 10 percent of the production was double marked with OTC and an adipose-maxillary clip; 20 percent OTC marked only; 20 percent clip only; and 40 percent were unmarked fish.

The sockeye were released into Lake Wenatchee in the fall of 1962, and migrated out in the spring of 1963. At the time of out migration, samples were taken and the ratios between the groups of marked fish at recovery compared to the ratios at time of release.

Our most important comparison for determining the effect of OTC on survival was between individuals with a double mark (OTC plus clip) and those with clip only. Of 823 clipped fish recovered, 34 percent were double marked. At time of release from Leavenworth hatchery 35 percent of the clipped fish had been double marked. The difference in percent at release and recovery does not appear to be significant. Thus, it seems evident that during their stay in Lake Wenatchee OTC did not effect the survival of these fish. Also, the condition factors of clipped only fish and double marked fish were identical.

One other comparison between groups was made, the results of which is also shown in figure 4. The recovery ratio of OTC only marked fish to clip marked fish only, is significantly less at recovery than at release. In this case the fish marked with OTC only were recovered in fewer numbers than expected. However, we doubt that this difference is attributable to OTC marking.

At present we offer three possible explanations for this significant difference: (1) the adipose-maxillary clipped fish, through removal of what is assumed to be a relatively minor structure, may not be able to avoid the fyke nets used for sampling as readily as unclipped fish; (2) the handling mortality involved in clipping may have eliminated the less viable among the clipped fish prior to release, while the corresponding group of less viable among the OTC marked fish would not succumb until after release; (3) the sampling procedure used at time of out migration allows a greater chance for sampling error to occur when comparing OTC only against other groups.

The results of the above two studies indicate that tetracyclin antibiotics are deposited permanently in the skeletal structure of the fish, are readily detectable after a period of rapid growth, and do not effect growth or survival.

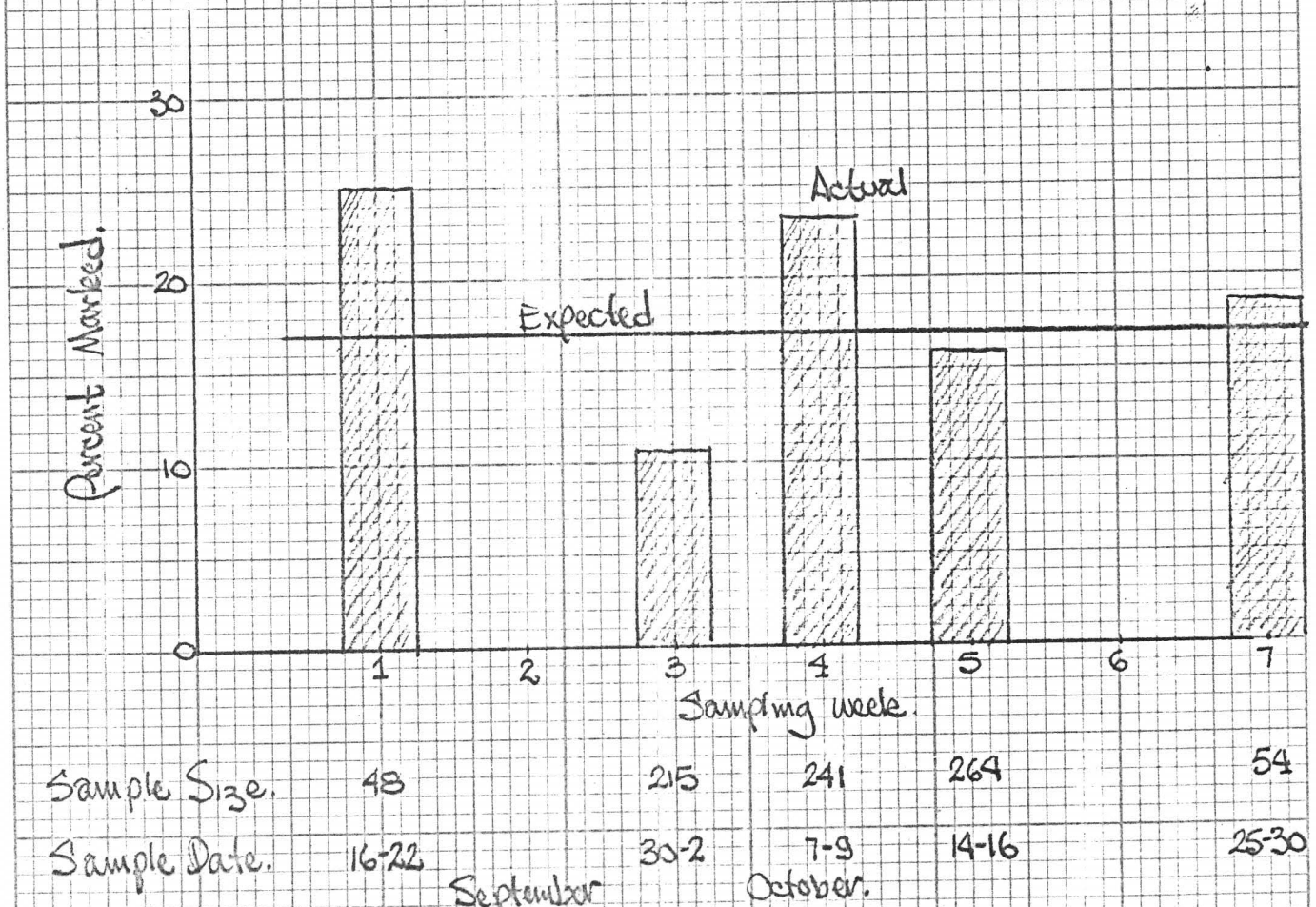

Figure 1. Expected and actual percent tetracycline marked silver salmon taken by Youngs Bay Commercial Fishery 1963.

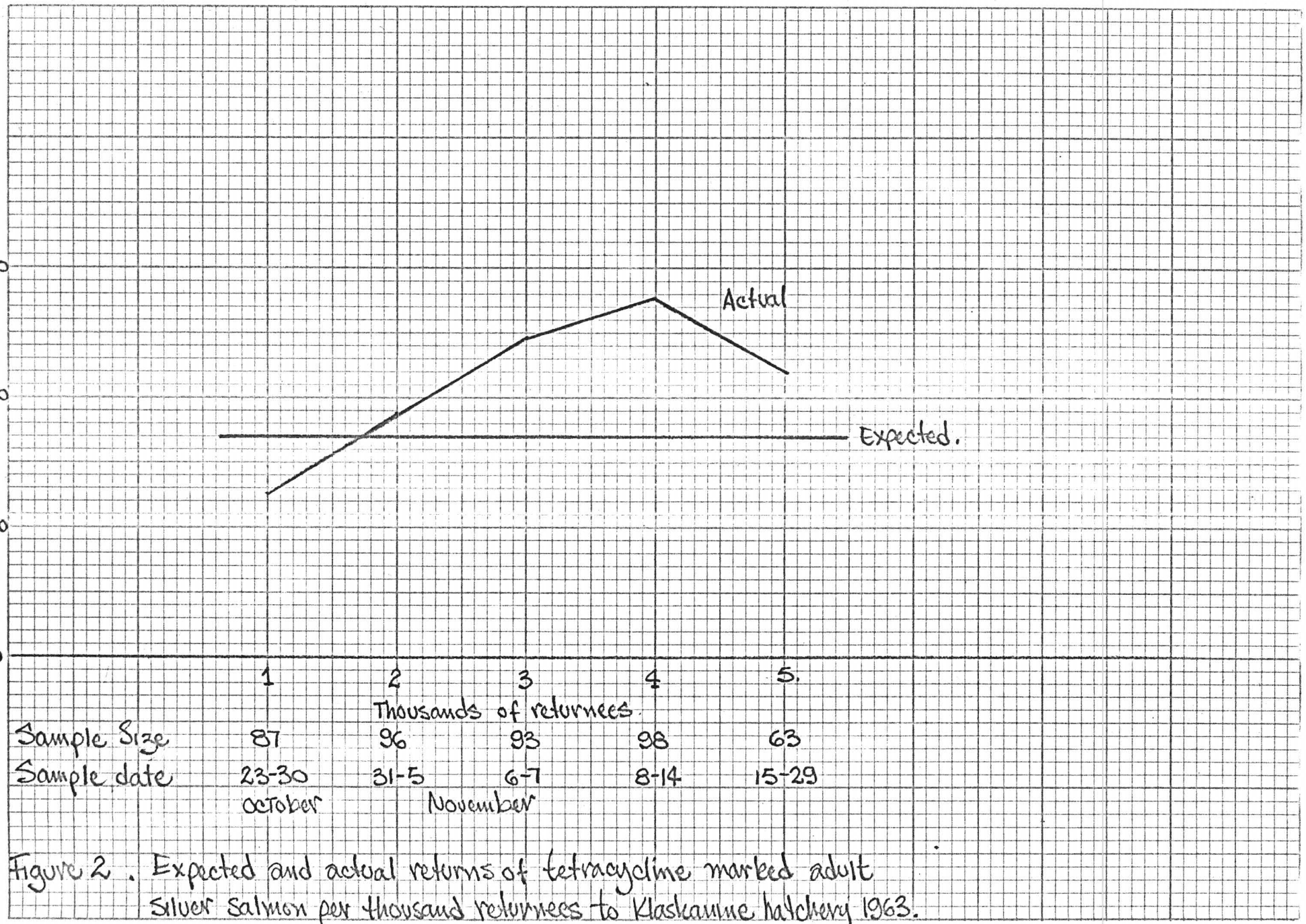

Figure 2. Expected and actual returns of tetracycline marked adult silver salmon per thousand returnees to Klaskanine hatchery 1963.

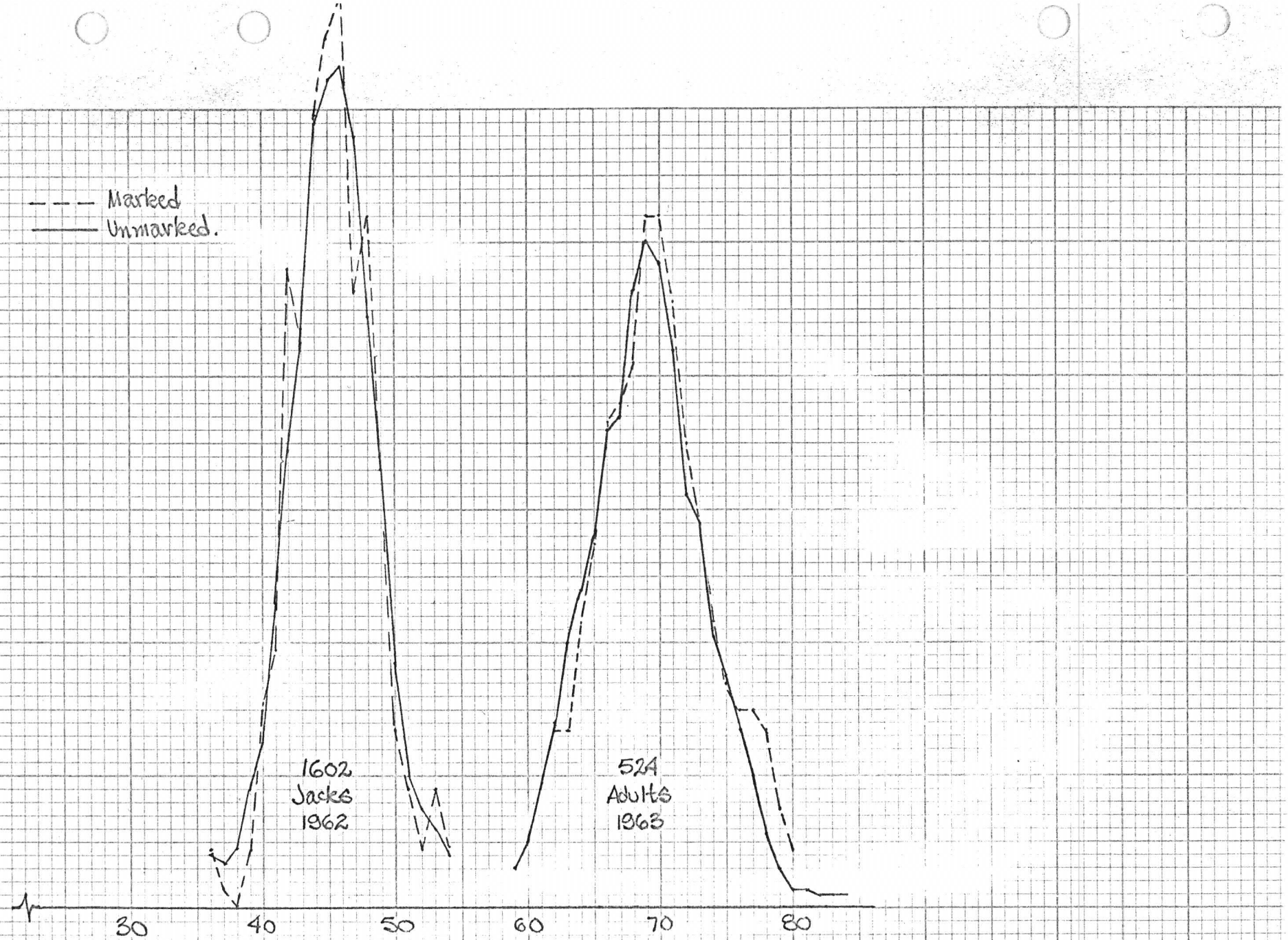

Figure 3 . Size frequencies in percent of tetracycline marked and unmarked jacks and adult silver salmon returnees to Klaskanine hatchery.

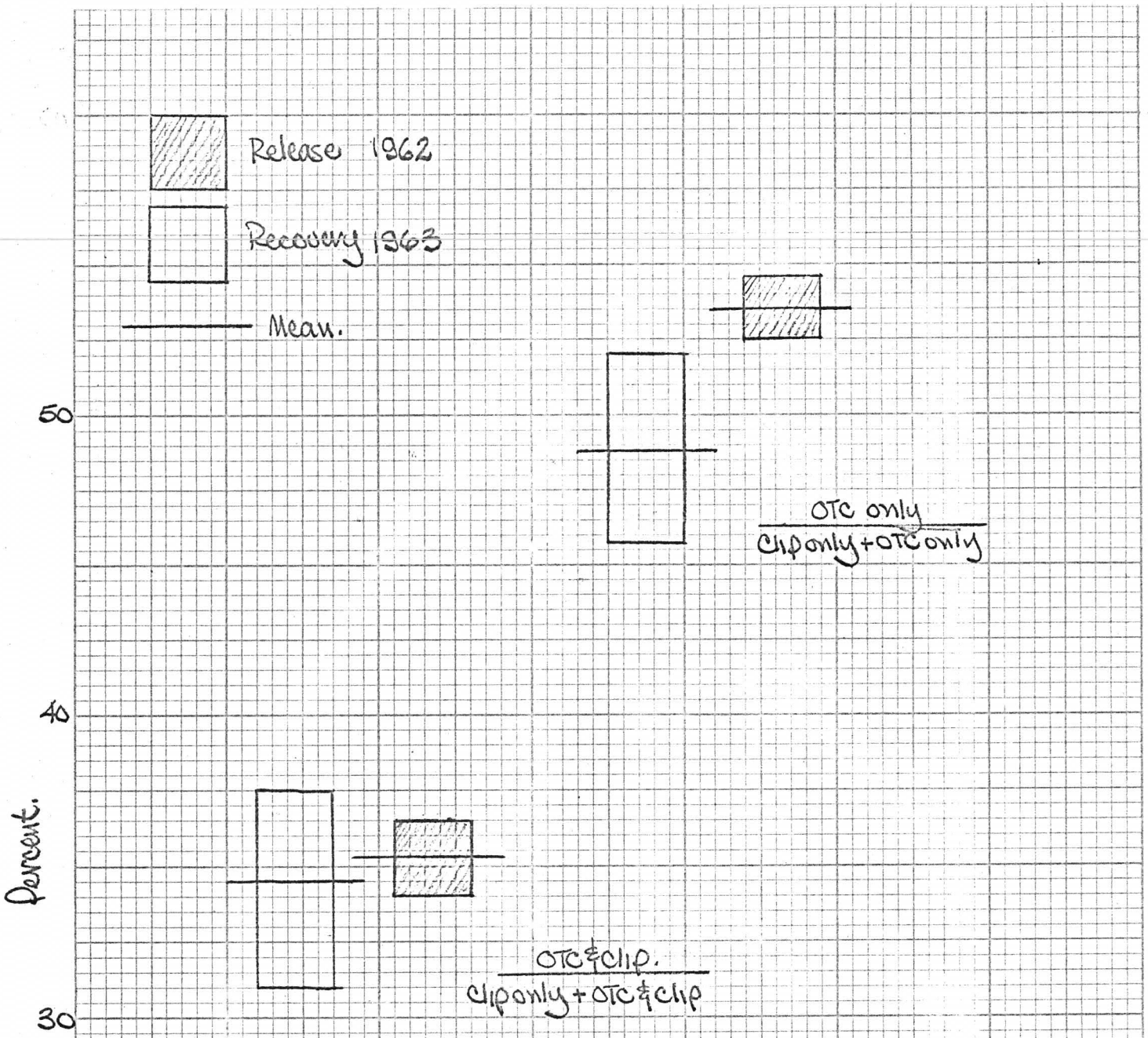

Figure 4. Release and recovery means and 95 percent confidence intervals around means for percentages of two comparisons of sockeye salmon marked at Leavenworth hatchery in 1962, and migrating out of Lake Wenatchee in 1963.

ELOKOMIN ADULT STEELHEAD TAGGING PROGRAM

Washington State Department of Game
Robert E. Watson

In an attempt to obtain information for the better management of our steelhead resources, a program of trapping and tagging returning adult steelhead was initiated 1/17/62 on the Elokomin River. This river was selected because it is a typical southwest Washington stream, and on it is located a trapping facility capable of trapping a complete run of fish. Due to a late start and trap failure, this initial program produced only 452 tagged steelhead. The experience gained was useful, but this sample was too small to be statistically valid. This year we were able to benefit from the experience gained and started the 1963 program on firmer ground.

The 1963 program was initiated on Nov. 7, 1962, when four steelhead were tagged and released at the Washington State Salmon Hatchery, located on the Elokomin River. These fish were trapped incidentally during the Department of Fisheries Salmon trapping program. These four fish represented the first fish of the winter steelhead run to enter the river. Fifteen more steelhead were tagged on Nov. 27, 1962. The Washington Department of Game took over operation of the trap officially on the 1st of December, 1962. The trap was in continuous operation until the 1st of April.

Although the Elokomin River attained its highest flow in the 31 years that records have been kept, the trap and weir held, and we succeeded in tagging 1,686 steelhead.

A dart type tag was used. Each tag was numbered and a record kept on each fish. This record consisted of date trapped, length, sex, origin and tag number. This is very useful in evaluating a tag recovery at a later date.

During the trapping period, there were several times when water was flowing completely over the weir and trap. This allowed fish to escape upriver with little difficulty. These periods totaled 184 hours. This is equivalent to 7.7 days of continuous flow. It is significant in that this spill was during the periods of peak fish movement. Of the total number of fish tagged, 51% can be accounted for on ten individual days. These days coincide with the periods of high water. Needless to say, many fish escaped upriver untagged.

Every day that there were fishermen on the river, an intensive creel check was made. 129 tagged steelhead were seen in these creel checks, along with 133 untagged fish. The total tag recovery from fishermen, including all sources, was 205 tags. There were 115 tagged fish trapped at the Beaver Creek Hatchery.

In calculating the total numbers of steelhead that moved past the trap, only the fish actually observed during the creel check were considered. The ratio of tagged fish observed to untagged fish observed times the ratio of fish tagged to x (fish not tagged) gives 1,271. This represents the calculated number of fish escaping untagged past the trap. This 1,271 plus the 1,686 tagged fish, gives a total calculated run past the trap of 2,957.

It should be noted that there was very extensive fishing below the trap site. Only incidental creel checks were made on this section of the river, but it was quite apparent there were many, if not more, fish taken here than in the river above the trap. During periods of low flow and clear water in the upper river, when there would be very light fishing with poor success, the tidewater area would have heavy pressure and good fishing. Practically all these fish were taken on the tides (outgoing). The increased velocity and volume of water apparently attracted the fish into this area. These fish were evidently milling in the estuary awaiting a rise in the river. Some fishermen felt the trap was responsible for holding the fish from going on up the river. The fact that the trap is located at the upper limits of the tidal effect accounts for its being blamed unjustly. This can be disproved by the large numbers of fish that moved through the trap immediately on the first rise of the river.

Out of the 1,686 fish tagged, 729 had imperfect dorsal fins. These fish are believed to be of hatchery origin. This is the criteria used to determine a hatchery fish versus a native fish. There were undoubtedly fish of hatchery origin that had perfect dorsal fins and were grouped as fish of native origin. Of the total fish tagged, 43% were of hatchery origin. Taking 43% of the run past the trap, we get 1,272 fish of hatchery origin. These returning fish were from a plant of 30,000 fish. This represents a 4% return. It should be pointed out this per cent would be much higher if the fish that were caught below the trap were considered.

There may have been a fair number of fish that moved up the river in addition to the calculated 2,957 fish, as on the day the trap was decommissioned there were 18 fish passed, indicating fish were still moving into the river at this time.

Numerous interesting observations were made during the course of this program. One of the most interesting was the recovery of four tags from last years pilot program (1962). One of these fish returned through the trap again this year. This fish was of hatchery origin. It started its life in the hatchery, where it spent a year. It was planted, migrated to sea, returned as an adult female from the sea, was trapped, tagged, released back to the river, escaped the fisherman's hook to spawn, went back to sea to return this year, was retrapped, retagged, then 13 unlucky days later was caught. Two other of the tags were recovered from fishermen -- one above the trap and one below. The fourth tag was recovered on the Grays River. These fish were of the 12 - 15 lb. class.

The first fish tagged this year, number 00000, was caught one day lacking three months later. The fish was a very bright spawned out female. There was much speculation as to whether these tagged fish would bite after having been handled and tagged. An example answers this question. A particular fish was tagged at 11 a.m. and recovered dead on the rack at 3 p.m. the same day with a hook deep in her throat and two feet of leader hanging from her mouth.

On Feb. 4 a tagged native male was trapped in the upper trap on Beaver Creek. It was transported several miles up the Elokomin River and released. On April 2 it was retrapped in the same trap. On Feb. 14 a tagged hatchery male was trapped at the holding pond trap at Beaver Creek hatchery. It also was trucked up and released into the Elokomin River. 42 days later this same buck was back in the trap. Both these fish had dropped back down the Elokomin several miles and found the mouth of Beaver Creek and traveled up and selected the same trap they had originally chosen. The fish with the hatchery origin rechose the hatchery water, and the native fish rechose the natural Beaver Creek water.

There were two instances of tagged fish showing up in the Columbia River commercial salmon catch during the May fishing season. One fish was reportedly caught in the lower 17 miles of the Columbia River. The other was caught a short distance from the mouth of the Elokomin River.

It should be noted that during the course of this program, the tags seemed to hold in the fish well. However, there were several tags seen in returning spawned out fish, and fish trapped at the Beaver Creek Hatchery traps that were broken. The numbers were gone. It was thought at first that someone was deliberately cutting the tags, but upon microscopic examination it was seen that they had not been cleanly cut. It was then postulated that the printing process was the cause of the tag weakening. Examination of all available broken tags disproved this when it showed they broke in a completely random pattern from 1/4 inch to 2 inches from the end. To eliminate this problem the tags were printed this year (1963) with the number on the barb end.

The 1963-64 tagging program is now under way with only minor changes having been made. This year the total river will be under intensive creel census.

SEX RATIO CONTROL IN HATCHERY PROPAGATED RUNS OF CHINOOK SALMON
(Progress Report)

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

As many of you will recall at the 1962 Northwest Fish-Cultural Conference, I reported on a sex-control experiment in which the hormone estrone was used as a means of stimulating sex reversal. The hormone was introduced to the newly fertilized eggs in an aqueous solution during the time of water hardening. The results reported were extremely encouraging with several of the treated lots producing one hundred percent female populations. Some reference was made to technical difficulties in the preparation of the aqueous solution of estrone but we were reasonably sure we were going to solve this problem and confirm the reported results in the experiment which was underway at the time of our 1962 meeting.

In concluding I stated that we believed that sex ration control could be accomplished by the hormone method described, but that considerably more work needed to be done before the method could be applied with confidence. I also pointed out the necessity for marking all fish from eggs that had been so treated so as to avoid genetic complications resulting from sex reversal.

The results of the confirming experiment were anything but confirming: none of the treated lots deviated significantly from the expected one to one male to female ratio, with one exception which had more males than females. This was not at all the intended situation. The reason for the failure of this experiment to duplicate the reported findings is not absolutely known, but the virtual insolubility of estrone in water is probably the prime source of our problem.

Because of the failure of our confirming experiment as well as the inherent genetic complications of the sex reversal approach, we have, at least temporarily, discontinued our hormone-induced sex reversal investigations. We have now shifted our approach to the pH of the media of sperm passage and to the longevity of the male and female gene-bearing sperms as possible means of sex selectivity in fertilization.

Our interest in the pH of the sperm passage media as a possible sex selecting factor was stimulated by an article by Manuel Gordon in the 1958 Scientific American in which it was suggested that an acid condition in mammalian fertilization may favor female progeny while an alkaline one may favor males. The mechanism through which pH would function is believed to be due to a differential in the stimulation or toxicity between the male and female gene-bearing sperms, or between the electrolytic attraction between the two sperm types and the ova.

Our application of this principle has been to adjust the pH of the milt prior to its introduction to the eggs. The pH adjustment is accomplished through the addition of acetic acid or NaOH whichever is necessary for the intended pH. The range of pH's used here was from 5.8 through 9.9 in a series of thirteen tests. In another series we used the milt as it came from the fish but rinsed the eggs in a series of buffered pH solutions, ranging in pH from 6.0 through 12.0; thirteen increments were used in this experiment. In a third series we treated the milt as in the first series and the eggs as in the second. The pH range in this experiment was from 6.0 to 9.5 in a series of nine increments. Preliminary tests indicated that the range of chinook salmon sperm activity was from pH 6.7 through 12.0.

The second means of possible sex-selection tested this year is based on the work in the medical field which indicates that there is a difference in longevity and activity between the two sperm types. The indications are that the male gene-bearing sperm is a more active but short-lived sperm than the female gene-bearing sperm.

In applying this principle we have stimulated a unit of milt to activity by the addition of an activating solution and allowed a time lapse before the milt was introduced to the eggs. The time lapses were from 5 through 70 seconds after stimulation to activity before the milt was introduced to the eggs. Six increments of time were used. In this manner it may be possible to introduce female gene-bearing sperm in an active state while the male sperms have already ceased their activity.

The fingerling from these treated groups will be held separate until they reach a size of about two inches when the resulting sex ratios of the treatment lots can be determined by examination of their gonads under the compound microscope.

If any of the methods are successful it will be a far more convenient procedure than the one discussed last year.

AN ELECTRICAL GRID FOR CONTROLLING TREMATODE CERCARIAE IN HATCHERY WATER SUPPLIES

Bobby D. Combs
Bureau of Sport Fisheries and Wildlife
Longview, Washington

Many Pacific Coast streams in Washington, Oregon, and California support large populations of snails which are the intermediate hosts of a number of trematodes. At least two species of these flukes, Nanophyetus salmincola and Sanguinicola sp. are known to be harmful to salmonid fishes and may present a serious problem in artificial propagation. The deleterious effects may be due to gill or other tissue damage or to bacterial infections which were provided easy ingress after penetration of the trematode cercariae. There is no practical method, at present, for eliminating snails from streams, however, electrocution has proved to be an efficient method for reducing the numbers of N. salmonicola cercariae in the hatchery water supply at the Salmon-Cultural Laboratory.

A parallel plate-type electrically charged grid was operated during the 1962 rearing season. This grid was designed to treat 5 cfs of water and operated on 60 cycle AC current at 240 volts per inch with a one-second exposure period. Although the grid proved successful, laboratory tests conducted during the summer of 1962 indicated that 310 volts per inch was more efficient when both rate of kill and economy of operation were considered. At that voltage, 80 per cent of the cercariae were killed or disabled with an exposure period of less than 0.5 second. A new and more efficient grid was designed for operation during the 1963 season.

The 1963 grid differs from the previous model in that it operates at 310 volts per inch and has an adjustable capacity for greater efficiency of operation. The grid is devided into three sections designed to accommodate water flows of 2, 4, and 6 cfs respectively. These sections may be operated singly or in combination to provide peak efficiency at water requirements of 2 to 12 cfs. The grid will treat 12 cfs of water for approximately the same cost as the previous model would treat 5 cfs.

The new grid was installed and activated on May 15, 1963, and was operated continuously until the chinook fingerlings were liberated on August 21, 1963. During this period pond-held fingerlings accumulated an average of 81 metacercariae per posterior one-third of the kidney. During the same period

cercariae-free target fish held in the creek for consecutive 7-day periods accumulated, theoretically, over 1,400 metacercariae. Experiments have shown that approximately 60 per cent of the incidence of metacercariae found in the stream-held fish were reflected in the pond-held fish when the grid was not operating. The pond-held fish would have accumulated approximately 840 metacercariae if the grid had not been operated. These results indicate, therefore, that the grid killed or disabled over 90 per cent of the cercariae in the water supply.

The adjustable capacity electrical grid at the Salmon-Cultural Laboratory was designed for a particular installation. A non-adjustable grid would perform equally as well if it were installed in a constant velocity flume. The depth of immersion of the plates and the power demand would change up or down in unison with the change in water requirement while the effectiveness of the grid would remain constant at the design value.

OPERATION OF THE HUMBOLDT STATE COLLEGE FISH HATCHERY:

1957 - 1960

Richard L. Ridenhour
Division of Natural Resources
Humboldt State College
Arcata, California

Humboldt State College has had a fish hatchery as a component part of its facilities for over 20 years. The first hatchery was built by interested faculty members and students. This hatchery had four troughs and operated with a very insecure water supply. A modern hatchery was completed in 1957 as an integral part of the fish and game facilities which had been built at the College in 1956.

The present hatchery was unique when it was constructed because the water was used over and over through a system of recirculation (DeWitt and Salo, 1960). Water is obtained from a 10 a.f. reservoir supplied by a small creek. A 50,000 gallon storage tank serves as the immediate water source. The water enters the system via a main sump from which it is pumped at a rate of about 200 g.p.m. All water is filtered by passing through one of three 500 gallon sand-gravel filters and then it passes through an aerating-cooling tower. From the tower the water flows by gravity to the 16 troughs. Two raceways, 6 circular ponds, and a 40,000 gallon earthen pond. The water returns to the main sump by gravity flow to be recirculated. The only demand for new water is for backflushing the filters and refilling facilities. Maximum use of all facilities can be obtained with no more than 10,000 gallons per day which would require less than a 14 g.p.m. water supply.

A refrigerated salt water system is available which operates on the same principle as the hatchery except that it is much smaller. Four 500 gallon aquaria are in the salt water system. These aquaria also may be operated with fresh water as part of the hatchery system.

The basic purpose of the hatchery at Humboldt State College is to provide part of the education of fisheries students. This use of the hatchery is not unique except that it has been conducted successfully with a recirculating water system.

A fish culture class uses the hatchery as a laboratory. Students obtain eggs at the beginning of the course, care for the eggs and fish, and stock the fish at the end of the semester. This assignment provides invaluable experience to students learning the rudiments of fish cultural techniques.

Many of the fish reared in the hatchery have been used as specimens in the classroom.

An interesting use of the hatchery has been to provide specimens for the College fish collection. Eggs from most of the North American salmonids have been obtained, hatched, and reared in the hatchery. Chinook, coho, sockeye (kokanee), chum, and pink salmon as well as masu salmon have been reared. The pink salmon were difficult to rear past about five inches even though they were held in salt water but the others did well. The masu salmon were obtained recently and the survivors are just starting to button-up. Atlantic salmon, rainbow, brown, and cutthroat trout as well as brook and lake trout have been reared successfully.

This process of rearing specimens has provided an invaluable opportunity to observe morphological and behavioral differences between species. It would be difficult to learn some of these differences in any other way.

Fish have been reared for research as they have been needed. Some have been used in the hatchery and others have been stocked.

Studies at the hatchery have included diet experiments with rainbow trout and coho salmon fed frozen daphnia and ostracods. The results of these studies are being analyzed and will be published by Dr. John DeWitt.

Dr DeWitt also has used numbers of rainbow trout reared in the hatchery for bioassay as part of his research on sewage oxidation ponds.

Some projects have involved stocking rainbow trout and chinook salmon in oxidation ponds. Dr. George Allen currently is considering further research of this sort.

Large numbers of chinook salmon were reared at the College hatchery and stocked by Dr. Ernest Salo to evaluate the productivity of saltwater lagoons in Humboldt County.

A secondary contribution of the hatchery has resulted from overproduction. Rainbow, brown, cutthroat, and brook trout broodstock maintained in the hatchery have produced far beyond the needs of the College. This surplus of many thousands of fish has been stocked in local waters under the authority of the California Department of Fish and Game.

The Humboldt State College hatchery has served its purpose very successfully. Growth and mortalities experienced by the species reared in the hatchery have been good even though there have been few attempts to operate on a production basis. However, a recirculating water supply does have some serious limitations.

The size of the hatchery is limited by the amount of water that can be circulated. The 200 g.p.m. of this hatchery does not allow the simultaneous operation of all facilities.

The water temperature in the hatchery is a function of air temperature. In areas where the average daily air temperature exceeds the 70° F. maximum of Arcata, this type of system would be handicapped. A refrigeration unit, such as the one in the small salt water system at the College, would be very expensive for much larger installations. Cold temperatures also may be a problem although heating units have been used successfully on small hatchery systems (Leitritz, 1962).

Another serious problem of a closed hatchery system is disease control. Ichthyophthirius were introduced accidently during the early operation of the College hatchery. Periodic outbreaks were controlled by malachite green and salt treatments (Johnson, 1961) until a severe outbreak occurred when over 200,000 chinook salmon fingerlings were in the hatchery. The "Ich" problem has been eliminated by sterilizing the entire system with calcium hypochlorite (70% available chlorine) and either by holding new fish for an extended quarantine or by obtaining eggs.

Pathogenic organisms being recirculated through the system makes disease research difficult or impossible at this hatchery. For the same reason, diet studies may be confounded by soluble nutrients which would be distributed throughout the system.

Although there are limitations with a recirculating hatchery water system, there are great advantages of limited water requirements. This type of hatchery system has served satisfactorily the needs of the fisheries teaching and research programs at Humboldt State College.

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PRELIMINARY EXPERIMENTS IN WATER RE-USE

Bobby D. Combs
Bureau of Sport Fisheries and Wildlife
Longview, Washington

The principal factor limiting hatchery production is the available water supply. If water could be efficiently re-used for fish rearing, production could be increased at many hatcheries and construction of new hatcheries in areas with little available water would be feasible.

Experiments were initiated in 1963 to determine some of the problems associated with water re-use. A recirculating water system was set up which consisted of a fish-holding trough, a water-collecting trough, an aeration tank, and a motor-driven pump. The pump forced the water through an aspirator into the aeration tank maintaining an oxygen level of near saturation at all times. During a trial run the temperature in the system rose from 54° to 72° F. in 20 hours. The installation of a 4-foot-long water jacket with 53° F. cooling water held the temperature below 67° F. The entire system contained 117 gallons of water which was circulated at the rate of 9 gpm. Approximately 0.7 gallons of water was added daily to replace that lost through evaporation and leakage.

The first experiment utilized 39 chinook fingerlings, weighing 2 pounds, in well water at pH 8.05. At the end of 28 hours the ammonia level in the water was 1.03 ppm as determined by direct nesslerization. Under these conditions the fish became distressed and the experiment was terminated.

The second experiment was conducted with 33 fish, weighing 2 pounds, in creek water at an initial pH of 7.3. Although the ammonia level in the water increased rapidly, the fish appeared normal and fed actively. On the 17th day of the experiment the ammonia level was 27 ppm. and the pH had risen to 8.05. The fish began showing signs of distress and ceased feeding. A fish transferred from fresh water to the re-use trough at this time died within 16 hours.

After a high of 27.6 ppm ammonia was reached on the 19th day, both the ammonia level and the pH began to decrease. On the 24th day when the experiment was terminated, the ammonia had decreased to 14.6 ppm and the pH to 6.7. During the last week the fish became lethargic and some mortality occured.

These preliminary experiments indicated that:

1. A heat exchanger was required to hold the water temperature in the recirculating system within tolerable limits.

2. An ammonia level in the water of 1.03 ppm was toxic to chinook fingerlings when the initial pH was 8.05.

3. Chinook fingerlings adjusted to increasing ammonia levels when the initial pH of the water was 7.3.

4. A condition was developed in the re-use system which caused a decrease in the amount of ammonia and lowered the pH. A bacterial growth is suspected as the cause.

The results of the experiment utilizing creek water are puzzling and attempts to duplicate the experiment have failed thus far. The quality of the fish used in the later experiments may have been the reason for the failure. It appears unreasonable that fish could survive at the high ammonia level measured especially at a pH above 7.5. However, little is known of the chemistry of the creek water or its effect on the ammonium compounds formed in the re-use system. Much further work is necessary and additional experiments will be conducted during 1964.

A PRELIMINARY REPORT ON A NEW MULTIPURPOSE FISH MOVING MACHINE

K. E. Morton
Oregon State Game Commission

Speaking of artificial propagation in general, it has always seemed to me that one of the major bottlenecks in hatchery operations has been the crude implements we have had to use to manipulate millions of pounds of fish and water in order to grade, thin, transfer, inventory, consolidate and liberate many tons of live fish during the course of the production year. I am sure that most of you will agree that wheelbarrows, carts, washtubs, buckets, hand scales, hand dip nets and a generous amount of back-breaking labor have been the standard tools of the trade for many more years than we care to remember.

As the two major costs in producing a pound of fish are food and labor and as pelletized food is rapidly reducing food costs to a near minimum, it seems to me that there must be a substantial increase in the pounds of fish produced per man employed if we are to achieve a substantial reduction in the cost per pound of fish produced.

For a number of years the writer has been working on a machine which would be easily adapted to a wide variety of different hatchery designs and greatly reduce this most laborious phase of our work. While the device herein described is not the "Piscatorial Combine" so facetiously referred to at our Portland meeting two years ago, it is a major step in that direction. The present machine has demonstrated the soundness of the basic principles involved and has been put to practical use.

As the present machine had to be built with very limited funds, it was not possible to include all the mechanisms desired. With adequate funds, a device with increased capacity and greater range and flexibility can be built.

I have color slides to show the various operations and many of the construction details. Briefly, the machine has been designed to handle fish from two and one-half to twelve inches in length in practically all phases of hatchery operations. The device is operated on a vacuum principal by a three-inch Homelite 300 g.p.m. water pump. A 16 foot snorkel tube, 5 inches in diameter, is attached to the top of a vacuum tank, which is 36 inches in diameter, 60 inches tall, and holds approximately 250 gallons of water. The snorkel tube is submerged into the pond.

After pumping the system full of water and purging all air from the system through the air-purging valve on top of the tank, the flow of water is reversed instantaneously by two lever-operated three-inch Homestead three-way valves. As the water is now being drawn from the bottom of the tank from below a brass-screened mechanical crowder, which has a special rubber seal around its perimeter to prevent small fish

from slipping by into the water pump, the fish are drawn into the tank via the snorkel tube from between crowders in the pond.

When sufficient fish have entered the tank, as determined by visual observation through two plexiglass windows in the tank, the pump is slowed to idle speed, the air-purging valve is opened to break the vacuum, and the three-way valves again immediately reversed. The special "Fabri" stainless steel, lever-operated, gate valve on the snorkel intake is closed. The end of the snorkel is raised a few inches above the pond surface allowing the fish and water remaining in the tube to run back into the pond, and it is at this time the oxygen is turned on at about eight liters per minute. The oxygen flows into the tank through two 1-1/2 by 24 inch carborundum tubes attached to the inside bottom. The snorkel tube is now raised by means of a block and tackle attached to an overhead boom to either a tank truck strainer-fish grader or transfer box. The flow of water is increased to about one-fourth pump capacity, the knife gate opened, and fish and water flow out the snorkel tube, assisted by a gentle nudge from the crowder as it is slowly raised. This completes the cycle, and the crowder drops back to its lowest position by gravity as the safety winch is turned in reverse.

With this system the fish are never touched with a hand dip net and are always submerged in water except for a second or two as they pass through a fish strainer or over a grading bar. There is always a heavy flow of water passing through the tank except for a few brief seconds when water flow is being reversed. Pure oxygen can be administered during these periods or at any times when the fish are being discharged from the tank. The fish leave the tank in water saturated with oxygen in active condition and, consequently, there is a noticeable improvement in the way they grade.

It will be recalled that a number of years ago Mr. Cliff Millenbach of the Washington Game Department showed a movie of his tank truck vacuum loading system. It was also about this time that Mr. Earl Leitritz of the California Department of Fish and Game published information on their fish loader. While both of these ideas were certainly very commendable, it seemed to me they still left much to be desired.

With the Washington system it was still necessary to hand seine, hand dip net, and weigh all fish into a live car before they were sucked into the tank truck by its vacuum system. Nor did it seem possible to use this system in grading operations. With the California system it was a major break-through to be able to measure the entire truck load of large fish by the displacement system. It seemed to me, however, this system still required hand seining and hand dip netting the fish on to the conveyor. The long ride up the conveyor on a hot day and having been scraped dry from water perhaps already low in oxygen is hard on fish and would, it seems to me, place a rather heavy burden on the tank truck's aeration system as the nearly exhausted fish drop into the tanker. It was also stated that the displacement weighing system was not used on fish running smaller than sixteen fish per pound.

It was this information that motivated me to try to develop a system that would encompass the best of both these ideas into a truly multipurpose fish handling device and, if possible, be equally effective in handling much smaller fish. It should be borne in mind that practically all lots of fish are handled numerous times during the course of the production year before final liberation. My only regret is that insufficient funds have prevented the development of a more elaborate device.

I might also mention that I have suggested the development of a special liberation trailer which would have two 2,000 pound separate compartments and be loaded by the displacement system. There is no question in my mind that this same trailer could be used for grading and inventory operations. It would be a matter of raising the grader to a sufficient elevation to permit the graded fish to flow by gravity to their proper size group tank through a strainer and be measured by displacement in the tank. When sufficient poundage of a particular size group had accumulated it could be discharged into its proper location, graded, weighed, and transferred, all in one operation. The only hand work required would be that of making sample counts of each size group.

While we have not had our machine in operation long enough to become thoroughly familiar with all of its idiosyncracies, all of the tests made so far have been most encouraging. The following test, made to check both the machine and our displacement weighing system on small fingerling, was so accurate that the results were a little difficult to believe. The test was as follows:

Pond eight, a circular pond 25 feet in diameter, contained 44,302 spring rainbow fingerling, according to our inventory of September 1. The fish were ungraded and varied from two and one-half to five inches in length. We made three 35 pound sample counts to determine number of fish per pound. These counts varied from 34.5 fish per pound to 38.9 fish per pound with an average count of 36.3. Using the count of 36.3, we calculated there should be 1,220 pounds of fish in the pond.

Backing the displacement weighing tanker up to the machine, the initial charge of water was pumped into the tanker by the machine's pump--it serves this purpose, too--until the meniscus rested on the 300 pound mark. We then counted up 1220 pounds on the gauge and made another mark. If our inventory was correct, the fingerling should raise the water level in the tanker to this point. The entire pond of fish was pumped into the tanker through the strainer in three lifts. This transfer probably could have been made in two lifts easily, but we were cautious in our first handling of fingerling. After all the fish were in the tank, we looked at our gauge; it sat exactly on the 1220 pound mark. Perhaps this was just a coincidence so, to make sure the poundage recorded was accurate, we moved the tanker to pond ten which contained no fish, discharged the entire load between crowders, and weighed very carefully by the dry method all fish back into pond eight. This weight was exactly 1,223 pounds, an error of less than three-tenths of one per cent.

As these fish were half the size of those recommended by Leitritz in the California system, we felt that a significant accomplishment had been made.

To make certain the machine would have no harmful effects on fish, we weighed out 700 pounds of mixed yearlings that varied from three to twelve inches in length and placed them in a pond by themselves. This same group was put through the machine eleven different times to run various tests, make minor modifications of equipment, and to learn how to use the machine. Only one fish died from all of this handling, and it is possible it was injured by a crowder in the pond.

On two different occasions during these tests the pump was shut off and the fish held in the tank by the oxygen system alone for fifteen minutes. In neither case was there any mortality. The maximum time the fish can be held in the tank with pure oxygen has yet to be determined. We still have much to learn on the maximum capabilities of such a device.

The oxygen system was installed for several reasons. The first and most important is that it serves as a safety factor in case of pump motor failure. The oxygen will hold the fish until the motor can be restarted or, that failing, it will allow time to return the fish to the pond through a special door, heavily-hinged and held firmly in place by twelve quick-release high compression clamps. Secondly, it was felt that it might permit heavier loads in the machine than have so far been attempted. Of more importance is the fact that the fish that do come out of the machine are in saturated water which definitely assists the grading operation and quite possibly places the fish in the tank truck in better condition, especially in warm weather. The possibility exists that the machine might also serve for administering certain external treatments for disease control.

To date we have put approximately 18,000 pounds of yearling rainbow trout through the machine with no more loss than would normally be expected in previous grading procedures.

There is one minor problem in grading fish with the machine and that is occasionally a surge of fish will come out of the tank at a faster rate than the grader can accommodate them. The fish have not always been evenly distributed across the grader intake apron. I have just recently designed a new diffusion chamber which will stop the forward motion of the fish and water and cause an even spread across the head of the grader. Unfortunately, we have not had time to test the new addition, but it is not felt that the problem is serious.

The machine will also pump fish uphill. We have raised them four feet above the top of the device; however, it does not work too efficiently as another set of internal crowders are necessary to force the fish to move out of the machine. The fish could, no doubt, be pumped to practically any height providing the equipment was specifically designed to withstand the pressure.

The present model works best when placed on a platform forty-eight inches high for loading tank trucks. This permits a gravity flow of fish down the outlet tube.

For grading, the machine sets on the ground. The machine can be set on a flat bed truck or placed on a timber crib that can be moved about by a forklift truck. It might also be handy to have it mounted on a specially-built trailer for easy transfer and inter-hatchery use.

The present design is mounted on a steel angle base and specifically designed for moving by a forklift truck. The entire unit, hoses, pump and the works can be quickly moved to any hatchery area and is ready to go to work in a matter of minutes.

In one test we extended the snorkel tube to fifty feet and successfully pulled water through the machine. In order to use such an extension, it would be necessary to have Kam-Lock quick connect couplers, and we must wait for additional funds to develop this angle. We are confident that fish can be moved from twelve of the Wizard Falls ponds in one setting of a more elaborate model within a seventy-five foot radius of the machine.

The device can also serve as a potent fire-fighting machine in case of fire on the hatchery grounds.

* * *

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"A NEW TOOL FOR SPAWNING"

MARVIN HULL - WASH. DEPT. OF GAME - SKAMANIA HATCHERY

CHARLES HILTZ - U. S. FISH AND WILDLIFE SERVICE - EAGLE CREEK HATCHERY

This new tool for spawning is air pressure. There are stories of others having tried it and Mr. James Wharton of Australia stimulated the present trend to acceptance during his visit last year. Air spawning can be a slow procedure without a tranquilizer to relax the fish or using hand air pumps. With any type of proper equipment and a simple routine established air spawning can be surprisingly fast, easy and efficient.

The big factor causing Mr. Hiltz and I to try something new is the difficulty in spawning steelhead which for our purpose might be described as large active fish with thick body walls biologically capable of re-spawning many times. Such spawning is tiring work and it is easy to break eggs hand stripping these fish. It is hard to get all the eggs and often the female will fungus up around the caudal peduncle and along the sides after spawning.

Where the air enters the body cavity isn't too important. Any veterinary hypodermic needle selected for length to penetrate the body wall suffices and will force loose eggs out the cloaca. Mr. Wharton shows his choice for needle insertion at a point about midway between pectoral and ventral fins and midway up the body cavity. This had two drawbacks: A fish might twitch and the needle occasionally puncture the kidney and it interferes with massaging the eggs out of the ovary skein if the fish is not completely ripe. For these reasons I prefer the most ventral position just forward to the ventral fins.

A very low pressure suffices to spawn the ripe fish. After it has been relaxed with a tranquilizer such as methyl pentynol or m s 222, four to two pounds pressure works nicely. The maximum pressure can be adjusted with a welding type regulator on a high pressure air line or with a smaller regulator on a portable paint sprayer unit. The variation to a lower pressure is easily attained by finger tip control on a bypass from the air line about a foot before the needle. Trying a pressure range of eight to six pounds on a few fish performed faster. The initial pickoff on these may have been a little higher although the overall percentage showed no difference.

It appears necessary to relieve the spawned fish of the excess air in the body cavity or it will float helplessly near the surface of the water. There are many simple ways to do this. A short supplemental line thru the arm sleeve to attach to the needle in the body wall makes the draw off possible by mouth. Too much of this will make the mouth quite sore. The air may be forced out by hand as a quick check is made for any remaining eggs.

Here are a few random remarks. Dipping the needle each time in a jar of rubbing alcohol resulted in no infection complication with several hundred fish involved. It was not tried without dipping. The clear thick walled plastic welding tubing is just the right size to self thread a universal type hypodermic needle base such as is used in a 25cc veterinary syringe. Occasionally a bit of fish scale may plug the needle. The new needles, such as a 16 gauge - one inch, come with a wire to clear them.

Mr. Hiltz has some slides and a training film all made at the Federal Eagle Creek station which will illustrate the new tool in action.

SOME EFFECTS OF HANDLING ON FISH BLOOD VALUES

Cecil M. Whitmore
Oregon Fish Commission
Clackamas, Oregon

INTRODUCTION

The Oregon Fish Commission conducts routine blood tests to measure the physiological condition of hatchery fish during the rearing period and at time of release. During rearing, early diagnosis of anemia or other disorder can permit treatment that would lead to recovery; whereas blood tests at liberation give a historical record for each group of fish. In order to fulfill these purposes, tests must be reasonably accurate and rapidly conducted.

Biologists of this group, as well as those from other fisheries agencies, have been concerned with unreasonable differences in blood values between individual fish and groups of fish, test equipment, and numerous other factors. It is my contention that an adequate explanation may be found for most deviations in blood quality or quantity, but it is another thing to find that explanation. Each possible deviation needs to be brought to the laboratory for investigation. Changes may be due to seemingly unimportant details, i.e., the manner in which tests are conducted, limitations in presently used methods, measurement of the wrong parameter, or to more obscure items involving the biology of fish being tested.

This presentation involves one possible source of variation - the period of time between collection and blood testing of chinook salmon. The study was necessitated by finding extreme changes in blood values of anemic chinook with a delay of 24 hours or more between fish collection and blood sampling.

MATERIALS AND METHODS

Immediate and delayed blood samples were taken from yearling spring chinook at our McKenzie, Willamette, and Marion Forks hatcheries. Those at McKenzie Hatchery were experiencing an anemia while the Willamette and Marion Forks fish were considered normal. In order to test the effect of delayed sampling as many consecutive blood tests were run as possible for a 1-man operation. These included red cell count, hemoglobin estimation, hematocrit, measurement of mature red cells, and examination of stained blood smears.

The fish were rapidly anesthetized with MS 222 and wiped dry. Then, blood samples were obtained by severing the tail and collecting the blood in 2 specially heparinized microhematocrit tubes. Tails were re-cut for filling the second tube and for making the blood smear. After

thorough mixing in the capillary tube, blood was transferred to red cell and hemoglobin pipettes. Sufficient blood was generally collected in the first tube for the red cell count, hemoglobin estimation, and hematocrit; the second capillary was centrifuged with the first hematocrit sample and served as a comparison. Red cell counts were made with a hemacytometer. Hemoglobin estimation was by the acid-hematin method using a Spectronic 20 colorimeter.

RESULTS

Results of immediate and delayed sampling are shown in Table 1. It will be noted that immediate test values are not too abnormal even in fish considered anemic. However, changes occurred with delayed sampling. The first test of the anemic group was taken during the period of most severe anemia. The MCHC increased markedly in the delayed samples due to lowered hematocrits, which in turn resulted from reduced size of red cells. Apparently red cell numbers were similar between groups tested immediately and those tested after a delay. This is shown by the similarity in hemoglobin level and uncompensated drop in hematocrit.

Test 2 was conducted after a 2-month recovery period. Both hemoglobin and hematocrit values increased in delayed samples but red cells became small as in the first test. The increase in hemoglobins and hematocrit was due to a greater number of red cells, which partially compensated for small red cell size. The amount of hemoglobin increased beyond the effect of the increased numbers of red cells. These results compare favorably with those from normal fish except for red cell size.

Tests 3, 4, and 5 with normal chinook include delays of 72 hours, 3 hours, and 1 hour. The tests were conducted with different time periods in order to reveal possible satisfactory time limits between collection and sampling. For practical purposes these tests show very small differences. However, the MCHC was significantly higher in tests 3 and 4 but not in test 5. Cell size was only significantly different in test 3, which had the highest delay period. In apparently healthy chinook (test 3), the decreased MCH reveals that high hemoglobins and hematocrits were due to increased red cell counts. The reduced MCV also shows the effect of the increase in red cell numbers in addition to reduced erythrocyte size.

SUMMARY AND CONCLUSIONS

Immediate and delayed blood tests were conducted on samples of normal and anemic yearling spring chinook salmon collected from hatchery populations. Delays of several hours between collection and sampling caused only slight changes in blood quality and quantity in apparently healthy fish whereas considerable differences were observed in anemic fish. Delay in sampling anemic fish increased MCHC's and decreased red cell size. Hematocrits dropped as a result of the decrease in red cell size. During recovery stages, sampling delay reflected increases in hemoglobin, and to a lesser extent hematocrits, due to release of additional red cells.

Table 1. Immediate Vs. Delayed Blood Tests of Spring Chinook Salmon.

Test No.		No. Fish	HB 1/	HT 2/	RBC's $X10^6$ 3/	MCHC 4/	MCH 5/	MCV 6/	RBC Area 7/
Anemic (McKenzie)									
1	Immed.	38	8.9	33.9	N.S. 8/	27.4	N.S.	N.S.	95
	24 Hrs.	40	9.1	24.0	0.9	38.4	102	269	83
2	Immed.	20	10.6	32.9	1.2	32.2	90	282	97
	24 Hrs.	20	12.2	34.8	1.3	35.0	97	278	83
Normal (Willamette & Marion Forks)									
3	Immed.	12	13.3	40.4	1.3	32.9	102	314	104
	72 Hrs.	12	14.5	42.0	1.5	34.6	96	279	99
4	Immed.	23	13.3	40.4	N.S.	31.8	N.S.	N.S.	108
	3 Hrs.	24	13.0	38.5	N.S.	34.3	N.S.	N.S.	108
5	Immed.	24	12.5	36.5	N.S.	34.6	N.S.	N.S.	105
	1 Hr.	23	12.0	34.4	N.S.	35.6	N.S.	N.S.	104

1/ Hemoglobin - grams/100 ml.
2/ Hematocrit (%).
3/ Red blood cells per cu. mm.
4/ Mean corpuscular hemoglobin concentration (%).
5/ Mean corpuscular hemoglobin in micromicrograms.
6/ Mean corpuscular volume in cu. microns.
7/ Mature red cell area in sq. microns.
8/ No sample.

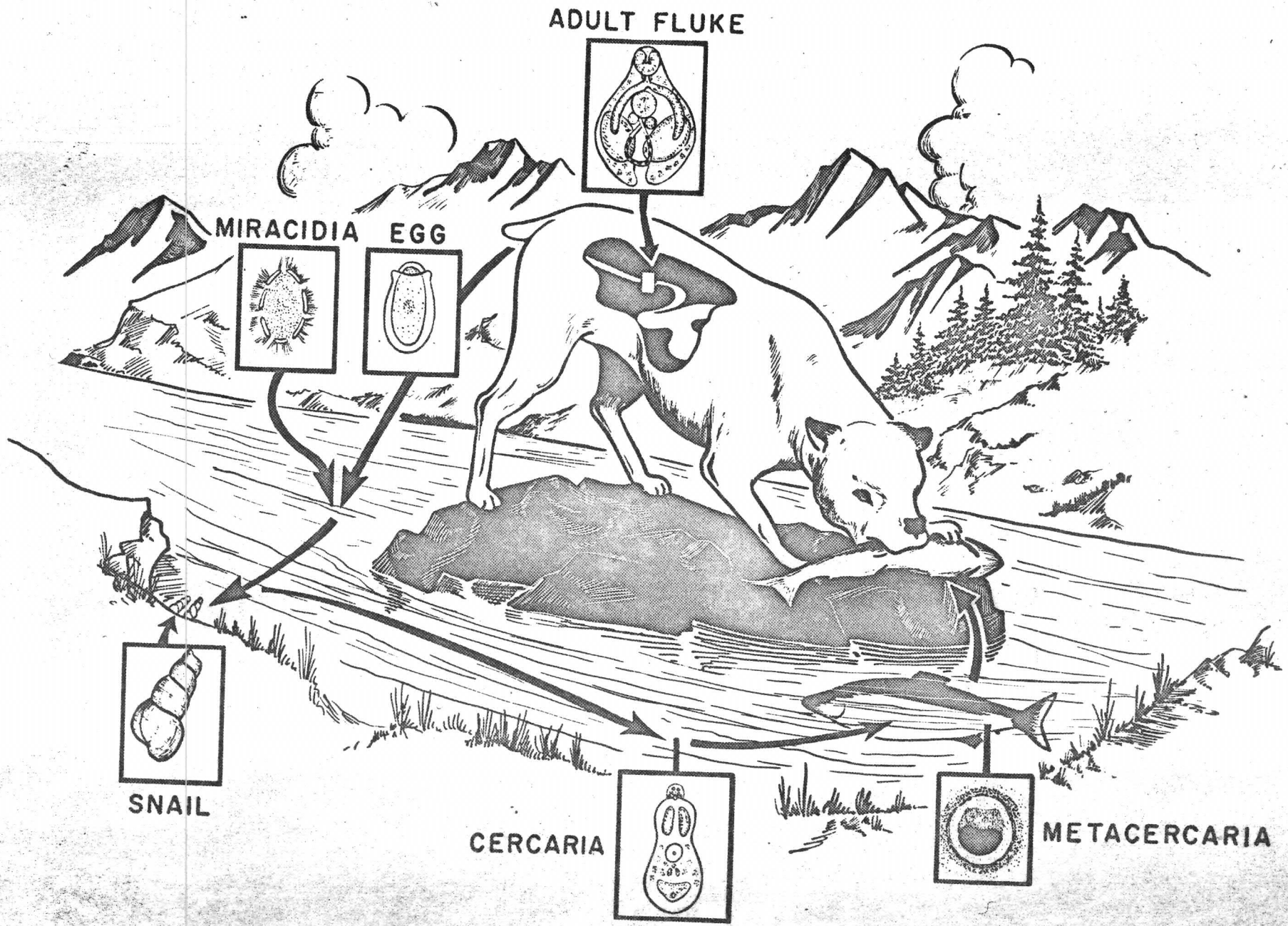
THE SALMON DISEASE FLUKE - PAST AND PRESENT

R. Keith Farrell
Pullman, Washington

The salmon disease (poisoning) fluke (Figure 1) is a naturally-occurring parasite of salmon and salmonoids from the 59th parallel north (Alaskan coast) to as far south as San Francisco, California, and as far east as Idaho. Contrary to existing literature, salmon are not cleansed of the fluke when they migrate to the ocean. The parasite has been rapidly increasing in numbers since 1927. It is a transmission and reservoir mechanism for two infectious diseases of dogs. Naturally-occurring infections with adult flukes have been found in coyotes, bears, raccoons, pigs, bobcats, mink, man and dogs.

Reproduction occurs in the adult fluke and a large number of eggs are passed from each fluke. These eggs must reach water to develop. After a period of time (up to one year), the eggs hatch into a free-swimming form which is called the miricidium. The miricidium then invades the snail, Oxytrema plicifer. Here it establishes itself and becomes a redia. The miricidium rapidly dies after hatching from the egg if a snail is not found. The redial stage is of interest to us because it, like the adult stage, is propagative. One redia can give birth to many individuals called cercariae. Once a snail is infected, it remains a potent source of infection for the stream in which it resides. The rediae in infected snails have been known to shed cercariae (the stage infective to fish) for at least four years. Snails less than 7/8 of an inch long seldom shed cercariae. Once a cercaria escapes the snail, it has approximately 48 hours in which to find a fish, if it is to survive. The cercariae secrete a mucous rope and are carried along with the stream until the rope contacts a suitable fish. Penetration can occur less than 30 seconds from the time of contact. Invasion is by penetration and is associated with the release of an enzyme which actually digests the tissues of the fish. This enzyme causes a local inflammation of the skin and tissues of the fish in the area of penetration. It is postulated that large numbers of cercariae penetrating simultaneously may cause a fatal toxemia in the fish. Microscopic examination of gills and fins of fish dead after subjection to overwhelming fluke infections clearly demonstrate severe inflammation and necrosis in the localized area of penetration.

Several people have described the acute death of fish by cercarial penetration (Ward and Mueller[1] and Bennington and Pratt[2]). One hundred percent mortality was seen in a 24-hour period in an experiment by Farrell and Lloyd using a snail-fish ratio of one to one in an aquarium containing 25 snails and 25 rainbow trout. A control group of


ADULT FLUKE
MIRACIDIA
EGG
SNAIL
CERCARIA
METACERCARIA

25 fish was maintained under identical conditions with 25 snails that were not shedding cercariae. No fish were lost in the control group. To be certain that the cercariae themselves caused the mortality, the negative control fish were placed in the aquarium with the shedding snails and new controls were placed with the non-shedding snails. The results were identical. It is fortunate that cercarial concentration seldom reaches this magnitude under natural conditions. However, during the season of high cercarial release in massively infected streams, it seems likely that mortality might occur. It also appears that massive destruction of the natural skin barrier by overwhelming penetration may serve as a predisposing factor to surface and systemic bacterial and mycotic infections. The role of the fluke in the transmission and persistence of diseases of fish has not been studied.

The seasonal cercarial periodicity is presented in Figure 2. The figures represent the average number of metacercariae over a 2-year period found in the posterior 1/3 of the kidney of target fish allowed to remain in infected water for one month.

Seasonal periodicity of Cercariae.
Posterior 1/3 kidney count.

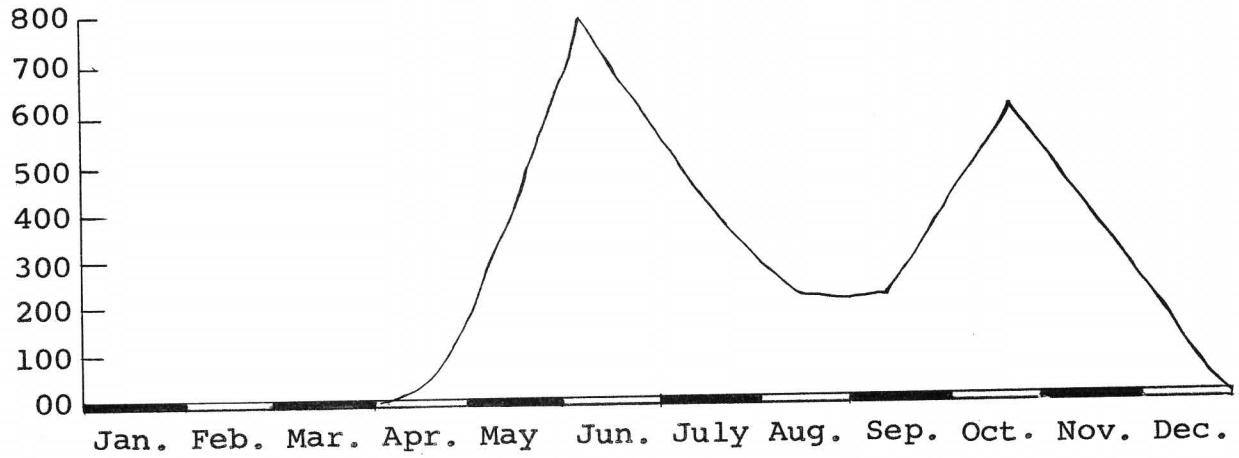


Figure 2

Microscopic examination of the tissues around the resting flukes after encystment reveals no untoward damage or sign of irritation from the presence of the fluke. This resting stage (metacercarial) survives in the fish for as long as four years and probably longer in spite of sea migration. It is believed that the flukes are not damaging to the fish after they become encysted unless they are of such numbers as to replace most of the functional kidney. The parasite cycle is completed when a warm-blooded animal eats the fish. At this time, the cyst walls are digested, the fluke sets up housekeeping in the intestinal tract of the animal, and start the propagative period again.

The worm itself causes little damage to dogs, raccoons, bears and other animals which have been infected experimentally. However, if the worm is harboring salmon disease, a fatal disease of dogs, or the disease referred to as Elokomin fluke fever, it is capable of transmitting the disease to the dog.

From a marine biologist's point of view, it would be wise to evaluate the information pertaining to the prevention and control of infection of fish with this parasite. In 1927, workers found no infected salmon in the Columbia River, and a careful survey of ocean-caught fish revealed no metacercariae at that time. Evidence from as recently as 1944 would imply that there has been a marked increase in infection since that time.

It appears that the most important warm-blooded animal for the dispersion of fluke eggs into the water is the raccoon. The logical approach seems to be to reduce the number of infected flukes available for raccoons to a minimum. Although raccoons feed on a certain amount of fish in nature, it is not as easily accessible as we make it by our hatchery procedures. Ponds are skimmed every morning and the infected fish are discarded into streams which makes "easy picking" for raccoons the following night. Spawning operations make large amounts of infected fish flesh available to raccoons. It appears likely that when a raccoon ingests a metacercaria, it results in an adult fluke which may spend as long as a year in the intestinal tract shedding eggs. Some thought might be given to freezing all fish prior to discard.

The snail, surprisingly enough, has a rather active pattern of migration. Many snails are washed or migrate down streams in the winter and in the spring and summer migrate up the smaller streams again. Snail barriers can be effective in preventing upstream migration. The most effecient barrier is created by water flowing over an angled screen which prevents the adherence of the snail's gastropod. If screening is to be effective, it must be borne in mind that each trickle of water must be screened, for snails will migrate on the concrete wings of a dam during rainy weather. Copper strips have been shown to prevent snail migration in soft water only. Even in soft water, they "silt over" or acquire a layer of organic material and are therefore ineffective. Molluscacides have been used effectively in killing snails already present in feeder streams. I regret to say that I have no selective molluscacides to recommend which kill the snails and leave the fish unharmed. In Clear Creek we were able to completely remove the snail population by the use of copper sulfate, but the fish were destroyed also. In another attempt, we tried to remove the snails from Beaver Creek, Washington with Copper sulfate at a level which prevented loss of all the fish. Many fish were lost in this attempt but the snail population was reduced to a very low level. Only the largest snails remained. The snails which remained seemed to increase their cercariae releasing activity. Each snail must have been shedding cercariae at a tremendous rate to account for the degree of infectivity

which occurred in this stream in spite of the snail reduction.

Cercariae have been inactivated experimentally by subjecting the water to an ultrasonic beam at 15 watts, cm_2 at one megacycle frequency. The practical adaptation of this to large scale hatchery operations has not been attempted.

During the time I have been studying salmon disease of dogs, there has been a northward migration of infectivity. We should attempt to prevent the spread of snails when stocking new hatcheries outside the area in which the disease is indigenous. Small snails are easily transported to non-infected areas in hatchery trucks. Some thought might be given also to the introduction of infected fish in disease-free areas.

Salmon disease (poisoning) has become the third most important disease of dogs in some areas. The cycle of the fluke represents a complex problem to the fisheries biologist. The recent finding of the parasite in ocean-caught fish may cause some concern in the public health circles. At present, there is no indication that the fluke is harmful to man, and it should be emphasized that freezing and cooking inactivates the parasite. People eat pork every day that is infected with trichina without becoming concerned. Each new discovery often brings a flurry of consternation perpetuated by frightened individuals who make noises far beyond that warranted by the problem.

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STUDIES ON COTTONSEED MEAL SUBSTITUTES, BINDERS AND A MODIFIED VITAMIN MIX

Oregon State University Seafoods Laboratory
Duncan K. Law

There have been persistent indications that cottonseed meal could be a factor in fish hepatoma. Cottonseed meal is present in the Oregon Pellet in considerable quantity. For this reason it was important to find a substitute. Safflower and corn gluten meals were tested in an attempt to find such a substitute. The results of this study indicate that corn gluten meal would be excellent. It provided a significant increase in growth and decrease in mortality over cottonseed meals. Safflower meal was found to be significantly inferior in terms of growth and mortality.

The commercial manufacturer of the Oregon Moist Pellet is sometimes hindered by variations in the viscosity of the mix. An adequate binder would stabilize the viscosity. This study indicated that either gum guar or kelp meal would be excellent binders. Kelp meal, in addition to binding properties, appears to have a growth stimulating factor, or factors.

The attempts to modify and develop a more economical vitamin supplement mix were inconclusive although statistical data indicated the possibility that a number of the vitamins could be decreased in amount, or eliminated. Despite the statistical inference, however, the observers felt there were some unexplicable differences between the presently used vitamin levels and the suggested modifications. It is apparent from this experiment that vitamin modifications must be approached with caution.

In addition to the studies mentioned in the title a study was made on variables in the Oregon Test Diet. The presence of crab or shrimp meal in the Oregon Test Diet has sometimes been subject of criticism since neither of these materials are of a standard nature. The importance of limiting such variables in a test diet was realized but earlier attempts to delete crab or shrimp meal were unsuccessful. Improved techniques in diet preparation, handling, and storage have shown that crab or shrimp meal may be deleted from the Oregon Test Diet.

OBSERVATIONS ON STRESS-DISEASE CORRELATION IN JUVENILE COHO SALMON

John F. Conrad
Oregon Fish Commission
Clackamas, Oregon

Most fish culturists and allied workers are aware of various stress factors that may adversely affect fish rearing, such as seasonal high water temperatures, inadequate water supply, heavily stocked ponds, low dissolved oxygen, high concentrations of metabolic waste, handling, disease, etc. These are all common phenomena, however, sometimes the obvious may be overlooked. The following information collected at the Siletz Hatchery during 1963 seemingly substantiates this premise.

At Siletz, coho salmon are reared in concrete ponds 10-$\frac{1}{2}$ feet wide by 58 feet long at a water depth of approximately 33 inches, and in a dirt impoundment containing approximately 1/3 surface acre of water with depths ranging from 2 to 6 feet. The hatchery water supply is from Rock Creek, a small stream with a temperature range of 32-72° F. with much reduced flow during summer months. The concrete ponds have individual water supplies; while the impoundment is partially supplied with unused water from the creek and a small spring plus additonal water pumped from the tailrace of the concrete ponds during periods of low stream flow. In addition, 3 upright pumps are located in slack water areas of the impoundment to provide aerations during critical periods.

Fingerlings are started in the concrete ponds and maintained there until size increase necessitates thinning (usually about mid June) when the excess fish are transferred to the impoundment. Approximately 400,000 fish were transferred this year.

Furunculosis outbreaks have occurred in Siletz Hatchery coho during each of the last 3 rearing seasons. Each year the disease has been first diagnosed during or just after pond thinning and fish transfer. This year transfer of fish from the ponds to the impoundment commenced about June 8, and furunculosis was first detected in both pond and impoundment fish on June 14.

Starting June 25, sulfamethazine was administered in the diet, to both groups of fish, at 5 grams Sulmet per 100 pounds of fish per day for 10 consecutive days. Mortality was sharply reduced in both rearing areas. However, in late July the loss started to increase in the impoundment and furunculosis was again detected on August 2 in these fish only. Once again mortality was sharply reduced by Sulmet therapy started on August 9, only to increase again in early September with furunculosis still present. The mortality pattern from June through October 1963 for each group of fish is shown in Figure 1.

The expense of 2 Sulmet treatments without disease eradication and the possibility of the appearance of a sulfa resistant strain of Aeromonas salmonicida prompted consideration of other methods of control. Kingsbury (1961) working with New York hatchery trout, reported the low available oxygen may often be a critical stress factor favoring the development of furunculosis.

Table 1 shows that dissolved oxygen values found in the Siletz impoundment on September 10, 1963 ranged from 3.5 to 5.2 p.p.m. with higher values in the upper part of No. 6 concrete pond and Rock Creek. The low dissolved oxygen values found in the impoundment were not completely unexpected, but the oxygen reading of 3.7 p.p.m. near the outlet screen in the concrete pond was shocking. Apparently the concrete-pond fish were able to escape areas of low oxygen by congregating in the upper parts of the ponds; whereas, the impoundment fish were forced to remain in inadequately oxygenated water favoring the persistence of furunculosis.

Installation of 2 additional pumps, better aeration methods, and cooler water temperatures resulted in more favorable rearing conditions, and mortality in the impoundment fish sharply declined and remained comparable to that in the concrete-pond fish.

Very similar mortality patterns to the one shown here for 1963 existed at the Siletz Hatchery during 1961 to 1962. Each year after high mortality associated with hatchery wide furunculosis was controlled by Sulmet therapy; the loss remained low in the concrete ponds but continued high in the impoundment.

It appears that each year the first furunculosis outbreak was triggered by handling during fish transfer, and the persistence of the disease in the impoundment fish was favored by low available oxygen. Inadequate oxygen should have been suspected but oxygen values were not determined until 1963.

Obviously here is a situation where the obvious was overlooked.

Literature Cited

Kingsbury, O. R. 1961. A possible control of furunculosis. Prog. Fish-Cult. 23 (3) : 136-137.

DISSOLVED OXYGEN VALUES, SILETZ HATCHERY,
SEPTEMBER 10, 1963

SAMPLE DESCRIPTION (TIME--9-12 A.M., SURFACE WATER TEMPERATURE -- 64° F.)	O_2 PPM SURFACE	O_2 PPM BOTTOM
Impoundment		
(a) End of Plank Walkway Near Impoundment Center	4.5 & 4.9	3.5 & 3.6
(b) Northwest Corner of Impoundment near Boat Tie-Up	4.1	4.2
(c) Inlet of Unused Water (Not from Ponds)	5.2	4.2
(d) Inlet of Water Pumped from Tailrace of Concrete Ponds	4.8	-
#6 Concrete Pond		
(a) Upper End of Pond Adjacent to Water Inlet	6.9	-
(b) Immediately above outlet screen	3.7	-
Rock Creek Above Hatchery		
(a) Immediately above Dam at Hatchery Pipeline Intake	9.0	-

THE USE OF BACTROVET (SULFADIMETHOXINE) FOR CONTROL OF FURUNCULOSIS ON ADULT SALMON

Daniel B. Romey
Oregon Fish Commission
Clackamas, Oregon

INTRODUCTION

Sulfonamides have been used to control bacterial fish diseases by a number of fish-cultural organizations for several years. The Oregon Fish Commission includes Sulmet (sulfamethazine) in the diet of juvenile salmon for controlling "cold-water" disease. This sulfonamide is administered on a daily basis until the fish have reached a given size and the water temperature has risen to a point where precludes the disease.

Now the possibility of administering sulfa to adult salmon to control furunculosis, a disease caused by Aeromonas salmonicida, is being investigated. The drug undergoing the most extensive tests is sulfadimethoxine and is known by the trade names of Bactrovet (Pittman More) and Madribon (Roche). This sulfa was selected because it is retained longer in the blood, and according to Amend et al.[1] gives a zone of growth inhibition equal to the other sulfonamides tested: Sulmet, Gantrisin (sulfasoxizole), and S.E.Z. (sulfaethoxypyridazine). Bactrovet is more expensive than the other three but less is used because longer retention in the vascular system requires application only every 3-5 days as opposed to daily administration of the less expensive sulfas.

METHODS AND MATERIALS

Two species of adult salmon were treated, chinook and coho.

Chinook Treatments

Three routes of administration were explored: oral force feeding, intestinal injection, and intramuscular (I.M.) injection. Tablets and gelatin capsules were fed with a modified veterinary balling gun. The intestinal injections utilized a 3-inch long piece of polyethylene tubing fitted to a 10 ml hypodermic syringe. Through this, a mixture of equal parts Bactrovet and sodium bicarbonate mixed with glycerin was forced through the anus directly into the intestine. The I.M. injections were of the commercial 10% solution injected into the caudal peduncle dorsal to the lateral line between the adipose and anal fin. All administrations were at about 100 mg of sulfa per pound of fish.

[1] 1962 unpublished data concerning in vitro tests on Aeromonas salmonicida.

The control fish received an equivalent volume of 0.85% injectable saline solution. MS 222, technically designated Tricane-methane sulfonate (Sandoz), was used as the anesthetic but was later superseded by Methyl Pentanol (Airco) as the latter produced no false sulfa readings in blood samples.

Blood samples were taken every 24 hours for 5 days following treatment. The sample from each lot was the combined sum of the individual 0.5 ml samples. Blood was drawn from the anterior terminus of the dorsal aorta at the junction of the first gill arch. Collection vials and syringes were heparinized to prevent clotting.

Coho Treatments

Coho jacks were used, treatments consisted of: (1) force-feeding tablets, capsules, and injectable 10% and 25% solutions; (2) injecting intramuscularly 10%, 25%, and 50% solutions; and (3) intestinal injections of 10% and 25% solutions. The technique for force feeding the coho was the same as that used for chinook. The I.M. injections on these fish were midway between the lateral line and the insertion of the dorsal fin. Two water-soluble, injectable solutions were prepared in the laboratory as only a 10% injectable was available commercially. The 25% and 50% solutions were prepared by mixing the free acid Bactrovet with sodium hydroxide in enough distilled water to form an injectable solution. All treatments were administered at the rate of 100 mg of sulfa per pound of fish. The controls received the equivalent volume of 0.85% injectable saline solution.

Blood samples were taken every 24 hours for only 4 days following treatment, first from the dorsal aorta at the first gill arch, then because of hemorrhage, by cardiac puncture. All syringes and blood collection vials were heparinized. All blood samples were analyzed for sulfa content by the Bratton-Marshall method utilizing a Spectronic 20 colorimeter. All fish were autopsied at the termination of each experiment.

RESULTS

With both chinook and coho the Bactrovet tablets and capsules fed into the stomach failed to be absorbed or to pass from the stomach to the intestine. The tablets disintegrated and the capsules became flaccid in 4-5 days; the capsules disintegrated in 6-7 days. The intestinal injection of the Bactrovet-sodium bicarbonate-glycerin suspension failed to be absorbed into the blood. In brief, all nonwater-soluble sulfa forms forced into the G.I. tract were not utilized. This tends to indicate that digestive enzymes or peristalic action is nonfunctional in the G.I. tract of the chinook or coho adult salmon at this stage. Consequently, no sulfa blood levels were observed on these force-fed fish.

The I.M. injections in both chinook and coho produced satisfactory therapeutic blood sulfa levels of 12-18 mg per 100 ml blood (mg%) within 24 hours. These values diminished to 7-8 mg% in 72 hours after treatment at which time an identical "booster" shot raised the sulfa concentration up to 12-16 mg%. These values again dimished to 9-14 mg% in 3-4 days and did not reach minimal levels of 4-7 mg% for perhaps 5-6 days. However, the success of the therapy is not without side effects. All fish receiving the sulfa I.M. injections developed hemorrhagic abscesses at the injection site. These abscesses do not apparently hinder the fishes mobility or metabolism even though autopsy revealed the abscesses to be comprised of blood and disintegrated myotome musculature. Amend et al. (unpublished) experienced this identical condition on coho and through culture tests found these abscesses to be sterile. In most cases the injuries were usually visible from the fishes exterior and were about the dimension of a lead pencil 2-inches long. These abscesses were at first believed to be the result of the excessive volume of the 10% solution (the only one available commercially) required to achieve the necessary sulfa blood level. However, the laboratory-prepared 50% concentrated injection of only one-fifth the volume of the 10% solution produced trauma of the same and sometimes greater severity, thereby indicating tissue sensitivity.

The water-soluble injectable 10 and 25% solution extruded into the G.I. tract produced sulfa concentrations of a minimal value of 3.6 mg% at the end of 24 hours diminishing to zero in 48 hours. This occurred only in the fish receiving the 10% injectable Bactrovet solution which had been forced into the stomach by the syringe. The solution injected into the intestine produced no sulfa blood levels on either sample. Continued experimentation will be employed on this technique, as autopsy revealed no adverse side effects.

CONCLUSIONS

Force feeding of drugs to adult salmon is mechanically and physically feasible but chinook and coho do not utilize ingestible forms of Bactrovet. The intestinal injection of Bactrovet suspension is no more successful. I.M. injections produce therapeutic blood sulfa levels but only at the expense of tissue damage at the injection site. However, these abscesses do not apparently hinder the fishes physical mobility and seem to be the result of tissue sensitivity and not volume rupture.

By force feeding a 10% water-soluble injection solution directly into the stomach, moderate blood sulfa levels might be obtained without injurious side effects.

1963 FEEDING TRIALS
SALMON-CULTURAL LABORATORY

Laurie G. Fowler
Bureau of Sport Fisheries and Wildlife
Longview, Washington

The 1963 feeding trials conducted at the Salmon-Cultural Laboratory, Longview, Washington, were designed to maintain fall chinook fingerlings for a 24-week period. Twenty experimental diets and one control diet were fed to determine the amount of crystalline vitamin supplementation necessary for adequate maintenance and to determine both the optimum caloric intake and protein calorie-energy calorie relationship. A composite meal consisting of salmon carcass meal, dried skim milk, cottonseed meal, and wheat germ was supplemented with four levels of crystalline vitamins ranging from 100 per cent to 200 per cent of the maximum levels recommended by Halver. Peanut oil was used to increase the levels of caloric intake which ranged from 1,650 calories to 3,050 calories per kilogram of diet. Water was used as a dilutent to maintain the protein level at 25 per cent and to give the feed a mush-like consistency similar to a raw-products diet. All diets were bound by the addition of CMC and ricer fed. Composition of the diets and other ingredients are presented in Table 1. Briefly, the results are as follows:

1. All of the experimental diets maintained fish for the entire 24-week period with exceptionally low mortality rates.

2. Fish receiving a meat supplement of 30 parts meat to 70 parts meal deposited higher levels of protein than did fish fed an all-meal diet.

3. Soybean oil meal was an inadequate replacement for cottonseed meal when fed as a component of the basal ration.

4. A single level of crystalline vitamin supplementation proved to be adequate.

5. Increasing the caloric level of a diet produced a sparing action on the protein requirements of the fish. Average protein deposition at various caloric intakes are presented in Figure 1. A caloric level of 2,350 calories per kilogram of diet appeared to be optimum under the conditions of this experiment.

Figure 1.--Average values of protein deposited in fish fed five different calorie levels and at a protein intake of 25 percent, Salmon-Cultural Laboratory, Longview, Washington. Protein utilization factors enclosed in parenthesis.

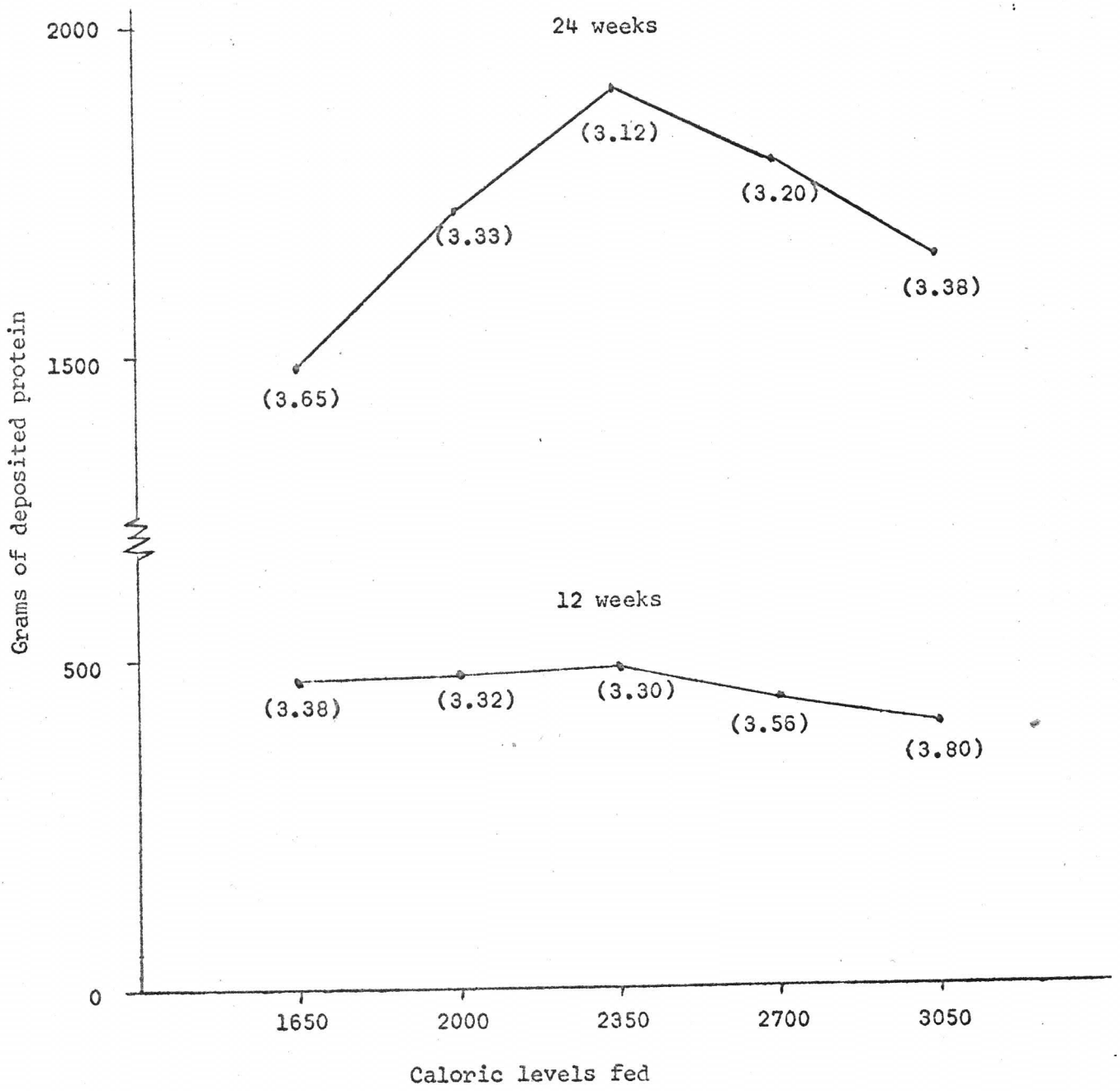


Table 1.--Composition of diets of the 1963 feeding trials

Salmon-Cultural Laboratory, Longview, Washington

Diet number	Percent protein	Meat-meal ratio	Crystalline vitamin supplement level	Calories per kg. of diet
1 *	21.3	90:10	--	1467
2	25	30:70	V-1	2350
3	"	30:70	V-3	2350
4	"	100% A-2 meal	V-1	2000
5	"	100% A-1 meal	"	1650
6	"	"	"	2000
7	"	"	"	2350
8	"	"	"	2700
9	"	"	V-2	1650
10	"	"	"	2000
11	"	"	"	2350
12	"	"	"	2700
13	"	"	"	3050
14	"	"	V-3	1650
15	"	"	"	2000
16	"	"	"	2350
17	"	"	"	2700
18	"	"	V-4	1650
19	"	"	"	2000
20	"	"	"	2350
21	"	"	"	2700

*Control Diet	100.0%
Beef liver	30.0%
Hog liver	30.0%
Beef spleen	30.0%
Salmon carcass meal	5.0%
Distiller's solubles	5.0%
A-1 Meal Mixture	100.0%
Salmon carcass meal	35.0%
Dried skim milk	30.0%
Cottonseed meal	20.0%
Wheat germ	15.0%
A-2 Meal Mixture	100.0%
Salmon carcass meal	35.0%
Dried skim milk	30.0%
Soybean oil meal	20.0%
Wheat germ	15.0%
Meat Mixture	100.0%
Beef liver	50.0%
Hog liver	50.0%

V-1 Vitamin Mix = 100% of maximum recommended level.
V-2 " = 125% "
V-3 " = 150% "
V-4 " = 200% "

SPRING CHINOOK PRODUCTION AT WILLAMETTE HATCHERY

Joe Wallis
Oregon Fish Commission

The Willamette Hatchery, located on the Middle Willamette River, was rebuilt and expanded to compensate for lost spawning and rearing areas blocked by Lookout Point Dam, a Corps of Engineers project. The strict purpose of the hatchery is to maintain the spring chinook run in the Middle Willamette River. This presentation is a preliminary report on the relative success of that operation.

The annual escapement of spring chinook over Willamette Falls has been estimated by Oregon Fish Commission biologists beginning in 1946 and continuing to date. The escapement varies considerably from year to year and has ranged from a maximum of 76,400 in 1953 to a minimum of 14,400 in 1960 (Figure 1).

Before 1953 adult salmon were captured at racks in Salmon Creek near the hatchery and in the Middle Willamette River near Oakridge, both areas upstream several miles from the dams. In 1953 and 1954 fish were captured at racks downstream from Lookout Point and Dexter Dams, and since 1954 they have entered adult holding ponds immediately below Dexter Dam. The enumeration of adults from 1953 to date are generally comparable, although not strictly so. The numbers of adults handled at Dexter have ranged from a maximum of 6,019 in 1963 to a minimum of 802 in 1960. Although the numbers of adults handled at Dexter have shown a long-term trend similar to that of the escapement over Willamette Falls, the trend has not been the same from year to year (Figure 1).

From 1953 to 1956 the numbers of adults in the run at the Dexter site totaled from about 2,600 to 4,400 fish. There were some hatchery-produced fish in the run during these years, but the run was predominately naturally-produced. Essentially all the adults returning since 1956 have been hatchery-produced fish.

We have sufficient data from Willamette Hatchery to evaluate production by brood, rather than assuming either a 4 or 5 year cycle. A comparison of parent runs to returning progeny as

both 4- and 5-year olds is shown in Figure 2. It is obvious that the 1953 through 1957 runs were not replaced, but the 1958 parent run was about doubled. In addition, based on past records and the number of 4-year olds returning in 1963, the return of the 1959 brood progeny should be about twice as great as the parent run, after the return of 5-year fish in 1964.

There were two primary reasons why the 1953-57 runs were not replaced. First, there were excessive pre-spawning losses of adults, and secondly, fingerling survival was relatively low. In 1953 and 1954 when fish were captured with racks, 83.7% and 56.4% respectively of the adult females were lost before spawning. From 1955 through 1960 from 35.8% to 68.3% of the adult females which entered Dexter Ponds died before spawning. For most of these broods fingerling survival was great enough to replace the females which survived, but was not great enough to compensate for the excessive pre-spawning loss.

The 1958 run was replaced and there is enough data to show that the 1959 run will also be replaced. The reason for this change was an increase in fingerling survival as a result of eliminating raw salmon viscera from the diet. Pasteurized salmon viscera was incorporated into a meat-fish diet fed to the 1958 brood, and Oregon pellets have been fed since, beginning with the 1959 brood. Before the 1958 brood; returns to Dexter had ranged from 0.019% to 0.164% of the number of fingerlings released. One exception was the 1953 brood which provided a 0.316% return; kidney disease was not found in fingerlings of this brood whereas it was in all other broods before 1958. The return of the 1958 brood represented 0.346% of the number released, and preliminary data indicate that the survival of the 1959 brood may be similar to that for the 1958 brood.

Two groups of 1958 brood fingerlings were marked to compare the survival of fish fed Oregon pellets with that of a group fed a wet diet containing pasteurized salmon viscera. The pellet-fed group produced a return (to Dexter) of 0.345% and the group fed the wet diet provided a return of 0.163%. There were differences in size at time of release and the noted difference in survival may have been partly a result of this.

Several diseases of adult salmon have been noted at Dexter, and have been reported previously. Routine treatments with malachite green were started in 1960 and the success in controlling and healing fungus infections was spectacular. However, the excessive loss of adults continued in 1960. Since

1961 the water entering the holding ponds has been cooler than in former years (maximum of 59° F. compared to maximums of 64 - 66° F. in earlier years), and the loss of adult females has been reduced to 5 - 10% the past three years.

Egg losses during the incubation period have also been reduced since cooler water has been available during the holding period; 5 - 10% now compared to 15 - 20% formerly.

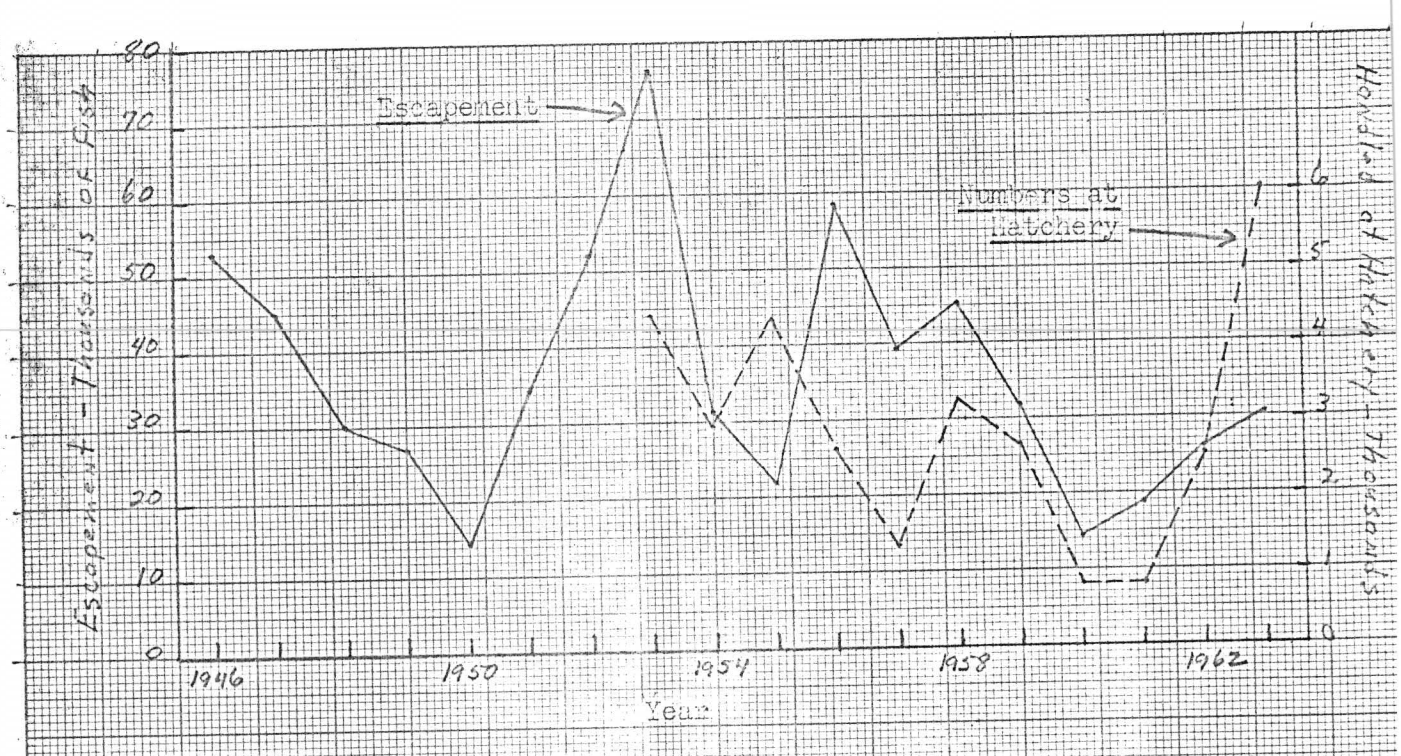


Figure 1. Escapement of Adult Spring Chinook over Willamette Falls and Numbers Handled at Willamette Hatchery.

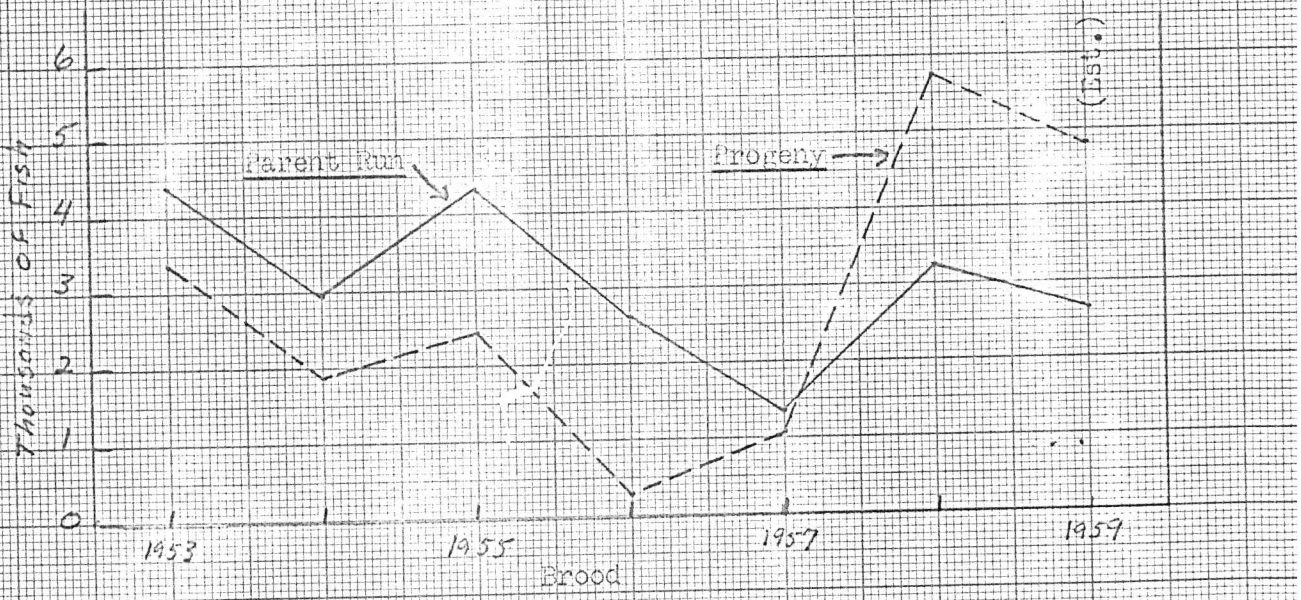


Figure 2. Comparison of 1953 - 1959 Parent Runs of Spring Chinook at Willamette Hatchery with Returning 4- and 5-Year Progeny.

PATHOLOGY AND HATCHERY EVALUATION

Edward M. Wood, M. D.
USFWS Salmon-Cultural Laboratory
Longview, Washington

The prime objective of hatchery evaluation is the identification of those factors responsible for good or poor survival. Included among these factors are a host of variables--water quality, length of rearing, rearing facilities, time of release, predation, nutrition, disease and ad infinitum. The fish cultural field has recognized the essential necessity for determining the effect of these variables and many programs are in progress in an attempt to define their individual importance. It is apparent that definative evaluation of the hatchery product can be based with validity on one criteria alone: survival to adulthood and subsequent contribution to the total fishery. Survival data, however, give little or no information as to the reasons for success with one hatchery population or failure with another. The application of pathology in the evaluation of hatchery fish is an attempt to determine the physiological status of the population at the time of release.

Two major variables subject to some degree of control in the hatchery environment are disease and nutrition. Without a comprehensive evaluation of the effect of these two factors, the valid comparison of other variables is impossible. A pathology examination is one step in this evaluation.

In a preliminary study to determine the usefulness of pathology, 5 groups of fish from the USFWS Salmon-Cultural Laboratory were submitted for evaluation. These fish were from the nutrition research program of 1962-1963 and represented groups reared in both river and well water, the latter considered essentially free of disease organisms. All groups had made excellent weight gains and were considered free of significant disease problems.

The pathology examination revealed:

(1) The river water fish were heavily parasitized by a variety of organisms. Of particular significance was a high incidence of sporozoans in the kidneys and dorsal muscles. The kidney organism caused marked tissue damage which undoubtedly represented a physiological handicap. The muscle organism potentially represents an inhibiting influence and should be so considered in the evaluation of stamina data.

(2) The well water fish were also infected with the muscle sporozoan and with Schizamoeba salmonis. This suggests that either the well water is not disease free, that the organisms were introduced with the feed, or that the infestation occurred during a short early period in river water. All three are subject to investigation.

(3) The diet fed each group could be identified by tissue changes in the liver and pancreas alone. The significance of these changes is unknown. Their correlation with survival, however, will be an important objective in the search for criteria of "good" hatchery fish.

(4) Marked liver lesions were observed in fish fed the "standard-control" diet. These changes suggested an abnormality of fatty-acid metabolism. Following this observation it was learned that the diet had contained rancid fish meal (high TBA number) for a short period. Since similar lesions are frequently seen in hatchery fish, this finding may represent a significant etiological clue.

In summary it may be stated that the pathology examination revealed marked abnormalities in 5 groups of fish believed to be in an excellent physiological status. The effect of these abnormalities on survival represents the challenge to progressive fishery research of the present and future. The recognition of these abnormalities is the role of pathology.

FURUNCULOSIS IN ADULT SALMON AND STEELHEAD TROUT

by

John Fryer, Oregon State University

and

John Conrad, Oregon Fish Commission

INTRODUCTION

Furunculosis is one of the best known bacterial diseases of salmon and trout. Since first described at a German fish hatchery in 1894 it has been observed to cause severe losses in stocks of trout and juvenile salmon. Aeromonas salmonicida, the causative agent of this disease, has received extensive investigation in both Europe and America as a result of mortalities occurring in populations of juvenile salmon, juvenile trout, and brood-stock trout. This organism has not, however, been generally considered a serious infectious agent of adult salmon and steelhead trout. Wales 1/ isolated this bacterium from adult coho salmon and steelhead trout which had entered Waddell Creek in California. Wood 1/ observed a gram negative bacterium believed to be A. salmonicida on slides prepared from tissues of fall chinook in the Snake River. Later he was able to isolate a bacterium from spring chinook salmon held at the Dexter Ponds on the Middle Fork of the Willamette River and identified it as A. salmonicida.

The purpose of this paper is to report a series of observations made since 1961 concerning the occurrence of this disease in adult fish. While there is very little of the quantitative information required to establish the true importance of the disease for adult fish, it is believed the following material coupled with previous observations will prove useful in future investigations.

METHODS

Fish examinations were conducted under field and laboratory conditions. Bacteria were isolated and cultured in accordance with standard bacteriological techniques and identification carried out employing the diagnostic procedures outlined in Bergey's Manual of Determinative Bacteriology.

1/ Personal communication.

OBSERVATIONS

Spring chinook salmon examined from three different locations have been found to be infected with A. salmonicida (Table 1). The disease has been diagnosed from fish at Dexter Dam on the Middle Fork of the Willamette River for the past three years. The incidence does not appear to be high; however, diseased fish examined have been badly infected and often exhibit the characteristic pathology associated with this condition. Spring chinook obtained from the Eagle Creek National Fish Hatchery and transported to the Oregon Fish Commission Clackamas research laboratory were subsequently found to be infected with this bacterium. The location at which the infection occurred is not known; however, it may very well have taken place after the fish were received at the Clackamas laboratory. A serious mortality of adult spring chinook salmon in 1963 from the upper Trask River prompted examination of these animals and the bacterium responsible for furunculosis was observed. The importance or extent of the infection is not known.

In 1961 adult fall chinook salmon held at the Oxbow Dam receiving pond on the Snake River experienced heavy losses. Examination of 75 mortalities obtained from this location revealed that 63% had furunculosis. Adult steelhead trout examined here were also infected with this bacterium (Table 1).

Above-normal mortalities in the fall chinook run held at the Trask River Hatchery resulted in the examination of these fish. Mortalities were found to contain the bacterium causing furunculosis and to exhibit the characteristic pathology of this disease. The course of this infection was curtailed with the advent of spawning.

Mortalities resulting in adult coho salmon during 1962-63 at both the Klaskanine and Big Creek hatcheries have in part been attributed to furunculosis. The loss of females was excessive in 1962, perhaps exceeding 5% during the period of infection. Autopsy of dead and morbid fish indicated the presence of A. salmonicida. Recently this organism has been detected in adult coho salmon at the Sandy River Hatchery by members of the Oregon Fish Commission pathology staff. The extent of this infection is at present under investigation.

Table 1. The Occurrence of Aeromonas salmonicida in Adult Salmon and Steelhead Trout.

Species	Year	Location
Spring Chinook Salmon	1961-62-63	Dexter Dam--Willamette River
" " "	1962-63	Eagle Creek National Fish Hatchery 1/
" " "	1963	Trask River
Fall Chinook Salmon	1961	Oxbow Dam, Snake River
" " "	1962	Trask River Hatchery
Coho Salmon	1962-63	Klaskanine Hatchery
" "	1962-63	Big Creek Hatchery
" "	1963	Sandy River Hatchery
Steelhead Trout	1961	Oxbow Dam, Snake River

1/ Disease diagnosed after these fish had been transported to the Clackamas research laboratory.

SUMMARY AND CONCLUSIONS

The importance of A. Salmonicida as a disease agent of adult salmon and steelhead trout is not known. Determination of this point is made difficult by the fact that these animals are often inflicted with multiple infections, any one of which could cause death. Individual fish have often been observed with infections of Columnaris, Ceratomyxa, Aeromonas sp., and A. salmonicida. One heavily fungused spring chinook salmon was examined and found to be infected with Columnaris, A. salmonicida, kidney disease, fish tuberculosis, and Ceratomyxa.

Furunculosis is, however, a well established disease of salmonids and the presence of this infection in adult fish from the various locations indicated is believed to be important.

THE IODIDE REQUIREMENT OF CHINOOK SALMON

A. N. Woodall
Gilles LaRoche
Western Fish Nutrition Lab., Cook, Wash.

The iodide requirement of chinook salmon was determined in a 15 month feeding trial. 5 lots of chinook salmon were fed a low iodide test diet with added sodium iodide to give diets containing: 0.1, 0.6, 1.1, 5.1 and 10.1 micrograms iodide per gram dry diet. Growth and food efficiency was excellent for all lots. From an evaluation based on maximal storage of iodine by the thyroid, it was concluded that the iodide requirement of chinook fingerlings at 24 weeks was approximately 0.6 micrograms per gram of dry diet while the requirement of advanced parr at 15 months was approximately double this figure.

PRODUCTION RECORD OF KLASKANINE HATCHERY

George Smalley
Oregon Fish Commission
December 3, 1963

The Klaskanine Hatchery is located on North Fork of Klaskanine River. The Klaskanine flows into Youngs Bay which enters the Columbia River at Astoria. This hatchery was remodeled in 1953 with funds from the Columbia River Fishery Development Program. As soon as our feeding program was changed to pasteurized viscera and Oregon pellets (1958 brood) it was apparent that we would have more adult coho back to the station than were necessary to provide the rearing stock. The present rearing capacity is about 1.5 million yearlings requiring about 600-650 females, or about 1,200 adults total.

Adult carcasses are given to public schools, state institutions, Indians, and some to rendering plants. An improvement in the use of adults would be made if more could be caught by either the ocean sport or commercial fisheries. The last few years these fisheries have also been increasing, however, a surplus still exists at the hatchery.

In 1961 it was proposed to reopen a commercial fishery in Youngs Bay for test fishing. There had been a fishery in this area about 30 years ago. The purpose of the test fishing would be to determine the timing of the coho through the bay, to find out if gill netting was possible since the area was relatively shallow and filled with snags, and to help to harvest surplus fish.

One gill netter was hired to fish during the regular open Columbia River fall season. Table 1 shows the yearling coho production from the hatchery, the adult returns, and those caught in the fishery.

Table 1. Klaskanine Hatchery Production and Youngs Bay Commercial Catch

Brood Year	Yearlings Liberated	Age Group of Returning Coho to Hatchery		Youngs Bay Catch		
				Numbers		Estimated
		Jacks	Adults	Jacks	Adults	Pounds
1953	-0-	71	110			
1954	699,000	18,368	9,740			
1955	288,000	205	306			
1956	203,000	1,312	966			
1957	356,000	631	968			
1958	410,000	2,616	3,323	131	459	5,377
1959	788,000	6,813	4,086	663	2,056	20,000
1960	1,124,000	15,234	5,602 1/	2,166	4,074	42,000
1961	1,079,000	6,669 1/				
1962	1,638,000 (on hand)					

1/ to date - November 30, 1963

The experiment appeared to be successful so in 1962 Youngs Bay was opened to gill netting during the regular Columbia River season. The catch was 2,056 adults and 663 jacks with 4,086 adults to the hatchery, more than 3 times those required.

In 1963 it was again opened to commercial fishing, however, during some of the closed weekends small meshed gill nets were allowed to harvest more jacks. One full week of the Columbia closed season was opened in the bay. The catch in 1963 increased to 4,074 adults and 2,166 jacks. Counts at the hatchery as of November 30 showed 5,602 adults - over 5 times more females than is necessary to provide the hatchery capacity of yearlings. Hatchery counts are minimal since there were large numbers which returned and which were refused entry and either spawned below or were caught by sport anglers. Sufficient adults were allowed upstream to seed that area.

This has been accomplished at the hatchery with low flows in the summer of less than 4 c.f.s.

Plans are to reopen the commercial season next year and to further liberalize the fishing.

Plans are also underway to find funds to construct a dam upstream to provide additional water in the summer to allow further increase in numbers reared and more contribution to the fisheries.

EFFECT OF FEEDING TWO CALORIE LEVELS ON QUALITY OF FINGERLING PRODUCED

Allan E. Thomas
Bureau of Sport Fisheries and Wildlife
Longview, Washington

The Abernathy Salmon-Cultural Laboratory has a continuing program to define the fingerling characteristics necessary for maximum adult survival. Under this program we impart characteristics we think may be important to survival into relatively large groups of fish, mark and release the fish, and evaluate the characteristics by the adult returns to the laboratory. These characteristics are used as the single variable between otherwise identical populations of fish. We yearly alternate characteristics imparted by nutrition with those from environmental conditions.

1961 Rearing Season - Two groups of fall chinook fingerlings were fed different diets to produce at least a 3% difference in body fat. The diets used were (1) the Cortland #6 diet known to produce fat fish and (2) the Entiat Production Diet which produces a lean fish. Each group was given a different mark and released. Returns this fall totaled 40 three-year fish and included 6 females. Returns by mark were 15 fish from the low-fat group and 25 fish from the high-fat group. Evaluation will be made after the four-year fish return the fall of 1964.

1962 Rearing Season - This experiment was discussed at the 1962 Northwest Fish-Cultural Conference. It was where a difference in stamina between two groups of fish was imparted by the rearing environment. Five two-year fish returned this fall from this experiment.

1963 Rearing Season - This experiment was similar to the 1961 experiment in that different levels of fat were tested. In this case, however, an identical basic diet composition was used and differences in fat levels were obtained by supplementing the diet of one group with additional energy calories.

On May 22, 1963, a homogeneous group of fish was randomly and equally distributed into identical rearing environments so that approximately 180,000 fall chinook fingerling were in each lot. The basic diet composition is listed in Table 1. The

caloric intake was controlled by the substitution of 7.37 per cent peanut oil in the high-calorie diet for 7.37 per cent water in the low-calorie diet. In this manner both lots receive 25 per cent protein and equal protein calories per kilogram of diet, but the energy calorie intake in the high-calorie diet is increased by approximately 700 calories by the addition of peanut oil.

The two groups were reared for a three-month period, marked by fin clipping, and released on August 20 and 21. The measurements made on the two groups are summarized in Table 2. Differences existed in survival, gain, conversion, protein utilization, and fat deposition. All these differences indicate a superior performance by the fish fed the high-calorie diet.

Stamina tests were conducted on samples from each group throughout the rearing season, usually at bi-weekly intervals. No significant difference in performance was found between the two groups of fish during this time. It had been expected that the larger size of the high-calorie fish and the increased energy calories available would produce significantly higher performance results above that of the low-calorie group before the rearing season was over. The flow patterns of the rearing pond types used for this experiment do not restrict performance as the fish size increases as does the conventional raceway pond type (Thomas, 1962 NWFCC).

A number of possible reasons for this lack of performance differences between the groups can be listed. Both groups, and especially the high-calorie group, went through a period of extremely rapid growth. We have data from other experiments showing less than the expected performance level for fish growing rapidly. This period of rapid growth for both groups eventually produced an evercrowding effect in the rearing environment. The irritation to the gills by a build-up of waste products, chiefly ammonia, had earlier been found to reduce the swimming ability of fish.

Disease may also have been a factor affecting the stamina results. Both groups were exposed to equal numbers of the cercariae of the salmon-poisoning fluke (Nanophyetus salmincola) which are extremely abundant in Abernathy Creek. The numbers in the hatchery water supply are kept at a low level by the electric grid (Combs, 1962 and 1963 NWFCC) and high levels of infestation are usually necessary before the fish are affected.

High water temperatures, 70° F. and above, were reached in the creek water during the late summer. To reduce possible stress to the fish from this warm water, the creek water was diluted with well water of a constant 53° F. The combination of over-crowding of fish in the ponds and a slightly increased pH level from the dilution with well water enabled a low level of bacteria gill disease to become established. It was detected only in the low-calorie group, but probably was in the high-calorie group as well, especially since the high-calorie group had more weight of fish per pond.

Low levels of furunculosis, Costia, gill Amoeba, and cell proliferation of the lamallae were found in both groups of fish from cursory examination. All had previously been found to reduce the performance of fish in the stamina tunnel. Treatments were Terramycin added in the diets for furunculosis and formalin and lignasan in the ponds for the external parasites. These treatments can also cause at least a temporary decrease in performance and no stamina tests were conducted for at least several days after a treatment period. Dr. Wood (1963 NWFCC) examined samples from both groups of fish and found a muscle sporozoan in 40 percent of the fish from each sample which may have a definite effect on swimming ability.

All the fish had an equal exposure to the diseases. At least in the case of furunculosis and the external parasites, there was a greater resistance to disease by the high-calorie fish. The low-calorie group had recurrent infestations, especially of the external parasites, and within two weeks after treatment would be "flashing" vigorously and the mortality would increase. The fish in the high-calorie group showed no such degree of infection. Treatment mortalities were nil in the high-calorie group and as high as 300 or 400 fish per pond for the low-calorie group.

Gross examinations were made of samples from both groups at the end of the rearing season. Seventy percent of the fish from the high-calorie group had heavy visceral fat and 30 percent had medium fat. Of the low-calorie samples, 10 percent had heavy fat, 70 percent had medium fat, and 20 percent had light visceral fat. Examination of samples by Dr. Wood produced fatty liver and pancreatic tissue in the high-calorie group, less fat in the low-calorie group, and the liver glycogen appeared to be lower in the high-calorie group.

Electrophoretic seperation of the plasma protein were conducted on samples from each lot. The best and worst ten fish from stamina tests on each group of fish were used as samples. Figure 1 shows the results for the analytrol method. The figure shows that the high-calorie fish had slightly more gamma

globulin, less beta and alpha 2 globulin, and considerably more albumin and alpha 1 globulin. The alpha 1 globulin and albumin could not be separated with the analytrol method. The average A/G ratio (albumin to globulin) was .31:1 in the high-calorie group and .16:1 in the low-calorie group.

The final phase of the effect of feeding two calorie levels on the quality of fingerling produced will be the comparison of the numbers of marked adult fish from each group returning to Abernathy Creek. Adult returns by the fall of 1966 should indicate if differences in survival may be induced by the calorie level fed the fingerling.

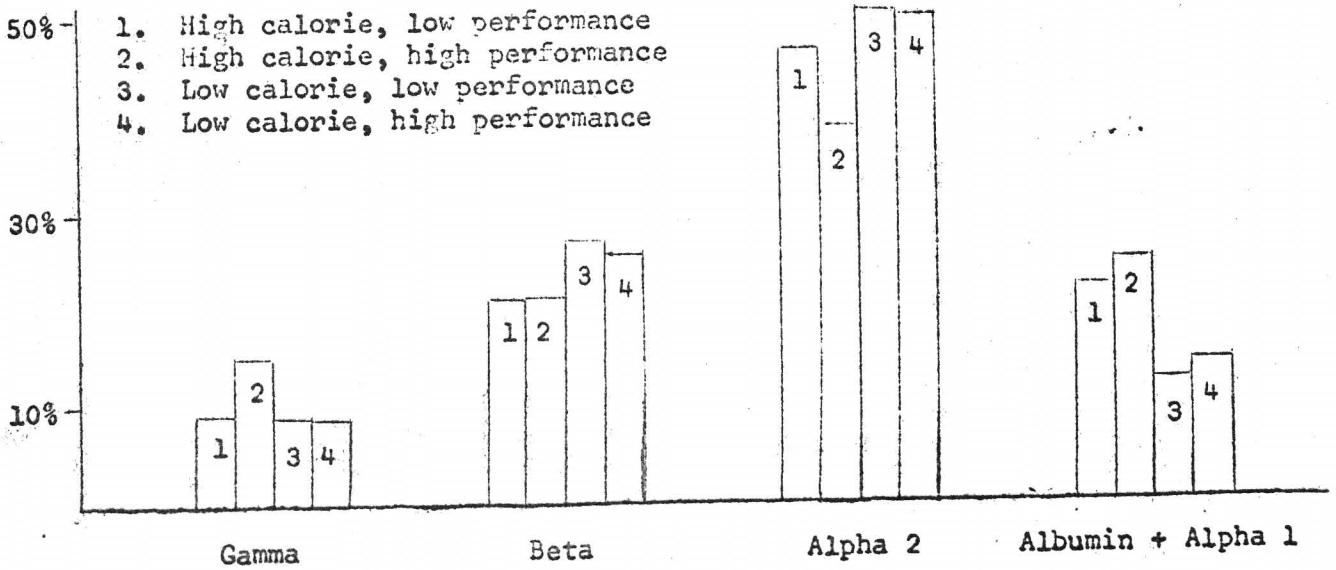
EFFECT OF FEEDING TWO CALORIE LEVELS ON QUALITY OF FINGERLING PRODUCED

Table 1.--Basic Diet	
Beef liver	(9.26%)
Hog liver	(9.26%)
Lung or spleen	(21.30%)
Turbot	(23.16%)
A-1 meal mix.	(29.64%)
Peanut oil(1) or water(2)	(7.37%)

A-1 Meal Mix.	
Salmon carcass meal	(35%)
Dried skim milk	(30%)
Cottonseed meal	(20%)
Wheat germ	(15%)

Table 2.--Results		High Calorie(1)	Low Calorie(2)
No. calories per kg. diet		2350	1650
No. fish released		170,660	170,940
Percent mortality		4.3	5.5
Total weight		11,428	8,944
Percent gain		465	342
Conversion		2.35	2.91
Protein utilization		3.5	4.2
No. per pound		15	19
Average weight		30 gm.	24 gm.
Proximate analysis of fish	water	74.26	77.54
	protein	16.75	17.27
	fat	7.86	4.14
	ash	2.22	2.44
Corpuscular count		1,574,000	1,514,000
Percent small cells	pre-stress	10.5	14.4
	stressed	14.5	20.6
Hematocrits		38.9%	37.9%

Figure 1.--Electrophoresis results - Analytrol method



IDAHO PRODUCTION DIET TESTS, 1963

Paul Cuplin
Idaho Fish and Game Department

The Idaho Fish and Game Department began the use of an open-formula dry fish feed for trout production during 1963. The diets used were developed in cooperation with Dr. A. M. Dollar, University of Washington College of Fisheries.

To evaluate the open-formula diets and to test the results of ingredient substitution in the diet, a series of tests were carried out by fish hatchery production personnel during April through September, 1963.

All diets consisted of a vitamin concentrate (Table II) and an ingredient formula (Table I). Minor changes were made in the formula and vitamins on July 1, 1963, as indicated. Test Diets I and II were the fry and grower diets, respectively. These diets were our open formulas for purchase of all fish feed for fish hatchery production during 1963. Diet III is predominately meat-scrap meal. Diet IV is Diet II with 150 percent of the vitamin concentrate. Diet VI is Diet II with the removal of all meat-scrap meal and substitution of liver meal and the substitution of soybean flour for soybean meal. Diet VII has a greater amount of soybean meal, reduced amount of wheat middlings, less whey, and two items not used in the previous diets, Distillers Solubles and dried beet pulp. Diet VIII is the same as Diet VII,, with the addition of 1,200 grams per ton of Inositol.

15,000 rainbow trout were used in each test, with the exception of Hagerman and Twin Falls Fish Hatcheries where 60,000 and 48,000 were used in each test, due to production requirements.

The number of pounds per cubic foot of tank space was maintained at .8 in all tests except Mackay, where .4 was used as a standard. The poundage was reduced by random selection and the fish were not graded during the experiment.

Diet Cost and Calculated Percent Protein
Results of Feeding Tests

The maximum percent protein and cost of Idaho production and experimental diets is outlined in Table III.

The results of the six-month feeding tests for rainbow trout on several dry diets is summarized in Tables IV and V.

Diet I gave the most promising results of the diets tested. Diet III, the meat-scrap meal diet, gave good results under limited testing;

further testing is planned. Adding 150 percent vitamin concentrate to Diet IV was of no benefit. Diet VIII, with 1,200 grams of Inositol per ton of feed, gave poorer results than did Diet VII, an identical diet with no Inositol added. Diet VI, which contained 15 percent liver meal, gave better growth response in relatively cold water at Ashton Fish Hatchery than did Diet I with 10 percent meat-scrap meal. Diet VII gave good growth response at Hagerman and Mackay Fish Hatcheries.

TABLE I

COMPOSITION OF DIETS, Idaho Production Diet Tests, 1963

Diet	Fish Meal	Meat Scrap Meal	Liver Meal	Soybean Meal	Flour	Whey	Wheat Mids.	Yeast	Kelp Meal	Dist. Sol.	Beet Pulp	Animal Fat	A&D Oil	Salt	Vit. Conc.
I	31	10	5	10		10	20	5	3			2		3.8	.2
II	27	10	5	10		10	24	5	3			2		3.8	.2
III		94											2	3.8	.2
IV		Diet II 150% Vitamin Concentrate													
VI	31		15		10	10	20	5	3			2		3.8	.2
VII	28	6	10	16		5	16	5	3	6	1.5		2	1.0	.5
III		Diet VII plus 1,200g/ton Inositol													

Formula Adjustments, July 1, 1963

1. 2 percent 2250A and 300D feeding oil substituted for animal fat.
2. Vitamin concentrate 4 lbs/ton to 10 lbs/ton (filler adjustment).
3. Salt 3.8 to 3.3 percent.
4. Condensed fish solubles 0.2 percent added.

TABLE II

Vitamin Concentrate, for One-Ton Feed Mix

Vitamin A [1/]	2,000 USP Units
Vitamin D_3 [2/]	360 IC
Vitamin E	60,000 IU
Riboflavin	90,000 milligrams
D - Calcium Pantothenate	50,000 milligrams
Niacin	100,000 milligrams
Choline Chloride	250,000 milligrams
Vitamin B_{12}	20 milligrams
D - Biotin [3/]	400 milligrams
Ascorbic Acid [4/]	400,000 milligrams
Thiamine Hydrochloride	90,000 milligrams
Pyridoxine Hydrochloride	20,000 milligrams
Folic Acid [5/]	2,000 milligrams

Weight of Vitamin Concentrate, plus carrier; 4 pounds

Adjustments, July 1, 1963

1. Removed.
2. Removed.
3. D - Biotin increased to 600 mg/ton.
4. Ascorbic Acid reduced to 200,000 mg/ton.
5. Folic Acid increased to 3,000 mg/ton.

TABLE III

MAXIMUM PERCENT PROTEIN AND COST IDAHO PRODUCTION AND EXPERIMENTAL DIETS, 1963

Diets	Cost/lb.	Percent Protein
I	.089, .0845	43.6
II	.084, .082	41.2
III	.056	52.8
IV	.10	41.2
VI	.10	44.8
VII	.10	44.9
VIII	.10	44.9

TABLE IV

Results of Rainbow Trout Feeding Tests, 6-Month Period
Idaho Production Diets, 1963

Hatchery	Diet	lbs. Food/ lb. Fish	Cost/lb. Gain	Total Mortality In %	Average Hematocrit	Water Temp.
Hagerman	I	1.3	.106	3.1	40.5	58°F.
	II	1.8	.147	3.2	41.5	
	VI	1.7	.165	2.9	41.5	
	VII	1.4	.144	3.1	41.3	
	VIII	1.6	.161	3.3	38.7	
Mackay	I	1.3	.111	5.0	42.8	52°F.
	II	1.4	.086	3.2	43.5	
	VI	1.4	.144	4.6	43.3	
	VII	1.3	.132	4.8	44.4	
	VIII	1.4	.144	4.6	40.1	

TABLE V

Results of Rainbow Trout Feeding Tests, 6-Month Period
Idaho Production Diets, 1963

Hatchery	Diet	lbs. Food/ lb. Fish	Cost/lb. Gain	Total Mortality In %	Average Hematocrit	Water Temp.
American Falls	I	1.9	.16	8.9	44.2	56°F.
	II	2.1	.176	8.0	37.2	
	IV	2.0	.201	10.1	35.0	
	VII	2.2	.221	7.9	31.3	
	VIII	1.9	.191	5.5	33.3	
Ashton	I	1.8	.151	26.6		47-53°F.
	II	1.7	.141	29.8		
	VI	1.3	.129	18.7		
Eagle	III	1.5	.082	4.7	42.8	55°F.
Twin Falls	I	1.4	.12	0.1	37.5	55°F.
	II	1.4	.12	0.2	38.4	
	IV	1.6	.16	0.1	42.1	

CHROMOSOMES AND SPECIES EVOLUTION IN SALMONIDS

Ray Simon
U. of Washington

Information was introduced to document a wide variability in diploid chromosome number in 14 species of Salmonidae (range 52-84). Consideration of mechanisms of chromosome alteration has let to the reduction of this seemingly untoward complexity by the recognition that some chromosomes are the equivalent of twice as much hereditary information as are other single chromosome units.

This equivalence is clarified by consideration of two examples, the rainbow trout (2n = 60) and the pink salmon 2n = 52). The latter species has chromosomes of a single type termed metacentrics. The manner in which chromosome numbers have apparently changed in these species strongly favors the joining of rod-like elements in pairs - these form the double-weighted metacentric chromosomes. The pink salmon thus has the equivalent of twice its diploid number in terms of single elements and is said to possess 104 "arms". The rainbow trout has 44 of the double-armed metacentrics (= 88 arms) and 16 single-armed chromosomes to make up its number of 60 chromosomes which are exactly the equal of the pink in terms of arms (also having 104). It is postulated that species have evolved by a shuffling of arms possessing very nearly the same genetic information but differing in their freedom to assort randomly due to linkage of some arms to others.

Since all species considered agree very closely to the above example, it was postulated that all species are very similar genetically. Examination of generic characters such as shape of some skull bones has lead to the recommendation that Salmo be nominally combined with Oncorhynchus and the latter name considered to be invalid. Suspicion is also cast on the validity of the genus Salvelinus but rejection of the genus is withheld until more information is available on the variability of the toothless shaft of the vomer, a bone in the roof of the mouth cavity. If the shaft of this bone is invariably found to be toothless and if the shaft of the same bone in Salmo (Salmo plus Oncorhynchus) invariably possesses teeth, then the genus Salvelinus must be retained.

Genetic consequences of linkage, position effect and general genetic similarity were discussed from the standpoint of hybrid success in many salmonid species.

THE WEIGHT OF SOCKEYE FRY IN RELATION TO PHYSICAL FACTORS DURING DEVELOPMENT

International Pacific Salmon
Fisheries Commission

Ernest L. Brannon

The embryos of sockeye salmon, Oncorhynchus nerka (Walbaum), were held from fertilization of the eggs to yolk absorption of the alevins under different conditions found in the hatchery environment. Four figures are presented showing the effect of feeding, of velocity, of natural light and of oxygen saturation.

A feeding study was initiated to determine whether or not late stage alevins should be fed prior to yolk absorption. Sockeye alevins had food presented to them from hatching, hatching plus 200 temperature units, hatching plus 400 thermal units and so on until the last group had commenced feeding at hatching plus 1200 temperature units. Feeding did occur among those fry having food presented to them from hatching. However, prior to the time of emergence (shortly before buttoning up) this food did not influence the weight of the fry. Beyond emergence, any delay in feeding did result in a reduction in the weight of the fry. The fry fed on liver (Figure 1) prior to emergence showed poorer growth (groups 1 & 2) than those fed at or near the time of emergence (groups 3 & 4).

The velocities tested ranged from the gravel to the hatchery environment. The weights of alevins and fry were relatively constant at low velocities, but at velocities in excess of 15 mm/sec the weight was less (Figure 2). The weight difference was attributed to exercise forced on the fish between hatching and yolk absorption. The decline in the curves was due to weight loss from starvation. Mortality was higher among those alevins held in velocities greater than 15 mm/sec.

Alevins exposed to the range of velocities were also raised under conditions of diffuse natural light and compared to those raised in darkness. Light exposed alevins were smaller than those raised in darkness, although some of the difference occurred prior to hatching. Light exposed alevins reached the time of "swim up" much sooner and were noticeably more active.

Three levels of oxygen saturations were studied and the resulting weights of fry compared. (Figure 4). Eggs and alevins incubated at high oxygen saturations yielded larger alevins. The groups incubated at lower oxygen levels were not only smaller but were delayed in reaching the time of emergence.

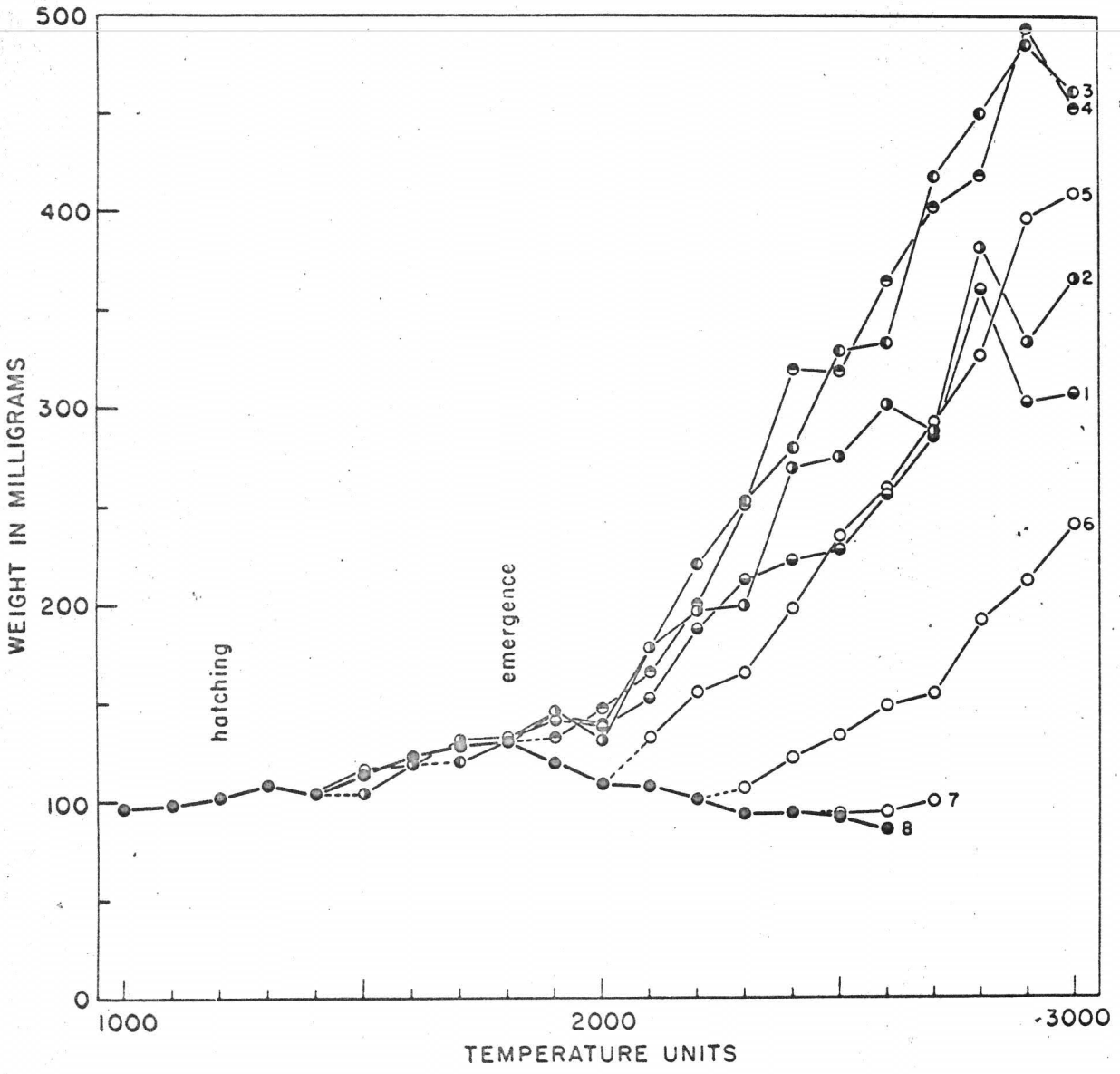


FIGURE 1. Weight of sockeye fry fed from hatching, hatching plus 200 temperature units, hatching plus 400 temperature units, etc. (Line 8 represents the base curve of growth of fry not fed during the course of the study.)

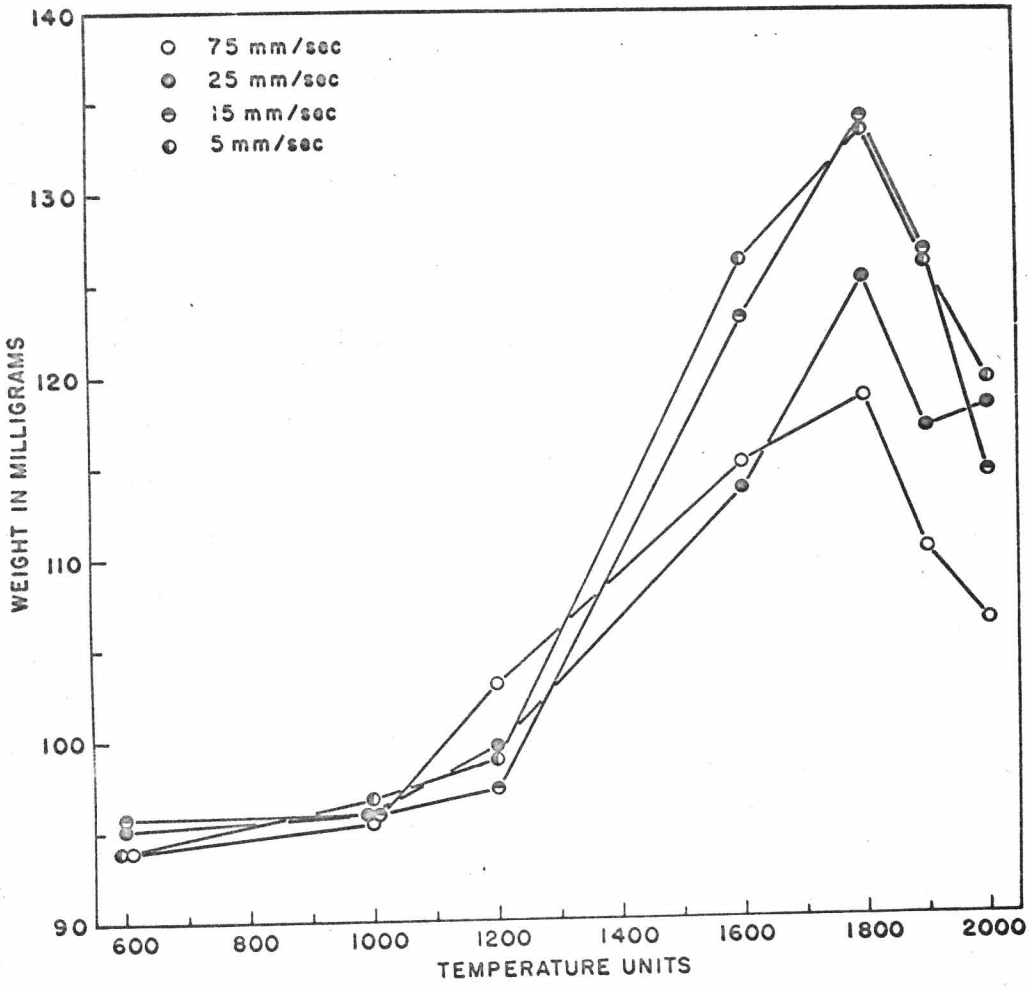


FIGURE 2. The weight of sockeye fry in relation to velocity during incubation. (75 mm/sec approximates 20 gal/min in the trays in the deep hatchery tanks)

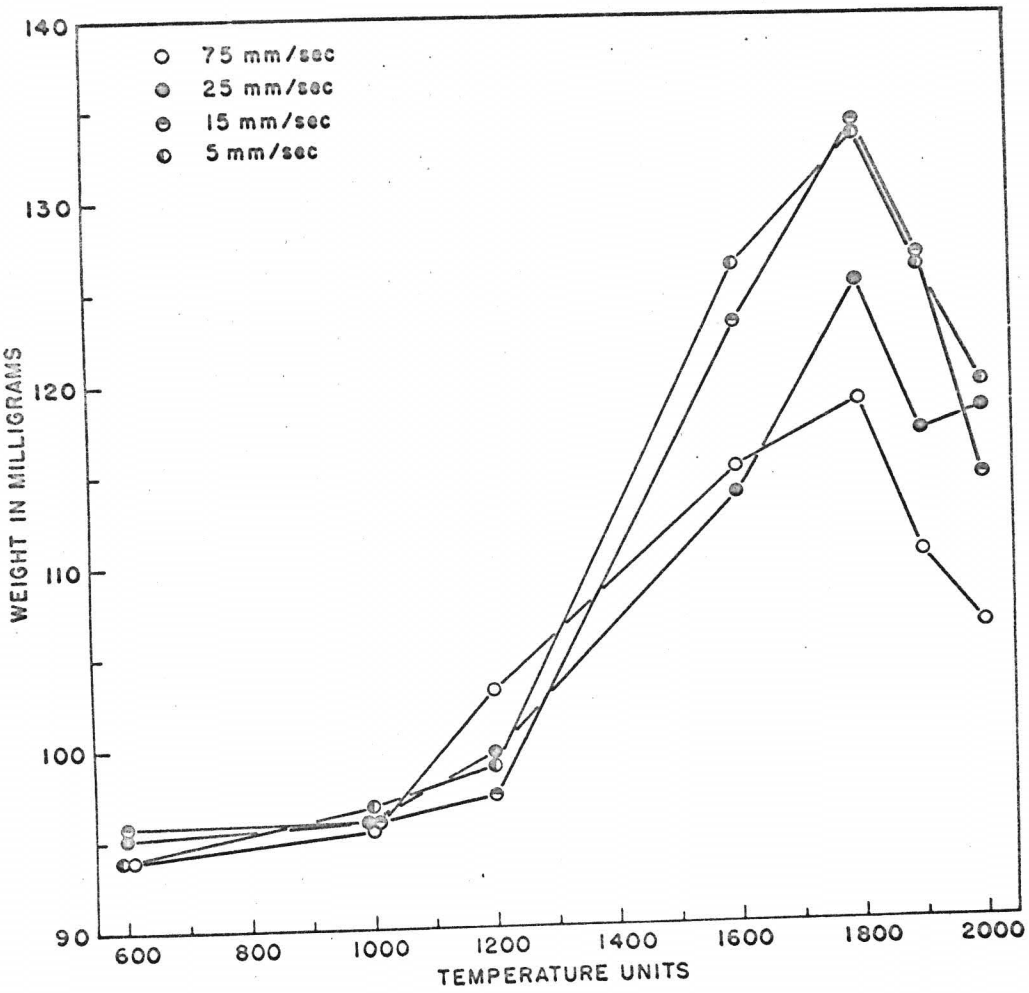


FIGURE 2. The weight of sockeye fry in relation to velocity during incubation. (75 mm/sec approximates 20 gal/min in the trays in the deep hatchery tanks)

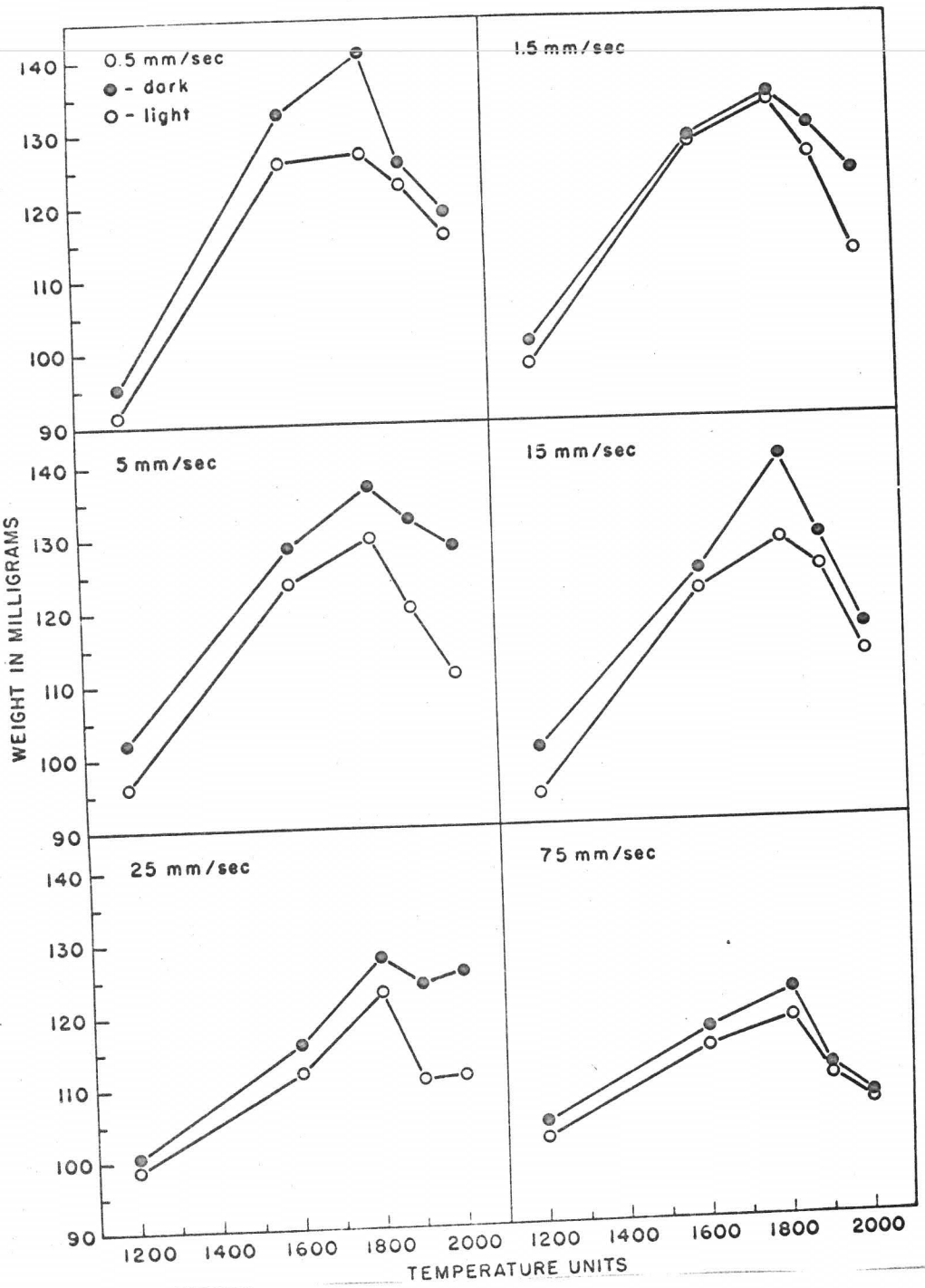


FIGURE 3. The effect of natural daylight on the weight of sockeye fry held in six velocities.

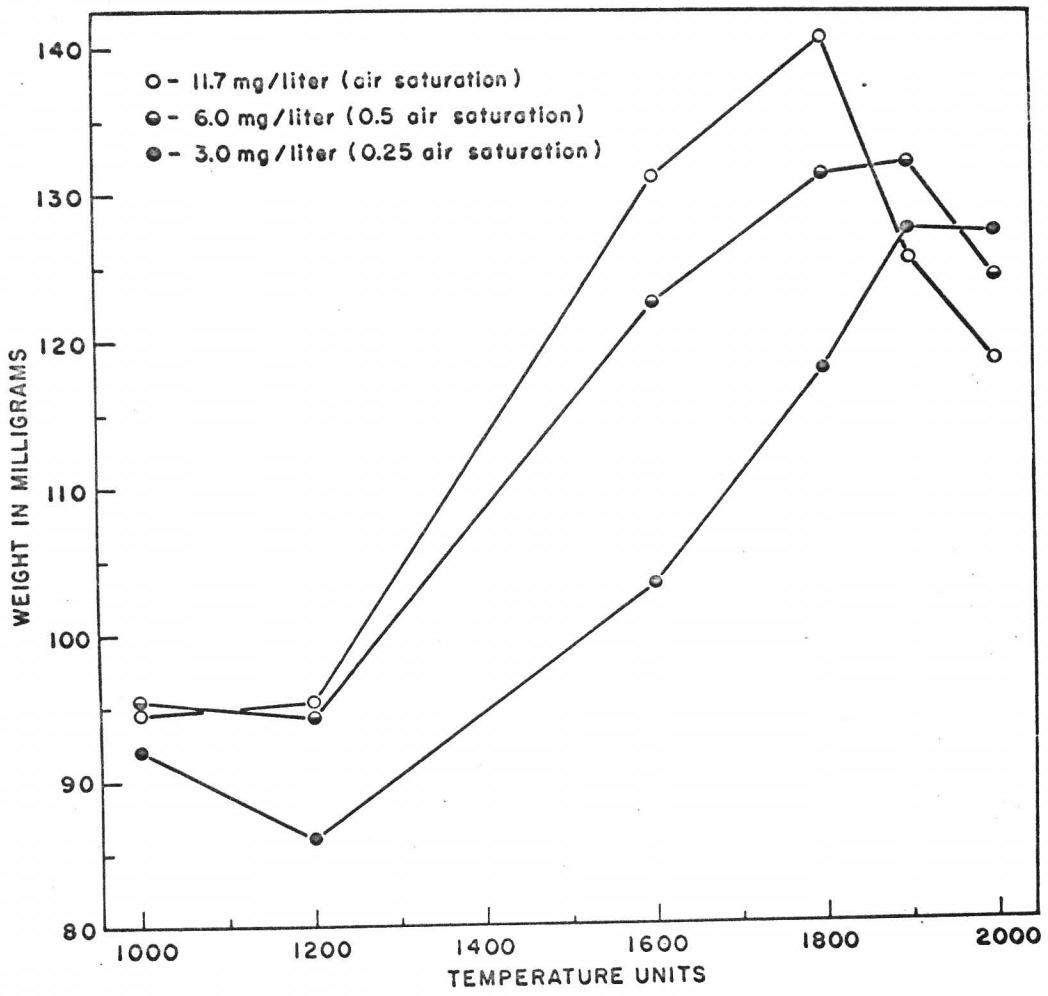


FIGURE 4. The effect of oxygen saturation on the weight of sockeye fry during incubation.

AGE AND SIZE OF STEELHEAD TROUT OUTMIGRANTS FROM MARION FORKS HATCHERY BY SCALE ANALYSIS

Mark De Cew
Oregon Fish Commission
Clackamas, Oregon

In 1951, construction of Detroit Dam closed the upper reaches of the North Santiam River to salmon and steelhead trout. The Marion Forks Hatchery was built to compensate for lost spawning grounds and rearing area. The number of female steelhead adults returning to Minto Pond, the adult holding facility, seriously diminished from 896 in 1951 to 129 females in 1963. However, hatchery management procedures were changed in 1960 from liberating small (about 200 fish per pound) yearlings to the liberation of larger (about 12 fish per pound) 2-year-olds. Adult returns in 1964 and after should reflect this change in liberation size.

Water temperatures of 40° F. or less severely inhibits growth for six months of the year at Marion Forks. Consequently, it is necessary to rear the steelhead at Willamette Hatchery for one year and then transfer them back to Marion Forks for the second year to obtain the desired size. The expense incurred in rearing for two years is considerably more than in hatcheries where steelhead are reared to a large size in one year. Another disadvantage is that rearing of Marion Forks steelhead at Willamette Hatchery occupies pond space which could be utilized for spring chinook salmon production. Considering the declining returns while using former hatchery procedure, present rearing disadvantages, and lack of hatchery contribution evaluation, a better knowledge of North Santiam steelhead migratory habits was considered necessary before further improvement could be made in management practices.

In 1962, a preliminary life history study of North Santiam River steelhead was initiated through scale interpretation of hatchery juveniles and adults returning to Minto Pond. It was assumed that the adults, of either hatchery or wild origin, would indicate a history which could be used as a basis for improved hatchery management. Size, age, and seasonal time at sea entrance as smolts were interpreted from adult scales and compared to size and age of the hatchery liberation stock. Analysis of adult scales was confined to the 1958 and 1959 brood years.

A body-scale relationship was computed from scale radial measurement of juvenile hatchery stock. The resultant formula is: $Y = 2.02X$, where Y is the predicted body length and X is the scale radial measurement. The calculated mean body length for 1958 and 1959 brood adults at time of sea entrance was 7.5 inches. Age analysis of the 1958 brood adults indicated that 77% entered the sea as 2-year-olds

and 23% entered as 3-year-old smolts. The 2-year-old smolts had a first annulus mean length of 3.37 inches compared to 2.87 inches for the 3-year-old migrants. The 1959 brood adults recovered to date migrated to sea at 2 years of age. Analysis also indicated that all fish spent two years in the ocean before returning to the parent stream. Comparison of adult scale interpretations with hatchery liberation data (Table 1) shows the smolts had obtained at least twice the size and age at time of sea entrance as the hatchery stock at time of liberation.

Circuli counts of adult scales were used to indicate time of sea entrance. An average of only 4.3 circuli were formed between the final fresh-water annulus and the last circulus of the fresh-water zone. This was only about one-fourth the number of circuli formed the previous year, indicating a short period after annulus formation. Examination of Marion Forks Hatchery juvenile scales indicated that annulus formation occurs approximately in the month of May. Comparison of these factors between adult and juvenile scales indicates sea entrance during spring and summer months.

Comparison of a sample of the 1959 brood hatchery stock with adults of the same brood year by circuli enumeration indicated a significant difference between these groups (Figure 1). The frequency distributions indicate that juveniles which were of the smaller body sizes at the time of first annulus formation did not contribute to the adult population returning to Minto Pond as 4-year-olds; 5-year fish will return in 1964. There is a possibility the hatchery sample was not representative of the liberated stock; consequently, it is not possible to say that the juvenile and adults represent two different populations. This evidence that the smaller hatchery liberation stock did not contribute to the adult run is further supported by the mean body length (2.70 inches) of the liberation stock, measured in June, being less than the mean body length (3.09 inches) of North Santiam adult steelhead of the same brood at the time of first annulus formation.

SUMMARY

Analysis of North Santiam steelhead scales indicated that returning adults of the 1958 and 1959 broods migrated seaward at a mean length of 7.5 inches, at not less than 2 years of age. Data is presented which shows that the former hatchery practice of liberating very small yearlings had little chance of contributing substantially to the adult runs. Current hatchery practices coincide fairly well with the early life history portrayed by adults of the 1958 and 1959 broods. Examination of adults returning in 1964 and later will reflect to what extent recent changes in hatchery procedure has affected survival.

4. CIRCULI ENUMERATION AT FIRST ANNULUS, NORTH SANTIAM ADULT STEELHEAD TROUT AND HATCHERY LIBERATION SAMPLE, 1959 BROOD

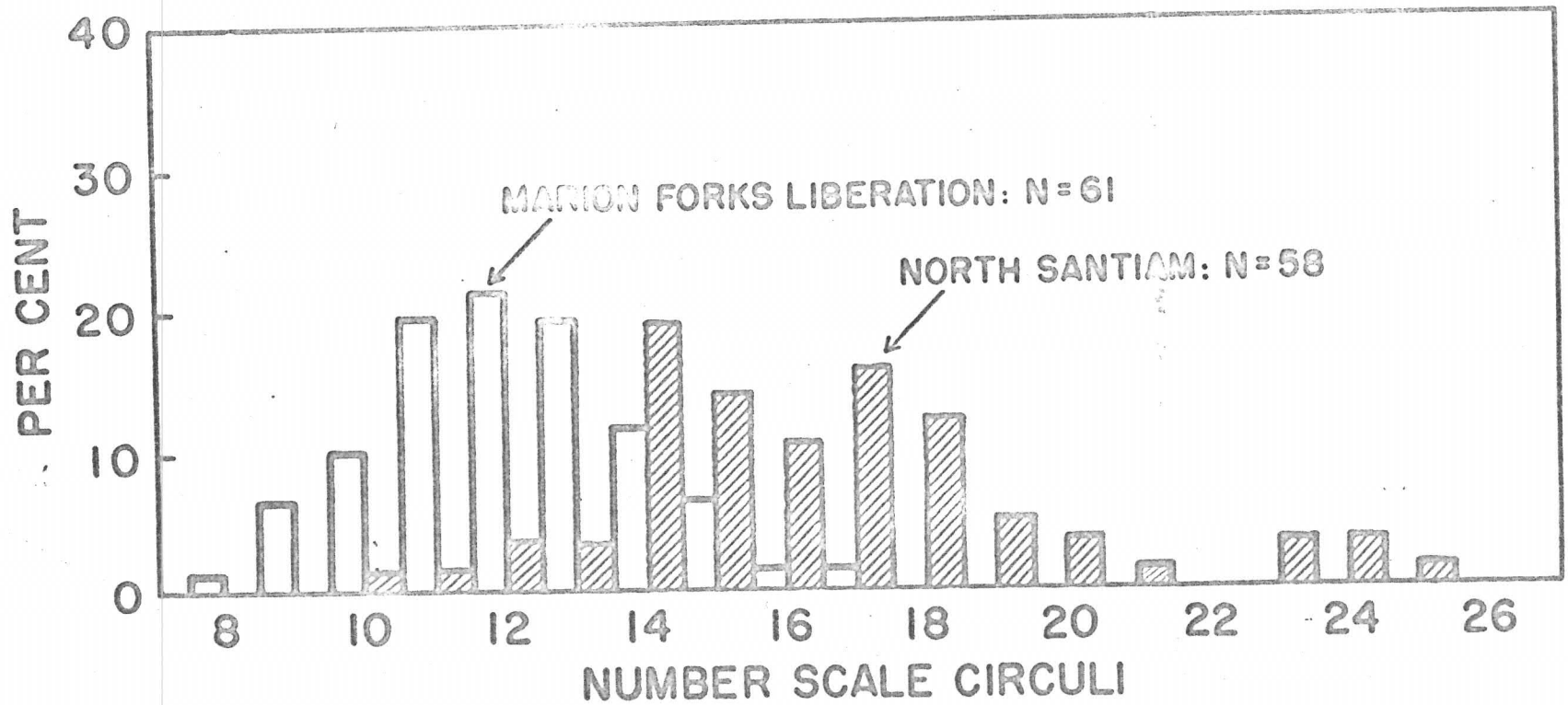


Table I SIZE AND AGE AT LIBERATION AND SEA ENTRANCE, NORTH SANTIAM RIVER STEELHEAD TROUT

BROOD	MEAN FORK LENGTH (INCHES)		AGE GROUP (PER CENT)			
			LIBERATION		SEA ENTRANCE	
	LIBERATION	SEA ENTRANCE	1	2	2	3
1958	2.69	7.34	100	0	77	23
1959	2.70 (4.54) 1/	7.53	83	17	100	2/

1/ 2-YEAR-OLDS.
2/ DATA NOT AVAILABLE UNTIL 1964.

TRINITY RIVER HATCHERY

David Ward
California Dept. of Fish and Game
December 6, 1963

The last time I attended the Northwest Fish Cultural Conference was in 1958.

At that time I reported on the operation of the Trinity River Trapping Station.

I am happy to say that these temporary trapping facilities are no longer needed as Trinity River Hatchery has been completed and is now in operation.

The Trinity River in Northwestern California is one of the few remaining river systems in the State whsch has not been completely altered by man. The construction of Trinity River Dam and the Lewiston regulating dam cut off much of the spawning area used by salmon and steelhead runs in the upper reaches of the Trinity River drainage.

Temporary trapping facilities, with an incubator house, were used to accommodate the Trinity River fish runs from 1958 until the present year when the hatchery was completed and put into operation.

The Trinity River Hatchery and trapping facilities were built by the U. S. Bureau of Reclamation at an overall cost of $2,600,000. The hatchery is the result of the combined thinking and planning of the California Department of Fish and Game, the U. S. Fish and Wildlife Service, and the engineering staff of the U. S. Bureau of Reclamation. It is being operated by the California Department of Fish and Game under contract with the Bureau of Reclamation.

The hatchery was designed to handle up to 35,000 adult salmon and steelhead for spawning in any one season and has an overall capacity to accommodate the following numbers and variety of fish raised to fingerling size: 31,000,000 king salmon, 4,000,000 silver salmon, and 5,000,000 steelhead.

It is also planned to raise 1,000,000 steelhead, 500,000 silver salmon, and 500,000 spring king salmon to a yearling size of from 5 to 7 inches in length before planting.

A crew of 10 permanent employees and from 4 to 6 temporary employees for seasonal duties will operate the hatchery.

The primary objective in the design of the hatchery was a compact unit where a large number of adult salmon and steelhead could be trapped, held, and spawned with a minimum of effort. It was planned to reduce excessive handling of fish of all sizes, reduce manpower necessary to operate it, and minimize maintenance costs.

The water supply for handling adult fish has been arranged and constructed with flush gates, mechanical lifts, and circular ponds, so that the fish are always moved in water and never handled by nets. The water currents throughout the spawning facilities can be reversed and fish can be attracted and moved from one place to another by the directional flow of the water.

Aluminum has been used wherever possible for strength, durability, and ease of handling.

Description of Operational Facilities

Slide No. 1. Lewiston Dam and Water Diversions.

a. Height of dam -
b. Length of dam -
c. Earth fill.

Slide No. 2. Aerator.

a. Capacity -
b. Size of pipe -

Slide No. 3. Fish Ladder.

a. The ladder consists of 30 pools with a one foot rise between pools. The ladder has a flow of 10 c.f.s. at the top with an additional 15 c.f.s. added about one-half way down to increase flow for attracting fish to the ladder entrance.

b. An added attraction to the public is the viewer windows in the side of the ladder.

Slide No. 4. Trap and Receiving Tank.

a. This tank, where the fish are trapped is 70 feet long, 12 feet wide, and 7 feet deep. The fish are held here until they are sorted for species, sex, and degree of ripeness.

b. Green fish from the holding tanks are moved back into this tank so that they may be handled mechanically for restocking.

Slide No. 5. Sweep and Brail.

a. This is used to crowd the fish into the anesthetic tank for sorting or spawning. The sweep, operated by a gasoline engine, moves along the steel rails on top of the tank walls, crowding the fish towards the spawning house.

b. At the end of the tank the brail is raised and the fish slide through the openings in the end of the spawning house into the "dope" tank.

Slide No. 6. Anesthetic Tank and Spawning House.

a. The "dope" tank is filled with a solution containing an anesthetic known as Quinaldine, which renders the fish immobile for handling. The tank is equipped with a false bottom which can be raised mechanically to waist height for ease in handling the fish.

b. Green fish are sorted in the tank and placed into flumes on either side of the spawning house where they swim out into one of the six circular holding tanks. Ripe fish are moved out to spawning tables in the center of the house.

c. In spawning and sorting, green fish are again moved back into the spawning house from the holding tanks. The spawning house is equipped with mechanical hoists, pressure water system for washing eggs, and space heaters for the comfort of the employees.

d. After washing, eggs are put into incubator trays. When an incubator stack is filled it is moved to the hatchery with a battery operated fork-lift truck.

Slide No. 7. Circular Holding Tanks.

a. The six circular tanks are for holding adult salmon and steelhead. They are 30 feet in diameter and 5 feet deep, and are supplied with a flow of 1,125 gallons of water per minute from directional pipes in the floor of the tank.

b. Each tank has an estimated holding capacity of 600 adult salmon or 800 adult steelhead at one time.

c. Circular ponds with bottom inlet and center outlet keep the fish quieter. We are very pleased with the performance of the tanks. There is very little jumping and mortality is negligible.

Slide No. 8. Hatchery Building.

a. This building is 80 feet wide and 160 feet long, and has a steel framework covered with aluminum. It contains 200 aluminum troughs and 960 "flow through" incubator trays.

b. The hatchery building facilities have a maximum capacity of 6,000,000 fingerling salmon at one time and 7,800,000 salmon eggs or fry until they reach the swimup stage.

Slide No. 9. Nursery Tanks.

a. These 44 tanks will be used to rear salmon and steelhead until they reach a size of 2 to 3 inches. The tanks are 22 feet long, 4 feet wide, and 27 inches deep. Each one will hold 50,000 fingerlings.

Slide No.10 Raceway Ponds.

a. These 72 ponds are for rearing yearling salmon and steelhead; each pond will hold approximately 28,000 yearling fish.

Slide No.11 Food Storage Bin.

a. This bin is designed to hold 84,000 pounds of pelleted fish food which will be purchased in bulk and delivered directly into the bin.

b. Food is taken into the pond feeder hoppers by gravity and then is distributed to the fish in the ponds mechanically by a blower mounted on a pickup truck.

WYOMING'S PROJECTED TEN YEAR FISHERIES PROGRAM

Don O. Terry
Wyoming Fish and Game Dept.
Tensleep, Wyoming

Fisheries Personnel expressed concern over the impact of the fisheries resources by the increasing numbers of people utilizing Wyoming's fishing waters. At the Annual Fisheries meeting, a program was planned to evaluate past years program and project a future ten year program.

The responsibility in assembling this ten year program was assigned to committees under the following headings.

1. Administrative Problems:

    a. Reorganization of present lines of authority.
    b. Five day work week.
    c. Update records, reports and accounting.
    d. Professional improvement of personnel.
    e. Liaison with State Personnel Board.

2. Management:

    a. Stocking.
    b. Regulations.
    c. Lands Access.
    d. Environmental Control and Habitat Improvement.
    e. Manipulation of fish populations.
    f. Research.

3. Fish Culture:

    a. Brood Stock.
    b. Improvement of Hatchery Facilities.
    c. Feed Purchases.
    d. Distribution.
    e. Field Spawning Operations.
    f. Hatchery Research.

Each of the above topics were discussed at our Annual meeting. Recommendations were made from the group, and the committees will evaluate these suggestions and submit a final draft to Cheyenne for consideration by the Commission.

RANKING OF SELECTED WET INGREDIENTS FOR OREGON PELLETS

John Westgate
Oregon Fish Commission
Clackamas, Oregon

Oregon pellets have been fed at Fish Commission hatcheries since 1959 and use of this diet has spread to other agencies.

The pellet is presently composed of 60% dry ingredients, such as cottonseed, herring, and wheat germ meals, and 40% wet fishery products. Tuna viscera together with turbot or pasteurized salmon viscera, each at 20% of the complete diet, has been successful as the wet component.

Future production of Oregon pellets, if they are widely accepted, may exhaust the supply of these waste products. In addition, there are certain problems in pellet manufacturing that are related to the wet ingredients. For instance, viscera containing active digestive enzymes is often too liquid and forms a soft sticky pellet. This has resulted from locally caught tuna and Puget Sound or ocean-caught salmon.

It is apparent, therefore, that additional alternate ingredients would be helpful, and the efficiency of alternates should be known.

Dogfish meal had produced good results during laboratory tests; however, high levels of urea and vitamins A and D were assumed to prevent the use of whole dogfish as a wet ingredient without further trials.

The objective of our work in 1962 was to rank the nutritional quality of tuna viscera, turbot, dogfish, and pasteurized salmon viscera when fed as the wet component of Oregon pellets.

The experiment was conducted at Clackamas Laboratory for 24 weeks, using replicate lots of 400 spring chinook. These fish averaged 224 per pound before the trial.

Test pellets were compounded at the Astoria Seafoods Laboratory. Formulation was identical to the 1962 production pellet, excepting the wet component. Each wet ingredient was tested singularly, as the entire wet portion, or 40% of the Oregon pellet. Dogfish was tested both whole and de-livered because of the danger of hypervitaminosis A and D. The control was the standard production pellet containing equal quantities of tuna viscera and turbot. The lots were fed on a timed appetite basis, that is, all the fish would readily consume in a certain time period.

Results (Table 1) were statistically evaluated by analysis of variance and ranking with Duncan's New Multiple Range Test at the 5% level of significance.

Weight gains from tuna viscera, whole dogfish, or de-livered dogfish were not significantly different. Each produced more gain than turbot or salmon viscera. Position of the control was not clearly defined.

Tuna viscera and whole dogfish produced longer fish than either turbot or salmon viscera. De-livered dogfish and the control appeared intermediate.

Whole dogfish was converted to weight more efficiently than the others. Position of the remaining diets was not clearly defined.

When diet efficiency was measured by calories needed to produce 100 grams of fish, salmon viscera and de-livered dogfish were superior to tuna viscera and the control, while whole dogfish and turbot were intermediate.

No significant differences were noted in mortality, hemoglobin, or hematocrit. Mortality was not excessive and the fish were not anemic.

A comparative summary of the ranking analysis is presented in Table 2. Criteria for comparison of diets were limited to weight gain, length, conversion, and caloric efficiency--measurements that the analysis of variance indicated had significant differences. A plus signifies that the diet indicated along the top produced a significantly better result than the diet on the left. A minus indicates an inferior result, while a zero means the difference was not significant. One point was given for each plus and 1 point was subtracted for each minus, resulting in a numerical total for each criterion and a final score for over-all comparison of diets.

Dogfish, both whole and de-livered, compared favorably with the other products, particularly in regard to food conversion. No problem appeared in this test from urea or vitamins A or D, and no advantage was realized from de-livering the dogfish. Tuna viscera produced excellent growth, probably related to its apparent taste appeal and the relatively large quantity consumed. Tuna viscera was somewhat weak in diet efficiency. Pasteurized salmon viscera, turbot, and control diets produced good results, but they did not equal dogfish or tuna viscera in the over-all comparison.

There was some indication that dogfish was not accepted well when the fish were small, and growth lagged until the last 2 months of the experiment. It was recommended that dogfish be tested with a production feeding trial, which will be reported on by Howard Drago.

COMPARATIVE SUMMARY OF RANKING, OREGON PELLET WET INGREDIENT TESTS, CLACKAMAS, 1962-63

COMPARISON	DOGFISH, WHOLE				DOGFISH, DE-LIVERED				TUNA VISCERA				SALMON VISCERA				TURBOT				CONTROL			
	WT. 1/	LGT. 2/	CV. 3/	C. 4/	WT.	LGT.	CV.	C.	WT.	LGT.	CV.	C.	WT.	LGT.	CV.	C.	WT.	LGT.	CV.	C.	WT.	LGT.	CV.	C.
Control	0	0	+	0	0	0	+	+	+	+	0	0	0	0	+	+	0	0	+	+	———			
Tuna Viscera	0	0	+	0	0	+	+	+	———				+	+	0	+	+	+	0	0	+	+	0	0
Turbot	+	+	+	0	+	0	+	0	+	+	0	0	0	0	0	–	———				0	0	–	–
Dogfish, Whole	———				0	0	–	0	0	0	–	0	–	–	–	+	–	–	–	0	0	0	–	0
Dogfish, De-Livered	0	0	+	0	———				0	+	–	–	–	0	0	0	–	0	–	0	0	0	–	–
Salmon Viscera	+	+	+	–	+	0	0	0	+	+	–	–	———				0	0	0	+	0	0	–	–
Total 5/	2	2	5	-1	2	-1	2	2	3	4	-3	-2	-3	-2	0	2	-3	-2	-1	2	-1	-1	-4	-3
Score			8				5				2				-3				-4				-9	

1/ Average Weight Gain (GM).
2/ Average Fork Length (MM).
3/ Food Conversion (Dry Weight).
4/ Calories Per 100 GM Weight Gain.
5/ 1 Point for Superior (+), 0 for No Difference, and -1 for inferior (-).

SUMMARY OF RESULTS, OREGON PELLET WET INGREDIENT TESTS
CLACKAMAS, 1962-63

DIET 1/	MEAN WT.GAIN (GM)	MEAN LENGTH (MM)	CON-VERSION (DRY WT)	CALORIES/ 100 GM WT. GAIN	MORTAL-ITY (%)	HEMO-GLOBIN (EST.)	HEMATO-CRIT (%)
Control	13.6	111	1.2	406	0.6	11	42
Tuna Viscera	14.8	113	1.2	402	0.8	11	43
Turbot	13.3	109	1.1	392	0.8	10	40
Dogfish, Whole	14.2	112	1.0	391	0.4	11	42
Dogfish, De-Livered	14.2	110	1.1	384	0.5	10	38
Salmon Viscera	13.4	109	1.1	376	0.3	10	41

1/ Replications Combined.

PRODUCTION FEEDING TRIAL OF SPRING CHINOOK WITH OREGON PELLETS CONTAINING DOGFISH SHARK

Howard Drago
Oregon Fish Commission

Our progression in diet studies is from experimental tank to pilot pond to full production. As John Westgate pointed out in the preceding presentation, dogfish as the entire wet fish ingredient in the Oregon pellet proved very satisfactory in the experimental trials. The next step then was to evaluate dogfish in pilot production to substantiate or refute the experimental findings. Since dogfish had not been used in crumbles fed to fish 700 to 300 per pound, the pilot production trial was designed to include this size range.

The feeding trial was conducted at Marion Forks Hatchery for 18 weeks - May 21 to September 17, 1963. Each of four 25 - foot circular hatchery ponds were stocked with approximately 34,000 spring chinook fingerlings averaging 700 per pound. Two ponds (26 and 27) were designated the test ponds and received Oregon crumbles and pellets containing 40 per cent (the entire wet fish portion) dogfish shark, prepared at the Astoria Seafoods Laboratory. Two ponds (28 and 29) were fed the standard production Oregon crumbles and pellets containing 20 per cent tuna viscera and 20 per cent turbot or pasteurized salmon viscera as the wet fish portion, commercially prepared by Bioproducts Incorporated in Warrenton, Oregon. Ponds 28 and 29 were designated as controls.

Frequency of feeding, amount to feed, and diet particle size followed the normal procedure for spring chinook at Marion Forks Hatchery. Mortalities were picked and recorded daily and fish behavior observations made.

It appeared to us at the hatchery that the dogfish pellets were a little heavier and went to the bottom more quickly. This required a little more time for the actual feeding. It may have resulted from the difference between the regular pellets which were made at Bioproducts and the dogfish pellets made at the Seafoods Laboratory.

The taste factor also was apparent in feeding. The pellets did not have equal taste appeal while the fish were just starting to feed and this was noticeable throughout the test. Taste factor was not apparent in the results as the table will show.

All four groups were sampled for weight gain and blood condition at monthly intervals. The complete lots were weighed and extensive blood studies made at the termination of the experiment. Length frequency measurements were made at the beginning and the end of the feeding trial.

A summary of the results is shown in the accompanying table. The fish of all lots grew well, converted their food efficiently, suffered low mortality, and maintained satisfactory blood values. Statistical analysis revealed no significant difference in any tests made.

Table 1. Summary of Data, Production Feeding Trial of Oregon Pellets Containing Dogfish Shark, 1963.

Measurements	Dogfish Pellets	Control Oregon Pellets
Weight Gain (lbs.)	1,380	1,275
Weight of Food Fed (lbs.)	2,204	2,270
Food Conversion (as fed)	1.6	1.8
Mean Fork Length (mm)		
Start	42.1	40.7
End	93.5	92.9
Mortality		
Number	1,195	1,016
Per Cent	3.5	3.1
Hematology-terminal		
Hemoglobin 1/		
Mean	12.3	12.6
Range	(9.9-14.1)	(8.1-15.7)
Hematocrit (%)		
Mean	36.6	35.0
Range	(33-45)	(25-45)

1/ Hemoglobin estimation in grams 100 ml. blood.

COLEMAN VIRUS DISEASE

John Pelnar, Manager
Bureau of Sport Fisheries and Wildlife
Fish and Wildlife Service
U. S. Department of the Interior

Operational procedures to reduce the incidence of the Coleman virus disease during the past year consisted chiefly of temperature controls of the water. Studies seems to indicate that temperatures above 54 degrees F. retards the virus outbreaks. We again found the usual outbreak of virus among fingerling chinooks reared in production water obtained from Battle Creek, having a varied temperature of 44 to 52 degrees; the mortality will be about 35% to 80% of the total number. When chinook fingerlings are hatched and reared in 56° F. water to a sixty day feeding period the mortality is reduced to 15% of the total number. A thirty day feeding period results in about a 40% of normal occurrence, or 20% mortality.

When eggs are incubated in production water, hatched and reared through the sac in 56° water the incidence of virus is reduced to 50% of normal. Eggs incubated in normal production water, then hatched and the sac fry held in 60 degrees water, then reared in 56 degree water, the virus does not occur. However, the use of 56 degrees and higher temperature water has caused a type of yolk sac trouble, particularly where we held feeding fish in re-used water in which other eggs had hatched. This hatching residue appears to have some effect on feeding fish, particularly very young fish, causing a yolk sac difficulty. Feeding chinooks reared in fresh 56° water did not develop the yolk trouble and for that reason we feel the re-used hatching water to be largely the cause of the yolk sac trouble.

Studies have disclosed that re-used and re-circulated hatching water of 56° temperature is extremely toxic to eggs, fry and fingerlings; heavy or total loss can be expected at Coleman under these conditions.

Incubating and rearing the spawn of individual females has given us an insight into the possibility that only a few female fish are carriers of the virus. During the past season twenty-four females were held separately, from these two groups of eggs two females developed the virus.

In the course of various studies we have observed a repetition of the fact that eggs held separately during the period of hatching seldom developed the virus. This brought attention to a change in hatching procedures so that no residue from hatching eggs will pass through the

water supply and over other eggs. The individual female study will be carried out further to note the incidence of virus as it may occur in 125 females, the eggs of each will be held and reared separately.

It has been observed that the egg taking, fertilization and hatching methods have a long time effect on the resulting fingerlings.

Efforts at reducing the virus are progressing and it is felt that methods developed to-date will save many fish and also produce better methods of hatchery operations.

Our program for the coming season will deal with laboratory studies, individual female study, use of warm water at hatching time, study of hatching residue, fluctuating temperatures and a change in hatching procedures in the operational program. An automatic frozen pellet fish feeding device has been developed which is displayed in the color slides.

This device which feeds fish in the 8' x 80' ponds automatically, consists of a rolling pipe containing the food and is operated by means of a cable drive and electric controls. Food to be fed is controlled by size openings in the feeder.

Kamloops trout, originating from sources in British Columbia in 1950, with offsprings reared at Coleman over a number of generations and offsprings from these, which were marked and released in 1961 as ten per pound fish into the Sacramento River have gone to sea and returned in 1963 as spawning adults. These returns to the hatchery number 33 and weigh from four to eleven pounds. While no records of recovery from fishermen were attempted, reports of many others being caught have come into us at Coleman. Two females of the 1961 release returned in 1962 as four pound fish and produced 3,200 at six per pound yearlings.

This work indicates the possibility of supplementing the steelhead run with these fish.

EFFECTS OF FISH SIZE AT RELEASE ON SURVIVAL AND LIFE HISTORY PATTERN OF HATCHERY-REARED STEELHEAD TROUT

Harry H. Wagner
Oregon State Game Commission
Division of Wildlife Research
December 4, 1963

The effects of fish size at time of release on adult return of hatchery-reared steelhead has been well documented in recent years, although in some cases it is difficult to separate the effects of size from other variables as stocking time, marking effects, viability, etc. The general picture which emerges, however, is one of increasing survival with the stocking of larger migrants. Also, of considerable importance is the influence of size at release on the life history pattern, that is, saltwater age. Time spent in the marine environment influences the number and size of the fish returning.

The data presented is for yearling steelhead trout released from the Alsea Trout Hatchery into the North Fork of the Alsea River from 1956 to 1961.

Adult return in relation to size at release is depicted in Figure 1. The curve shows the general survival picture in relation to size at release. It does not take into consideration other variables effecting survival. Returns are a composite of estimated catch (+ standard error) and observed escapement. Catch and escapement are considered to be minimal measures of return. The breaking point in terms of survival appears to be around 10 fish-per-pound at the lower end. There are not sufficient numbers of releases at the upper end of the scale to determine at what size adult returns decline or remain static.

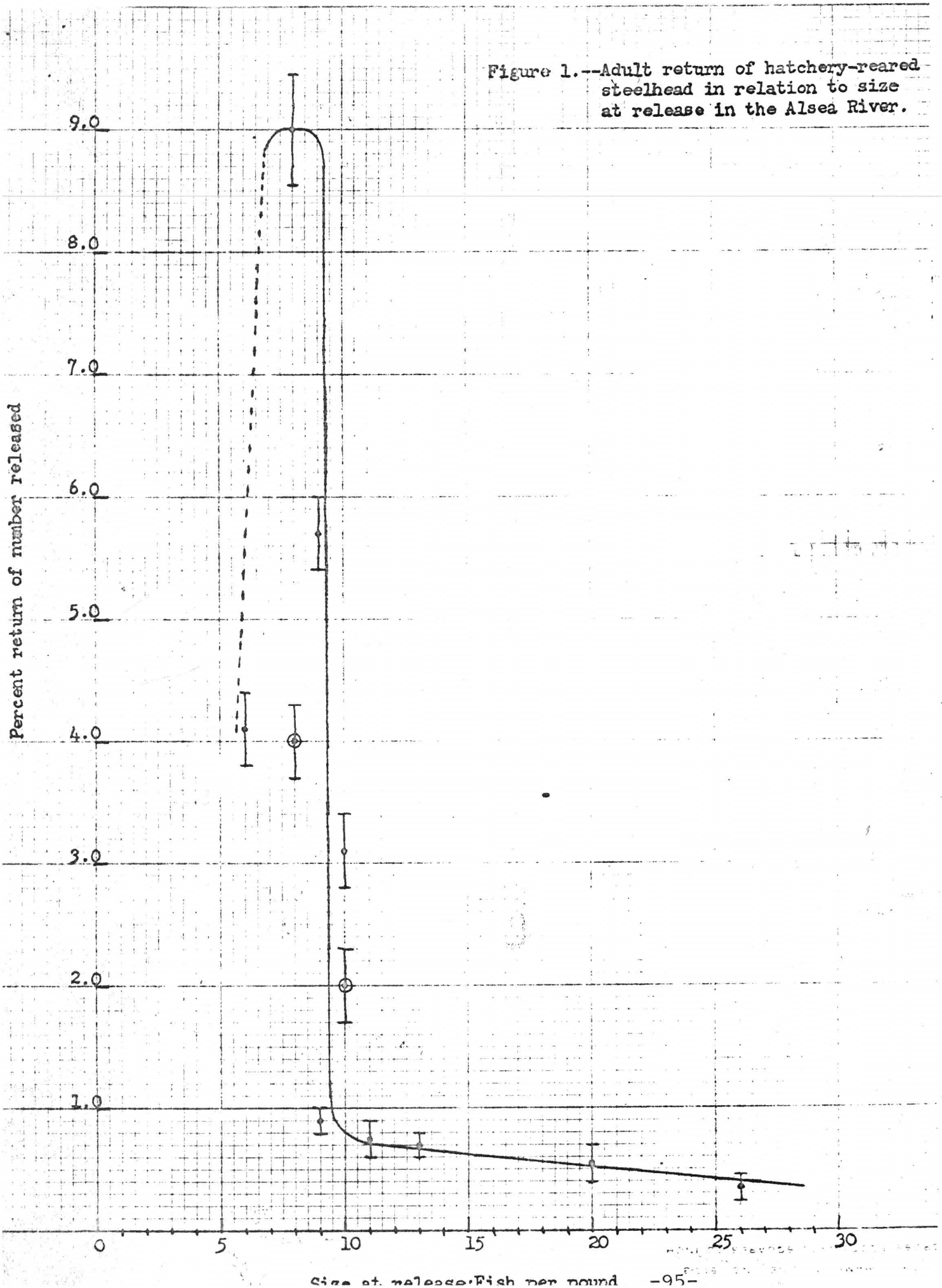
The life history pattern in relation to size at release for returning hatchery-reared steelhead trout is pictured in Figure 2. The life history patterns presented are saltwater ages of first time spawners. The following patterns 1/1, 1/2 and 1/3 make up approximately 98 percent of the fish returning to spawn for the first time.

With increasing size at release the percentage of jacks (2-year-old fish) in the total return increases. For three-year-olds, the percentage increases until a release size of around 9 fish-per-pound is reached but decreases at that point with releases of larger juveniles. The percent of three-year-olds in the total return is apparently related to the number of jacks at one end of the curve and the number of four-year-olds at the other end. The percentage of four-year-old steelhead increases as the size at release decreases, that is, fish released at a smaller size spend a longer time in the sea before returning on the first spawning migration.

Smolts released at a size of around 8 or 9 fish per pound have produced the maximum number of adults (3-year-olds), the minimum number of jacks, and have shown some of the highest survival rates.

Additional experiments are needed to define more closely the upper size limits as regards adult survival and saltwater age.

Figure 1.--Adult return of hatchery-reared steelhead in relation to size at release in the Alsea River.



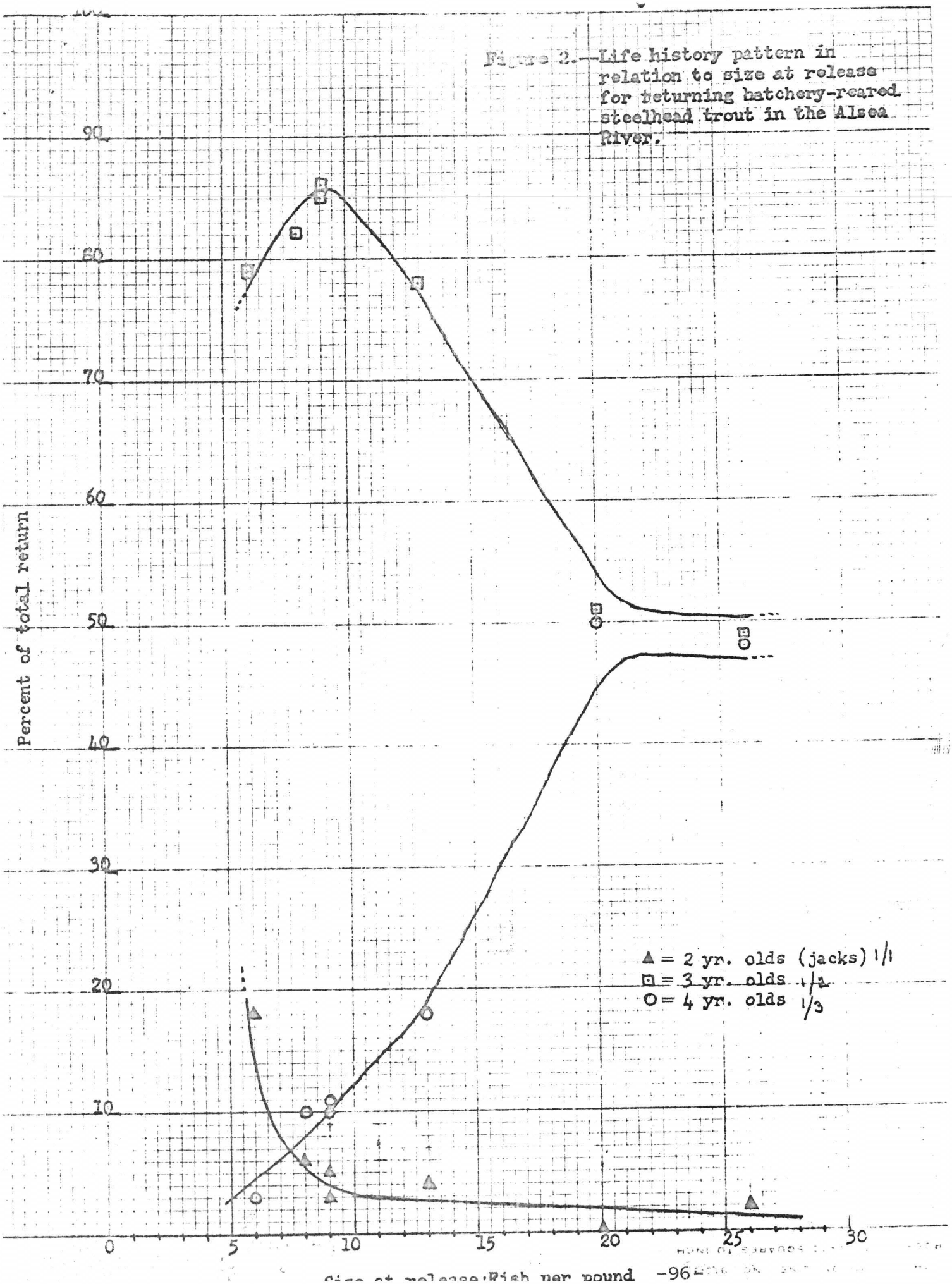


Figure 2.--Life history pattern in relation to size at release for returning hatchery-reared steelhead trout in the Alsea River.

Size at release:Fish per pound

SALINITY ADAPTATION IN STEELHEAD TROUT AND COHO SALMON IN RELATION TO MIGRATION DISPOSITION

Frank P. Conte
Harry Wagner
T. O. Harris
Oregon State University and Oregon Game Commission

Purpose:

Attempts are being made to understand the nature of the biological changes which are associated with seaward migration of juvenile salmonids; especially those species applicable to the Columbia River fisheries.

Results:

Slide
(1)

Salinity survival in steelhead trout has been shown to be dependent upon size of fish and not chronological age. Temporal or seasonal factors have been demonstrated in relation to saltwater adaptation. The number of steelhead juveniles surviving increased in February for all size groups above 10 centimeters in length but decreased in June, even though the fish had grown to a large size, to the pre-February levels. Resident rainbow trout, which demonstrated none of the visual changes associated with smoltification showed a similar pattern of salinity survival. Therefore, saltwater adaptation is one biological change which is associated with seaward migration and preceeds migration by six to eight weeks. An increase in salinity adaptation cannot be used by itself as an index to migration disposition for the purpose of determining time of release. However, decreasing salinity survival can be used as an index because it corresponds to the termination of downstream migration.

Coho salmon show a greater and earlier saltwater tolerance than do similar sizes of steelhead. Coho 9 to 11 centimeters in length are showing a 95 percent survival in seawater as early as October. The results from the above experiments suggest at present that downstream migrant steelhead and coho salmon are adapted to the marine environment prior to migration and would require little time in the estuary for adaptation purposes before entering the sea.

GAS BUBBLE DISEASE IN ADULT CHINOOK SALMON

Richard L. Westgard
Washington State Department of Fisheries

One third of the adult chinook salmon impounded at McNary Spawning Channel in 1962 were blinded in one or both eyes. Pre-spawning mortalities of 88 per cent occurred in the blinded fish compared to six per cent in those not afflicted.

Hemorrhaging of the iris followed by blood clots in the aqueous humor and/or corneal opaqueness was the most frequent damage. Occasionally, gas bubbles appeared in the aqueous humor which gave a clue to the cause of blindness.

Waters where the fish were impounded were analyzed for dissolved nitrogen by the Van Slike technique. Nitrogen saturation of 119 per cent occurred when entrained air in cascading water was forced into solution at depth.

Two test lots of adult salmon were held in 104-107 per cent and 116-130 per cent nitrogen saturated water respectively. No trouble developed in three weeks of holding in the water of low nitrogen saturation. All test fish held in the water of high nitrogen saturation developed the typical above described symptoms and were blind within 10 days.

CHEMICAL DIFFERENCES OF HATCHERY-REARED CHINOOK SALMON

Joseph W. Elliott
Bureau of Sport Fisheries and Wildlife
Longview, Washington

Hatchery rearing of salmon fingerling is essential for maintaining and enlarging the salmon industry. As participants in a four-year evaluation of hatcheries, we are attempting to define and measure fish quality. In this study we are measuring the inherent differences of hatchery fingerling. These differences we hope to correlate with physical performance and ultimately with the return of adult salmon.

One area of the research is measurement of chemical differences. Because the products involved are interdependent the level of many components must be determined initially to detect an inbalance. A screening program has been underway since 1962 to determine which components can be used on a practical basis as indices of quality. The screening program, refinement of procedures, and evaluation of results are concurrent studies.

The results obtained to date in the selection and evaluation of chemical indices for defining fish quality are:

1. The blood plasma of hatchery fingerling chinook salmon shows variable levels of total protein, glucose and cholesterol and the levels of these components can be associated with the hatchery fish-cultural procedure. The differences in blood plasma composition of fish from various hatcheries are indicated in figure 1 and table 1. The values are compared on a percentage basis with the lowest 1963 value.

2. The body water and body lipids of hatchery fingerling vary depending on hatchery fish-cultural procedure, figure 2 and table 2.

3. There is a positive correlation at the 1 percent level of 0.61 between fish weight and physical performance (performance rating) and of 0.79 between fish weight and body protein.

4. There is a significant positive correlation at the 1 percent level of 0.79 between plasma protein and plasma inorganic phosphorus and of 0.78 between body fat and plasma cholesterol.

The results obtained in the measurement of chemical differences will be correlated with return of adult salmon. Evaluation of the data

can not be concluded until return figures are received but measurements can be refined and the work load can be readjusted by discontinuing useless measurements and adding others as out knowledge in this area of study is increased.

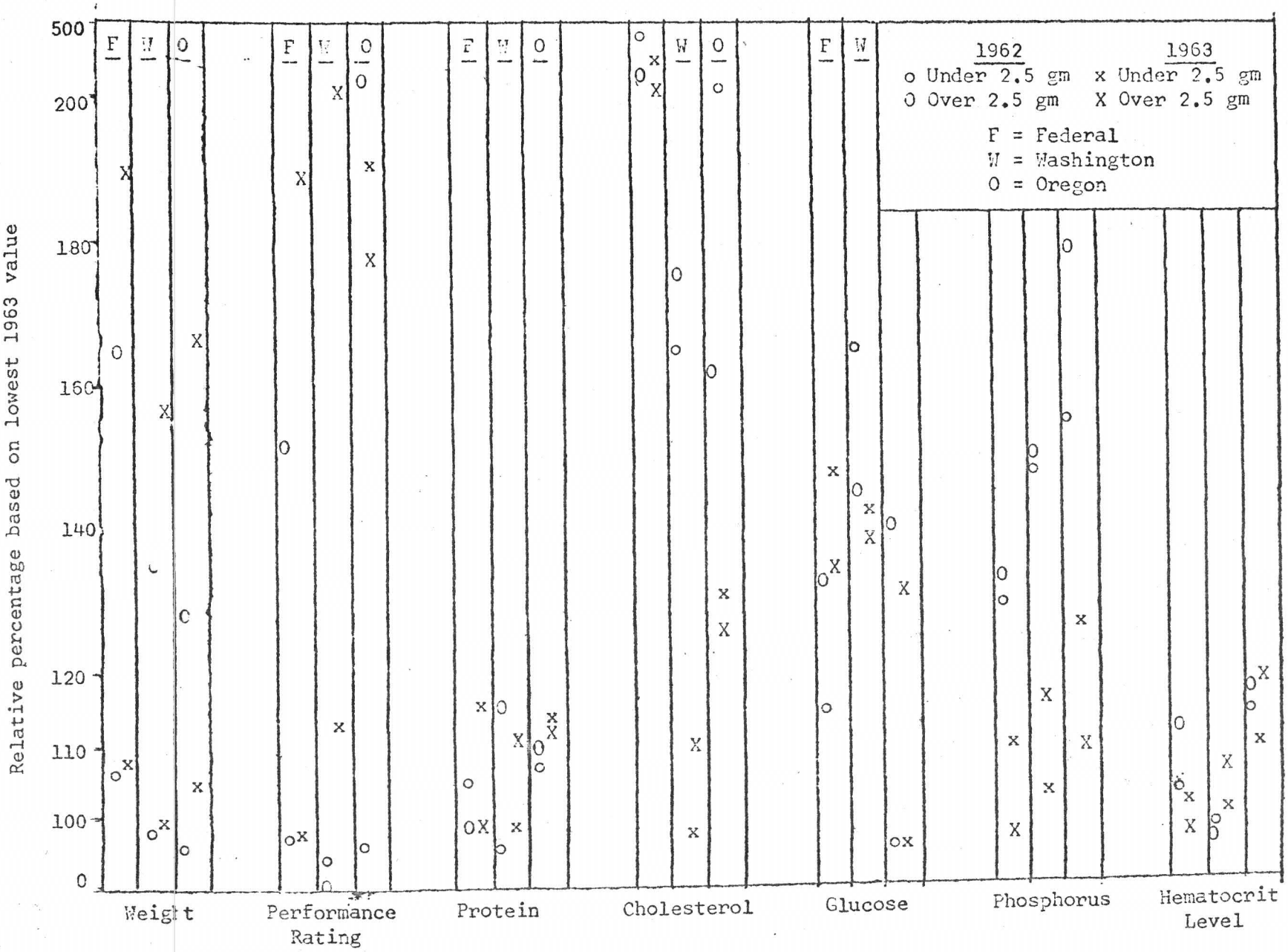


Figure 1.--Comparison of Differences in Weight, Performance Rating and Blood Plasma Composition of Hatchery Salmon Fingerling.

Table 1.--Comparison of weight, performance rating, and blood plasma composition of hatchery salmon fingerling.

Hatchery Group			Weight	Performance Rating	Plasma				Hematocrit
					Protein	Cholesterol	Glucose	Phosphorus	
Federal	1962	*	109	150	108	465	115	132	106
		**	164	94	102	271	134	136	113
	1963	*	110	100	117	297	137	114	104
		**	190	194	102	203	152	100	100
Washington State	1962	*	96	74	95	164	167	149	101
		**	135	39	116	174	146	151	94
	1963	*	100	114	100	100	143	106	104
		**	156	205	111	112	139	117	108
Oregon State	1962	*	87	91	107	252	98	158	117
		**	131	245	110	160	141	181	117
	1963	*	104	195	116	142	100	131	110
		**	167	180	111	133	133	112	118
Base Value			1.92	54.4	2.58	.195	.061	.0132	35.5
			gm.		%	%	%	%	%

All values are relative percentages of the lowest 1963 value which is taken as the base value.

* Under 2.5 gm. - in excess of 180 fish per pound.

** Over 2.5 gm. - less than 180 fish per pound.

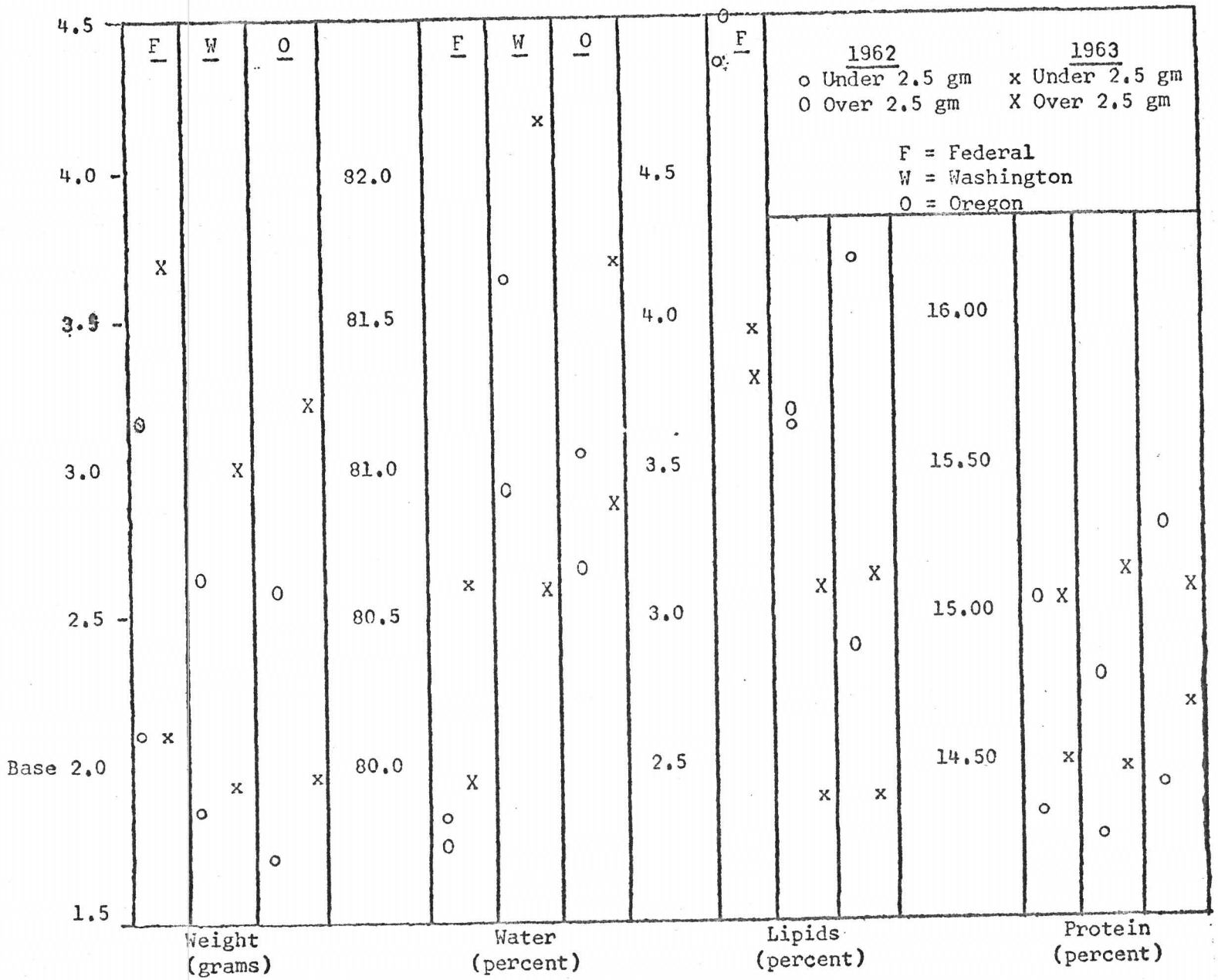


Figure 2.--Comparison of Differences in Body Composition of Hatchery Salmon Fingerling.

Table 2.--Comparison of differences in body components of hatchery salmon fingerling.

Hatchery group		Units above base level			
		Weight	Body protein	Body lipid	Body water
		gm.	%	%	%
Federal	1962 *	.10	-.14	2.33	-.15
	**	1.14	.54	2.49	-.26
	1963 *	.12	.04	1.46	.61
	**	1.64	.54	1.31	-.06
Washington State	1962 *	-.15	-.20	1.10	1.63
	**	.60	.31	1.11	.87
	1963 *	-.08	-.01	-.10	2.14
	**	1.00	.66	.61	.55
Oregon State	1962 *	-.32	0.05	1.72	1.04
	**	.52	.81	.38	.67
	1963 *	0	.20	.08	1.70
	**	1.20	.59	.65	.85
Base		2.00	14.50	2.50	80.00
		gm.	%	%	%

* Less than 2.5 gm.-in excess of 180 fish per pound.

** More than 2.5 gm. - less than 180 fish per pound.

PERCENTAGE OF SMALL BLOOD CELLS AS AN INDEX TO QUALITY IN FALL CHINOOK FINGERLINGS

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

As part of the 1963 Hatchery Evaluation program blood corpuscular counts were made on samples of fall chinook salmon released from many of the lower river hatcheries. The corpuscular counts were made using the recently developed Coulter Particle Counter and Size Frequency Distribution Plotter. With this instrument system we have been able to acquire data not only on the numbers of cells per unit volume of blood but also on the size frequency distribution of these cells.

A study of the size frequency distribution plots made by this instrument system revealed that the small cell portion of the plots showed considerable variation from one group of test fish to another. In attempting to determine the meaning of this variation in the cell distribution pattern we arbitrarily selected the first twenty- five percent of the total range of cell sizes counted, as the small cell portion of the distribution.

The percentage of the total count found in the first twenty-five percent of the cell size distribution was then calculated from the plotted data for the whole size range. The percentage small cell data obtained from the hatchery evaluation samples was correlated with the performance rating obtained for the same hatchery samples in an attempt to evaluate the significance of this variation in relation to the quality of the fish.

Briefly, the performance rating is an index of the stamina of fish as determined in our stamina tunnel (Thomas, NWFCC, 1962). The relationship between these two factors, the performance rating and percentage small cells, was found to be an inverse one in which the performance rating increased as the percentage of small cells decreased. Figure 1, the resulting coefficient of correlation has a negative "r" value of -0.498 which is statistically significant at the ninety-five percent confidence level (Snedecor, 1946).

It seems logical that the stamina of fish at the time of release will have a profound influence on their potential for survival; therefore, we postulate that the percentage small cells will also be an index to the quality of fall chinook fingerlings. The true meaning of both the performance rating and the percentage small cells, however, will not be known until the marked returns come in from which the test-samples were drawn.

Figure 1.--The percentage of small cells in the blood of hatchery evaluation samples of fall chinook salmon fingerlings as related to their performance rating.

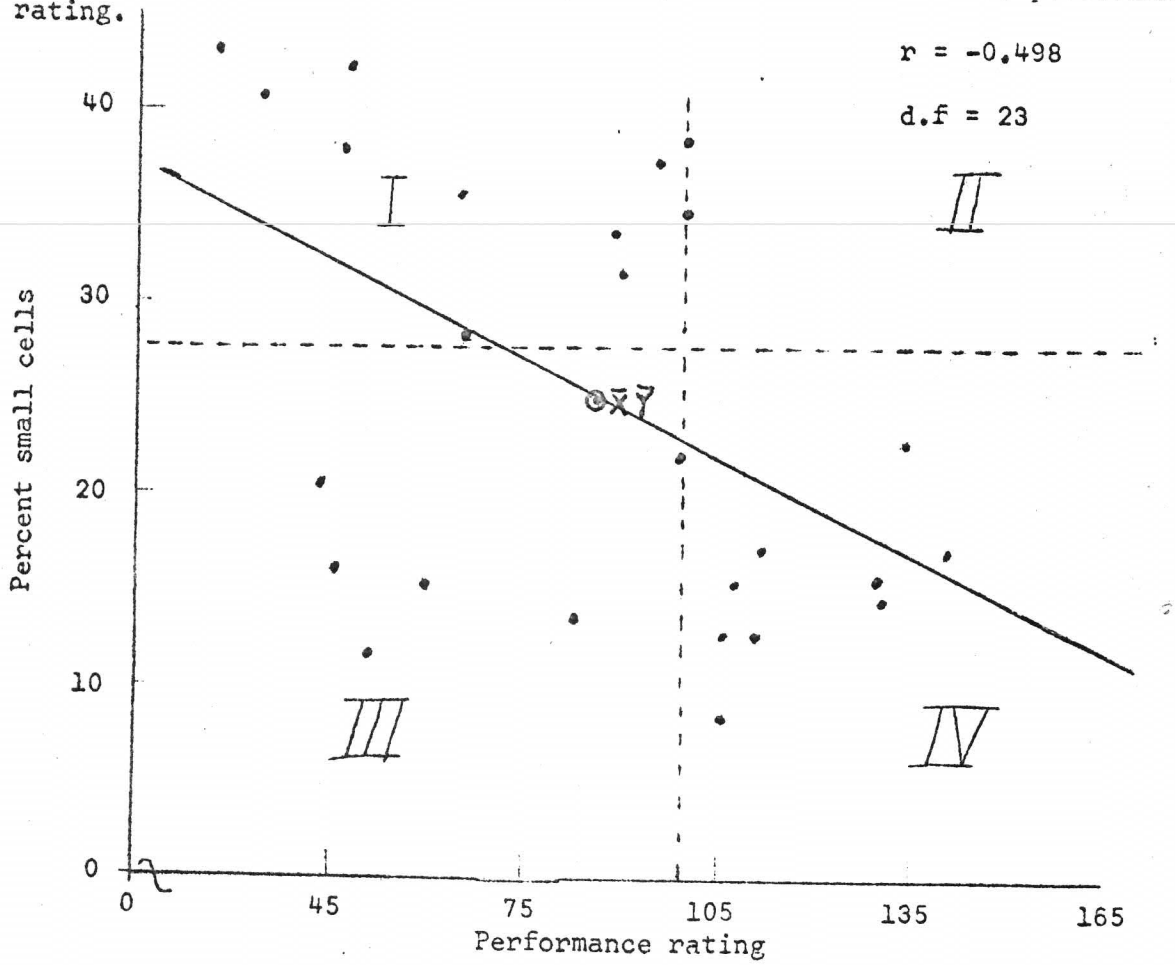
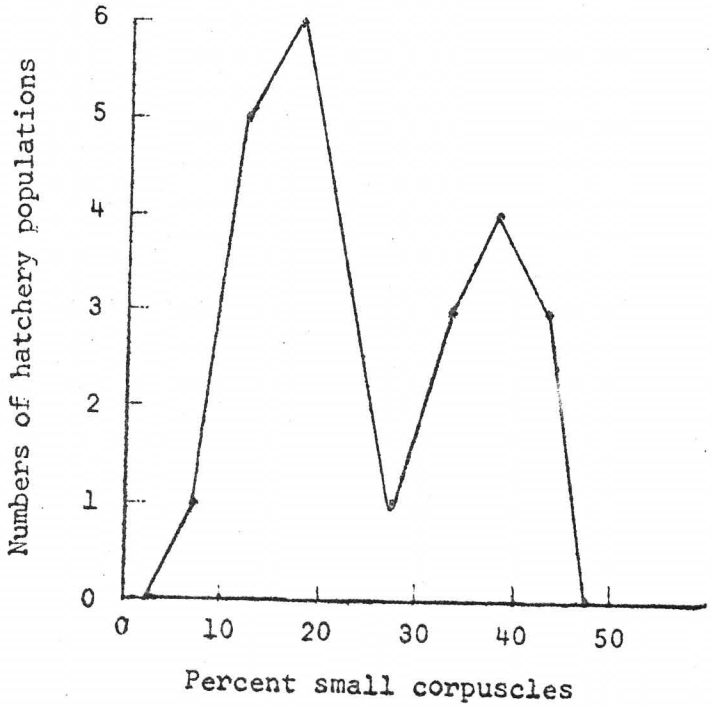


Figure 2.--Frequency distribution of the percentage of small cells in the blood of twenty-six hatchery populations of fall chinook fingerlings.



INFECTIONS OF *AEROMONAS LIQUEFACIENS* IN ADULT CHINOOK SALMON

M. D. Knittel*
Oregon Fish Commission
Clackamas, Oregon

INTRODUCTION

A gram negative rod-shaped bacterium identified as *Aeromonas liquefaciens* was isolated from the kidnesy of over 70% of the adult fall chinook salmon which died at the Oxbow Hatchery on the Snake River in 1962. Work was undertaken to determine if the bacterium was pathogenic to chinook salmon and its relative importance in chinook mortalities.

During initial isolation a phage was isolated for one of the cultures. The indicator strain was designated as stock culture number 2035H and the phage numbered 2035H Ø.

MATERIALS AND METHODS

Cultures were isolated and maintained at room temperature on Meuller-Hinton medium and transferred weekly. Liquid cultures were made in a modified Meuller-Hinton broth containing 11.5 g/l Bacto Casitone, 50 g/l brain-heart infusion, and 1.5 g/l soluble starch. PH of the broth was adjusted to 7.0.

Cultures were purified by standard techniques and carried on Meuller-Hinton slants. Determination of flagellation was done by electron microscopy and by Baileys flagella strain. Final identification was made using the criteria in Bergey's Manual of Determinative Bacteriology, Vol.7.

Cells of 2035H used for injection were centrifuged from liquid culture, washed twice with sterile saline, and suspended again in sterile saline. Cells were injected as a saline suspension. Cells for feeding experiments were harvested by centrifugation, and the packed cells added to the food.

Oregon moist pellest were thawed, and the cells from an 18-hour broth culture were added plus other test ingredients (such as ground glass) where indicated. New pellets were made by re-pelleting through a home food chopper and refreezing. Fish were fed at the rate of 1.5% of their body weight per day.

Juvenile spring chinook salmon and juvenile coho salmon were used as experimental animals. They were weighed and distributed into groups

*Present address: Department of Microbiology, Oregon State University Corvallis, Oregon.

of 10 fish. Water temperature was raised, after introduction of the fish, to the experimental level (15° C.) over an 8-hour period.

The phage was prepared and maintained according to Clark (1962). The phage indicator strain was used in all experiments of infectivity. When a mortality occurred, cultures were made aseptically from the kidney onto Meuller-Hinton medium, and the resulting growth tested for phage sensitivity by the agar overlay method (Adams, 1959). This was a rapid and concise method for identification of the isolated organism. This pahge was found to be specific for the indicator strain 2035H when tested against 20 other isolates of A. liquefaciens from the same area.

RESULTS

Table 1 lists the experimental routes of infection which were tried. When the organism was injected intraperitoneally or subcutaneously the fish began to die about 9 hours after injection, and all were dead by 15 hours. The infection which resulted was systemic in that the test organism could be isolated from the liver, heart, blood, and kidney of the mortalities. These methods of infection are, of course, quite severe and do not demonstrate any invasive powers on the part of the organism.

The next experiment was designed to demonstrate if the organism could enter by way of an abrasion of the skin. The slime layer was first scraped away from the side of the fish and the skin lightly abraded with fine sandpaper. This area was "painted" with a 5-hour culture. The first deaths occurred at 65 hours after infection and continued until 81% of the test fish were dead at 140 hours after infection. Four control fish also died between 135 hours and 140 hours, however, in this case the test bacterium was not isolated.

It is apparent that a fatal infection with A. liquefaciens can be produced by abrasion or some similar trauma. In similar experiments with A. salmonicida fatal infections could be produced most successfully in this manner, giving a consistently uniform and reproducible measurement of infection.

In a number of cases Ceratomyxa sp. was diagnosed from organs of dead chinook salmon at the Snake River. This information prompted the use of some agent whereby an infection with A. liquefaciens could be established by way of the intestinal route. Wolfe and Dunbar (1959) attempted to establish a route of entry through the intestine for the agent of kidney disease by using ground glass in the food. A similar experiment was designed using 4 lots of fish. The control received untreated Oregon pellets while another group had the bacterium incorporated in the diet. A third lot received 5% ground glass in the diet while the fourth group received the ground glass plus the bacterium. If infection by Ceratomyxa of the intestinal tract had provided the route of

Table 1. Experimental Routes of Infection for Aeromonas liquefaciens.

Route of Inoculation	Number of Fish Tested	Number of Survivors End of 24 Hr.	Size of Inoculum Cells per ml
Intraperitoneally	10	0	100×10^7
Subcutaneously	10	0	100×10^7
Feeding 1/	10	10 1/	10.9×10^7
Water	10	10	5.9×10^4
Control (IP)	10	10	0
Control (Sub Q)	10	10	0
Control (Feeding)	10	10	0
Control (Water)	10	10	0

1/ The experimental fish were fed the diet containing the bacterium for a total of two weeks at the end of which all experimental fish were sacrificed and examined.

Table 2. Thirty-Day Feeding 1/Experiment Using Aeromonas liquefaciens (2035H) Isolated from Chinook Salmon from the Snake River.

Diet	Number of Fish Tested	Total Number Mortalities	Number of Mortalities 2/ Caused by A. liquefaciens (2035H)
Control (Oregon Pellet)	10	0	0
Oregon Pellet Plus Bacterium	10	0	0
Oregon Pellet Plus Ground Glass	10	3	0
Oregon Pellet Plus Ground Glass and Bacterium	10	5	5

1/ Rate of 1.5% of body weight per day.
2/ The bacterium used in this experiment was the phage sensitive strain of A. liquefaciens, therefore the isolates from the mortalities were identified by sensitivity to phage infection.

entry for the bacterium which established a systemic infection, then the ground glass in the diet should have reproduced experimentally a similar intestinal lesion.

Table 2 gives the results of this experiment. The group receiving the ground glass plus the bacterium contracted the infection while the fish which received the bacterium only in the food did not. The group that received the ground glass but no bacteria incurred some mortalities, but the infection was not caused by the test organism 2035H. Here again the phage sensitivity of 2035H was of practical value.

DISCUSSION

Phage typing for identification of cultures has been used for a number of years in medical microbiology, a field particularly interested in typing Shigella, Salmonella, and Staphlococci for epidermiological reasons. The method used here is essentially a reverse of this technique in that the experimental infection is produced using the bacteriophage-sensitive bacterial strain. Any bacteria isolated from mortalities are then identified by phage typing. Only those bacteria which are susceptible to the phage are designated as causing the mortality from which they were isolated.

The bacterium which is discussed here was identified as a member of the Aeromonas genus by the Presumptive Identification Chart published by Bullock (1961). The bacterium was then classified by use of Bergey's Manual of Determinative Bacteriology and from published results of others (Kulp and Broden, 1943; Miles and Miles, 1951; Snieszko, 1962; Stanier and Adams, 1944; and Stanier, 1943). Several isolates were later compared to other members of the Aeromonas genus obtained from the American Type Culture Collection.

From the evidence presented, it is concluded that this bacterium can cause death of juvenile salmon upon intraperitoneal injection or subcutaneous injection. When the organism is fed to fish in a diet containing groundglass the infection can be established, but no infection occurs by general water contact alone. The results indicate that this bacterium lacks the power of invasion necessary to establish an infection but must be introduced into the tissue. Once the bacterium has passed the natural surface barriers an infection is established. These observations are further supported by the demonstration of infection by Ceratomyxa in these fish from which the isolation of A. liquefaciens cultures were made.

During the summer of 1963, further observations were made on a group of adult spring chinook salmon to determine the presence of A. liquefaciens and Ceratomyxa infections. Again the bacterium was isolated and in a majority of the cases Ceratomyxa was also found. Though the life cycle of Ceratomyxa is not known, it seems likely that this organism gives A. liquefaciens its portal of entry, and that the

death of the fish is then caused by a systemic infection by this bacterium.

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A SPINAL CURVATURE RELATED TO AN ICHTHYOSPORIDUM INFECTION IN RAINBOW TROUT

The Snake River Trout Company
J. David Erickson

During September, October, and November of 1963 trout growers in Southern Idaho became aware and concerned over a spinal curvature in commercially grown rainbow trout. The spinal curvature was found to have a possible disease origin. Although just one trout farm was severely affected with spinal curvature in trout, several others were involved. The live transfer of affected trout to other states prompted investigation and study of the problem.

The parasitic organism in question is an Ichthyosporidium infection of uncertain species. In brief, Ichthyosporidium is an internal fungus which is commonly found miscroscopically in visceral organs. The organisms appear as spheres of variable size (mostly of 10 to 20 microns in this study) or unbranched hyphae. Mortalities from these organisms were noted in the past in rainbow trout in Southern Idaho, particularly during winter and early spring of 1962. The 1962 mortalities in most cases appeared to be the result of damage to kidney and liver tissue. The causative organism was believed to be identical with that described in 1953 by R. R. Rucker and Paul V. Gustafson[3] which caused an epizootic in a western Washington trout farm.

In September of 1963 the spheres of Ichthyosporidium were found in the brain of rainbow trout which exhibited severe scoliosis. Similarly infected specimens were later found with a lordosis. In the United States there have been no reports to my knowledge of Ichthyosporidium causing spinal curvature in trout. The German literature contains a description by Plehn[1] in 1924 of Ichthyosporidium in the brain which he says "can cause curvature of the spine". Also from German literature, Schaperclaus[2] in 1954 reported the organism as occurring in brain as well as kidney, liver, fins, and the eyes. From the United States, Rucker and Gustafson reported that the organism occurred rarely in the brain in the Western Washington incident.

Tissue sections of brain and other trout anatomy have now shown an apparent relationship between the organism Ichthyosporidium and the spinal curvature incidence. The spheres replace much brain tissue and could put pressure on or cause damage to motor nerves which govern myomeric musculature. Atrophic muscle bundles were located in tissue sections from the myomeres. X-Rays of brain-infected fish indicated that the curvature was not a result of a difficulty within the skeletal system. Rather, it appears that atrophic musculature has pulled the spine out of its normal attitude.

It is believed that the spinal curvature discussed will present no serious problem in hatchery management if safe feeding practices and sanitation are observed. In 1962 Ichthyosporidium was located in carp,

Cyrinus carpio, which was taken from northern Utah to be ground and fed to commercial trout in southern Idaho. The elimination at trout farms of the causative organism from three sources will insure that symptoms of Ichthyosporidium infections, including spinal curvature, will not develop. These sources are:

1. Food. Infected fresh water trash fish, viscera, infected ocean fish.

2. Pond bottom materials containing infective spheres - feces, mud, or debris.

3. Infected, living trout.

[1]Plehn, M. (1924)

[2]Schaperclaus, W. (1954)

[3]Rucker, R. R., and Gustafson, Paul V., (1953)
An Epizootic Among Rainbow Trout
Progressive Fish Culturist, Vol. 15, No. 4, p. 179-181.

PRODUCTION TRIALS UTILIZING SULFONAMIDE DRUGS FOR THE CONTROL OF COLD WATER DISEASE IN JUVENILE COHO SALMON

Donald F. Amend, Oregon Fish Commission 1/
John L. Fryer, Oregon State University

INTRODUCTION

"Cold water" disease, i.e. peduncle disease (Cytophaga psychrophila), is one of the most serious diseases presently encountered among juvenile coho salmon in Oregon Fish Commission hatcheries. Sulmet (sulfamethazine) is presently used prophylactically and therapeutically for control of this disease in both fish-meat and Oregon pellet diets. Customarily, the fish-meat diet is fed until the fish reach a size of 700/lb, then the Oregon pellet diet is given. Initially, a therapeutic dose (10 grams per 100 pounds of fish per day) of Sulmet is administered for 10 days in the fish-meat diet, then a prophylactic dose (2 grams per 100 pounds of fish per day) is administered until 7 days before feeding Oregon pellets. In this 7 day period the therapeutic dose is again administered. This is standard hatchery procedure and will hereafter be referred to as the 10-2-10 treatment. Medicated pellets are fed so that the fish receive a prophylactic dose of about 2 grams of Sulmet per 100 pounds of fish per day until the water temperature is above 50° F.

The Fish Commission has used the above-mentioned procedure for about 6 years. But in many instances the prophylactic use of Sulmet in the fish-meat diet did not appear to control cold water disease. Because the disease occurs throughout the state, and the use of Sulmet is not completely satisfactory, an experiment was conducted to determine the relative effectiveness of Sulmet in controlling the disease.

MATERIALS AND METHODS

Gantrisin (Sulfisoxazole) and S.E.Z. (Sulfaethoxypridazine) were compared with Sulmet. Gantrisin was chosen because it showed a high degree of activity in in vitro tests. American Cyanamid, the producer of Sulmet, suggested testing the experimental drug S.E.Z. because it showed more activity than Sulmet against bacteria susceptible to sulfonamides. Drug dosage was the 10-2-10 treatment and a continuous 4 grams per 100 pounds of fish per day. Previous experimentation with Sulmet indicated that the 4 gram level would give sufficient concentrations of blood-sulfa for prophylaxis.

The Siletz Hatchery was chosen for the production trials because it has had a high incidence of cold water disease in past years. The test utilized 720,000 coho salmon stocked in 10 cement raceway ponds

1/ Present Address: Department of Food Science and Technology, Oregon State University, Corvallis, Oregon.

over an 8-day period. There were 3 ponds for each drug used and 1 control pond. One pond in each set of 3 received the continous 4 gram treatment while the other 2 ponds received the alternating 10-2-10 treatment.

Treatment was initiated within 24 hours after the fish were ponded. The drugs were added directly to a premixed fish-meat diet and the medicated pellets were prepared by Bioproducts of Astoria, Oregon. The medicated food was fed daily over an 8-hour period. Weight samples were taken from each pond weekly and the amount of drugs and food adjusted accordingly. Each treatment lasted approximately 64 days; 25 days on the fish-meat diet and 35 days on Oregon pellets. Mortalities were collected daily and periodically samples were examined for cold water disease by preparing bacteriological cultures from the kidney or lesions on Myxo media[1]/. A positive culture of cold water disease on Myxo media is characterized by circular, convex colonies with a smooth surface and entire margin. The colonies are yellowish, translucent, and are of viscid consistency. Wet mounts reveal single long flexing rods, and the organism is gram negative.

Success of the various treatments was determined by fish mortality and incidence of cold water disease.

RESULTS AND DISCUSSION

As in past years, cold water disease was again present. The majority of the mortalities which occurred after the firstweek had symptoms of the disease and positive cultures were obtained from all lots within 10 days after the fish were stocked in the rearing ponds.

Usually mortality declines after the initial ponding loss, but when disease or other diminishing factors are introduced the mortality continues to rise. If the fish are infected before being ponded, initial mortality is higher and continues to remain at a high level. If drugs are administered and have any effect on the pathogens, the mortality should not increase after the first 2 weeks and if the group is heavily infected, mortality should drop.

In the control pond, mortality was high during the first week, continued to rise during the second week, and remained at a high level throughout the meat-feeding phase of the experiment. When pellet feeding was initiated, mortality dropped rapidly to a level which was comparable to the treated ponds in just two weeks (Figure 1.) There was no immediate explanation for the reduction in mortalities, for the pellets were non-medicated and the water temperature remained favorable for the disease, never exceeding 50° F. during the experiment. The incidence of the disease directly corresponded with the drop in mortalities. This same situation also occurred in the treated ponds, but the difference was not

1/ Myxo Media: 0.5% Tryptone, .25% Yeast infusion, .9% Agar.

as drastic. Therefore, it is believed that the effects of the various treatments can best be analyzed by comparing the results during the meat-feeding phase of the experiment only.

In all cases each of the drugs effectively controlled the disease. Figures 1 and 2 demonstrate the mortality from each of the treatments, and indicate that losses from each treatment were less than in the control ponds. Analysis of variance indicated statistically significant differences ($P = .05$) between treated and control ponds. There was no statistically significant difference found between the drugs used or the treatment levels. The continuous 4 gram treatment controlled the disease as well as the alternating 10-2-10 treatment for only 53-60% of the drug cost.

Even though statistical tests did not show a difference between the drugs, some interesting trends were observed. In the case of Sulment, mortality was highest during the first week, almost approximating the control pond. After the first week, mortality sharply declined while in the control pond mortality remained high. In past experiments Sulmet has been shown to be absorbed slowly, taking from 3 to 6 days to reach maximal blood concentrations. This is probably the reason why sulmet showed little control during the first week. Gantrisin, being absorbed quickly (less than 24 hours) appears to have controlled the disease somewhat faster.

It was mentioned in the introduction that there was some question of the efficacy of using Sulmet at the 2-gram level. This experiment indicated that the 2 gram level may be effective, but this level was only administered for 7 of the 25 days on the fish-meat diet. Hence it is not known if a greater difference would have resulted had the 2-gram level been used longer. The decline in mortalities during the 2-gram treatment period may have been only a reflection of the 10 gram level reaching effective blood levels.

CONCLUSIONS

1. The use of sulfonamide drugs can reduce the loss of fish due to cold water disease.

2. A continuous 4-gram treatment can control the disease as well as the alternating 10-2-10 treatment and requires less drug.

3. In this experiment, Gantrisin appeared to control the disease more quickly than did Sulmet. In other experiments Gantrisin as compared to Sulmet has been shown to be less toxic, more quickly absorbed, to have no adverse effects on growth when administered at recommended levels, and to have more activity against cold water disease, *in vitro*.

4. S.E.Z. does not appear to offer any advantages over the other drugs.

Figure 1 PER CENT AVERAGE DAILY LOSS WITH DRUGS ADMINISTERED AT 10-2-10 GM LEVEL IN THE MEAT DIET AND 2 GM LEVEL IN OREGON PELLETS, SILETZ HATCHERY, 1963

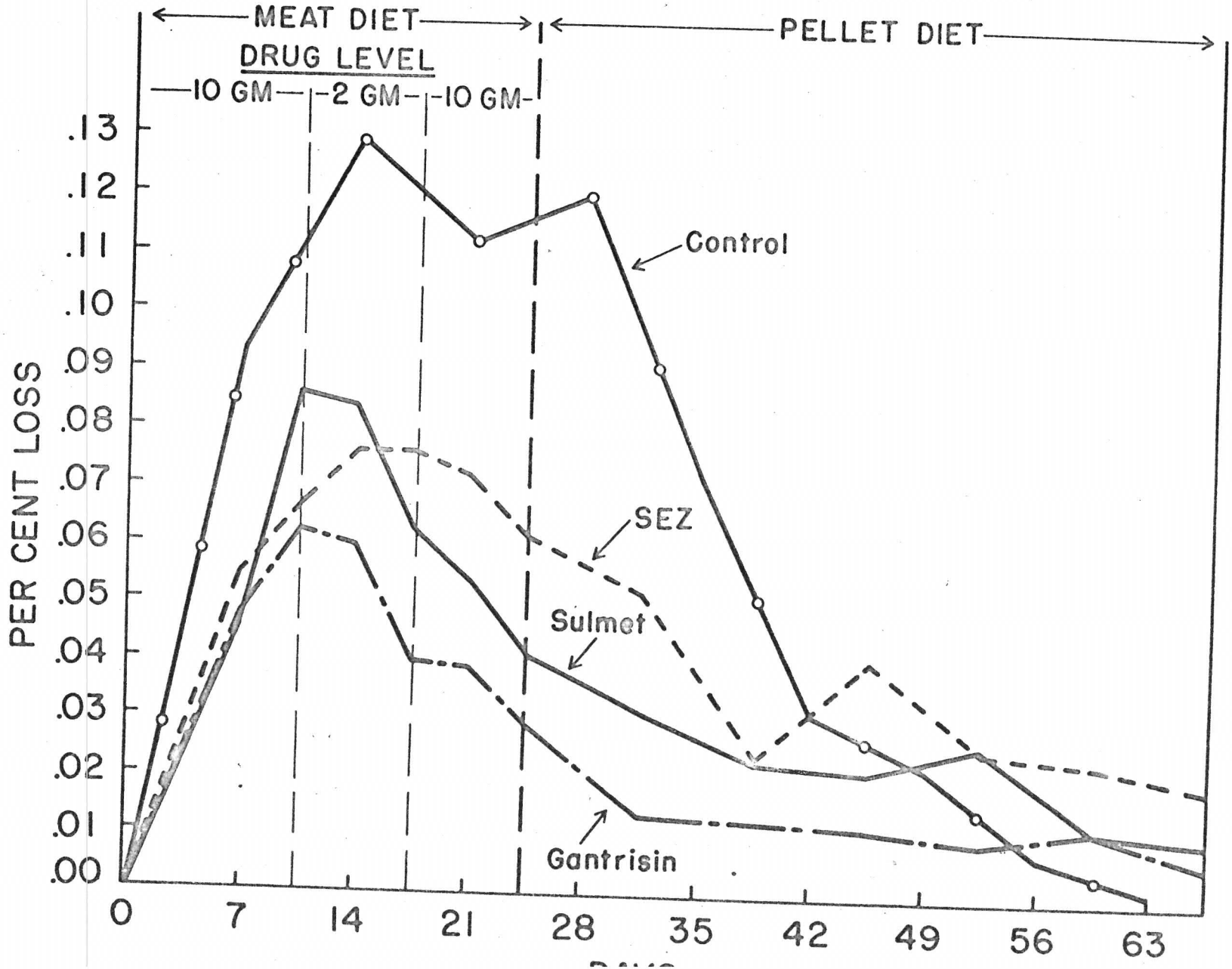
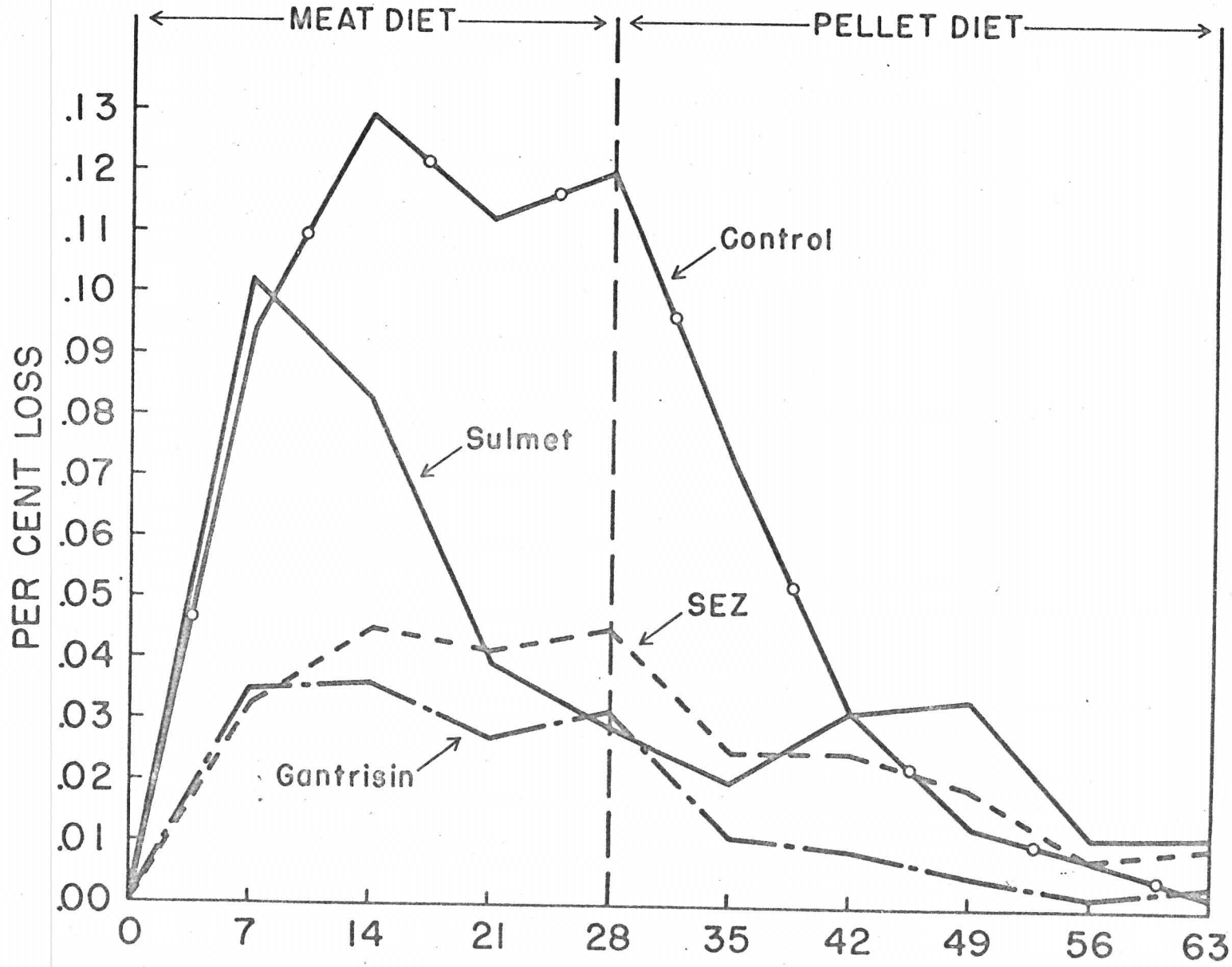


Figure 2 PER CENT AVERAGE DAILY LOSS WITH DRUGS ADMINISTERED AT 4 GM LEVEL IN THE MEAT DIET AND 2 GM LEVEL IN OREGON PELLETS, SILETZ HATCHERY, 1963



HEMATOLOGY AND FISH DISEASES

By

Western Fish Disease Laboratory
George W. Klontz, DVM

In September 1963, five of us, Tosh Yasutake, Larry Ashley, Charles Smith, Joe Wales and myself, got together to discuss the possible application of the various hematological techniques to studying fish diseases. Out of this meeting came a list of variables that must be considered when studying fish from this standpoint, plus a cursory examination of the various techniques and staining procedures. There was also a morphological description of the different types of erythrocytes and leukocytes in the rainbow trout.

The following is a list of variables that must be considered and minimized when one is studying the constituents of the circulating blood and of the blood-forming organs of fish.

1. Species of fish
2. Origin or source of the fish in question
3. Water temperature
4. Water constituents; i.e., pH, hardness, ions present, etc.
5. Diet being fed to fish
6. Age of the fish
7. Numbers of fish per unit water volume
8. Physical manipulations of fish during the study
9. Type of holding facility; i.e., pond, tank, raceway, etc.
10. Sampling techniques; i.e., method of killing, making imprints, etc.
11. Enumeration techniques; i.e., hemocytometer, Coulter Counter, etc.
12. Staining techniques; i.e., pH of buffer, differential staining, etc.
13. Personnel taking the samples.

The above listed potential sources of variations are but some of the difficulties encountered in attempting to determine deviations from "normal" with respect to blood studies. To minimize their influence and maintain them as constants, we made the following recommendations:

1. The rainbow trout should be the reference standard. In nearly every facility, whether State or Federal, there is easy access to stocks of this fish. Even though another species is being investigated, the data obtained can be interpreted in terms of that for the rainbow trout.

2. In determining the hematological data on the rainbow trout, various investigators should keep in mind that the available information should be interpreted only in the light of what they themselves find. That is, there is no such thing as a normal table of blood values for the rainbow - or any other species of fish, for that matter. We felt that one of the main factors suppressing the desire to study fish hematology has been the confusion arising when an investigator compares his work with that of someone else and finds no correlation whatsoever. Remember, a "normal" fish is a fish that is healthy in all respects in his particular environment. Also, the hematological changes that occur in disease are going to be similar in most respects from area to area, but they are not going to be the same. Not recognizing this has led many a person "down the primrose path".

3. With respect to the many techniques that may be employed to make the same determination, we recommend that a single technique be adopted for each type of determination. That is, there are many techniques for running a hemoglobin test: Cyanmethemoglobin, acid hematin, color comparator, and so forth. Of all these, the cyanmethemoglobin method seems to give the most consistent results. Thus, it should be adopted as the routine test, and all the other methods interpreted in terms of it. Another consideration with respect to the various techniques that may be used for measuring one blood component is the fact that they very often measure different compounds that are part of the component. That is, the acid hematin technique measures only the oxyhemoglobin while the cyanmethemoglobin technique measures the methemoglobin and oxyhemoglobin. Thus, the reason for variations when comparing two techniques to do the same thing.

In this write-up of the talk I will not include the morphological description of the trout red and white blood cells. During the conference of last September the five of us agreed on what cell would be called what. But, this does not mean to say we are right. We hope to discuss this more fully at the 1964 meeting, at which time many more workers in fish hematology will be present.

ORAL IMMUNIZATION OF RAINBOW TROUT AGAINST REDMOUTH

Western Fish Disease Laboratory
George W. Klontz, DVM

Before I go into the details of what we did and what we found, I would like to mention that John Ross and not I should be presenting this material. He is the one that put forth all the "blood, sweat, and tears" to make this work.

A little over a year ago we killed some organisms that had been isolated from an outbreak of redmouth at Hagerman NFH. These organisms were put into a pelleted diet and fed to a group of yearling rainbow from Quilcene NFH. Two months later we injected some "hot bugs" into ten of these fish and into ten controls. Only one of the controls survived, whereas nine of the test fish lived through it. At five more intervals (98, 304, 339, 360 and 408 days) we challenged fish with anywhere from 1 LD_{90} (that amount of organisms that will kill 90% of the unprotected fish) to 40 LD_{90}. The final result was that there was 88% survival in the test fish against 19% survival in the control fish.

This past month we were granted permission to set up a full-scale hatchery trial at Hagerman NFH. Fish are being fed food containing the vaccine. Now we are just sitting and waiting to see if in the spring we have more immunized fish than control fish - that would be success.

As a final note, John and I are putting the final touches on a manuscript for the Transactions of the American Fisheries Society in which we describe in detail what we did.

ACTIVE AND PASSIVE IMMUNIZATION OF RAINBOW TROUT, SPRING CHINOOK, AND COHO SALMON AGAINST AEROMONAS SALMONICIDA

Kemet D. Spence, Oregon Fish Commission*
John L. Fryer, Oregon State University
K. S. Pilcher, Oregon State University

The lack of extensive information on the immunological character of poikilothermic animals was emphasized by Hildemann (1962) who also states that with the exception of temperature (Cushing, 1942; Bisset, 1948; Elek, Rees, and Gowing, 1962), conditions effecting the formation of antibodies in cold-blooded vertebrates can be assumed to be the same as those in other animals. Techniques employed in the study of immunological reactions of other vertebrates are usually found adaptable to similar investigations with poikilothermic animals (Ridgway, 1962).

Anti-bacterial agglutinins have been produced in rainbow and brown trout at 10° C. (Smith, 1940), in cutthroat trout at averages of 7 and 9.0° C. (Duff, 1942), and in carp at both 10° C. (Smith, 1940) and 20° C. (Pliszka, 1939). Duff was able to show the formation of significant titers through oral administration of Aeromonas salmonicida vaccine, however, reasonably high titers occurred among many of the control fish.

Though isoagglutinins could not be detected in fish held at from 3-5° C., they were produced in sockeye salmon at 9-14° C. and in rainbow trout held at 15° C. (Ridgway and Klontz, 1960; Ridgway, 1962). Ridgway (1962) also showed the production of hemagglutinating antibodies by sablefish at temperatures as low as 5-7° C.

Ultimately, the question arises as to whether the vaccination of these animals is affording them some form of protection since neither agglutinating antibodies nor other circulating types of antibodies necessarily indicate disease protection. Duff (1942) was able to develop resistance to furunculosis disease in cutthroat trout by feeding an oral vaccine containing A. salmonicida. It has also been recently shown that newly hatched fry of Symphysodon discus and related species apparently receive some form of passive immunization by feeding on the epidermal mucous secretion of the parental skin (Hildemann, 1962).

Temperatures favoring the most rapid production of antibodies are at the higher temperature tolerance limits of cold-water fishes. Since most trout and salmon are reared in relatively cold water it becomes important to not only determine what concentrations can be expected under these conditions, but also the relationship of these antibodies to actual protection for the fish. This study was undertaken to investigate several aspects of these phenomena.

*Present Address: Department of Microbiology; Oregon State University; Corvallis, Oregon.

ACTIVE IMMUNIZATION

Rainbow Trout

Agglutinating antibodies were successfully prepared in 2-, 3-, and 4-year-old fish. The fish were held in a spring water supply which maintained an average of 12° C.

A formalin-killed vaccine was mixed 2:1 with Freund's (complete) adjuvant prior to inoculation, and injected intra-abdominally. Two-year-old rainbow received 3 injections, the second injection occurring 1 week after the initial inoculation, the third after an additional 5 weeks. Control fish in this group received either aqueous 0.85% NaCl or 0.85% NaCl plus Freund's adjuvant. Blood was removed from these fish 4 weeks after the final injection.

The first five injections of the 3- and 4-year-old rainbows were carried out 1 week apart, the sixth after an additional 2 weeks, the seventh after an additional week, and the final injection after an interval of 5 more weeks. Control fish received 0.85% NaCl each time the other group received vaccine. Serum was removed from this group one month after the final injection.

The relatively high levels of agglutinating antibodies attained in the 2-, 3-, and 4-year-old fish are shown in Tables 1 and 2. Both groups of vaccine-injected fish showed relatively high levels of agglutinin formation, while the controls all lacked similar antibody levels. The low levels found in some of the controls probably reflect the residual of previous nonartificial stimulation.

To eliminate the possibilities that there might still be A. salmonicida agglutinating antibodies present, though undetectable through lack of homogeneity between strains, a group of other isolates was tested with the serum. These included organisms from various Oregon Fish Commission hatcheries and an ATCC strain, 14174. All showed high agglutination titers with the anti-5000H serum making it unlikely that agglutinins against other A. salmonicida strains were present, though undetected. The lower titer experienced with the vaccine culture, 5000H, when compared with the other A. salmonicida can be explained by the fact that it had been repeatedly transferred throughout a period of two years, being used almost exclusively in these experiments. The other strains employed experienced no such vigorous culturing stress, most being isolated only recently. The homogeneity of the strains is apparent in Table 3.

There was also a second reason for the specificity test. Since we were planning a production scale oral vaccine experiment involving the use of a predictable natural infection it was necessary that we obtain some idea of the serological relationship between various strains.

Spring Chinook Salmon

Adult fish used in this experiment were obtained from the USFWS Eagle Creek National Fish Hatchery. Information was obtained from three fish, survivors of a group which received a single 9 ml injection of the same vaccine used for intraperitoneal inoculation of the rainbow trout. Controls received the same amount of a saline-Freund's adjuvant preparation. Blood was removed 8 weeks after the single injection and assayed for agglutinating antibodies.

As shown in Table 4, antibody formation was detected among the fish tested within 8 weeks following the single vaccine injection. Lack of additional test animals hampered this experiment although the results obtained clearly show a difference between the control and vaccine fish. These results are limited, however, and do not show the high antibody titers found among the rainbow trout which received a larger number of vaccine injections.

Production of antibodies is apparently slow among cold-water fishes, and this disadvantage is not helped by the fact that adult salmon have a limited life expectancy, nor by the degenerative physiological state in which they exist. There is a possibility, however, that although these fish may not prove to be good suppliers of antiserum, they may be able to produce sufficient antibodies to protect themselves until the eggs or sperm can be harvested.

PASSIVE IMMUNIZATION

It is well known that high levels of agglutinins do not necessarily indicate the existence of "protecting", or "immunizing" antibodies. The co-existence, and stimulation of both these factors may be shown by passive protection experimentation.

Juvenile coho salmon were experimentally infected using a combination of two methods which had experimentally proved to be effective and measurable. First, immediately after injection of the serum, while the fish were still anesthetized, an area of about 1 cm^2 (midway down the lateral line) was scraped clean of scales and slime, and a concentrated suspension of A. salmonicida 5000H applied with a swab to the scraped area. The fish were then immediately placed back in the fresh running water. After 24 hours a 50 ml suspension of the same strain (incubated for 24 hours at 18° C.) was added to each tank. Immediately after introduction of the bacterial suspension, the water to each tank was stopped and the water-contact treatment carried out for 1 hour with aeration. The same procedure was repeated again at 48 hours. All fish mortalities were posted to determine the specific cause of death.

The experimental fish were held at 13° C. and fed Oregon pellets until start of the experiment. Water temperature was slowly raised to

17° C. over 3 days after the fish had been transferred to 16-liter tanks in the laboratory.

Antiserum and control serum used for injection consisted of pooled twenty-fish samples (filter sterilized) from the same rainbow that were tested for agglutinating antibodies.

Three groups of coho were involved in the study: (1) negative controls which received no serum; (2) controls which received 0.5 ml undiluted sterile serum from control rainbows; and (3) fish which received 0.5 ml undiluted sterile anti-5000H serum from vaccinated rainbow. Serum was injected intraabdominally after anesthetization with 1:17,500 MS-222. Four hours after inoculation of the serum the fish received their initial exposure to A. salmonicida 5000H.

The protective value of immune rainbow serum is shown by the hourly mortality in Fugure 1. Each line on the graph represents an average between two samples giving a total of 37 negative control fish, 36 positive control fish, and 40 antiserum-receiving fish. The administration of antiserum prior to infection not only delays the onset of furunculosis disease, but also suppresses the normal mortality dynamics. The antiserum-treated fish follow a similar, though delayed cumulative mortality curve (Figure 2), from 36 to 48 hours behind those of the control fish. Total mortality is shown to reach 72.5% in the antiserum fish, and 88.9% and 91.9% in the positive and negative controls, respectively.

This experiment indicated that immunizing substances did exist in the rainbow serum, whether related or unrelated to the existence of the agglutinins, their presence conferring a relatively high level of protection on the juvenile coho. The success of this study could have been more absolute had the method of infection been less drastic, however, it had been earlier determined experimentally that this method of infection gave rise to sensitive and reproductible measurement of the disease progression resulting in a uniform mortality curve.

This discovery of passive protection with fish is not surprising, since it has been quite commonly employed for some time in combatting diseases of warm-blooded animals. Its effectiveness has been found to be of relatively short duration in the latter case, giving protection for up to 2-4 weeks. Its lack of practicality for treatment of juvenile fish is obvious; its best application appears to be in the treatment of adult salmon since no consideration need be made for continuing protection, nor long-range effects as in the case of juveniles. In addition, adult salmon are usually present in numbers which could be treated practically. A system of passive immunization or protection could be created where several diseases could be combatted simultaneously with proper serum preparations. Other animals could be utilized as a source of immune serum as with warm-blooded animals. Information

shown in this paper suggests that serum may possibly be prepared in the adult salmon themselves, perhaps in sufficient quantities to aid succeeding generations.

ORAL IMMUNIZATION

Although intra-abdominal inoculation of 2-, 3-, and 4-year-old rainbows, and spawning age salmon (to a lesser degree) proved to result in the production of significant amounts of A. salmonicida agglutinating antisera, the oral administration of similar vaccines proved ineffective when incorporated into juvenile coho diets.

Both laboratory and hatchery fish were orally treated. Four groups of fish were involved in the laboratory experiments: the first group received the oral vaccine over a 98-day period; the second received the vaccine over a 46-day feeding period; a third received the vaccine over a period of 22 days; and, a fourth consisted of control fish. Water temperature was held at 13° C. throughout the experiments. The fish were experimentally infected by introducing a 50 ml broth culture of A. salmonicida 5000H into the water supply daily through the first 15 days of the experiment. Although the survival and mortality data (Tables 5 and 6) would seem to suggest that some protection was conferred on the fish fed 98 days, statistical analysis X^2, indicated that the computed value (1.22 with one degree of freedom) was not significant at the 5% level.

Juvenile coho at the Siletz Hatchery experienced a natural infection predicted accurately from analysis of furunculosis infections occurring in previous years at this hatchery. Had the infection not occurred as predicted, fish would have been removed to the laboratory for challenge. The fish received the vaccine over a period of 81 days. There were 72,000 each of vaccine and control fish fed. The average water temperature was 15° C.

No protection was observed among fish involved in the Siletz experiment. This was expected since these fish received much lower levels of vaccine than those held in the laboratory. It was hoped, however, that a smaller effect might be more easily detected in the large populations of this experiment.

Non-significance of mortality differences is supported by the lack of agglutinins among these fish. No agglutination could be detected with serum removed from any of the fish involved in any of the oral vaccination experiments. Circumstances which might explain the lack of stimulatory effect by the oral vaccine include: insufficient time to allow antibody formation, lack of optimal temperatures, presence of vehicle inhibitors, sub-optimal antigen concentrations, and fish strain and physiological factors. The question also arises as to whether the vaccine was of a nature conducive to antibody formation. Although it is shown that this vaccine causes the formation of "protective" sub-

stances in the blood after intra-abdominal inoculation, it would not necessarily follow that this same preparation should elicit as dramatic a result by the more circuitous, and chemically vulnerable oral route.

CONCLUSIONS

1. Intra-abdominal inoculation of a formalin-killed vaccine preparation of A. salmonicida causes the formation of agglutinins, and whether related or unrelated, the formation of protective antibodies in adult rainbow trout.

2. This method of vaccine administration also results in the formation of agglutinating antibodies in adult spring chinook salmon after only one injection.

3. Passive immunization was found to give a relatively high degree of protection when adult immune rainbow serum was injected into juvenile coho salmon prior to contact with the disease.

4. Attempts at oral immunization with juvenile coho against A. salmonicida in laboratory and field experiments gave negative results.

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Table 1. Agglutination of Aeromonas salmonicida 5000H by Immune and Control Serum from 2-Year-Old (300 gm) Adult Rainbow Trout.

Serum Sample	No Agglutinins	Dilution 1:10	1:20	1:40	1:80	1:160	1:320	1:640
Vaccinated	0	0	0	0	0	0	2	1
Control (saline)	4	1	0	0	0	0	0	0
Control (Freund's adjuvant)	5	0	0	0	0	0	0	0

Table 2. Agglutination of Aeromonas salmonicida 5000H by Immune and Control Serum of 3- to 4-Year-Old (900-1000 gm) Adult Rainbow Trout.

Serum Sample	No Agglutinins	Dilution 1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
Vaccinated	0	0	0	0	0	2	5	8	6	1
Controls	20	7	1	0	0	0	0	0	0	0

Table 3. Specificity of Anti-5000H Serum.

Antigen	Serum	Agglutination 1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
5000H	Anti-5000H	4+	4+	4+	4+	4+	2+	2+	-	-
	Control	-	-	-	-	-	-	-	-	-
5006Z	Anti-5000H	4+	4+	4+	4+	4+	4+	4+	4+	2+
	Control	-	-	-	-	-	-	-	-	-
5007W	Anti-5000H	4+	4+	4+	4+	4+	4+	4+	3+	2+
	Control	-	-	-	-	-	-	-	-	-
5010B	Anti-5000H	4+	4+	4+	4+	4+	4+	4+	3+	3+
	Control	-	-	-	-	-	-	-	-	-
5012T	Anti-5000H	4+	4+	4+	4+	4+	4+	4+	3+	1+
	Control	-	-	-	-	-	-	-	-	-
5016K	Anti-5000H	4+	4+	4+	4+	4+	4+	4+	4+	2+
	Control	-	-	-	-	-	-	-	-	-
ATCC 14174	Anti-5000H	4+	4+	4+	4+	4+	4+	4+	3+	2+
	Control	-	-	-	-	-	-	-	-	-

Table 4. Agglutination of Aeromonas salmonicida 5000H by Immune and Control Serum from Adult Spring Chinook Salmon.

Serum Sample	No Agglutinins	Dilution 1:2	1:5	1:10	1:20	1:40
Vaccinated	0	0	0	1	1	0
Control (saline plus Freund's adjuvant)	0	1	0	0	0	0

Table 5. Oral Immunity of Juvenile Coho After 98 Days Treatment with Aeromonas salmonicida 5000H Vaccine.

Sample	No. Fish	Per Cent Cumulative Mortality Days after Initial Infection Attempt 0-6	7	8	9	10	11	12	13	14	15	16	17
Vaccine	(13)	0	15.4	15.4	30.8	30.8	30.8	30.8	30.8	30.8	30.8	30.8	30.8
Vaccine	(15)	0	0	6.7	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3
Control	(13)	0	7.7	7.7	15.4	23.1	30.8	30.8	46.2	46.2	46.2	46.2	53.8

Table 6. Oral Immunity of Juvenile Coho After 22 and 46 Days Treatment with Aeromonas salmonicida 5000H Vaccine.

Sample	No. Fish	0-6	7	8	9	10	11	12	13	14	15	16	17
Vaccine	(16) 1/	0	12.5	18.8	18.8	31.3	31.3	31.3	31.3	31.3	31.3	31.3	31.3
Vaccine	(14) 1/	0	0	0	0	7.1	14.2	14.2	14.2	21.3	21.3	21.3	21.3
Vaccine	(12) 2/	0	0	8.3	8.3	16.7	16.7	16.7	16.7	16.7	25.0	25.0	25.0
Vaccine	(11) 2/	0	0	0	9.1	9.1	9.1	9.1	18.2	18.2	18.2	18.2	18.2
Control	(21)	0	0	9.5	14.3	14.3	19.0	19.0	19.0	23.8	23.8	23.8	23.8
Control	(24)	0	0	0	0	4.1	20.8	25.0	25.0	37.5	37.5	37.5	37.5

1/ Fish samples which received vaccine over a period of 22 days.
2/ Fish samples which received vaccine over a period of 46 days.

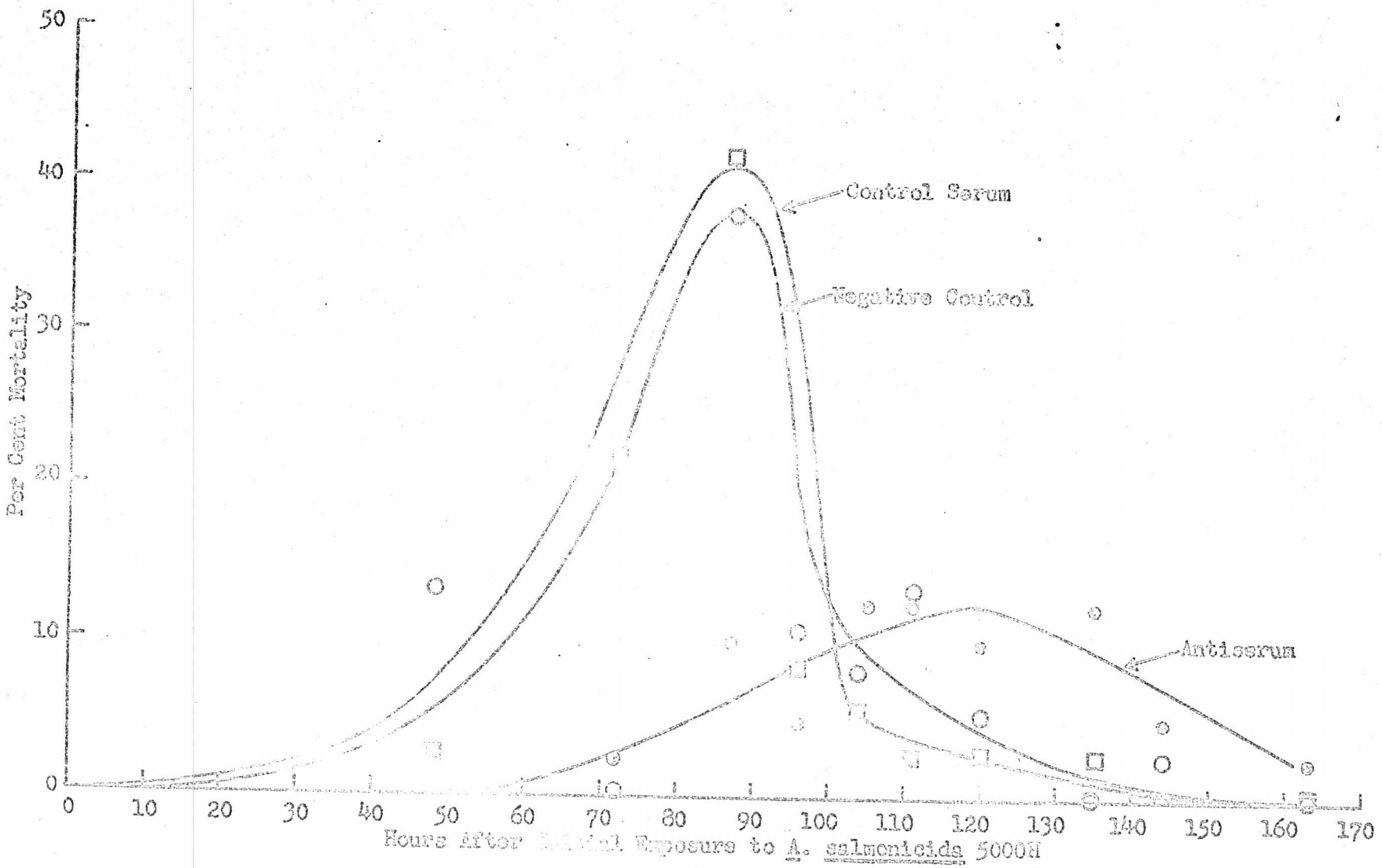

Figure 1. Hourly Per Cent Mortality of Negative Control, Serum Control, and Antiserum-Treated Fish.

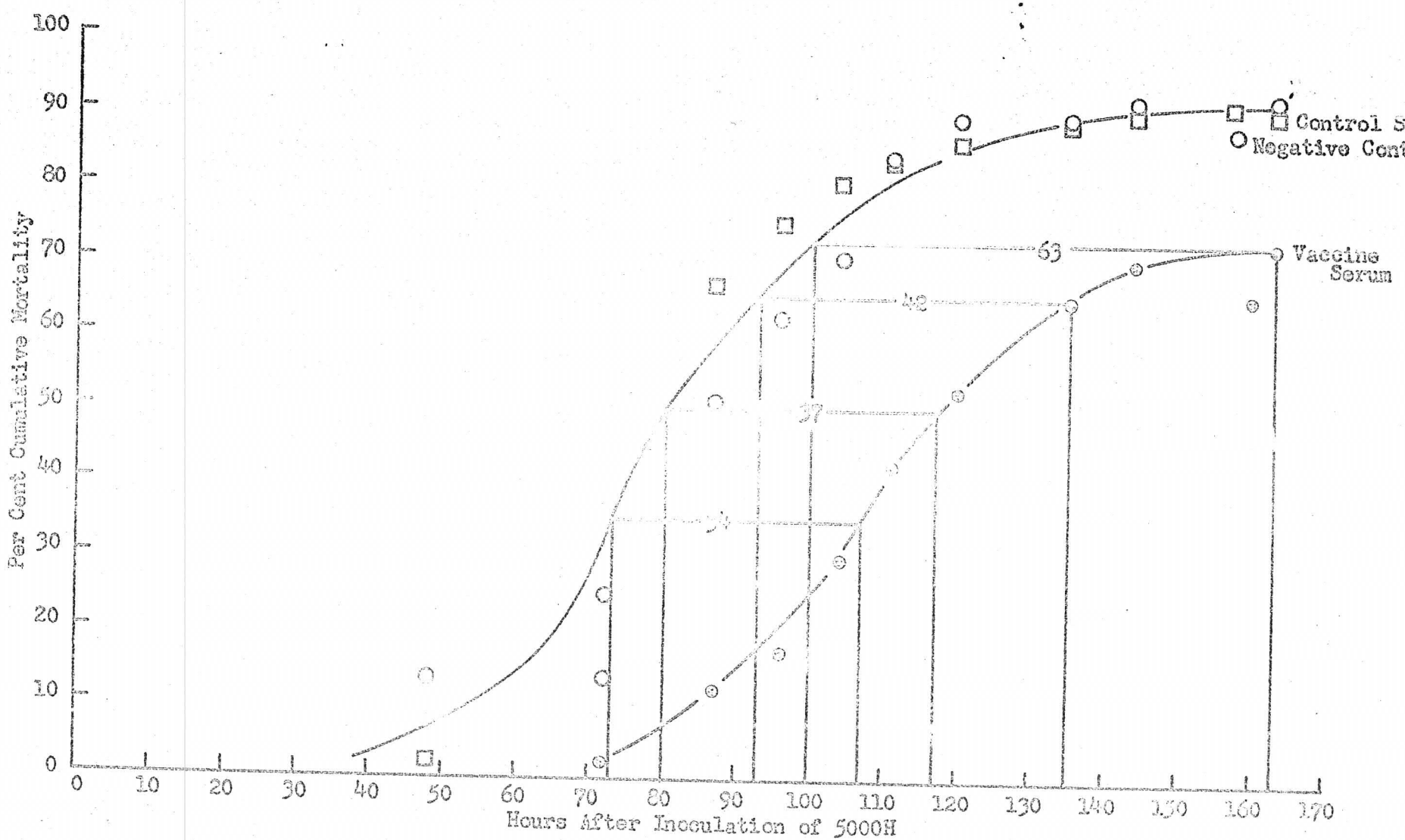

Figure 2. Cumulative Mortality of Juvenile Coho Infected with A. salmonicida 5000H After Receiving No Serum, Control Serum, and Antiserum From Adult Rainbows.