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VOLUME V

ADAPTABILITY OF SALMON TO NEW ENVIRONMENTS CREATED BY DAMS

C O N T E N T S

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PREDICTION AND CONTROL OF ENVIRONMENT

IMPORTANCE OF WATER TEMPERATURE IN THE MAIN STEMS OF THE COLUMBIA AND SNAKE RIVERS IN RELATION TO THE SURVIVAL OF SALMON

by

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INTRODUCTION

The relation between the distribution of separate species of poikilothermal fish and environmental temperature is very strong. Permanent shifts in the temperature regime can eventually cause changes in the species composition within the geographical area affected. The tilefish (Lopholatilus chamaeleonticeps) appeared off of the New England coast in large enough quantities to support a commercial fishery in the late 1800's. In 1882, a sharp cooling trend set in, causing mass mortalities of the species. The tilefish disappeared completely from this region.

Nikolsky (1963) states, "The cooling caused by the spread of the ice sheet at the end of the Tertiary and the beginning of the Quaternary periods enabled the representatives of the salmon family, which were adapted to cold water, to spread considerably southward as far as the Mediterranean Sea basin, including the rivers of Asia Minor and North Africa." The Pacific salmon, which has evolved as a cold-water species, is able to adapt to temporary shifts of the temperature regime it inhabits, although not without loss. A permanent temperature shift to higher levels during the fresh-water life history could result in minimal survival conditions.

The taxing demands of heavy fishing pressure, obstructions to upstream and downstream migrations, and the gradual loss of adequate spawning grounds have placed the upper Columbia River and the Snake River salmon runs in jeopardy. Instead of accepting rising temperatures in the river with resultant fish losses as a way of life, fishery agencies might well take an aggressive stand and seek the means to create beneficial rather than harmful temperature changes.

The purpose of this report is to point out the importance of water temperature as an environmental condition and the advantages to be gained by control.

TEMPERATURE REQUIREMENTS OF PACIFIC SALMON

Salmon are faced with immediate temperature problems in the main stems of the Columbia and Snake Rivers during two major stages of their life history. These are the juveniles (including fry) which have moved out of the spawning areas and the adults migrating upstream. Incubating eggs and fry in the spawning areas are generally not within reach of temperature control at the present time.

Effects of Increased Temperatures on Adult Salmon

Adult salmon make their final journey from the estuary to habitual spawning grounds on stored energy. The installation of dams on the Columbia River has eliminated the falls and rapids which may have required great expenditures of energy by the salmon during migration.

The storage of water, however, has created a situation which is difficult to assess. Water temperatures have increased due to increased atmospheric heat transfer, and adults migrating through these impoundments are, for various reasons, delayed in their journey.

The metabolic rate of all fishes is closely dependent on the temperature. The rate of metabolism will increase with an increase in temperature, even if the animal is motionless (Winberg, 1956). Synergistic effects are particularly noticeable with increased temperatures because (1) oxygen demands increase, (2) oxygen-carrying capacity of the water decreases, and (3) susceptibility to toxic substances increases. A rise in temperature from 60^{o} to 70^{o} F. could probably raise the active metabolic rate by onefold to fivefold (Brett, 1962). The possibility then arises that the total energy expended due to demands caused by increased temperatures and prolonged migration times may well equal or exceed that energy expended under prior conditions.

Elevated temperatures not only increase the metabolic rate and drain the reserves of energy but also drastically affect the total ability of the adult salmon to do work. Muscular activity increases, but the recuperative powers of the animal diminish.

In addition, there is evidence which shows that the sex products of adult salmon which are exposed to high temperatures during the prespawning period are adversely affected[1/]. Increased water temperatures decrease the rate of maturation and prolong the period of time in which the animal is exposed to fresh-water

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1/ Fulton, L. A. The effect of temperature on incubating eggs, juvenile, and adult salmon, a literature survey. Bureau of Commercial Fisheries, Fish-Passage Research Program, Seattle, Washington. Unpublished report (1963).

dangers before the eggs are deposited. Diseases such as *Chondrococcus columnaris* become highly virulent when the water temperatures reach the upper 60's and low 70's (^{o}F.), seriously infect large numbers of adult salmon, and have been suspected to be the principal cause of declines in the Redfish Lakes sockeye salmon runs (Ordal and Pacha, 1963).

Optimum Temperature Conditions for Adults

Maximum productivity of adult salmon during the fresh-water stage is achieved in the temperature range of 42.5^{o} to 55^{o} F. (Burrows, 1963). This is the optimum range for adult survival, egg viability, time of spawning, and spawning efficiency. Although fall chinooks in the Columbia and Snake Rivers are faced with, and actually survive, temperature ranges of 65^{o} to 75^{o} F., the maximum during migration and maturation should be 60^{o} F.

When other environmental conditions, such as turbidity and stabilized water flow, are optimum, there is no question that water temperatures in the optimum range of 50^{o} to 60^{o} F. can definitely benefit the adult salmon population. The most striking example of this is the chinook salmon runs in the upper Sacramento River after the construction of Shasta Dam and Keswick Dam (reregulating).

The most significant effect of Shasta Dam is the profound change in the temperature regime downstream (fig. 1). Prior to impoundment, temperatures dropped as low as 42^{o} F. in the winter months and reached a high of 72^{o} F. in the summer months. The temperatures below the dam are now higher in the winter and lower in the summer, maintaining a narrow range between 50^{o} and 60^{o} F. Other environmental features were also altered to the salmon's benefit. Flow patterns were smoothed, and turbidity decreased considerably. This significantly improved the more than 90 miles of excellent gravel bottom in the river bed below Keswick Dam. The decreases in river temperatures, in addition to the other advantageous environmental features, have influenced the spring chinooks to remain in the main river and spawn, avoiding the collection racks at Keswick Dam. In 1943, examination of dead females showed 95 percent spawning success. In 1944, spawning success was 67 percent, this lesser success being caused by mine pollution in the immediate vicinity of Keswick Dam; and in 1945, spawning success was 98 percent.

In 1946, the commercial catch of California king salmon was 13,700,000 pounds, a 31-year record. This phenomenal catch attributed to the success of the 1941, 1942, and 1943 year

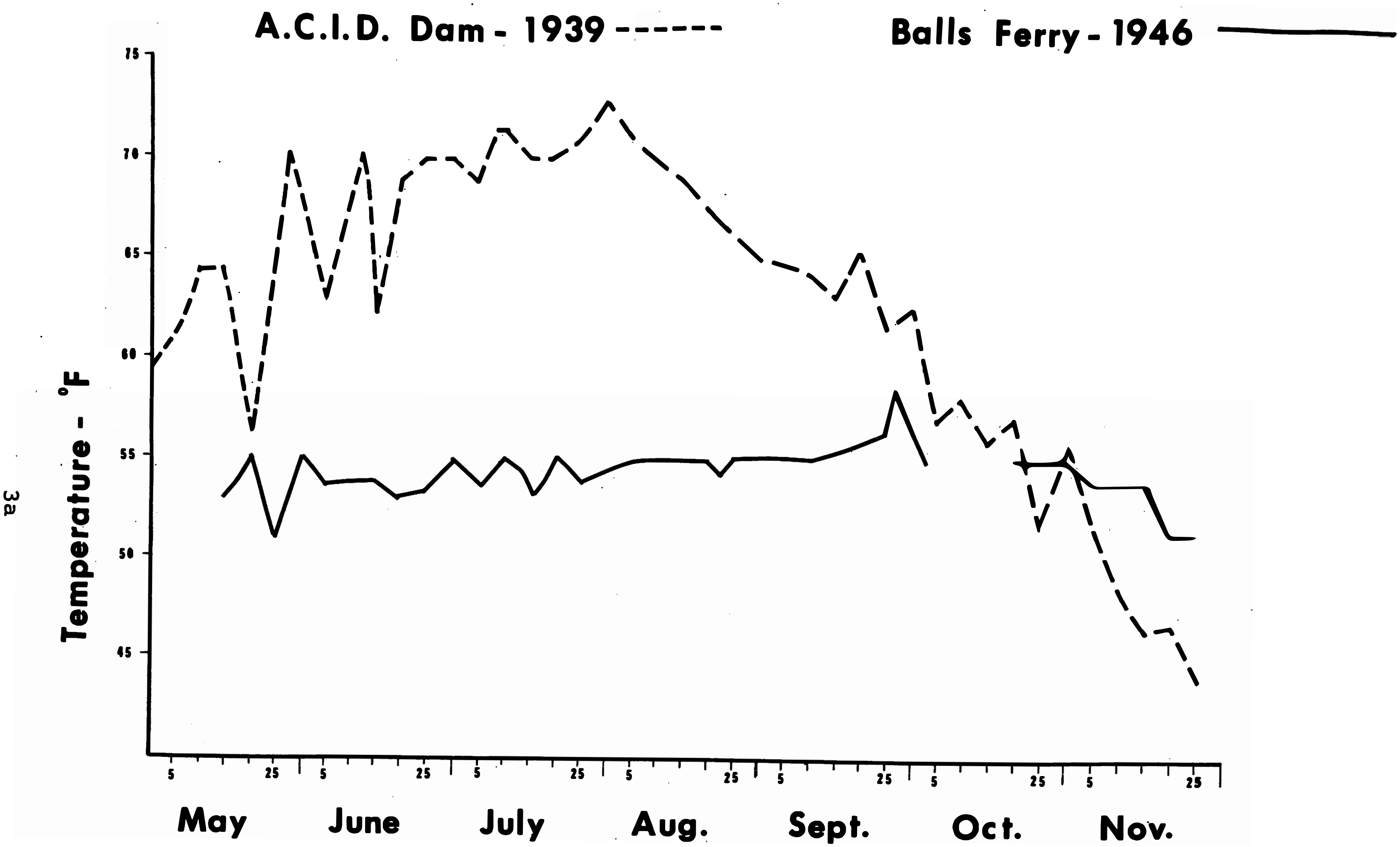

Figure 1.--Pre- and post-impoundment water temperatures of the Sacramento River below Keswick Dam. From Moffett (1949).

classes. In addition to advantageous conditions for adults, other factors of a desirable nature were noted. Egg incubation was accelerated. Time of migration of juveniles is now ahead of maximum irrigation usage. River food production for juveniles has increased, owing to higher winter water temperatures and stabilized flow. Most significantly, the scrap fish and predatory nonsalmonid species have virtually disappeared.

The thermal cycle of the upper Sacramento River was once borderline habitat for salmon. It now conclusively resembles thermal cycles of streams which are in the center of the geographical range of chinook salmon (Moffett, 1949). More recently, it has been noted that the Sacramento River is the only river on the continent which has definitely established three separate runs of king salmon: fall, winter, and spring (Slater, 1963).

The Effects of Increased Water Temperatures on Juvenile Salmon

The upper lethal temperatures for juvenile salmon of all five species do not vary by more than 1° to 2° C. However, the juveniles of each species have other temperature relationships which have evolved as adaptions to the environment. The optimum cruising speeds for young sockeye, for example, are found at 15° C., whereas coho performance is best at 20° C. In British Columbia, sockeye feed and live in the cooler limnetic water of lakes and migrate out before lake outflow temperatures reach 14° or 15° C. Young coho feed and live in the littoral zone of the lakes and ascend small tributary streams, where waters sometimes get quite warm. Outmigrations of coho may coincide with the juvenile sockeye, but will frequently continue on long after the sockeye have left (Brett et al., 1958).

Lower water temperatures reduce the effects of most diseases on juvenile salmon with the exception of *Cytophaga psychrophilia,* a myxobacterium. As the temperature rises above 15° C., the virulence of certain strains of *Chondrococcus columnaris* increases rapidly (Ordal and Pacha, 1963). Resistance to pollutants, both organic and inorganic, generally decreases with increased water temperatures. As water temperatures rise, the respiration and metabolic rates rise. Circulating blood is exposed at a faster rate and noxious chemicals enter the system at a rate which exceeds excretory removal. Here again, the synergistic attacks upon the physiological functions become complicated. With increased water temperatures, the oxygen carrying capacity of the blood decreases, metabolic demands for oxygen increase, and the oxygen levels in the water decrease. Subjection of fish to minute quantities of pollutants during these stresses can be disastrous.

Optimum Temperature Conditions for Juvenile Salmon

There are so many synergistic effects associated with decreased water temperatures in regard to juveniles that the absolute assessment of the benefits derived is a difficult task. Only the highlights are presented here.

The amazing results in the Sacramento River have already been noted. Hoar (1948) has stated that changes in the water temperature or changes in the periodicity of thermal cycles produce notable reactions in juvenile salmon, some of which are associated with seaward migration. Warmer water during the winter months throughout the habitat will accelerate embryonic and fry development and consequently alter the time of migration.

The effects of water temperature changes during downstream migration are of great importance. In recent years it has been demonstrated with pink salmon that the environmental temperature at the time of downstream migration through the estuary is correlated with the strength of the returning year class. There has been no such effort to measure or correlate the strength of the returning year class with estuarine environmental conditions in the Columbia River at the time of downstream migration.

Variations in evolved temperature zone habitation are confirmed by laboratory temperature preference tests. The final preferendum temperature for juvenile sockeye is 14.5° C.; for chinooks, 11.7° C.; for chums, 14.1° C.; and for pinks, 11.7° C. (Ferguson, 1958). Optimum growth of juveniles is achieved at the preferential temperatures, and for all species of Pacific salmon this would be within the range of 10 to 15° C. (Burrows, 1963).

TEMPERATURE REQUIREMENTS OF OTHER SPECIES OF FISH IN THE COLUMBIA AND SNAKE RIVERS

Increased changes in the temperature regime or shifts in the thermal cycle can influence the balance of other species of fish in the system as well as the salmon.

The classical effects of the influences of cold-water releases from Shasta Dam on the Sacramento River salmon are just as applicable to other fish species. Studies of sport fishing efforts show that the resident trout populations have increased, whereas species such as bass, catfish, and sunfish--which compete with trout for food and space--have decreased (Moffett, 1949).

Species Inhabiting Warm Temperature Zones

The predacious squawfish (Ptychocheilus grandis), once the dominant carnivore in the Sacramento River, has been driven from many miles of stream below the dam. It has become dominant in smaller streams which are experiencing a warming trend (Taft and Murphy, 1950). The preferred temperatures of squawfish, based on various field observations, appear to be from 16° to 25° C., with a lethal high of 29° C. The temperatures in the Columbia and Snake Rivers are well within the preferred range of the squawfish for at least several months each year (fig. 2). There appears to be a strong correlation between the incidence of highly virulent strains of C. columnaris and squawfish population buildups in the Columbia River near Richland, Washington, during high-temperature periods (Ordal and Pacha, 1963).

Cyprinids, such as Cyprinus carpio, are the dominant forage fish in the mainstems of the Columbia and Snake Rivers. Carp do best in extremely warm waters, near the upper limit of their preference range (20° to 32° C.). They will continue to feed actively until water temperatures drop to 8° C., and will not begin their annual spawning until the temperatures rise again to at least 15° C. (Nikolsky, 1963). They usually seek out shallow bays late in the spring as spawning sites. These are the first areas to warm. The many sloughs and the shallow littoral zone behind McNary Dam, for example, make this reservoir particularly productive. Largemouth bass (Micropterus salmoides) also favor waters in the upper twenty degree centigrade range and are common in the quiet back bays and the warm shore areas of some of the reservoirs. The smallmouth bass (M. dolomieui) is more commonly found in the rocky sections of the Snake River, especially in Hell's Canyon. Optimum spawning conditions are achieved when the water temperature suddenly accelerates to 16° C., or higher, and is held there for at least 3 weeks. Spawning successes below 16° C. are extremely doubtful (Macan, 1963). Both the smallmouth bass and the perch (Perca flavescens) prefer water temperatures in the low twenties (centigrade). Perch will spawn at lower temperatures, but members of the sunfish family--such as Lepomis gibbosus--require minimum temperatures of 22° C. for spawning, their habits being quite similar to those of the largemouth bass.

Species Inhabiting Intermediate Temperature Zones

Most of the aforementioned species would suffer the severest setbacks in those areas where water temperatures are held back to 16° C. or less during the early summer months, owing to disruption of spawning cycles. Trout, whitefish, and some species of suckers would undoubtedly benefit by the presence of colder water during the summer months (fig. 2). Carside and Tait (1958) found that rainbow trout which were acclimated to cold water preferred to move into warmer waters as the acclimation temperatures approached or exceeded 13° C. The final preferential temperature was 13° C. Brook trout, which are not common in the main stems, are most active between 13° and 16° C., and maximum growth is achieved at 13° C. (Macan, 1963).

Members of the bullhead family (Ictaluridae) are active at all temperatures between 10° and 34° C. Their population limitations are similar to those of the sturgeon. Both groups are bottom feeders and prefer silty conditions and deep holes, where dead insects and organic material collect.

CONCLUSIONS

The main stems of the Columbia and Snake Rivers are now, thermally speaking, borderline habitat. That is, the temperature ranges are not optimum at the present time for most of the warm-water species, nor are they optimum for the development of a highly productive trout and salmon environment.

On the basis of evidence at hand, there is no question but that fishery agencies must take advantage of every situation where cold water can be introduced into the system during the summer and early fall months. In addition, if warm waters can be introduced during the winter months, it is not unlikely that improved water quality similar to that of the Sacramento River will develop.

The present trend of gradually increased temperatures could continue upward with the addition of reactor power stations. VonGunten[1/] has stated that the installation of one 100-million-kilowatt thermal reactor power plant would raise the temperature of the Columbia River at The Dalles 9.4° C. (17° F.). A temperature

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1/ U. S. Corps of Engineers, Walla Walla District. Personal communication (1964).

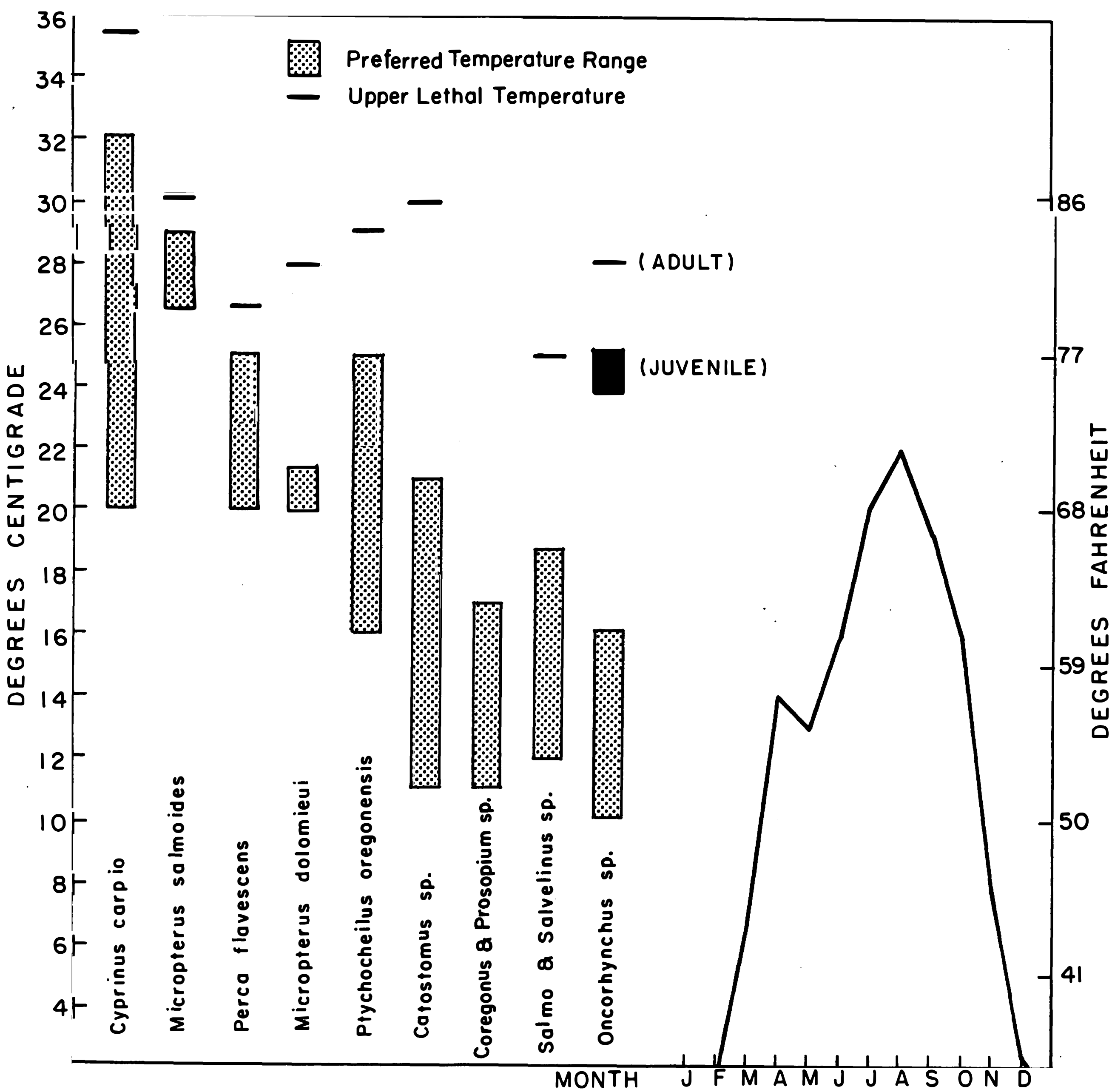

Figure 2. Preferred and lethal temperatures of some Columbia Basin fish compared with lower Snake River water temperatures. Temperatures were recorded at the mouth, and monthly averages for 1954 to 1957 combined.

rise of this magnitude would certainly place a large segment of the river beyond the range of the thermal requirements of the salmon.

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PRELIMINARY STUDY ON THE PREDICTED WATER CHANGES OF THE LOWER SNAKE RIVER DUE TO THE EFFECTS OF PROJECTED DAMS AND RESERVOIRS

PART I: FORECASTING WATER TEMPERATURE CHANGES DUE TO FLOW THROUGH INTERMEDIATE DEPTH RESERVOIRS

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Prepared for: Bureau of Commercial Fisheries
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Seattle 15, Washington

Contract Order No: 64B-RO-78
140.12A
July 25, 1963

Material Requested: 1. Preliminary Study on Predicted Water Quality Changes of the Snake River. Study to include an estimate of the possible effects of the following list of dams and also an evaluation of the potential for control of river temperatures and oxygen levels in the Snake River: Ice Harbor, Lower Monumental, Lower Granite, Asotin, China Gardens, High Mountain Sheep, Hells Canyon, Oxbow, Brownlee, Penny Cliffs, and Bruce's Eddy. The preliminary study will include a review of data required for such a study, the data presently available, an estimate of the time required to develop the information, and an estimate of cost.

Prepared by Wayne V. Burt with the assistance of W. Bruce McAllister.

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November 12, 1963

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ABSTRACT AND CONCLUSIONS

A method has been developed for forecasting temperature changes for cold water passing through intermediate depth reservoirs during summer and fall. Preliminary studies indicate that some benefits, in the form of lower downstream water temperatures, may be expected during critical times of the year, after all six projected medium depth reservoirs are in operation on the lower Snake River, provided that low temperature water is available from upstream.

The results of these studies indicate that water passing through the six reservoirs may be exposed to the atmosphere in a turbulent condition during at least 25% of the time of passage before it leaves the surface to flow as a density current toward the reservoir outlets. For this reason oxygen depletion should not be as serious a problem in the density currents passing through the reservoirs as it is in the subsurface waters of deep reservoirs such as Brownlee.

Previous temperature forecasts have been made for tailrace discharge temperatures for the projected High Mountain Sheep and Nez Perce reservoirs. These forecasts indicate that any combination of dams and associated reservoirs on the Snake River at its confluence with the Salmon River will deliver cooler than normal water into the projected China Gardens reservoir. China Garden is the first of the six medium depth reservoirs on the lower Snake River. Similar water temperature forecasts have been made in the past for the projected

Bruce's Eddy reservoir and the Clearwater River between Bruce's Eddy and Lewiston. These forecasts indicate that both the Bruce's Eddy and Penny Cliffs Reservoirs will lower the temperature of the Clearwater River as it flows into the projected Lower Granite Reservoir above Lewiston, Idaho. The beneficial effects of both reservoirs will increase with increasing discharge rates.

Past studies by the author on several deep reservoirs have all indicated that downstream temperatures may be lowered during critical times of the year by manipulation of discharge from outlets at several depths. Cold water may be conserved when it is not needed and then discharged when it is needed.

INTRODUCTION

The whole crux of studying the effects of the six medium depth lower Snake River reservoirs on temperature conditions in the river depends on the ability to forecast temperature changes within each individual reservoir.

The methods for forecasting temperature changes in reservoirs have been developed over the past decade. Raphael (1962) has developed a system of forecasting temperature changes in shallow reservoirs on the Columbia River. The shallow depths, small volumes and high discharge rates insure vertical mixing and turbulence from top to bottom over most of the year. On the other extreme, the author and others have developed and used several systems for forecasting temperature changes in deep reservoirs where layered or nearly layered flow occurs whenever a vertical density gradient exists.

Unfortunately, the six lower Snake River reservoirs are medium depth, medium discharge reservoirs and neither of the above systems may be used except for times when the vertical density gradient is very slight (or absent) or the rate of discharge is at an absolute abnormal minimum.

Up to the present time no one had been able to handle the problem due to lack of knowledge of flow and mixing patterns in medium depth reservoirs. Turbulent mixing and flow takes place in the upper end of these reservoirs. Some sort of layered flow takes place in the deeper lower end of these reservoirs whenever a vertical density gradient exists.

Recent work by Yih (1958), Duncan et al. (1962), Harleman (1961), Debler (1959) and others has furnished the theory and a beginning of the empirical verification of the theory for forecasting flow conditions in all reservoirs. Specifically, they show that a fundamental relationship should exist between the flow pattern within the reservoir and the vertical density gradient, the discharge and shape parameters of the reservoir. This heretofore missing relationship allows us to develop a system for forecasting temperature changes within the six medium depth reservoirs of interest. A discussion of the above series of studies on flow in channels and reservoirs appears in the Appendix. It should be emphasized that more field measurements in actual reservoirs are required before these relationships will be completely understood.

FLOW PATTERN IN INTERMEDIATE RESERVOIRS

For convenience we will define intermediate reservoirs as those similar to the six reservoirs on the lower Snake River beginning with Ice Harbor and running upstream to China Gardens. Figure I is a schematic drawing of the current structure in an intermediate reservoir during the late spring, summer, and early fall. This type of current structure should occur whenever there is a measurable vertical temperature change and the discharge rate is not at a maximum. The reservoir is divided into five zones for purposes of discussion.

Zone I

Zone I consists of the turbulent area at the up river end or head of the reservoir between the points A and B on Figure I. Water enters the reservoir from the river or next reservoir above at point A. The temperature of the inflowing water is determined by conditions upstream. Flow is relatively rapid and turbulent between points A and B. The flowing water is warmed or cooled from the surface depending upon its temperature and the surface heat budget at any given time. The heat budget is a function of meteorological conditions in the air over the reservoir. The exact distance from A to B (the length of Zone I) is constantly changing from hour to hour and day to day. The position of B depends on discharge, changes in discharge, changes in the rate of heating or cooling at the surface and changes in the water level in the

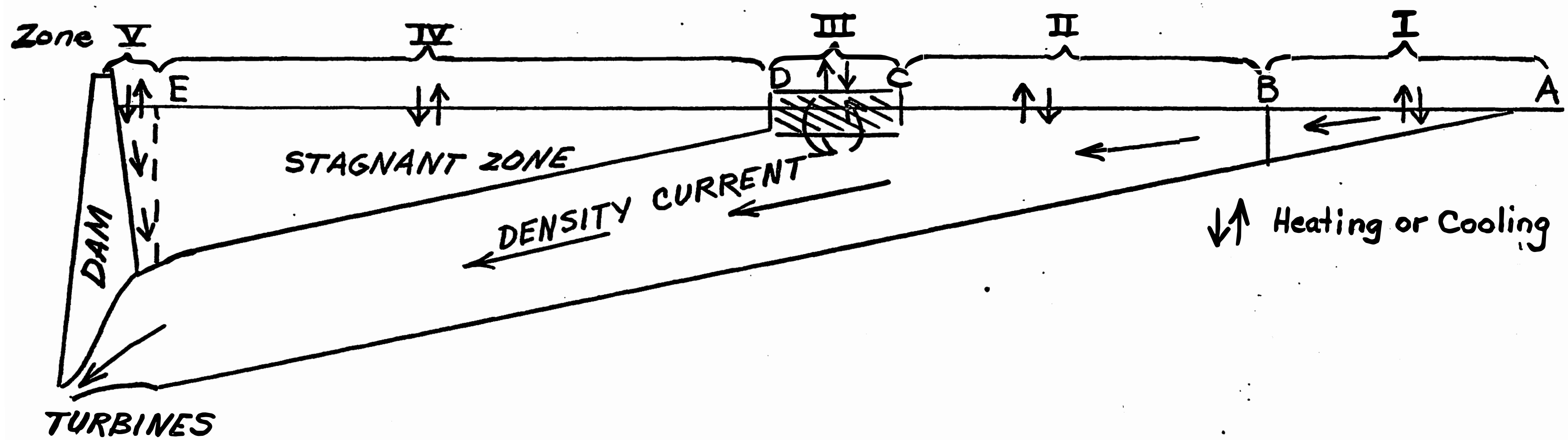

Figure 1. Schematic diagram of processes taking place in intermediate stratified reservoirs due to the combined effects of surface heating and cold water inflow.

reservoir. At point B the reservoir has widened sufficiently so that current will slow down and the turbulence will be reduced. Heating will not penetrate all the way to the bottom downstream from point B.

If the water in Zone I is low in oxygen content there will be a rapid movement of oxygen from the atmosphere through the surface into the water.

Zone II

Heating or cooling may be taking place in Zone II depending on the water surface temperature and the heat budget. If surface heating is taking place, a vertical temperature gradient will develop* as the water passes from B to C. If cooling is taking place over Zone II, the cooled surface waters will sink, causing some overturn and turbulence will increase. If cooling is sufficient to overcome vertical thermal gradients set up by previous heating, the overturn and increased turbulence will extend to the bottom. Whenever this occurs, Zones I and II will merge and the point B will move downstream to point C.

At point C the downstream current leaves the surface and dives under the surface layer as it flows as a sub-surface current toward the turbine outlets. After the current leaves the surface, we will call it a density current.

The discharge temperature from the reservoir is largely determined by inflowing water temperature and the heating or cooling that

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* The term temperature gradient is used to mean any temperature decrease with depth.

takes place at the surface in Zones I and II. After the water leaves Zone II some heating will take place as warmer water is entrained from above at the shear zone just above the density current. Some warmer water will also be entrained from above due to turbulence right at the face of the dam. If the water in Zone II is low in oxygen content, oxygen will move down from the atmosphere through the surface into the water.

Zone III

Zone III is a transition zone or boundary zone between the new water flowing in from upstream and older water which has been in the reservoir for some time. The new and old refer to length of residence in the reservoir. Continuous boundary changes will take place between the points C and D. Wind mixing, cooling from above, and inertia related to changes in discharge will all effect the position and extent of the zone. Increase in discharge will move the zone downstream. Decreases in discharge will move it upstream.

Zone IV

The stagnant zone between the points D and E (above the density current) contains water that is not participating in the major current pattern in the reservoir. The top layer in this zone will be relatively stagnant when compared to the density current. Wind stress on the surface will bring about some mixing and will cause currents in the surface layer of zone IV.

The temperature of the very surface layer of zone IV will be changing continually with day to day and hour to hour changes in the local weather over the reservoir.

Orographic conditions will bring about some mixing from below which may extend all the way from the density current, through the stagnant layer, to the surface. Sharp bends in the old river channel, the sides of islands or channel restrictions will all cause local turbulence with some vertical mixing between layers.

Zone V

The boundary zone right next to the dam will have some turbulence within it due to a variety of causes. Any vertical turbulence will cause some mixing between the two layers. Causes of turbulence may be inertial, related to discharge changes, or due to vorticity in the density current as it increases in velocity or entering the turbine intakes.

From a study of Figure I it is obvious that a large number of different factors are affecting the temperature and current structure in intermediate size reservoirs such as those on the lower Snake River. For exact forecasting we need a large number of current measurements over a variety of conditions to describe the expected current structure within intermediate reservoirs. In the meantime, the relationships discussed in the Appendix give us some quantitative idea of what to expect in the lower Snake River reservoirs. A forecasting system can be developed to show the general trends of what to expect in these reservoirs and then be corrected as more qualitative information on flow relationships becomes available.

It should be mentioned that both Army Engineer personnel and people employed by the Bureau of Commercial Fisheries have measured small vertical temperature gradients in the Ice Harbor reservoir during summer. Due to long exposure both the water coming downstream into the reservoir and resident water in the reservoir are either in equilibrium or very near in equilibrium with the atmosphere most of the time. Both river surface and reservoir surface are influenced by the same climatic conditions. Thus, one would not expect strong thermal gradients. The very fact that they are measured indicates that the reservoir would stratify to some extent if cold water were available at its upstream end.

FORECAST

All six of the intermediate reservoir systems that are or will be formed by dams on the lower Snake River have somewhat the same characteristics as to depth, volume, and area. The upper two will have less discharge than the lower four due to the inflow from the Clearwater River. The most important related factor is depth which enters as a square in the computation of the Froude number (see Appendix). Conditions are similar enough that a forecast for one reservoir will tell us what to expect from the other five. Such a forecast may be used to see if it may be worthwhile to make detailed forecasts for all six reservoirs.

The Army Engineers (Walla Walla District) do not have complete area, volume, elevation, and dam characteristics for any one of the reservoirs. The most data are available for Ice Harbor and Lower Granite. Ice Harbor was chosen because its depth, volume, and surface area are about the average for the depths, volumes, and surface areas of all six reservoirs. Missing area, volume, elevation data for Ice Harbor were inferred from available data for Lower Granite. Differences between the shape of the two reservoirs would not materially effect the conclusions reached.

Question to be answered:

If all six reservoirs were in operation and cold water were available from discharge into the upper reservoir, would any trace of the cold water be left by the time it was discharged through the last dam downstream? Another way of stating the question is: Would the cold water coming into the upper reservoir be heated up to equilibrium with the atmosphere by the time it left the last of the six reservoirs?

Let us assume that all six dam and reservoir systems have the same shape and other charactersitics as Ice Harbor. Let us further assume that the relationships between the thickness of the density current, discharge rate, and vertical gradients are the same for all six reservoirs. A last assumption is that the results of Debler's (see Appendix) model study apply to full scale reservoirs of the same conformation.

The above assumptions then allow us to forecast the temperature rise for cold water passing through 1, 2, 3, 4, 5, or 6 reservoirs with any given initial inflowing water temperature.

The exact numerical step by step process of the forecasting will not be discussed. It is similar to other forecasts by the author. The thickness of the density current was derived from Figure 2 which in turn was derived from the shape of the Ice Harbor reservoir, using Debler's (see Appendix) relationships. The area and volumes of zones I and II (Figure 1)were derivedfrom the shape of the reservoir and the thickness of the density current.

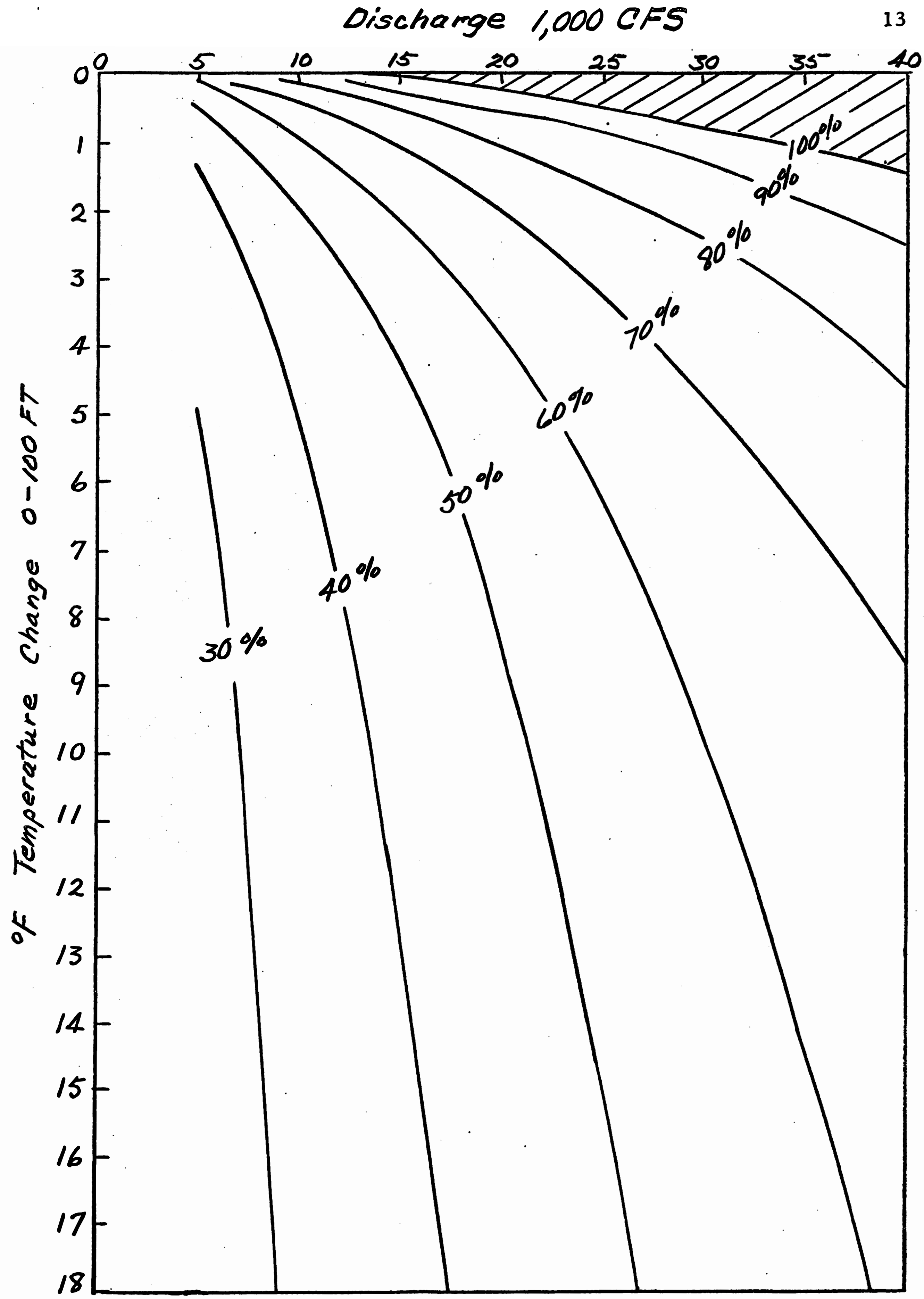

Figure 2. Thickness of density currents in stratified intermediate reservoirs as a percentage of the maximum depth of the reservoir.

The travel times from A to C (Figure 1) were derived from the shape of the reservoir and the discharge rate. The rate of heat flow across the surface of zones I and II was then computed from the surface temperature of the water and the heat budget for the area. Later revisions of heat budget graphs for the Lewiston area appearing in Burt (1958) were used for this calculation.

July 15 was selected for the forecast because it is near the time of maximum heating over cold water surfaces in this area. The water surface temperature in zone IV of the reservoir was assumed to be 72° from past surface temperature data for Ice Harbor. Three different water temperatures were selected for the water entering the reservoir from above: 67° F. $\Delta T = 5°$; 62°, $\Delta T = 10°$, and 52°, $\Delta T = 20°$.

In each situation the water was allowed to pass through one reservoir with the characteristics of Ice Harbor. The amount of heating was calculated and applied to the discharge water. The water was then assumed to enter the next reservoir (again with the characterisitcs of Ice Harbor) and the amount of heating calculated on the basis of the new discharge temperature. The calculations were then repeated for the 3rd, 4th, 5th, and 6th reservoir.

In all, seventy-two forecasts were made for the six reservoirs, the three original inlet temperatures and four different discharge rates (10,000-20,000-30,000 and 40,000 CFS). The 1925-1958 average July discharge (with 1985 depletions) is 34,000 CFS. The minimum monthly mean July discharge for the same period was 13,000 CFS.

CAUTIONS

The forecasting has been simplified for this report by not considering the effect of the heating taking place in any individual reservoir on the thickness of the density current. If further forecasts are made, a refinement will be added to take this effect into account. For this reason these forecasts should be considered as examples of what can be done in the way of forecasting rather than true forecasts of what will occur.

The accuracy of any forecast for reservoirs of the type under consideration will depend upon the applicability of the relationship developed by Debler (see Appendix). For this reason an effort should be made to locate reservoirs with similar characteristics to those under consideration and make actual current measurements within this reservoir. The reservoirs examined must have vertical temperature gradients to furnish useful data.

RESULTS OF FORECASTS

The results of forecasts are shown on Figures 3 and 4.

The vertical scale is discharge in cubic feet per second. The horizontal scale is the number of dams traversed. The curved lines show the number of degrees rise in temperature which should occur for any given number of dams. The top part of Figure 3 is for a temperature rise of 20° between the temperature of water flowing in at the

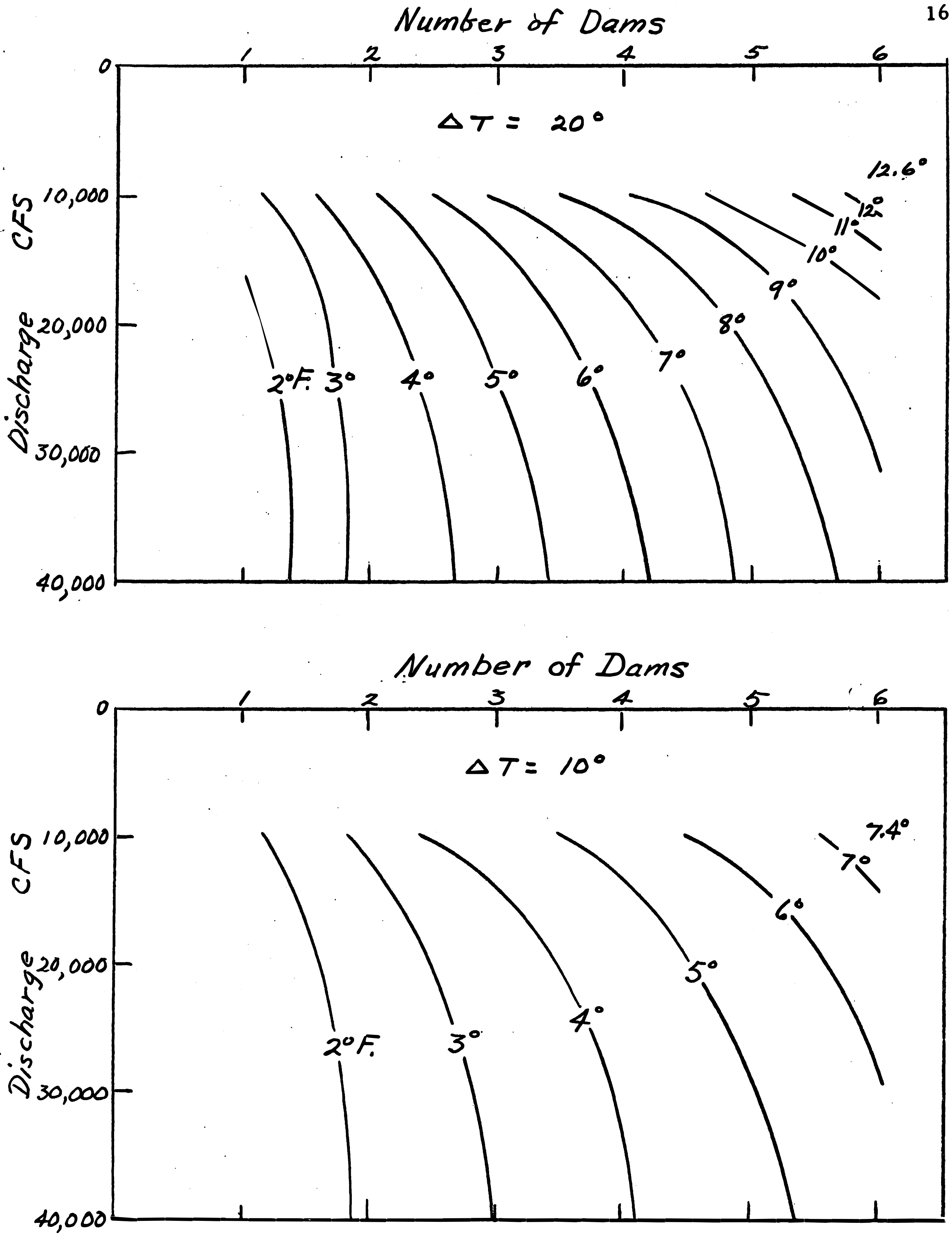

Figure 3. Temperature rise in degrees F. in water flowing through intermediate reservoirs with the characteristics of Ice Harbor reservoir. ΔT is the vertical temperature change per 100 feet just above the dam.

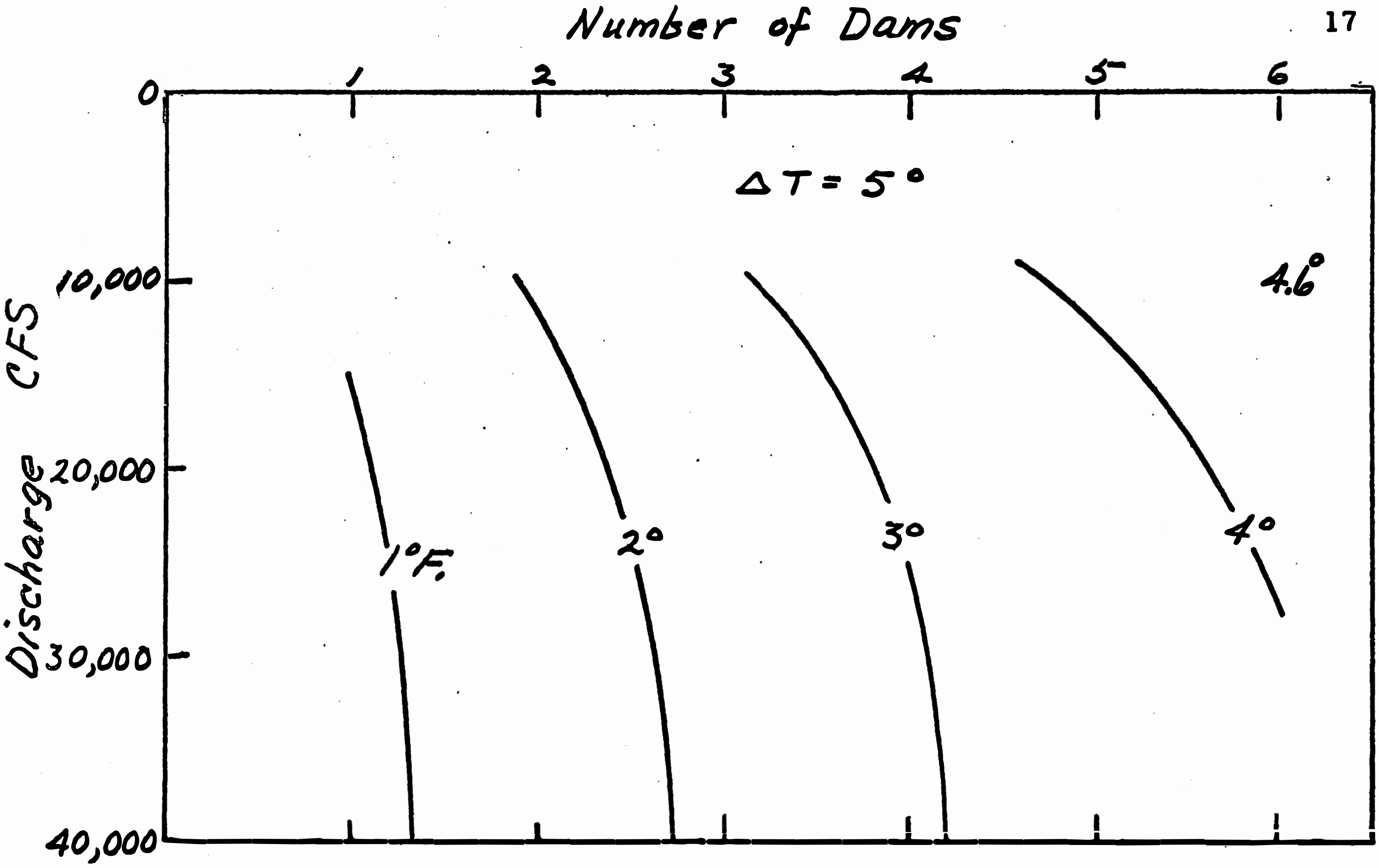

Figure 4. Temperature rise in degrees F. in water flowing through intermediate reservoirs with the characteristics of Ice Harbor reservoir.

upper end of the reservoir and the surface temperature of the water in the stagnant area near the lower end of each reservoir. The lower part of Figure 3 is for a ΔT of 10° and Figure 4 is for a ΔT of 5° F.

Example: What is the discharge temperature of the last dam if T = 10 and the discharge equals 30,000 CFSΣ. From the lower half of Figure 3 enter the diagram with 30,000 CFS and 6 dams and find the curve labeled 6° F. The surface temperature is 72° F. Ten from seventy-two is equal to the inflowing temperature of 62° F. Sixty-two plus a 6° rise in temperature equals 68° F., the discharge temperature.

FUTURE FORECASTS

If more detailed forecasts are made for the six intermediate reservoirs on the lower Snake River, the shape characteristics for each individual reservoir should be used.

The Walla Walla District of the U.S. Army Corps of Engineers has the basic data available for computing all the necessary parameters.

The following data are needed for more detailed temperature forecasts:

1. Area and volume versus elevation curves for the whole depth range of each reservoir.
2. Topographic maps for the area of each reservoir.
3. Surface area and total volume for segments of the reservoir. If these data are readily available for segments between each

river mile, such data would be ideal. A substitute would be the width and vertical cross sectional area at each river mile.

4. Expected daily and hourly discharge for different mean monthly discharge rates for each reservoir. Will one turbine run part of each day and several turbines for the rest of the day? Will discharge rates change over the weekend?

5. Transverse cross sections through the projected China Gardens and Asotin Dams.

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APPENDIX

Duncan, Harleman and Elder (1962) and Harleman (1961) made a study of withdrawal of water from vertically stratified reservoirs using temperature data from Fontana and other TVA reservoirs. They inferred the vertical thickness, b, of the internal density current flowing into the turbine intakes from a critical examination of the shape of the vertical temperature (density) depth curve at any given time.

The end product of the Fontana study is an empirical relationship between the ratio b/Z_o and the densiometric Froude number F_i, where Z_o is the vertical distance from the reservoir water surface to the vertical centerline or midpoint of the turbine intakes.

The densiometric Froude number, F_i, is

$$F_i = \frac{Q}{W Z_o^2} \sqrt{\frac{\rho}{g\beta}}$$

The densiometric Froude number is non-dimensional, hence any consistent system of dimensional units may be used.

Q = Turbine discharge

W = width of the reservoir where temperature observations are made and at the depth Z_o

Z_o = depth from the surface to the centerline of the turbine intakes

ρ = density of the water at Z_o

g = acceleration due to gravity

β = vertical density gradient at the depth Z_o

Yih (1958) had shown that a relationship should exist between the flow pattern and F_i for high Froude numbers. He and Harleman (personal communication) stated that there is a critical value of the Froude numbers of $1/\pi$ (0.318) for the stratified flow in a channel. For Froude numbers above $1/\pi$, water is drawn from all layers up to the surface and selective withdrawal cannot occur. Yih's (1958) theoretical study indicates that for Froude numbers below the critical value of $1/\pi$ stratification will occur in the channel and an internal density current will be set up and the flow out of the end of the channel will not extend all the way to the surface.

Yih's criterion was applied to the range of parameters expected in the lower Snake River reservoirs. This study showed that whenever there is no vertical temperature gradient, the flow will take place all the way to the surface (this is well known from other considerations). Flow may extend all the way to the surface with small density gradients, provided the discharge is great enough. For example, $F_i = 1/\pi$ when the temperature changes one degree per 100 feet (69°F. to 70°F.) and Q = 40,000 CFS for a reservoir with the average dimensions of the lower Snake River reservoirs. Thus, one would expect flow all the way to surface whenever the temperature gradient was approaching zero or whenever the discharge was well above average with a very slight temperature gradient.

Two problems arise in using the results of Duncan et al. (1962) and Harleman (1961) to predict what to expect in the low reservoirs on the lower Snake River.

Duncan et al's.(1962) data for TVA reservoirs covers a very restricted range in F_i from $F_i = 0.0035$ to $F_i = 0.016$. Their data is sufficient to show that the ratio of b/Z_o is greater than 0.5 for values of F_i greater than 0.016. This means that for reservoirs where the flow may be above or well below the centerline of the turbine intake, the thickness of the layer flowing into the turbine intake is numerically greater than half the vertical distance from the surface to the centerline of the turbine intakes whenever $F_i > 0.016$.

The TVA reservoirs are quite different from the lower Snake River reservoirs. Fontana and the other TVA reservoirs have outlets well above the bottom, permitting flow from well below the depth Z_o while most, if not all, of the Snake River reservoirs have or will have turbine intakes at the bottom of the reservoir. For this reason, you would expect a different numerical relationship between b/Z_o and F_i for the lower Snake River reservoirs. The principal use we can make of the TVA study is to help us realize that a relationship of this type should exist for intermediate depth reservoirs on the lower Snake River.

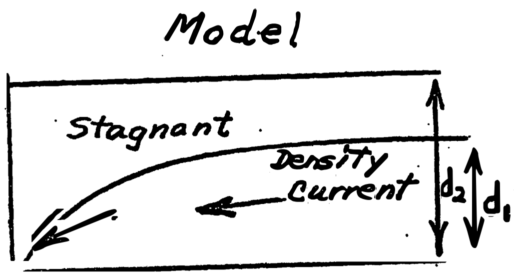
It is fortunate that we have both theoretical and full scale empirical evidence for a relationship between the thickness of the density current and the densiometric Froude number. We need full scale field measurements of internal currents for a variety of conditions to show the correct relationship for various types of reservoirs.

In the meantime, we will have to rely on the results of model studies. One advantage in the use of non-dimensional parameters such as the Froude number lies in the fact that the numerical relationships remain the same for any size of the scale of motion, providing that the physical processes (such as viscosity and surface tension) which are neglected in the Froude number do not assume major importance.

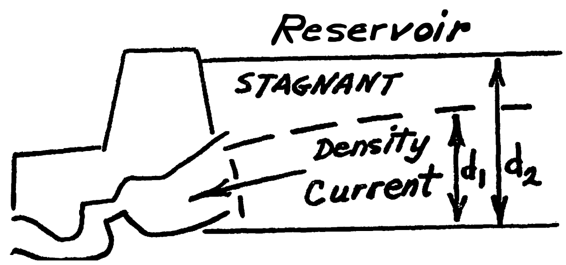
Debler (1959) supplies the information we need for this study. He made a very careful series of model tests at the Hydraulics Laboratory of the National Bureau of Standards Model Laboratory in Washinton, D. C. A vertical density structure was set up in the model by using colored layers of water with different densities. Water was then drawn off from a line sink across the bottom of one end of the flume tank. Different rates of discharge were used and the model photographed from the side. The photographs show the flow pattern as a density current flowing out through the outlet on the end of the flume.

Debler's model has the same conformation as that of the lowest Snake River reservoirs. Figure 5 shows a cross section of the model flume tank compared to a transverse section through a typical lower Snake River reservoir. In both situations the outlet is at the bottom

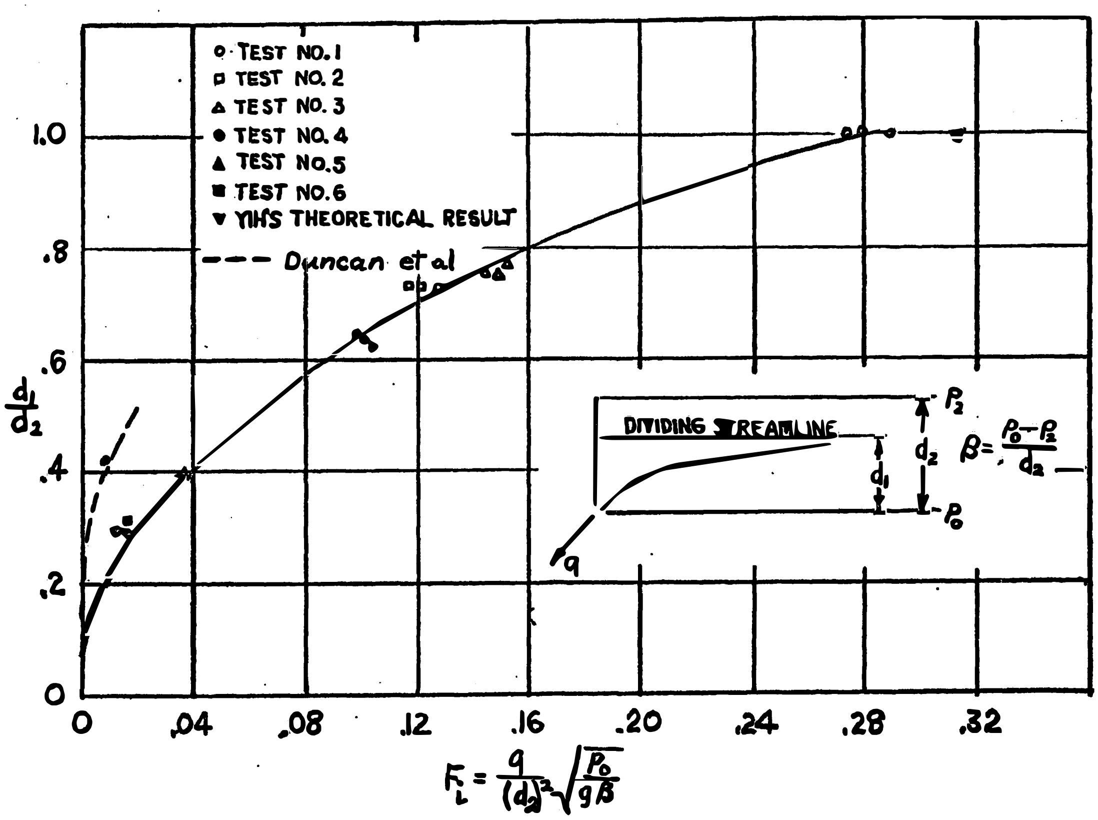
Figure 5.



a) Debler's model channel



b) Schematic diagram of density current in a stratified intermediate reservoir.



c) The ratio of the thickness of the density current to the depth of the channel (from Debler, 1959).

and the flow is restricted to elevations at or above the elevation of the bottom of the reservoir outlet or line sink of the model.

Note that Debler used d_2 the whole depth of the reservoir instead of Z_o (the depth to the centerline of the turbines used by Duncan et al, 1962). This difference in one of the depth parameters would add to the expected differences between Debler's relationship between F_i and d_1/d_2 and Duncan et al's. (1962) relationship between F_i and d/Z_o.

Figure 6 is redrawn from Figure 9 of Debler's paper. Debler's q is discharge per unit width of the model, or Q/W.

Debler ran his experiment through a large range in F_i up to the point where the flow extended all the way to the surface ($d_1/d_2 = 1$). The critical value of F_i was experimentally found to be 0.28 in good agreement with Yih's (1958) theoretical critical value of 0.318. On the lower part of the scale of F_i the values of d_1/d_2 are about half the values of d/Z_o found by Duncan et al. (1962). The over-all agreement between the results of the three investigations is remarkable, considering the differences in the three methods of approach.

Debler's (1958) relationship was applied to data for the Ice Harbor reservoir. The depth, d_2, was taken as 118 feet and the width, W, was taken as 2500 feet. The results are shown on Figure 2. The vertical scale on Figure 2 is the temperature difference between the surface and 100 feet, assuming that the surface temperature was 70°F. The horizontal scale is discharge in cubic feet per second. The curved lines on the graph are computed numerical values of the ratio d_1/d_2.

Example: At a discharge rate of 20,000 CFS and a temperature gradient of 2°F per hundred feet, what is the thickness of the current into the turbines? At the intersection between 20,000 CFS and 2°F, read 70%. 70% x 118 ft. = 83 ft.

Localized boundary conditions will cause differences between the actual flow in the reservoir and that predicted by Figure 2. The results will tend to make the actual thickness of the current greater than the predicted thickness due to turbulence effects. Vorticity will cause some disturbance right at the face of the dam with resulting turbulence and withdrawal from other than predicted depths.

Turbulence will increase the actual thickness of the current over that predicted for high flows with low thermal density gradients. The actual degree of increase in thickness can only be shown by current measurements in full scale reservoirs.

Despite the above limitation, the above can tell us a good deal about the expected flow pattern in the Ice Harbor and other low, lower Snake River reservoirs.

POTENTIAL SOURCE OF COOL WATER FOR FISH FACILITIES IN THE COLUMBIA RIVER BASIN

by

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September 1964

FISH-PASSAGE RESEARCH PROGRAM
U.S. Bureau of Commercial Fisheries
Seattle, Washington

INTRODUCTION

In the past decade, fisheries agencies in the Pacific Northwest have stressed the need for supplemental fish production facilities where dams have inundated substantial sections of the natural spawning ground of salmonids.

One of the problems associated with the development of such facilities, particularly in the Columbia River drainage, is that of securing an adequate supply of suitable water for the fish.

The critical stages for fish appear to be the pre-spawning, spawning, and incubation periods, all of which are currently subjected to relatively high water temperatures if the river is the only source of water.

There is a definite need for optimum water temperature production at each hydroelectric dam site. Eight more dams either are planned for, or are being constructed on, the Snake River and two more for the Middle Columbia River. However, at this time the cost of mechanically cooling fish facility water appears to be prohibitive.

The following paper describes an existing nonmechanical cooling system and some physical characteristics of the water found in the system. A discussion of future application of this cooling technique is included.

WATER COOLING SYSTEM

At Chief Joseph Dam on the Columbia River, a unique structure called a relief tunnel today collects and discharges approximately 40 c.f.s. of water at an average temperature of 50° F. In addition, the relief tunnel is strategically located for use as either a hatchery or spawning-channel water supply, or both.

In comparison, Spring Creek Hatchery, the Bureau of Sport Fisheries' most productive salmon hatchery in the Columbia River Basin, discharges 6 to 8 c.f.s. at a constant 46° F. ($\pm 0.5^{\circ}$ F.). In peak years, the Spring Creek Hatchery "take" exceeds 70 million eggs utilizing this cold water supply.

The Chief Joseph relief tunnel discharges cool water in an appreciable amount all year through a nonmechanical cooling system which purposefully intercepts and delays the groundwater flow. This system, which could benefit fish facilities planning in that it has potential application to the Snake River drainage, was intended as a protective measure to reduce downstream structural damage.

According to Stinchfield (1958), the water moves into the relief tunnel from river seepage that enters the right bank 2,000 feet upstream from the dam (fig. 1). "Intercepted leakage of relief tunnel water is influenced by reservoir and tailwater levels; leakage has ranged from a maximum of 93 c.f.s. at first filling of the reservoir, to approximately 50 c.f.s. at the present time." An impervious blanket has been installed by the Corps of Engineers which extends 2,000 feet from the dam on the right abutment. The impervious blanket and the relief tunnel protect against excessive abutment seepage in lieu of a positive bedrock cutoff.

COOLING WATER CHARACTERISTICS

Temperature records from Chief Joseph Dam, January 1962 to May 1964, show that the relief tunnel and the Columbia River penstock intake water had about the same annual average temperature (table 1). The 2-year average difference was 1.3° F. However, the relief tunnel water ranged from 45.5° F. to 55.8° F., whereas the Columbia River water ranged from 36.5° F. to 64.4° F. These data are based on weekly comparable temperatures.

Water moves from the river at a permeability rate of 0.324 cm. per sec. to a 1200-foot collection tunnel. Moving from the collection tunnel, this water falls into a 4-foot discharge conduit that leads through the left training wall and enters into the tailrace.

The time lag between the river and the relief tunnel water is 3 to 4 months (fig. 2). This time-lag phenomenon would allow for a higher total quantity of water to be used in a fish facility operation; i.e., cold river water can be added to a warmer relief-tunnel water in February and March, and cold relief-tunnel water can be added to warmer river water during July, August, September, and October. This mixing of river and relief tunnel water could almost double the present average discharge of 40 cubic feet per second while maintaining a constant 50° F. year-round temperature.

FUTURE APPLICATION

In order to consider the potential application of this structure to other sites, answers must be found to the following

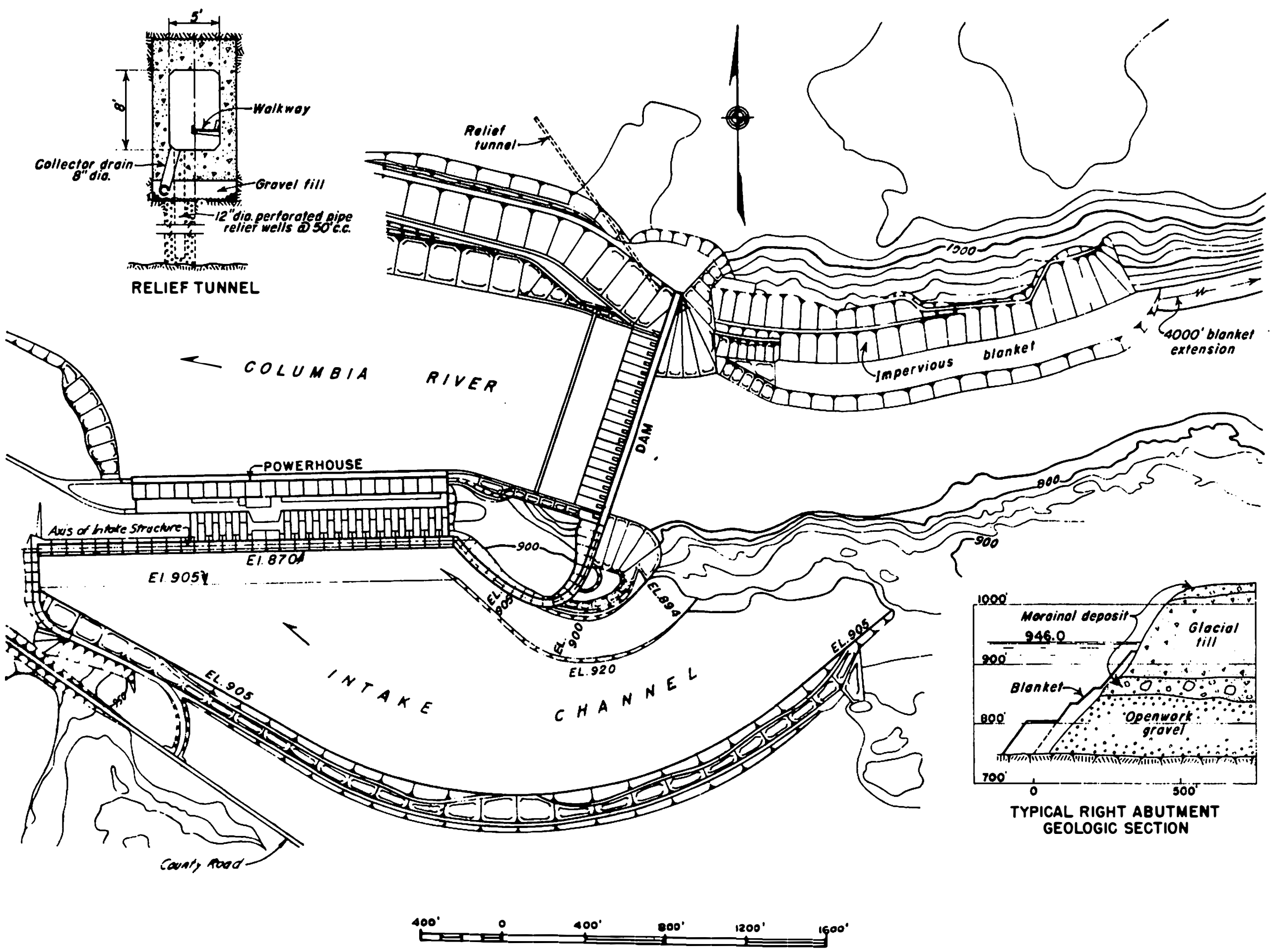

Figure 1.--General layout of Chief Joseph Dam with details of impervious blanket and relief tunnel required to control seepage in the right abutment.

Table 1.--Chief Joseph Dam "relief tunnel", average monthly temperature and discharge for 1962-1963.

Date	Average tunnel discharge	Average tunnel water temperature 1/	Average Columbia River temperature 2/
1962	c.f.s.	°F.	°F.
January	41.8	48.2	53.2
February	39.1	55.8	37.4
March	39.6	54.0	36.5
April	39.3	49.0	41.0
May	39.1	48.0	47.5
June	41.5	45.5	53.6
July	42.5	46.4	57.6
August	41.9	46.4	61.5
September	40.0	48.6	62.6
October	39.2	50.0	61.2
November	41.7	53.2	56.5
December	41.1	53.6	57.4
1963			
January	39.7	55.4	43.7
February	39.5	54.9	38.3
March	38.2	53.1	37.8
April	37.2	51.8	42.4
May	37.9	48.2	49.1
June	40.89	46.8	55.4
July	38.9	46.4	60.1
August	38.7	46.8	62.6
September	38.2	48.6	64.4
October	38.9	51.3	63.0
November	39.1	54.9	58.3
December	40.3	55.0	50.7
Averages			
1962	40.6	49.8	51.6
1963	38.9	51.4	52.2
2-year	39.8	50.6	51.9

1/ Water analysis of the relief tunnel water has been carried out by Douglas County Public Utilities District, according to George Atwood, Plant Superintendent, and has been found to be pure and usable for drinking purposes.

2/ Penstock intake.

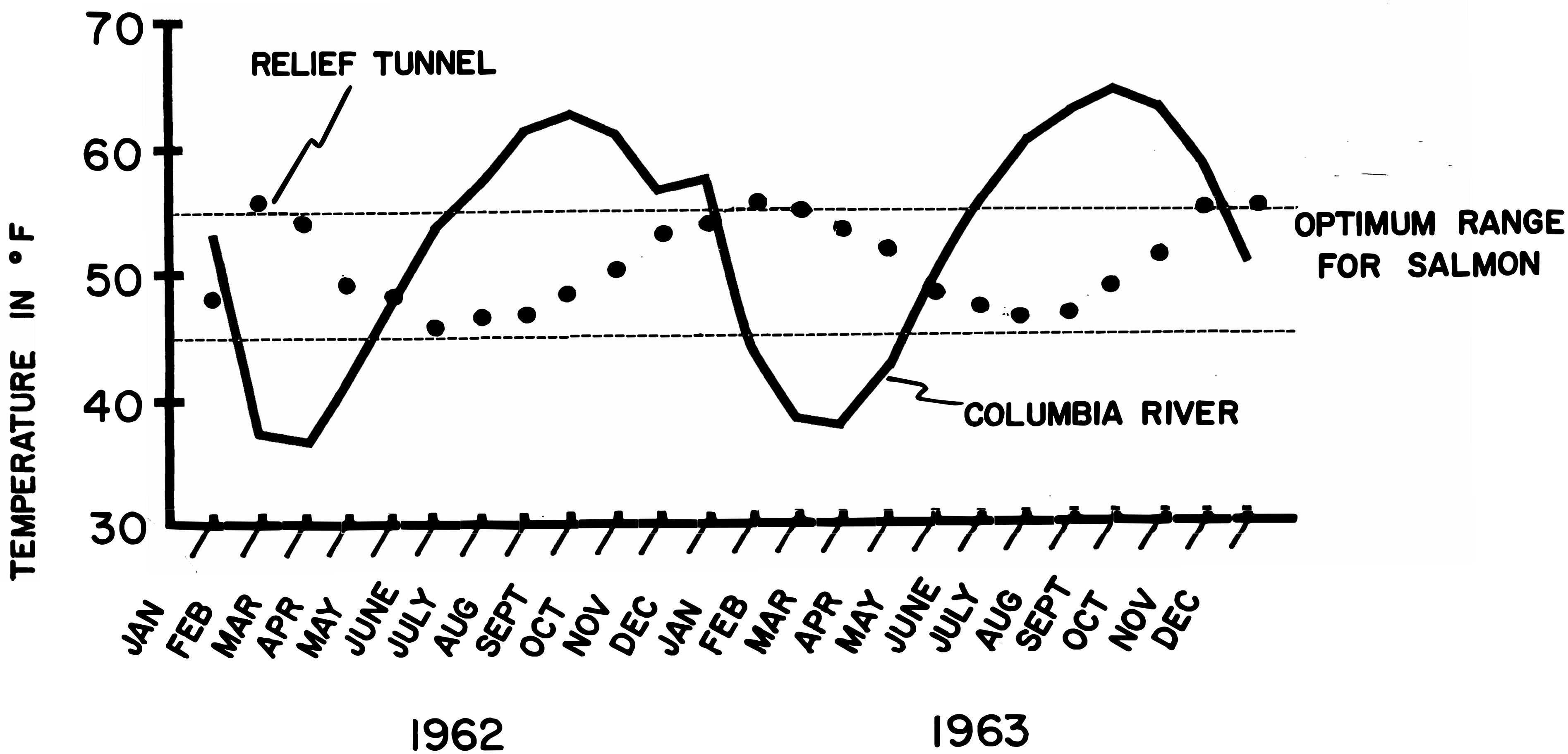

Figure 2.--Average monthly water temperatures, Chief Joseph Dam, 1962-1963.

questions: (1) What specific geologic structure is producing this cool temperature and uniform flow condition? (2) Does this structure have application to other hydroelectric sites or is it unique to Chief Joseph Dam? (3) Is it possible to predict the temperature and flow characteristics of such a relief tunnel at other sites?

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ADDENDUM

Ice Harbor Dam, Snake River Mouth, Average Monthly Water Temperatures, May 1962 - April 1964.

Average Snake River water temperature

Month	1962 °F.	1963 °F.	1964 °F.
January		37.5	38.6
February		38.1	38.0
March		44.2	40.8
April		49.6	47.5
May	53.9	55.0	
June	59.2	60.6	
July	69.3	68.3	
August	73.5	73.3	
September	68.3	69.5	
October	58.8	63.3	
November	50.5	50.2	
December	44.7	41.3	
Average	59.8	54.3	41.2

24-month average 53.9° F.

Possible tunnel water range, 49.1 -- 57.9° F.

PROPOSED WATER TEMPERATURE STUDIES RELATING TO HIGH MOUNTAIN SHEEP DAM

by

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U.S. Bureau of Commercial Fisheries
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INTRODUCTION

Seven more hydroelectric dams are scheduled to be constructed on the Snake River below Oxbow Dam. Each dam potentially could increase river temperature by increasing surface exposure and decreasing channel velocity. Increasing temperatures in this key area could be of serious consequence to all of the migrating anadromous populations of Salmon and Snake Rivers and their tributaries.

The Snake River contains both high- and low-head dams, either existing or in the planning stage. In general, the low-head dams produce an increase in river temperature, whereas high-head dams produce a decrease if properly planned and operated. Prediction of future water temperatures of on-river reservoirs and their effluents allows for the proper planning of hydroelectric structures to prevent undesirable thermal increases.

The purpose of this proposal is to have one of the key high-head dams investigated to determine what the physical characteristics of the water will be before, during, and after it leaves the dam complex. The ability to manipulate a water temperature control system that is inherent to High Mountain Sheep and the associated regulatory dams may make it possible to compensate for the temperature increases downstream.

Thirteen reports dealing with water temperature predictions have been prepared to date for the Snake River Basin (see bibliography). Dr. Wayne Burt, oceanographer at Oregon State University, has been the principal investigator in all of these studies. A brief summary of the subject matter in pertinent report follows.

Three reports are concerned with Brownlee Reservoir and downstream water temperature from Brownlee Dam. One report relates to Brownlee water effluent to and through Oxbow and Hell's Canyon Dams.

Two articles deal with temperature predictions in High Mountain Sheep Reservoir. The first report gave agencies some optimism in river thermal planning, for in the normal operation of single-level intakes, the river water would be cooled a maximum of 15^{o} F. In the second article, Dr. Burt reported on the use of a single high intake (one of ten) at this site (5,000 c.f.s. at 1,300 feet). Results indicated that a 2^{o} to 3^{o} F. temperature reduction would be achieved over a 7-day period during peak temperature conditions. More important was that minor temperature reductions of .5 to 1.0° F. could be gained during the month of October, with only one "multiple" intake. It is important that "multiple" intakes be considered in the planning of High Mountain Sheep Dam. A portion of this proposal deals with collection of prediction data needed for "multiple" intake planning decisions.

The last report considered water of an optimum temperature from High Mountain Sheep Dam moving down through all the completed lower Snake River dams. Based on extended predictions of mean water temperatures (mean flow year) through the lower six Snake River dams, it would appear that temperature increases would be expected in the magnitude of .8° F. for each of all six dams. What benefits would be realized from High Mountain Sheep, and how far downstream would a benefit be projected if actual hydroelectric installation scheduling is considered?

A tabular summary has been prepared (table 1) to show predicted temperatures at Brownlee, Oxbow, Hell's Canyon, High Mountain Sheep, and the mouth of the Snake River (mouth of Snake considered with all dams installed).

Recorded mean temperatures for Swan Falls, Oxbow, and Clarkston are included for comparison. Temperature benefits from the originally planned intakes at High Mountain Sheep Dam would be realized from March until September (until cool water storage in High Mountain Sheep is used up). It is anticipated from Dr. Burt's predictions that critical maximum temperatures would be present from mid-September through October.

PROPOSAL

The following studies are requested in order that we may understand fully the potential of High Mountain Sheep Dam for affecting the temperature regime of the Snake River.

1. Daily Flow Release Schedules at Hell's Canyon, High Mt. Sheep, and China Garden Dams.

In the past, the approach to the prediction of flow release schedules has been to project flow as a function of the annual mean. It is proposed in this study that flow be expressed in terms of the amount of flow and percentage of time at each flow for any given hour of any day (24 hours) for minimum, mean, and maximum flow years.

Table 1.--Estimated and recorded* temperatures for Snake River downstream of the respective dam sites

Date		(1959)* Swan Falls	Brownlee	Oxbow	(1959)* Oxbow	Hell's Canyon	High Mt. Sheep	(1959)* Clarkston	Mouth	Diff. between predicted mouth and Swan Falls
			6/	6/		6/	8/		13/	
Jan.	1	44	39	38.7	42	38.5	40.5	41.5	45.1	+ 1.1
	11	40	39	38.8	42	38	39.0	42	43.6	+ 3.6
	21	40	39	38.8	41	38.2	39.0	38	43.6	+ 3.6
Feb.	1	44	39	38.9	38	38.5	39.0	39	43.6	- .4
	11	42	39	39.0	38	38.8	39.0	38	43.6	+ 1.6
	21	44	39	39	39	39.1	39.0	41	43.6	- .4
Mar.	1	47	39.4	39.4	41	39.4	39.0	42.5	43.6	- 3.4
	11	45.5	40.0	40.1	42	40.2	40.5	42	45.1	- .4
	21	45.5	42.3	42.0	43	41.8	41.5	44	46.1	+ 1.4
Apr.	1	51	44.9	44.7	46	44.2	42.5	46	47.1	- 3.9
	11	54	48.1	47.9	50	47.5	43	48.	47.6	- 6.4
	21	54.5	50.7	50.6	52	50.3	43	--	47.6	- 6.9
May	1	57	53.3	53.2	53	53.2	43	51.5	47.6	- 9.4
	11	56.5	55.0	55.0	56	55.3	43	51	47.6	- 8.9
	21	58.5	57.0	56.9	56.5	57.2	44	--	48.6	- 9.9
June	1	62	59.6	59.6	59	59.7	45	--	49.6	- 12.4
	11	66	61.5	61.6	61.5	61.9	46.5	54.5	51.1	- 14.9
	21	71	63.5	63.6	63.5	63.8	48	61	52.6	- 18.4
July	1	69	65.6	65.8	65.5	65.8	49	61	53.6	- 15.4
	11	70	67.1	67.3	67	67.4	50.5	65	55.1	- 14.9
	21	73	68.3	68.4	69	68.4	52	71.5	56.6	- 16.4

Continued...

Table 1.--Estimated and recorded* temperatures for Snake River downstream of the respective dam sites (continued)

Date	Swan Falls (1959)*	Brownlee 6/	Oxbow 6/	Oxbow (1959)*	Hell's Canyon 6/	High Mt. Sheep 8/	Clarkston (1959)*	Mouth 13/	Diff. between predicted mouth and Swan Falls
Aug. 1	73	69.3	69.2	69.	69.2	54.	70.5	58.6	- 14.4
11	69.5	70.2	70.0	--	69.4	55.5	70.5	60.1	- 9.4
21	67	70.7	70.4	70	69.9	57	66.5	61.6	- 5.4
Sept. 1	66	70.6	70.3	71	69.8	59	67.5	63.6	- 2.4
11	66	69.9	69.6	69	69.3	60.5	67	65.1	- .9
21	62	68.5	68.4	69	68.3	62.5	64	67.1	+ 5.1
Oct. 1	57	66.1	66.0	64	66.0	64.5	57.5	69.1	+ 12.1
11	56	62.8	63.0	61	63.4	66.5	55.5	71.1	+ 15.1
21	55	60.6	60.6	58	60.3	61.0	55	65.6	+ 10.6
Nov. 1	52	57.2	57.2	56	57.5	57.5	49	62.1	+ 10.1
11	47	54.3	54.2	53	54.3	54.5	45.5	59.1	+ 12.1
21	44	51.2	51.2	50	51.3	52	44	56.6	+ 12.6
Dec. 1	43	48.1	48.2	45.5	48.2	49	42	53.6	+ 10.6
11	40.5	45.1	45.1	44	45.3	46.5	40.5	51.1	+ 10.6
21	39	42.0	42.2	44	42.5	43.5	40	48.1	+ 9.1

_/ Numbers indicate publication source for predicted downstream temperatures.

* 1959 Data - Water temperature studies for 1959 -- Middle Snake River drainage U.S. Dept. of the Interior, FWS, BCF, Portland, Oregon, Mat 1960, in $^{\circ}$ F.

It is proposed that this study be undertaken to determine:

a. Hourly flow release schedules throughout the year for

(1) Hell's Canyon before and after High Mt. Sheep.

(2) High Mt. Sheep before and after China Gardens.

(3) China Gardens.

b. Hourly flow release from High Mt. Sheep expressed as a percentage of maximum, minimum, and mean Salmon River discharges throughout the year.

c. Minimum flow releases for each project in terms of percentage to maximum, mean, and minimum flow (Hell's Canyon, High Mt. Sheep, and China Gardens).

Hourly information in terms of cubic feet per second should be presented in graphic form for yearly and daily flows for a minimum, mean, and maximum year and on IBM punch cards for predicted hourly flows and percentage time at each flow per hour in relation to daily release (24 hours) throughout the year for a minimum, mean, and maximum flow year.

2. <u>Projections of Reservoir Fluctuations at China Gardens.</u>

It is proposed that information on predicted reservoir fluctuation be projected in terms of feet elevation on an hourly basis throughout the year for a minimum, mean, and maximum flow year.

The above information should be presented in graphic form and IBM card form.

3. <u>Water Temperature Predictions.</u>

The present predictions of water temperature in the Snake River are based on mean flow years and mean water temperatures. It is proposed that predicted temperatures in this study be expressed in terms of $^{\circ}$ F. for hourly flow conditions expected during maximum, mean, and minimum flow years. Further, it is proposed that mean and maximum thermal conditions be imposed on maximum, mean, and minimum flows to determine the predicted river temperatures on an hourly basis throughout the year for areas immediately below:

a. High Mt. Sheep before and after China Gardens Dam. Predicted downstream river temperatures under the following conditions are requested:

(1) With low turbine intakes.

(2) In multiple intakes at the 1,450-, 1,400-, 1,350-, and 1,300-foot levels for 1 to 10 turbines.

b. China Gardens Dam.

Predictions are to relate to maximum, mean, and minimum Salmon River flows and maximum and mean temperatures.

c. Ice Harbor Dam.

Temperature predictions on an hourly basis throughout the year at Ice Harbor after High Mt. Sheep Dam is operational are requested for the following conditions, assuming this order to be the sequence of installation:

(1) With Lower Monumental Dam installed and operational.

(2) With Lower Monumental Dam and Little Goose Dam installed and operational.

(3) With Lower Monumental Dam, Little Goose Dam, and Lower Granite Dam installed and operational.

(4) With Lower Monumental Dam, Little Goose Dam, Lower Granite Dam, and Asotin Dam installed and operational.

The above information also should be presented both graphically and on IBM cards.

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13. 1963 (November)
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(PART 1: FORECASTING WATER TEMPERATURE CHANGES DUE TO FLOW THROUGH INTERMEDIATE DEPTH RESERVOIRS).
Water Research Associates, Corvallis, Oregon.

RATES AND TIMING OF MIGRATION

EFFECT OF DAMS ON THE UPSTREAM MIGRATION RATES AND TIMING OF COLUMBIA RIVER SALMONIDS

by

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September 1964

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INTRODUCTION

For some time fishery agencies responsible for the management of stocks of anadromous fishes in the Columbia River have been concerned with the possibility that dams--particularly a sequence of dams and inpoundments--may cause a serious delay in the upstream migration of salmonids. Evidence of delay was reported by Schoning and Johnson (1955). They concluded that the 2- to 3-day delay found at Bonneville Dam was insignificant, but they expressed concern over the cumulative effect of such a delay when spread over a sequence of 7 to 10 dams. With sockeye salmon (Oncorhynchus nerka), a delay of this magnitude could be very serious. Studies by the International Pacific Salmon Commission (Thompson, 1945; Andrew and Geen, 1960) revealed that a delay of 2 to 4 days is often detrimental, and that sockeye salmon delayed more than 12 days were unable to spawn effectively after reaching their spawning grounds. On the other hand, the timing of chinook salmon (O. tshawytscha) and steelhead (Salmo gairdneri) has not been found to be as critical. Spring chinook salmon often wait several months before spawning, and most steelhead migrating up the Columbia River in the summer or early fall do not spawn until the following spring. Therefore, emphasis in this paper has been placed on the effect of a sequence of dams on the timing and migration rates of the sockeye salmon runs in the Columbia River. The timing of chinook salmon runs is also considered.

APPROACH

Twenty-six years of fish counts at Bonneville (U. S Corps of Engineers, 1938-1963) and Rock Island Dams[1] provided the basis for examining the effects of four additional dams on the migration rates and timing of Columbia River salmon. The more recent dams include Mc Nary (1954), the Dalles (1957), Priest Rapids (1959), and Wanapum (1963). Starting with Bonneville and continuing upstream to Rock Island, these dams are 145, 192, 292, 397, 413, and 450 miles, respectively, from the sea.

The evaluation consisted of comparing the arrival time of salmon runs at Bonneville and Rock Island Dams. Our analysis was simplified considerably by the availability of fish counts in standard weeks[2]. The statistic used was the date on which the median fish passed each dam during the period 1938-1950, when only the two dams existed, and during 1951 to 1963, when more dams were being constructed or were in existence along this route of migration. In order to use this type of analysis, it was assumed that factors such as floods, changes in fishing regulations, inaccuracies in fish counts, etc., did not significantly bias the

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[1] Rock Island Dam salmon and steelhead counts, 1933-1963. Staff, Biological Laboratory, Bureau of Commercial Fisheries, Seattle, Washington. Processed.

[2] Prepared by staff, Biological Laboratory, Bureau of Commercial Fisheries, Seattle, Washington. Processed.

results.

Salmon Runs

Four species of salmonids travel up the Columbia River past Rock Island Dam. These include sockeye, chinook, steelhead, and coho (O. kisutch). This analysis is dependent upon accurate measurement of arrival time at each dam and of elapsed time between dams. To calculate elapsed time, runs of fish must be identifiable at each successive dam. This was possible only with sockeye.

Sockeye. --This species migrates up the river in a well-defined group over a relatively short period of time. Two major races (Wenatchee and Okanogan), both spawning above Rock Island Dam, comprise the bulk of the Columbia River sockeye run. Tagging studies[3] have shown no difference in the timing of these two races at Rock Island Dam. Thus, they were considered as one run for this report.

The proportion of the run reaching Rock Island Dam has varied somewhat over the years, primarily due to a fishery above Bonneville Dam. Prior to 1957, counts at Rock Island varied from 22 to 74 percent of the Bonneville tally. Following inundation of the Indian fishery at Celilo Falls in 1957 by the Dalles Dam and termination of all commercial fishing above Bonneville in the same year, this figure has increased to nearly 100 percent. The effect of the fishery, if any, on the timing of the sockeye run at Rock Island Dam was not considered in this analysis.

Chinook. --Chinook salmon runs are generally found in the Columbia River from mid-March to mid-October and consist of many races widely dispersed over the drainage. The species has been arbitrarily classed into three major runs: (1) "Springs", which generally spawn in upper sections of tributary streams; (2) "summers", which usually spawn in lower sections of these tributaries and in the main stem Columbia River above Rock Island Dam; and (3) "falls", which spawn primarily in the lower main stem of the Columbia River below Rock Island Dam. Most of the chinook passing Rock Island Dam are spring- and summer run fish. Unlike sockeye, however, only 5 to 8 percent of the spring run and 14 to 20 percent of the run at Bonneville, have been accounted for at Rock Island Dam. Lacking precise knowledge of the timing of Rock Island chinook at Bonneville Dam, no attempt was made to assess for delays in migrations due to dams. However, the arrival times for the spring and summer chinook at the two dams were compared.

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[3] The influence of Rocky Reach Dam on the migration of adult sockeye salmon by Major, Richard L. And James L. Mighall, Biological Laboratory, U. S. Bureau of Commercial Fisheries, Seattle, Washington. Manuscript in preparation.

For this analysis, chinook counts at Bonneville and Rock Island Dams were separated chronologically into spring and summer runs. These dates are March 1 to May 31 and June 1 to August 1, respectively, for Bonneville Dam, and April 1 to June 25 and June 26 to September 3, respectively, for Rock Island Dam.

Steelhead. --This species is available nearly the entire year. Two major migration peaks occur at Bonneville but only one peak is evident at Rock Island. Since it is difficult to identify individual runs and since only 3 percent of the Bonneville runs pass Rock Island, this species was not considered in the computations.

Coho. --The number of coho salmon have been relatively small and counting at the dams has not continued over the entire period of migration. For these reasons, the coho data were considered insufficient for this analysis.

RESULTS

Sockeye and Chinook Time of Arrival--Bonneville and Rock Island Dams

Generally, the arrival time for both sockeye and chinook migrations at the two dams has not changed appreciably in the last 26 years (Fig. 1). This is true even in recent years when fish were required to negotiate three to four additional dams. The only exception to the trend is the summer chinook run, which is now arriving about a week earlier at Bonneville Dam. This earlier arrival could have resulted from changes in fishing regulations or adaptation of the fish to new environments created by dams.

Sockeye Migration Rate--Bonneville to Rock Island Dam

The elapsed passage time of sockeye between the two dams is shown in Figure 2. Average elapsed time between Bonneville and Rock Island in the 1938-1950 period was 17 days, whereas in the subsequent period (1951-1963), elapsed time was 19 days. Average migration rates in these two periods were 17.65 and 15.79 miles per day, respectively. The indicated average delay for the latter period is less than 3 days, which if true, would not appear to be of serious consequence.

One method of examining the effect of delays would be to compare the returning year class strength of the "slow" and "fast" migrations in relation to the respective excapements. If delays seriously affect the population, then returns from a slow-moving spawning migration would be less than those from a fast migration. Five years in which the migration was slower than average were compared with 5 years of faster than average migration (Table 1). The 1941 migration one of the fast groups (Fig. 2) was excluded from this analysis because of an

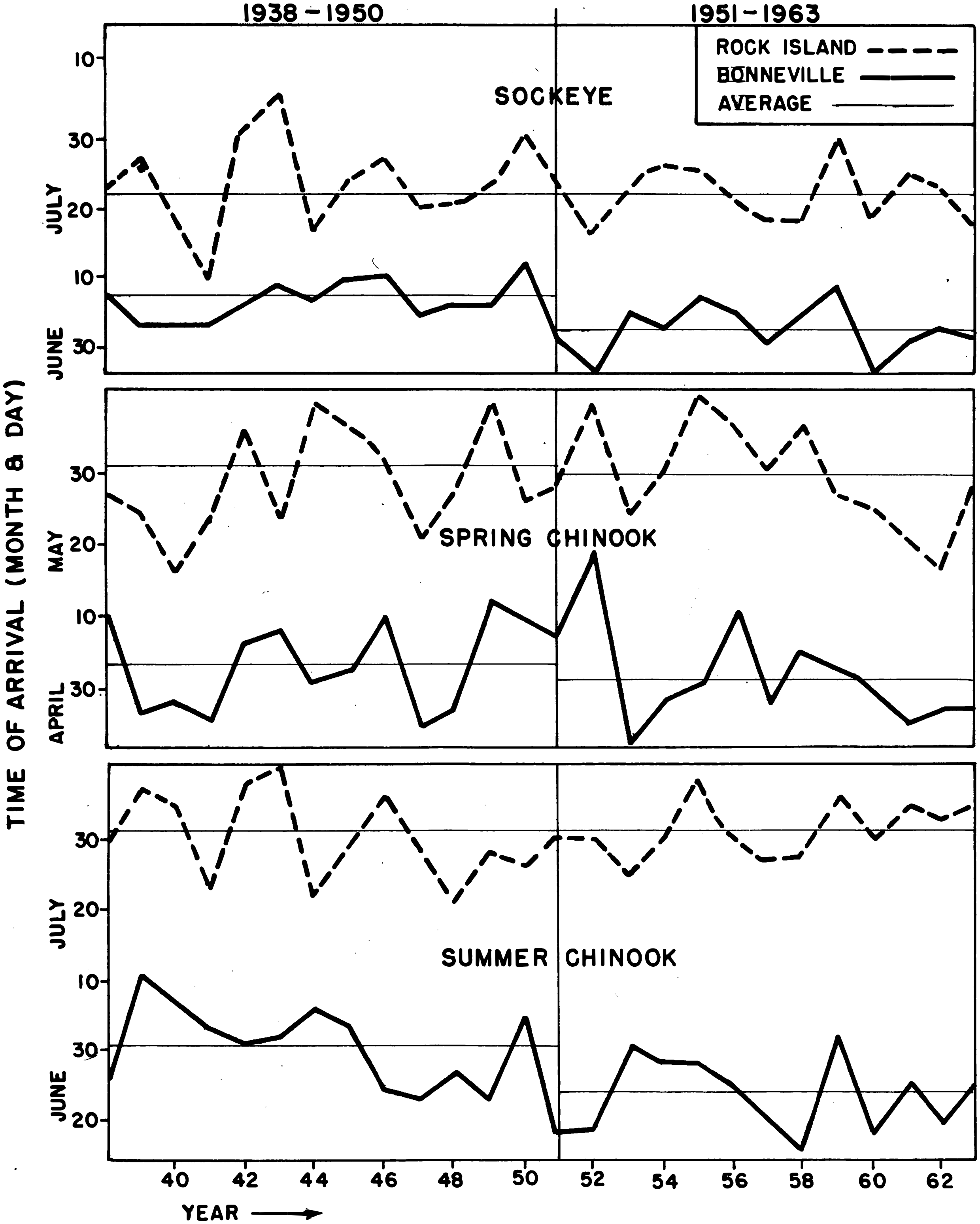

Figure 1.--Arrival times of median fish at Bonneville and Rock Island Dams for the periods 1938-50 and 1951-63.

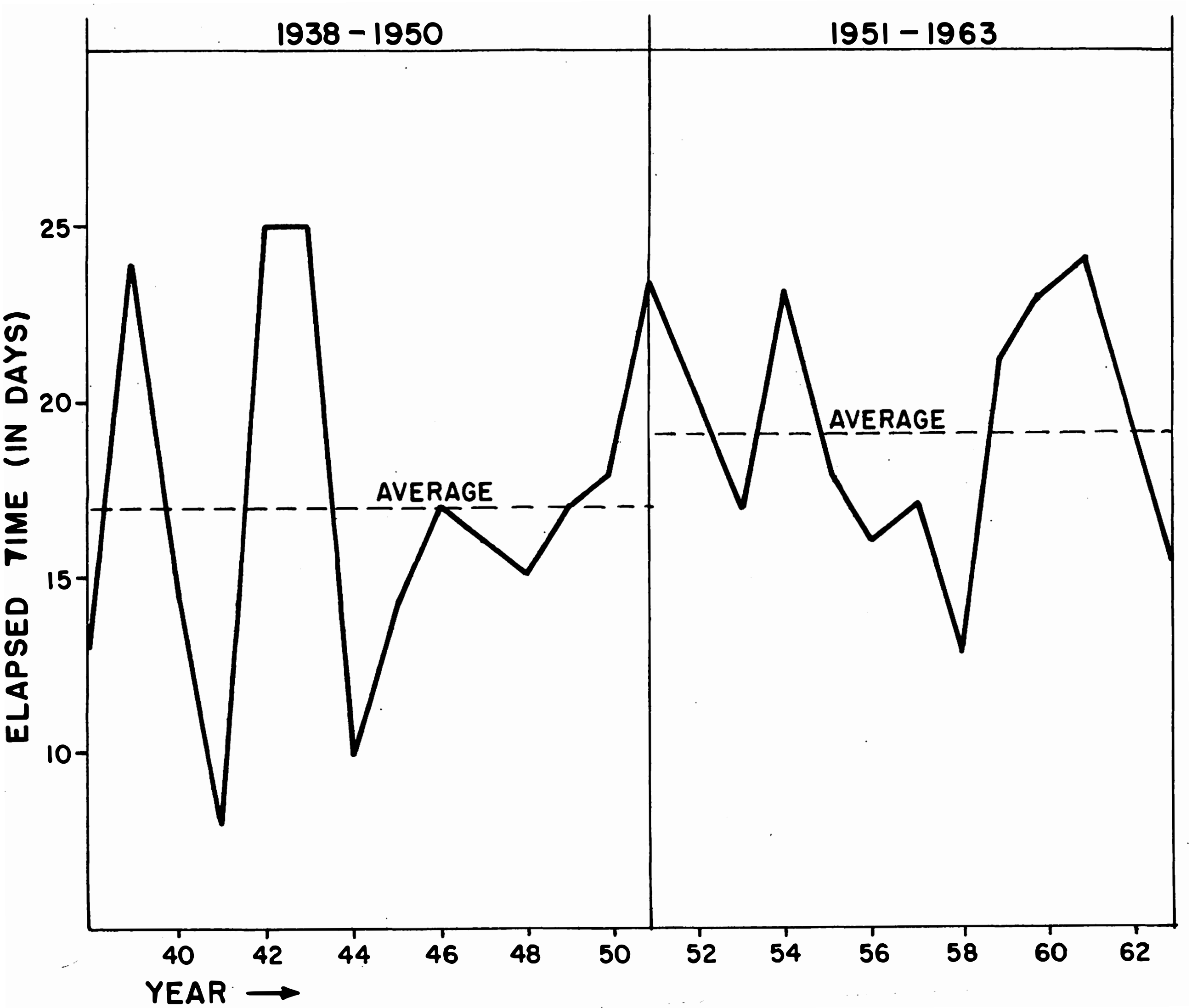

Figure 2.--Elapsed time of sockeye salmon between Bonneville and Rock Island Dams (300 miles) for the periods 1938-50 and 1951-63.

Table 1.--Parent migrations and adult returns 4 years later, showing adult returns per parent for slow and fast moving migrations of sockeye salmon past Rock Island Dam.

Slow Migration					Fast Migration				
Years	Migration rate (miles/day)	Parent fish past R. I. Dam (thousands)	Adult return[1] (thousands)	Adults per parent	Year	Migration rate (miles / day)	Parent fish past R. I. Dam (thousands)	Adult return[1] (thousands)	Adults per parent
1942	12.2	16	101	6.2	1938	21.8	17	94	5.5
1943	12.2	18	335	19.1	1944	30.5	5	143	28.6
1951	13.3	102	245	2.4	1945	21.8	7	53	7.5
1954	13.3	91	313	3.4	1956	19.1	92	181	2.0
1959	13.9	72	160	2.2	1958	23.5	99	38	0.4
Average	13	60	231	3.8	Average	23	44	102	2.3

[1]Catch plus escapement based on 4-year cycle, from 1963 status report of the Columbia River commercial fisheries, Oregon Fish Commission, Washington Department of Fisheries (January 1964).

abnormally low escapement (949 fish) over Rock Island Dam. These data show that the average return was actually somewhat higher for the slow moving runs. In fact, one of the better returns (1943) resulted from an escapement with the latest median arrival date (August 5) at Rock Island of all years on record.

SUMMARY

Fish counts at Bonneville and Rock Island Dams provided a means of studying the effect of dams on the timing of certain salmon runs in the Columbia River drainage. Periods compared were 1938-1950 and 1951-1963. In the early period, salmon passed only Bonneville and Rock Island Dams, but in the ensuing period, four additional dams were added within the network.

Sockeye salmon runs were distinguishable at both Bonneville and Rock Island. Hence, it was possible to assess both timing and migration rates for this species. The timing of spring and summer chinook runs was also considered in the analysis.

The comparison of fish migrations during the two periods indicated the following:

1. Additional dams hve not appreciably affected the timing or migration rates of sockeye salmon. Average elapsed time between Bonneville and Rock Island Dam was only 2 days more during the 1951-1963 period than in the 1938-1950 period when no intermediate dams were present.

2. The average arrival time of spring and summer chinook at Rock Island Dam was virtually the same over the two time periods. Spring chinook salmon arrived at Bonneville Dam an average of 1 week earlier in the 1951-1963 period than they did in the 1938-1950 period.

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USE OF TURBINE INTAKE GATEWELLS FOR SAMPLING OF MIGRANT JUVENILE SALMONIDS

by

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and

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FISH-PASSAGE RESEARCH PROGRAM
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INTRODUCTION

Research is needed to determine how seriously juvenile outmigrants would be affected by the series of dams and impoundments forthcoming in the Snake River. A comparison of the present magnitude and timing of individual races with that in succeeding years as the environment is changed would provide such a measure. A program of this magnitude is deemed too costly if present large scale fish-sampling equipment were to be utilized. To maintain costs within reasonable levels, more economical methods of collecting migrants must be found.

The possibility was considered of obtaining large samples of seaward migrants from turbine intake gatewells, already an integral part of the low-head dams. Fingerling salmonids have been found in the gatewells at Bonneville and McNary Dams, and samples obtained from them produced enough fish to warrant further investigation on the feasibility of using wells as collectors through an entire migrant season. If sufficient numbers of fish are caught, then the juvenile migrant sampling can be accomplished at a relatively low cost. Ice Harbor Dam, the lowermost dam in the Snake River, was selected for an exploratory study in the spring of 1964. This report summarizes the results of the first year's research.

METHODS AND MATERIALS

Ice Harbor Dam has three Kaplan turbine units through which approximately 40,000 cubic feet per second of water flows at a normal head of 93 feet. Each unit has three gatewells in which slots for both emergency and operating gates are provided to seal off the turbine from the forebay water during maintenance operations. The gatewells of each unit are designated as A, B, and C, starting from the south shore. The location and relative size of the gatewells and slots are shown in figure 1.

Fish were collected from all nine gatewells in the area between the emergency and operating gateslots. A hydraulic cylinder prevented access to the entire gatewell area, but the majority of the water mass was strained by the sampling equipment.

Downstream migrants were removed from the gatewells with a gatewell dipnet, 18-1/2 feet long by 11-1/2 feet wide, using a 6-foot bag. The net frame was constructed of tubular iron pipe which was hinged longitudinally in the center so that it could be closed as it was lowered into the gatewell. In operation, the net was lowered by a warehouse crane (fig. 1) in closed position until the desired fishing depth was reached. It was then opened and slowly raised by the crane to the water surface. At the surface, it was closed to permit withdrawal through the gate opening.

Fish were first dipnetted from the surface water of gatewells and then from successively greater depths. This was done to minimize mortalities due to crushing when large numbers of fish were present and to obtain data on the vertical distribution of fish in the gatewell. Each well was fished until less than 100 fish were captured in a sample.

A 2- by 2- by 6-foot holding tank was provided to retain the fish. Water was circulated continually through the tank to control temperature and provide aeration.

The fish were removed from the holding tank in small lots of 15 to 20 fish and anesthetized with MS-222 before processing. Approximately 25 percent of the fish collected were marked. A red fluorescent pigment tattoo and the new mark was used. (See "A thermal marking technique for juvenile salmonids", Groves and Novotny, vol. 5, Review of Progress, Fish-Passage Research Program). In accordance with laboratory findings, only fish greater than 90 mm. in length were marked. The remainder were examined for previously affixed marks, enumerated, and, at weekly intervals, fork-length measurements and scale samples were taken.

After the fish were processed, they were placed in a tank truck, in which the water was continually oxygenated, and transported to the release areas. The majority of the marked fish were taken upstream and alternately

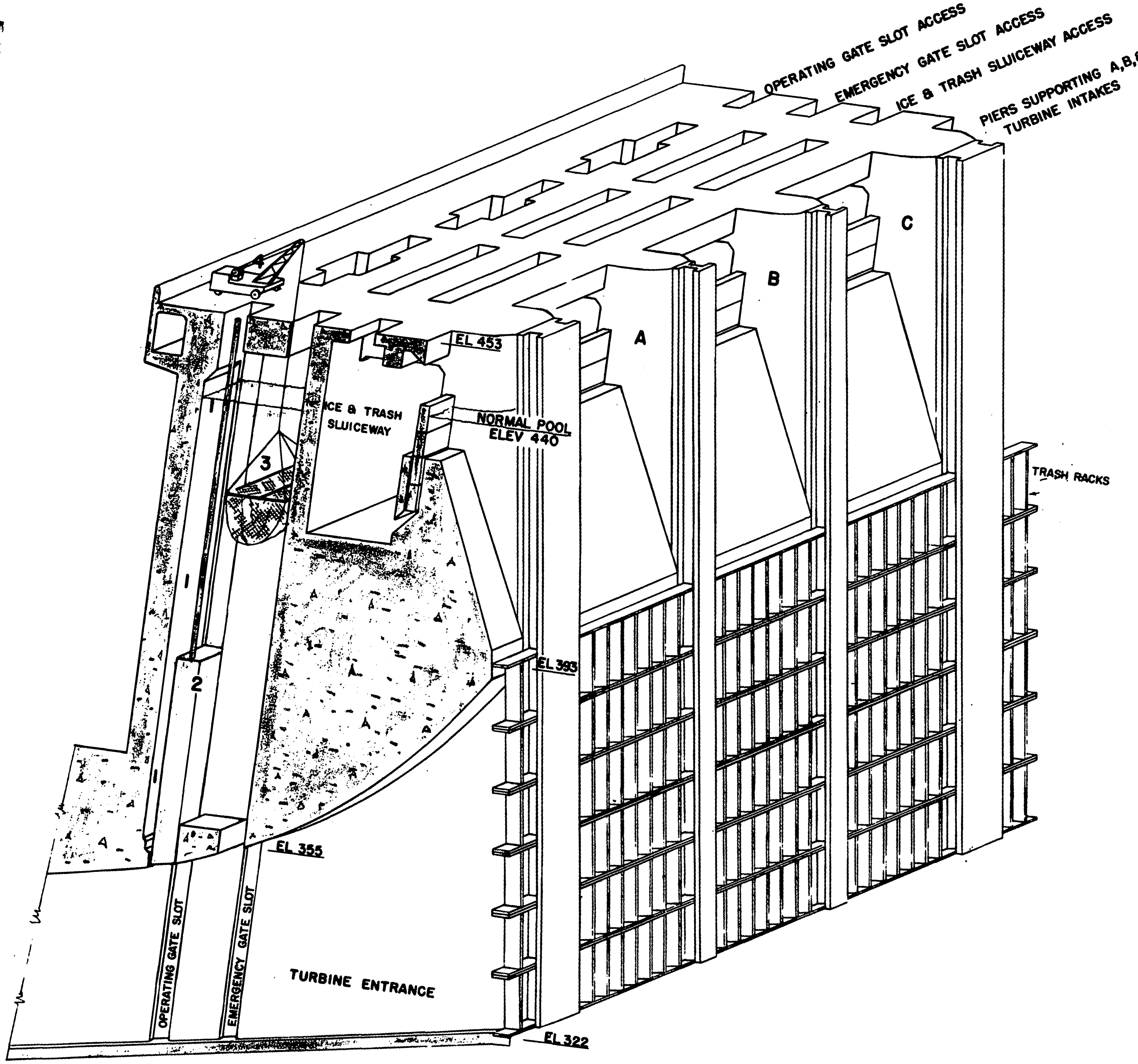

Fig I. Isometric View of Ice Harbor Dam showing:

1. Area Unavailable for Fishing
2. Operating Gate and Cylinder in Stored Position
3. Gatewell Net in Open Position
4. Warehouse Crane

released into the Snake River at Fishhook Park (south shore) and at Snake River Junction (north shore). (fig. 2). These sites were 8 and 13 miles, respectively, above Ice Harbor. The unmarked fish were transported below the dam and released in the tailrace.

The efficiency of the gatewells as collectors and the population estimates were based on the recoveries of marked fish released above the dam. Gatewell efficiency was assessed in terms of the percentage of mark recoveries, and population estimates were derived from the general formula

$$\hat{N} = \frac{nt}{s} \quad \text{(Chapman, 1948)}$$

where

$\hat{N}$ = the population estimate

n = number sampled

t = number marked

s = number recaptured

Population estimates of chinooks less than 90 mm. in length could not be made since these fish were not marked. However, the total estimate was not adversely affected since less than 10 percent of the total chinooks collected were under 90 mm.

Separate marks were used to identify fish released at the two sites. In addition, marks were changed each week. This was done to obtain a measure of mixing of marked fish within the population and to permit stratification of the data. It was assumed that, if proportionately equal numbers of fish were recaptured from each release site, then mixing did take place.

The suitability of the thermal marking technique for field use was demonstrated by comparing returns and survival of thermal marked fish and tattooed fish. Recovery percentages from the approximately 23,000 thermal marked fish released was slightly higher than for about 2,000 tattooed fish (2.5 vs. 2.0 percent). No difference in survival was noted among tattooed, thermal marked and unmarked fish held for observation for a week.

Exit rates from gatewells were explored by periodically introducing marked fish, in lots of at least 10 fish, into adjacent gatewells. Exit rate was determined by comparing numbers of marked fish remaining in a given well after periods of 1, 3, and 6 days.

To examine the effect of light intensity on fish accumulation and attraction of fish into the gatewells, tests were conducted in three gatewells of turbine unit no. 2 for a period of 2 weeks. One gatewell was used as a

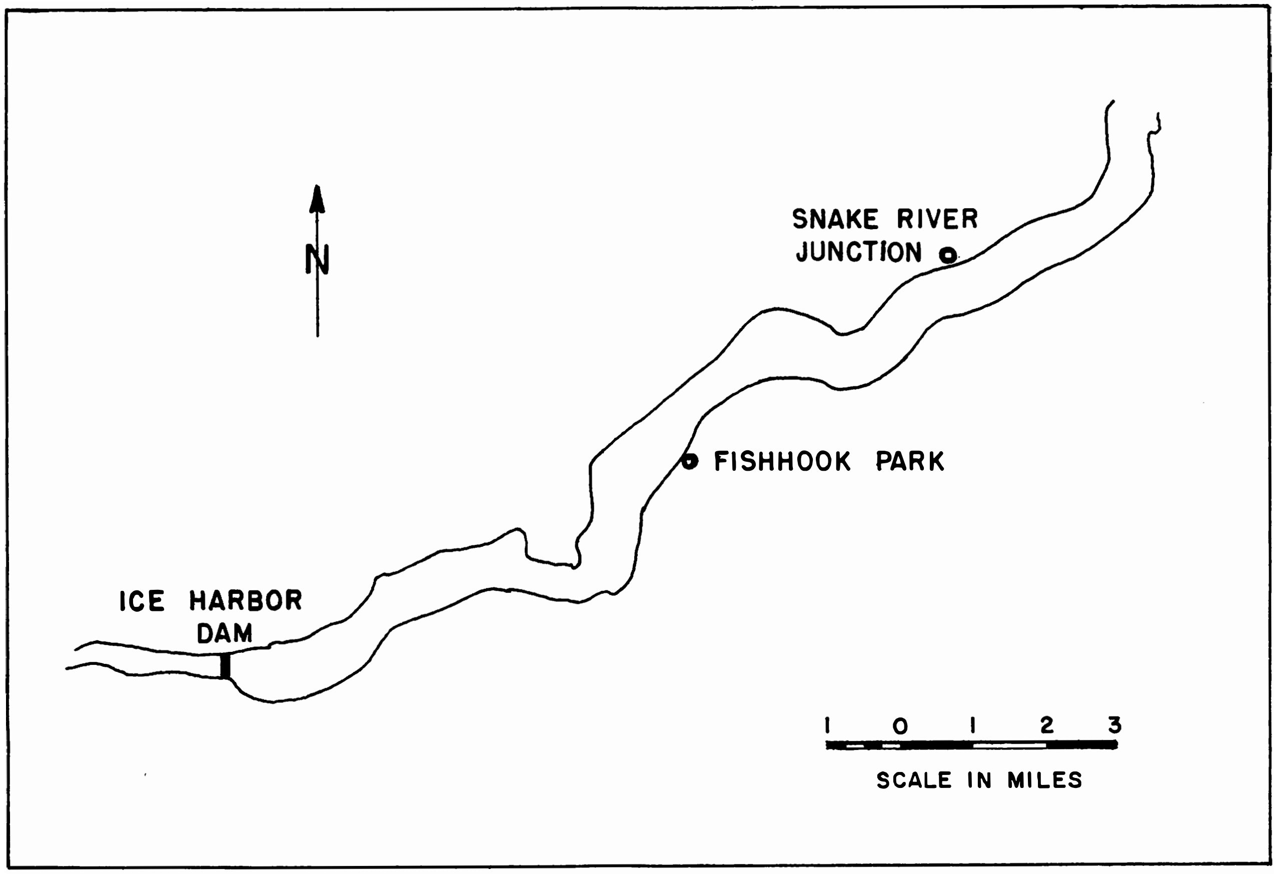

Figure 2.--Marked fish release sites at Fishhook Park and Snake River Junction upstream from Ice Harbor Dam.

control, one was completely covered with black (opaque) plastic sheeting and the third was artificially lighted with three 400-watt underwater lights. Conditions were rotated among the gatewells, usually once every 3 days in the following sequence.

Light	Dark	Control
B	C	A
A	B	C
C	A	B

Corps of Engineers activities during the experimental season prevented continuous sampling of all gatewells; unit 3 was unavailable for fishing until June 5, one month after the peak of the chinook run had passed. Therefore the results generally are based on data from units 1 and 2 only. Salmonids collected from unit 3 were, however, included in the overall totals of marked and unmarked fish.

RESULTS

Gatewell Collection Efficiency

Gatewell sampling from March 23 through June 30, 1964, resulted in the collection of 126,833 salmonid migrants: 76,350 chinook salmon (Oncorhynchus tshawytscha) over 90 mm. long; 6,450 chinook less than 90 mm.; 43,836 steelhead trout (Salmogairdneri); 106 sockeye salmon (O. nerka); and, 91 coho salmon (O. kisutch).

The gatewell collection efficiency for all salmonids throughout the season was 2.5 percent based on 644 recaptures out of 25,735 marked fish released. This percentage was obtained while fishing an average of 33 percent of the gatewells 30 percent of the time. With continuous collection, this percentage would have been substantially increased. Returns of this magnitude indicate that sufficient samples of downstream migrants can be collected from Ice Harbor gatewells to warrant expansion of the racial timing and magnitude studies.

Population Estimates

Population estimates were made for the total run and for chinook salmon and steelhead trout respectively. These estimates included only fish larger than 90 mm. passing Ice Harbor Dam between April 17 and June 10, 1964. The estimates of the population and the 95 percent confidence limits around the estimates were:

	Lower	Mean	Upper
Total population	4,172,477	4,518,521	4,888,177
Chinook salmon	2,744,596	3,036,917	3,333,614
Steelhead trout	1,270,088	1,481,604	1,664,144

In making a population estimate it is necessary to consider mortalities of marked fish and the degree of mixing of marked fish with the unmarked population between release and recapture. As the mortalities during handling and transportation were less than 1 percent, it appears the fish were released in good condition. However, if delayed mortality occurred, it may have been higher in chinook than in steelhead. Recoveries of marked steelhead amounted to 2.9 percent whereas those of chinook were 2.3 percent. If mortalities occurred, the foregoing estimates are high.

There was evidence that the marked fish became well mixed with the unmarked population since recoveries were similar from each release site (table 1).

Table 1.--Number and percent of marked fish released and recovered in relation to release site.

Release site	Chinook Salmon			Steelhead		
	Released	Recaptured	Percent recaptured	Released	Recaptured	Percent recaptured
Fishhook Park	8,210	203	2.47	3,634	109	3.00
Snake River Junction	9,530	203	2.13	3,632	101	2.78
Totals	17,740	406	2.29	7,266	210	2.89

Migration Rates and Timing--Chinook and Steelhead

The recapture of marked fish released in this study indicated an elapsed time of 1 to 2 days from the release sites to the dam. Additional migration rates in the Snake River were obtained from the recapture of 31 tagged fish released in the Brownlee-Oxbow Dam area. Five of these fish were from a group of 150 chinook fingerlings collected in Eagle Creek and transported and released below Oxbow Dam. The average migration rate of these fish was 15 miles per day for the 270 miles to Ice Harbor Dam. The remaining 26 fish had been released within Brownlee Reservoir, and since the proportion of time spent in the Brownlee Reservoir and the river is unknown, the river migration rates cannot be established. It is of interest, however, that these fish passed through both Brownlee and Oxbow impoundments and arrived in excellent condition at Ice Harbor Dam.

The magnitude and timing of salmonid migration is shown in figure 3. While some fish were caught as early as March, the main migration of chinooks began about April 17, and peaked about May 11. The steelhead migration started later and peaked about 10 days after the chinook. The peak of the chinook outmigration occurred about 30 days before the main flood. This agrees with results obtained by the Washington Department of Fisheries at Central Ferry (Mains and Smith, 1956). Migration peaks did not appear to be related to water turbidity and temperature.

Length and Age Composition

Fork lengths of 90 percent of the steelhead trout measured were between 140 and 190 mm. and averaged about 170 mm. throughout the season. By contrast, 90 percent of the chinooks collected were between 70 and 140 mm. Average lengths varied throughout the season (fig. 4).

Initial sampling of the gatewells in late March revealed a sizeable group of chinook salmon averaging 205 mm. (8 + inches). Analysis of scale samples indicated that these fish were mostly in their second year and that they showed extensive fresh water growth. These data on timing, size, and scale patterns were similar to data obtained from winter outmigrants from Brownlee Reservoir.

Two size ranges, averaging 43, and 117 mm., were observed in the first half of April. The smaller fish were believed to be the progeny of the 1963 brood-year fall-run chinook spawning in the Snake River system. The larger fish were considered the progeny of the 1962 brood of spring and summer chinook spawning in the tributary streams.

During the last half of April and the first half of May, a unimodel size range was observed. These fish averaged 117 mm. in length. While fish of this size dominated the picture most of the month, a group of fish averaging 75 mm. in length was observed entering the catch in the last 10 days of the month.

During June, this 75-mm. size group range continued to increase in numbers and by the middle of the month, 66 to 90 mm. fish dominated the catch. It is possible that this group could have originated from the Oxbow rearing facility as the timing of release at Oxbow and subsequent recovery at

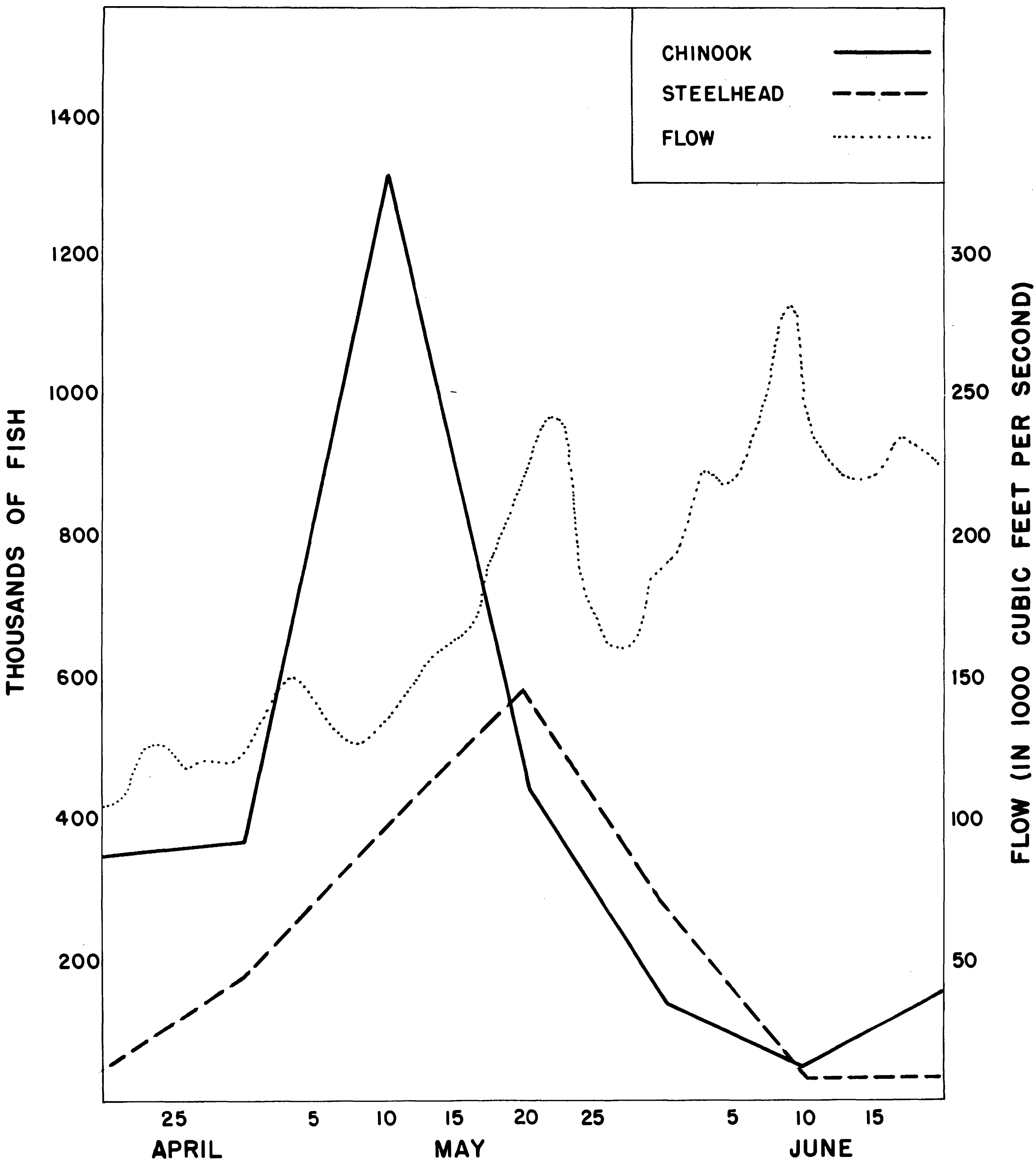

Figure 3.--Timing of downstream migration of chinook salmon and steelhead trout in relation to water flow at Ice Harbor Dam, 1964.

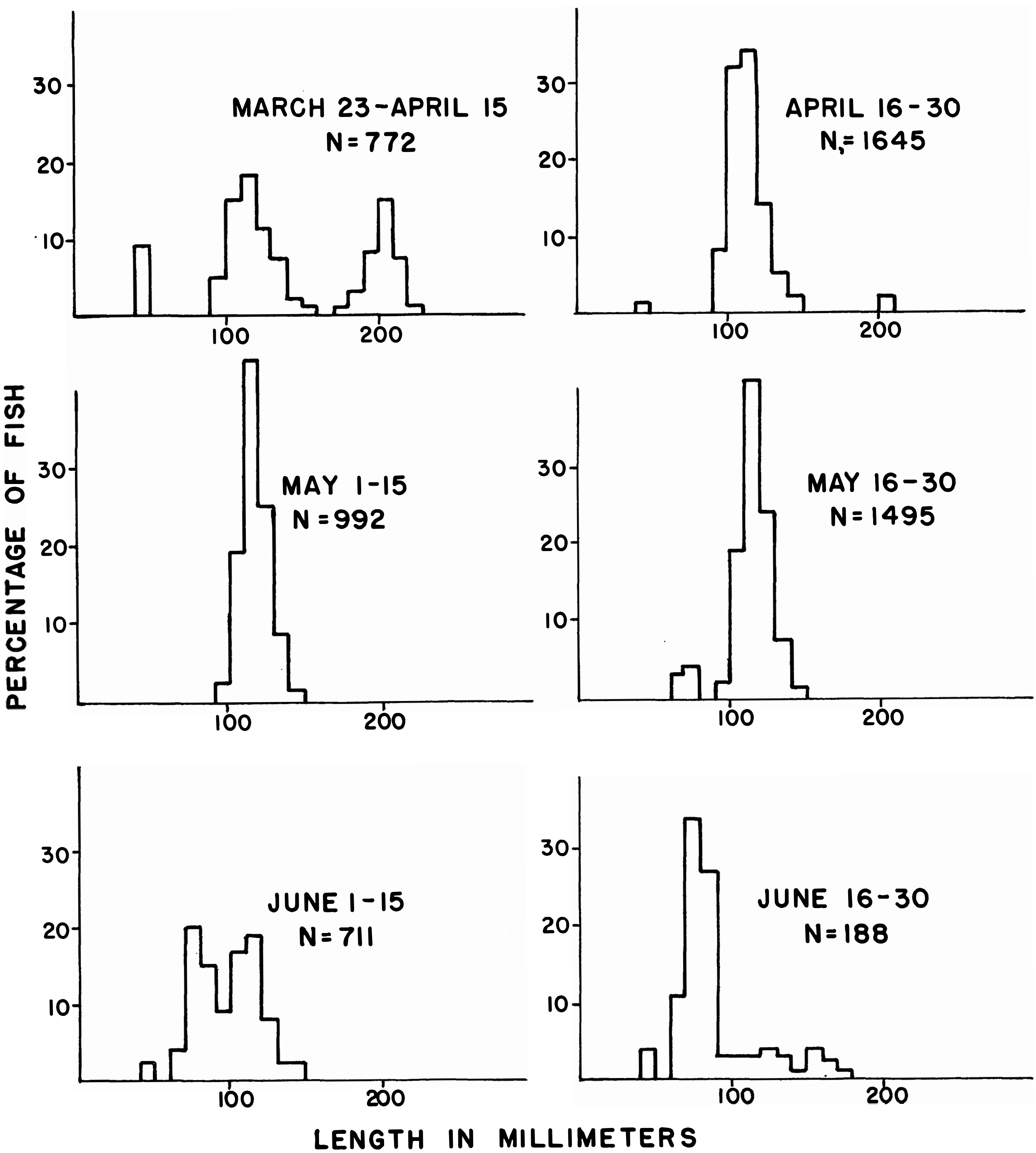

Figure 4.--Length frequencies of migrant chinook salmon, Ice Harbor Dam, March 23 - June 30, 1964.

Ice Harbor followed in logical sequence.

An analysis of chinook scale samples showed a general relation between size and age. 0 age fish were usually less than 90 mm.; yearlings ranged from 75 mm. to 214 mm.; and 2 year old fish ranged from 160 to 240 mm. The variation in time of the formation of annuli and numbers of circuli suggests the presence of several races of chinook.

Distribution in Gatewells

Sampling for vertical distribution of fish in the gatewells indicated that 84 percent of the chinook salmon were concentrated in the upper 20 feet of the gatewells, whereas only 50 percent of the steelhead trout were captured in the same area. This assumes that the net efficiency was the same for both species and that efficiency did not vary with depth.

The horizontal distribution of fish in the gatewells of an individual turbine unit revealed that Gatewell A consistently contained more fish than well B, and that well C produced far less fish than either A or B wells. Figure 5 shows this distribution in relation to the velocities taken at the emergency gate slots of each turbine intake. By inspection, it may be seen that the catches in the respective intake wells were in some degree related to velocity within these intakes; i.e., the higher the velocity the higher the catch. Since the gatewells in turbine unit no. 3 throughout most of the season were unavailable for fishing, this relationship could not be expanded across the powerhouse section.

Exit Rate from Gatewells

Exit rate tests during various times of the season showed that an average of 58 percent of the chinook and steelhead remained in the gatewells after one day, 45 percent after 3 days, and 36 percent after 6 days. However, the data suggest that exit rate is related to species and time of migration. The chinook exit rate appeared to be faster earlier in the run than just prior to the peak. Steelhead showed the reverse of this trend. A test conducted 3 weeks after the peak was of special interest; 87 percent of both chinook and steelhead had not exited after 3 days.

Lateral Movement in Gatewells

The lateral movements of 12 out of 300 marked chinook and steelhead placed in A and B gatewells of turbine unit 2 on April 7 and 9 are shown in figure 6. To accomplish this movement, fish had to sound 84 feet to leave the gatewell and then swim 35 to 40 feet upstream through the turbine intake to the forebay where they must have moved laterally and passed downstream into another gatewell (fig. 1). Under normal load, velocities at the gateslot of A or B intakes were in excess of 5.9 f.p.s. Powerhouse operation records during this period showed unit 2 operated under full load for all but one 6-hour

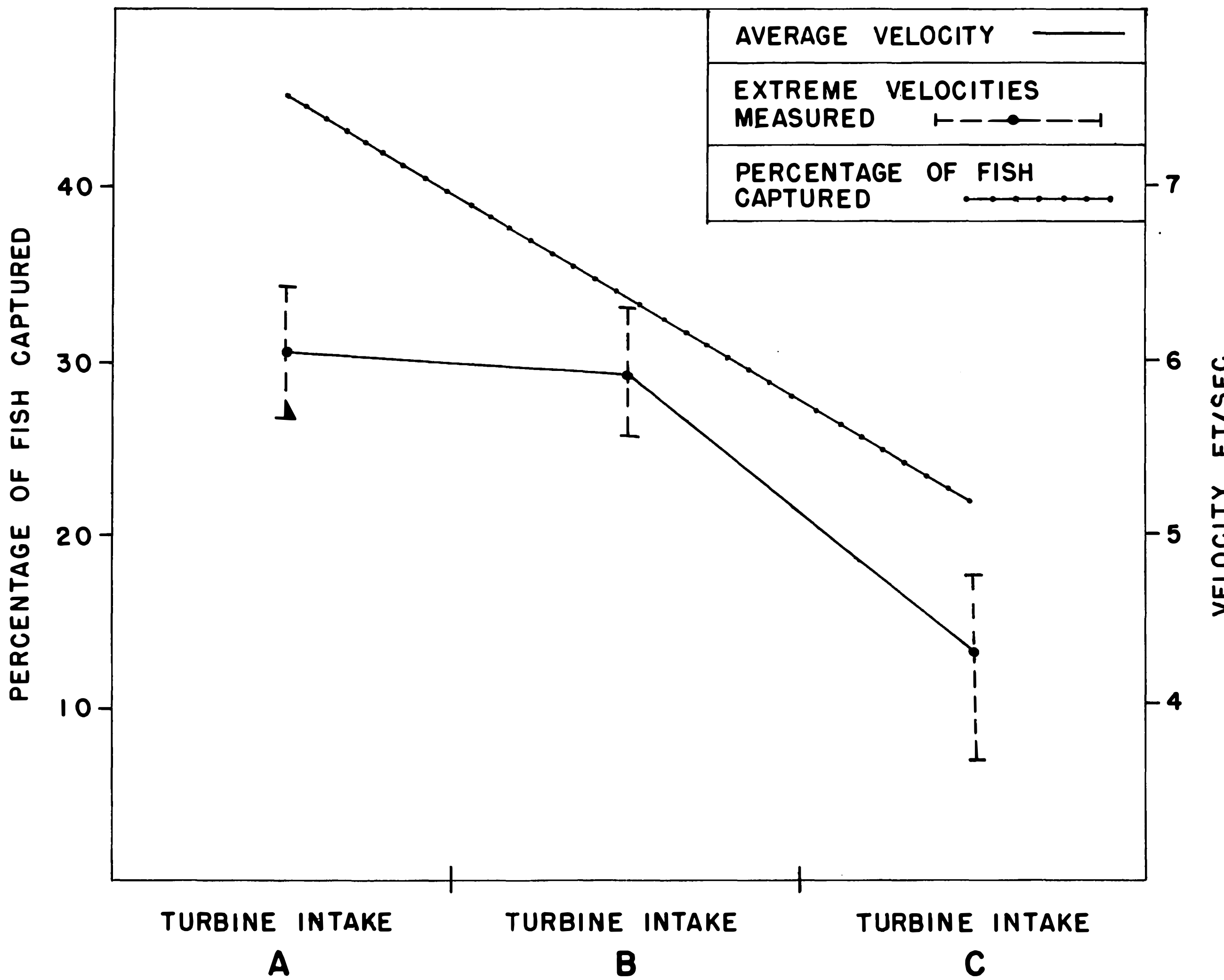

Figure 5.--Proportion of fish in gatewells A, B, and C in relation to velocities measured at the emergency gateslots within each gatewell.

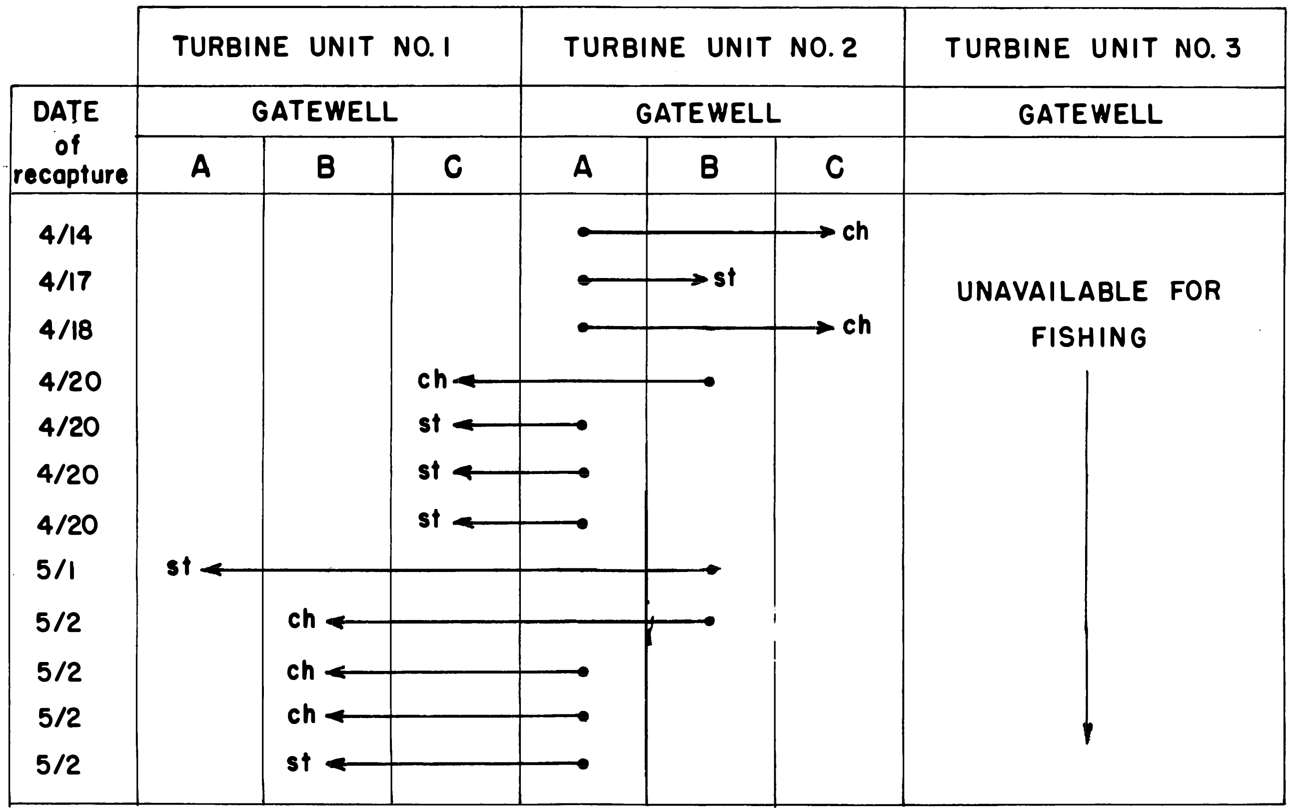

Figure 6.--Lateral movement of 12 salmonids released in A and B gatewells of turbine no. 2 on April 7 and 9.

period on May 1 when the turbine was off the line.

How were these fish able to perform the feat? One possibility is that velocities may be lower in certain areas of the turbine intake such as near the ceiling. Velocities within the upper foot of the intake could not be measured. Temporary accumulations of debris against the trash rack could create areas of reduced flows that might be traversed by these fish. However this movement took place 45 days prior to the flood when the debris load was low.

Lightened vs. Darkened Gatewell

Numbers of salmonids entering gatewells A, B, and C of turbine unit no. 2 under the different light conditions are shown in table 2.

Table 2.--Numbers of chinook salmon and steelhead trout captured in A, B, and C gatewells of turbine unit no. 2 under light, dark and control conditions.

	Chinook			Steelhead		
Test No.	Light	Dark	Control	Light	Dark	Control
1	131	72	132	301	278	338
2	170	133	147	454	248	276
3	99	104	233	215	293	350
4	93	22	57	66	62	100
5	147	51	52	44	22	27
6	53	100	45	67	144	44
	693	482	666	1147	1047	1135

Although more chinook salmon were recovered from both the control and lightened gatewells than from the darkened gatewells, statistical analysis showed no significant differences. No significant differences were observed in the steelhead catches. These results are in contrast to those observed by Fields, et al. (1964). They found at McNary Dam, that there was a significantly larger catch in each of the lightened gatewells than in unlightened wells, and that only one-tenth as many downstream migrants were obtained from the A, B, and C gatewells having solid covers as from the B well with steel grill covers.

Predation in Gatewells

Evidence of predation by steelhead was observed in gatewells at Ice Harbor Dam. Predation was predominantly on the smaller chinook salmon (less than 90 mm.) in gatewells where fish had been allowed to accumulate for several weeks.

Potential Use of Gatewells for a Fingerling Bypass

When it became apparent that large numbers of fish were utilizing the gatewells, an attempt was made to estimate the percentage of fish entering the turbine intakes that would be potentially available to a collector within a gatewell. If it could be demonstrated that sufficient numbers of fish would become available, then attention might be given to installing bypasses in gatewells to divert fingerling migrants safely around turbine areas of future dams.

During the migration period, an average of 40 percent of the total flow of the Snake River passed through the turbines. If the numbers of fish were in proportion to the flow of water, then approximately 2,000,000 fish entered the turbine intakes in the spring of 1964. Based on fishing effort, exit rate tests, and marked fish recoveries in this experiment, it was estimated that between 400,000 to 500,000 fish entered the gatewells. Thus, an estimated 20 to 25 percent of those fish entering the turbine intakes were available for diversion. With slight modifications in the design of gatewells in future dams, this percentage might be increased considerably.

SUMMARY

Turbine intake gatewells at Ice Harbor Dam were sampled with a specially designed dipnet during the spring of 1964 to determine the feasibility of utilizing such collectors for economically monitoring the Snake River juvenile outmigration.

During the period between March 23 and June 30, 126,833 salmonids were collected. This total included 76,350 chinook over 90 mm. and 6,450 less than 90 mm.; 43,830 steelhead; 106 sockeye; and 91 cohos. A mark and recovery program yielded the following results:

1. Gatewell collection efficiency was approximately 2.5 percent based on 644 recaptures out of 25,735 marked chinook and steelhead released upstream.

2. The Snake River spring outmigration for salmonids larger than 90 mm. was calculated to be 4,518,521. Of this total, 3,036,917 were chinook salmon and 1,481,604 were steelhead trout.

3. Salmonids averaged 4 to 13 miles per day in the Ice Harbor reservoir. Recoveries of fish marked in the Brownlee-Oxbow area indicated a migration rate of 15 miles per day in the 270 miles of river and reservoir between Oxbow and Ice Harbor Dams.

4. The peak of the chinook migration occurred on about May 11, 30 days ahead of the main flood. Steelhead peaked 10 days later on May 21.

5. Chinooks averaged 117 mm. in length, with 86 percent ranging from 70 mm. to 140 mm. Steelhead averaged 170 mm., with 90 percent ranging from 140 mm. to 190 mm.

6. Preliminary estimates of the exit rates from gatewells showed that, on the average, 58 percent of the fish remained after 1 day, 45 percent after 3 days, and 36 percent after 6 days. The length of time fish remained in the gatewells varied with the species and time of season.

7. There was evidence that fish moved from one gatewell to another. Since each well is a separate unit, not connected with adjoining units, these fish had to swim out of the wells and return upstream through the turbine intake to the forebay before entering an adjoining intake and gatewell. These occurrences were of interest because the fish were required to swim upstream through intake velocities approaching 6 f.p.s.

8. Artificial lighting in the intake wells did not appear to attract or hold more fish in the wells.

9. Vertical distribution studies in the gatewells indicated that the majority of the chinook (84 percent) were in the upper 20 feet.

10. Steelhead preyed on young chinook (less than 90 mm.) in the gatewells.

11. If fish passed through the spill and turbines in proportion to volume of water, 40 percent of the total run entered the turbine intakes. Based on fishing effort, exit rate tests, and mark returns, it was estimated that 20 to 25 percent of the fish entering the intakes passed up into the gatewells.

CONCLUSIONS

1. Turbine intake gatewells provide a convenient area in which to economically sample downstream migrant populations.

2. The large number of fish observed in gatewells suggests that these areas might well be incorporated into a bypass system that would divert substantial numbers of fish out of the turbines.

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A THERMAL MARKING TECHNIQUE FOR JUVENILE SALMONIDS

by

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and

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September 1964

FISH-PASSAGE RESEARCH PROGRAM
U.S. Bureau of Commercial Fisheries
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INTRODUCTION

Work with juvenile salmonids often calls for short term marking techniques by which individual fish or groups of fish may be identified for varying periods. Such recognition can be provided by various familiar means such as fin removal, attachment of markers or tags, tattooing, or perhaps whole body staining.

Although the above methods may each serve in specific applications, a simple, quickly learned technique is needed which will permit the rapid use of a variety of visible surface marks such as letters, numbers, or symbols, by which individual fingerlings may be recognized easily over intervals of weeks or months. The purpose of this report is to describe a marking system which appears to meet such needs. The method, developed in the Seattle laboratory of the Fish-Passage Research Program, U.S. Bureau of Commercial Fisheries, consists of marking juvenile salmonids by topical application of mild heat to dorsal skin surfaces.

Use of heat to mark or brand small fish has been attempted previously. In such instances the levels of applied heat have been relatively high. Buss (1953) marked young brook trout with a wood burning pencil. He reported that some brands remained visible after 21 months and one after 4 years. Johnson and Fields (1959) tried to mark fingerling steelhead by applying to the skin surface a nichrome wire electrically heated to white heat. According to the authors, this caused injuries which penetrated the skin. Though these were readily visible, they were slow in healing. After 5 months, no distinguishable marks or scars were left. Similarly, white-hot wire was used by Watson (1961) to mark young sea herring. He reported that scars were discernible after 7 months, but differences between marks were evident only during the first several days.

To mark fingerling salmon, we explored the use of a small electrical soldering iron with the heat tips shaped into various patterns. The trials were not satisfactory. Marks could not be produced consistently at heat levels other than those which caused lesions penetrating through the skin into the underlying musculature. Though the resultant injuries appeared as prominent dark gray marks, they bore no resemblance to the patterns of the heat tips and left no visible traces after a 3-month healing period.

These results indicated that substantially lower applied heat levels would be necessary to avert excessive damage to the contact areas. Such attempts were made, but no marks were produced. This lack of success may have been due to the limited thermal capacities of the marking tools.

Thereupon, new applicators were designed to enhance such characteristics as heat storage and conductance. To employ a level of heat considerably lower than that applied previously, boiling water was used to heat the applicators. This heat source and the specially designed tools have made practicable the effective marking technique described herein.

MATERIALS AND METHODS

The marking tools are made from metals having superior thermal conductance properties. A pencil-sized piece of copper tubing forms the handle. The marking tip is silver, smoothly polished to facilitate heat transfer. Individual flat-surfaced figures are cut from quarter-inch thick silver plate and soldered in reverse (fig. 1) onto a matching piece of one-eighth-inch thick silver. The plate with attached figure is then soldered to the end of the copper tube. This physical continuity maintains a relatively uniform heat flow from the handle to the marking surface when applied to a fish. The copper handle is insulated with polyvinyl tubing which covers all but the first 2 inches from the marking tip. Only the tip and about 1 inch of the uninsulated portion was immersed in the heating water. Currently, the tools are produced by a manufacturing jeweler at a unit cost of about eight dollars ($8.00).

Fish used in the tests were juvenile sockeye salmon (<u>Oncorhynchus nerka</u>) and rainbow trout (<u>Salmo gairdneri</u>) from the National Hatchery at Leavenworth, Washington; fingerling spring chinook salmon (<u>O. tshawytscha</u>) from the National Hatchery at Carson, Washington; and silver salmon (<u>O. kisutch</u>) from the National Hatchery at Quilcene, Washington.

Fish for marking were anesthetized and held in aerated solutions of M.S. 222. The actual marking procedure was simple. After the fish was taken from the anesthetic, the marking tool was removed from the heating water, shaken to remove any drops, and applied to the skin surface between the lateral line and dorsal fin. A light, positive, even contact was maintained for about one and one-half seconds (fig. 2). The fish was returned to water and the marker placed back into the heating container. No more than one application with the same tool was made without reheating. If a mark required the use of several figures, the fish was usually

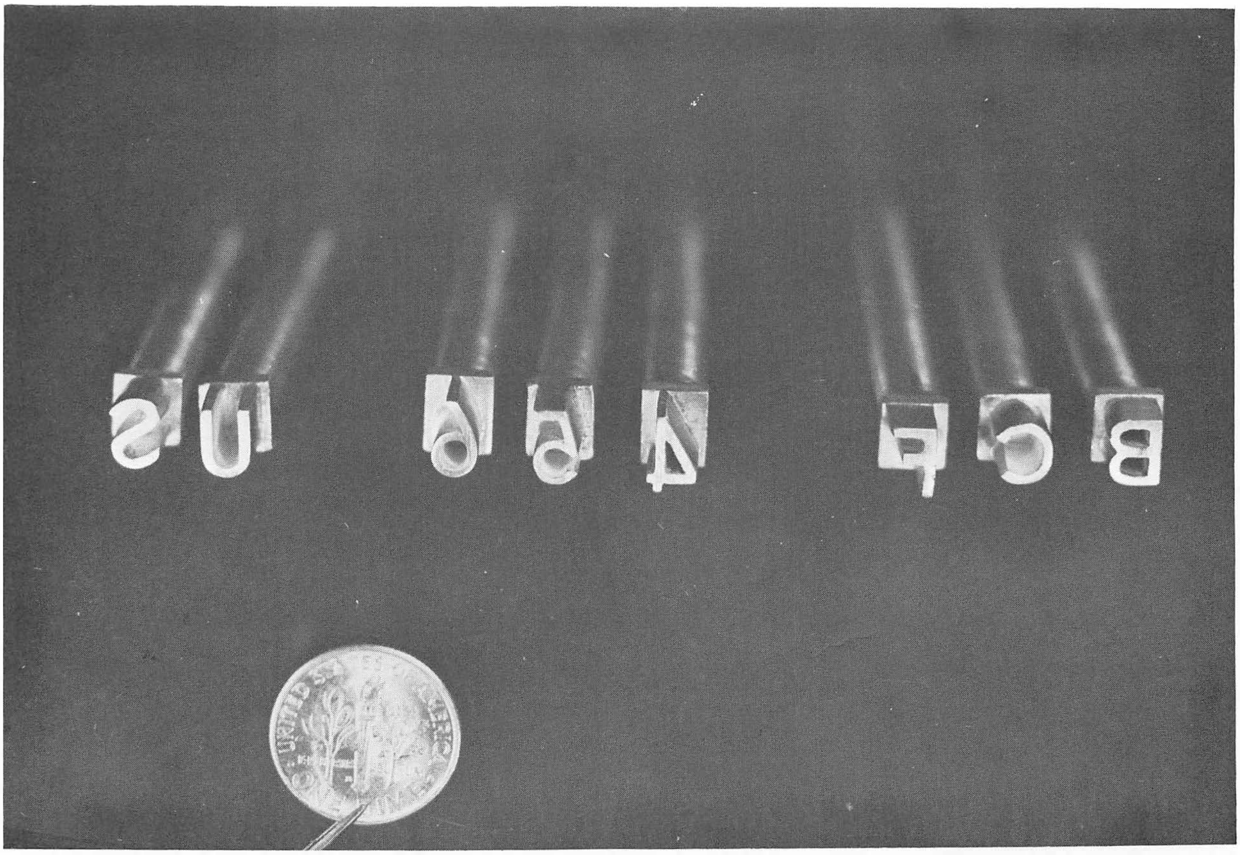

Figure 1.--Closeup of the solid silver tips of the marking tools. Ten cent piece in foreground shows size relationship.

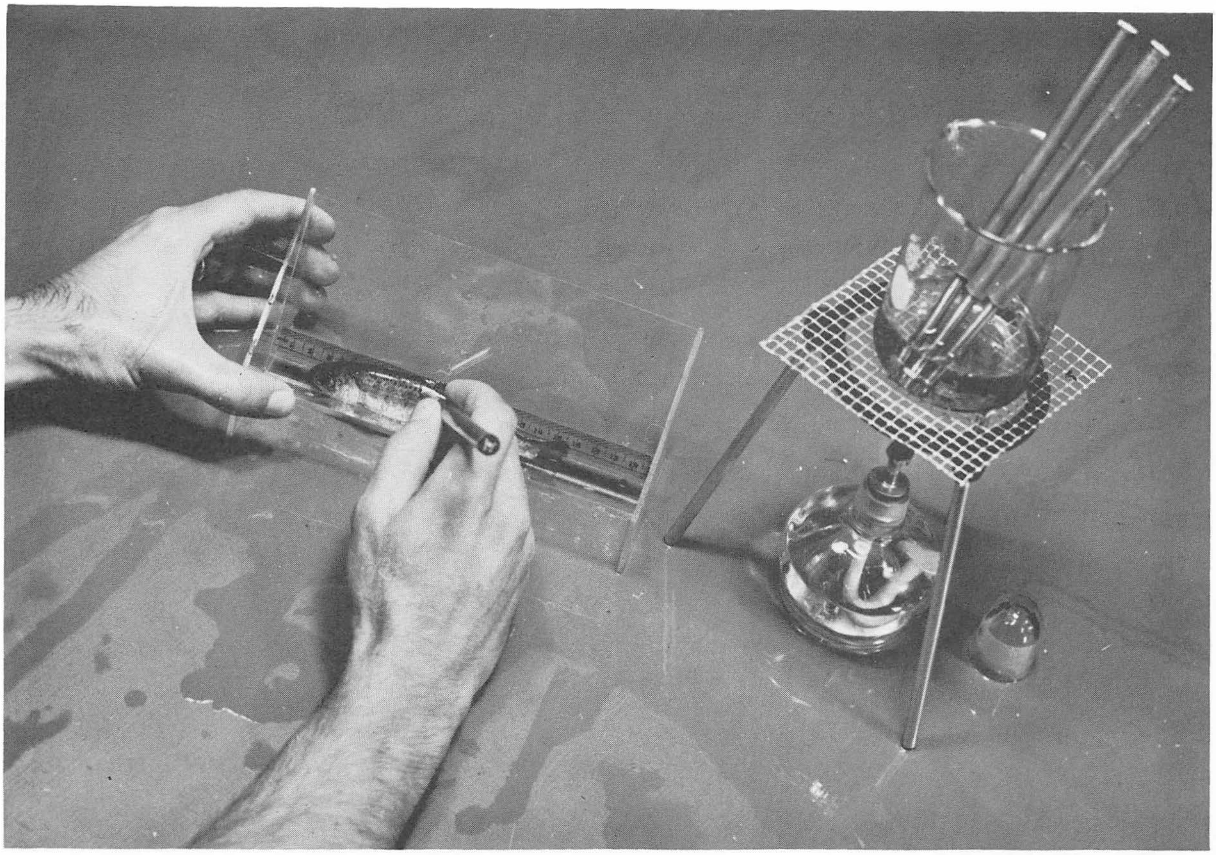

Figure 2.--Applying a thermal mark (dorsolateral) to a chinook salmon fingerling. Engraved plastic disc at top of copper handle identifies marking tool and insures proper alignment during marking. Photograph depicts the minimal equipment needed for thermal marking--marking tools, water, and a heat source.

held out of water for the full marking interval. In such instances the temperatures of the anesthetic solutions were held down to around 45° F. to help offset any possible increased handling stress.

Clear marks depended mostly on steadiness of the applicator during contact. Occasional blurring resulted from uneven contact, probably due to small amounts of mucus adhering to the marker. In most instances, however, the boiling water kept the figures clean. Immediately following application, little indication of marking was evident--other than a faint whitish outline of the mark. Minutes after the fish revived from the anesthetic, the mark usually appeared prominently dark against the normal pigmentation.

RESULTS AND DISCUSSION

In the laboratory the method has been used to place a variety of persistent marks (fig. 3) on fingerling salmonids ranging from 85 to 160 millimeters in length. Six months after marking, juvenile sockeye displayed the original figures with little loss of clarity. Similar results were obtained with spring chinook fingerlings.

Two-hundred forty-four juvenile rainbow trout were marked with individual serially consecutive arabic numerals. One month later any fish could be identified by its number. After 2 months, the numbers were still evident on all fish, but apparent local differentials in skin growth had distorted some numerals. Ten months later the fish had grown from about 100 millimeters to over 200 millimeters in length. All fish still had visible marks. These resembled the numbers two to three times their original size but many numerals were not distinctive and could not be identified. Numbers on some trout were outlined by a patterned alignment of dark pigment spots. A similar heat marking effect on rainbow was noted by Buss (1961).

Marking caused no mortalities in these tests. Overall comparisons between marked fish and control groups indicated no differences in general behavior responses or activity.

In the field, the technique has been used successfully to mark over 23,000 juvenile chinook salmon and steelhead trout migrating downstream in the Snake River. These ranged in length from 90 to 240 millimeters. Marked fish were identified easily upon recovery after varying intervals of up to 45 days.

Figure 3.--Year-old sockeye salmon fingerlings about 3½ months after marking. Differences in the apparent visibility of the marks are due to the variations in position of the fish with respect to the incident light.

The applied heat evidently alters normal pigmentation at the contact site. Initial mark visibility appears due to localized disruption of chromatophores and dispersion of dark pigments. This aspect usually lasts for a number of days. Later, the silvery pigment guanine seemingly disappears from the contact area, and the resultant reflective contrast gives a high accent to the mark. In laboratory tests, this quality has persisted for several months. With chinook and sockeye salmon, as the guanine reappears in the site, the distribution of darker pigment usually is less, so that the mark appears lighter than the surrounding area (fig. 4). Except with rainbow trout, as noted, pigment differences in the mark area fade gradually within a year. Upon close examination, however, local differences in reflection of incident light can be seen which still may be distinguished as the original mark. One sockeye and one silver salmon in the laboratory displayed such marks 15 months after application.

Results so far indicate that marking success is related to the condition of the fish. Sound, actively feeding, growing fingerlings usually can be marked as described, but if the fish are less than healthy, the probability of consistent mark production appears diminished. Fish size limits for which the technique is effective have not yet been determined. Though the observed pigment changes may be related to age factors, they may well be the results of heat alone. Therefore, duration of application or applicator size should be considered in relation to size of the fish. Also to be determined are possibilities that more permanent features of the skin, such as scales, may be influenced by the heat application.

Based on the testing to date, the method shows considerable promise as a simple system for placing an impressive variety of short term marks on fingerling salmonids. It may be of help in a number of laboratory or field applications.

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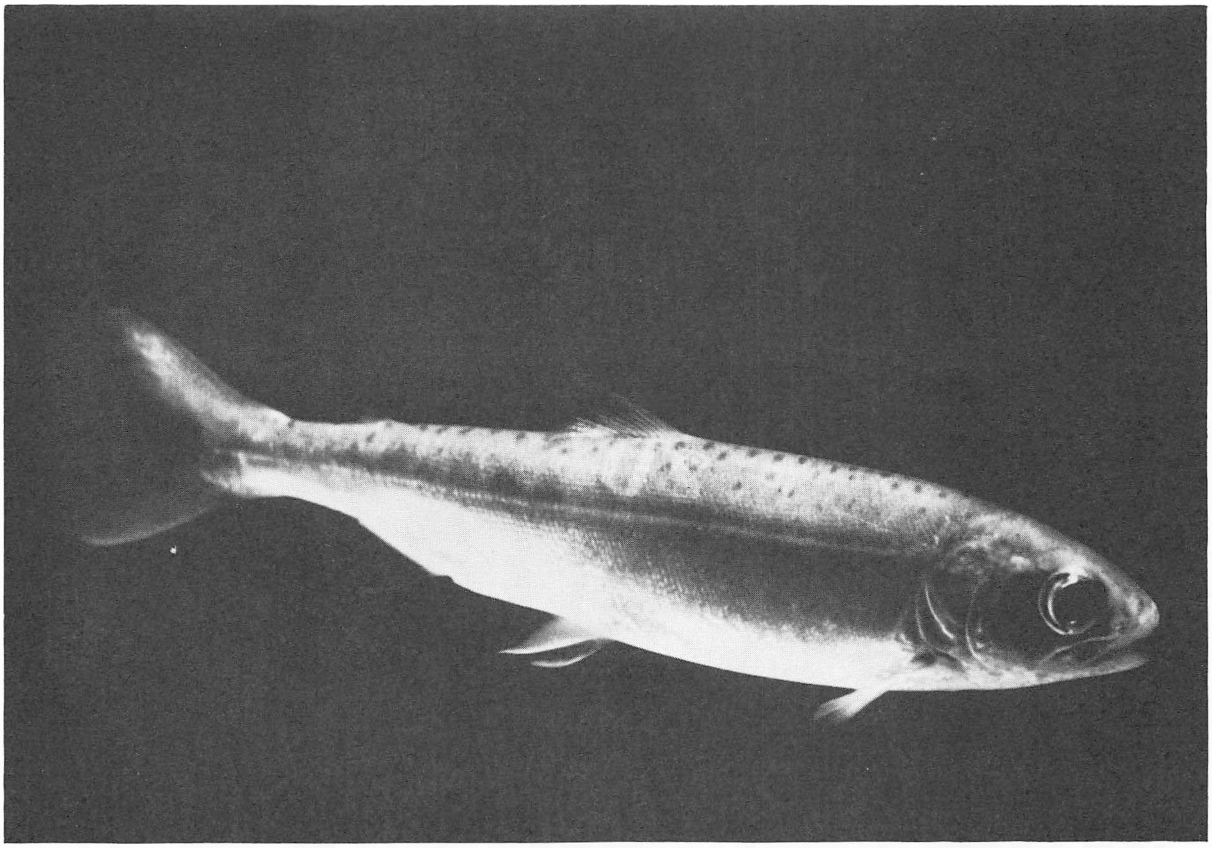

Figure 4.--Six-month old mark on one of the fish shown in figure 3. Although faded, the mark is still visible.

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PHYSIOLOGY AND BEHAVIOR

SENSORY FACTORS IN THE HOMING OF ADULT CHINOOK SALMON
(SUMMARY)

by

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October 1964

FISH-PASSAGE RESEARCH PROGRAM
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INTRODUCTION

Adult salmon, after feeding in the vast areas of the North Pacific Ocean, migrate back to their home river and then return rather specifically to their stream of origin. Information is needed on this homing phenomenon, especially in relation to its directing sensory mechanisms. A favored hypothesis is that the final critical migratory movements are directed by odor and olfactory responses.

An experiment was conducted in the fall of 1960 to explore the possibility that the olfactory sense does play a key role in the final homing of adult chinook salmon. This consisted of blocking sensory receptors of fish which had attained a spawning goal, displacing them away from the site, and then observing their abilities to return.

MATERIALS AND METHODS

The work centered at the Spring Creek Hatchery, at Underwood, Washington (fig. 1). This station, currently a major return site for fall-run chinook salmon, is located on the main stem of the Columbia about 20 miles above Bonneville Dam. Though situated on the river, the hatchery water source is distinctly separate, deriving from a series of springs which supply the buildings and ponds. The same source fills the holding pond and flows out the entrance fishway abruptly into the Columbia--a physical arrangement which suggests that homing to this site may involve detection of a specific water quality.

The experimental fish were all males, excess to the number normally needed in the hatchery spawning operation. Four treatments were used on the fish. These were: (1) Olfactory occlusion, (2) visual occlusion, (3) olfactory & visual occlusion, and (4) controls (no sensory occlusion, same handling procedure). Olfactory occlusion entailed insertion of a packing of petroleum jelly and cotton into the olfactory sac followed by sutures across the nares to retain the plugs. For visual occlusion, the optic lens were removed surgically and replaced with a mixture of petroleum jelly and carbon black. These techniques were selected to minimize operative shock and the time required for treatment.

All fish were anesthetized with M.S. 222 prior to treatment, then marked with numbered Petersen disc tags. After treatment they were transported, still anesthetized, in units of four (treatments 1, 2, 3, 4) to selected release points away from Spring Creek.

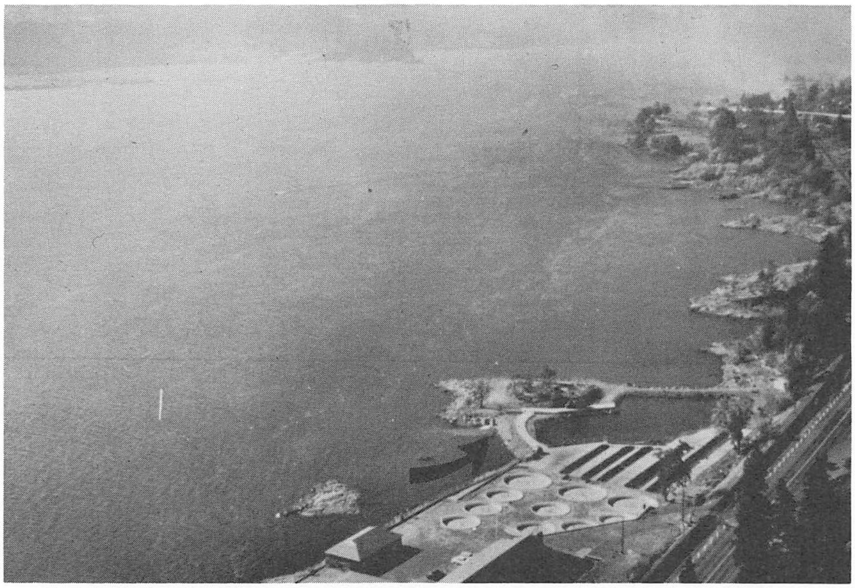

Figure 1.--Spring Creek Hatchery on the Columbia River. Holding pond for returning adults is enclosed from the river within the earth dike. Opening to the entrance fishway is left of the turn in the roadway on the lower dike. Attraction water (arrow) is flowing from fishway into river at about 9 cubic feet per second.

Three release sites were used (fig. 2): (1) Downstream north shore (Stevenson, Washington), (2) downstream south shore (Cascade Locks, Oregon), and (3) upstream north shore (Lyle, Washington). A sample of 100 fish for each treatment at release points 1 and 2 was planned. A smaller sample of 50 in each category was attempted at site 3.

RESULTS

From 866 fish treated, tagged, and released between September 2 and September 21, 1960, tags recovered from September 5 through October 7 accounted for 348 fish or about 40 percent (table 1).

Returns to Spring Creek

Half of all recoveries were from fish returned to Spring Creek, where tags were collected individually as fish reentered the hatchery fishway, beginning September 5. After September 20, when the treatment and release work was terminated, the remainder of Spring Creek tag recoveries were gathered in groups as the fish were used in spawn taking.

Total returns to Spring Creek by treatment category from each release point are shown in figure 3. Approximately 50 percent of the control fish made their way back from the downstream releases and 37 percent from the upstream release. About half as many blind fish as controls returned from each point.

Six olfactory occluded fish returned--five from the north shore downstream release, and one from the south shore downstream release. These fish returned after September 20, and were not checked for adequacy of olfactory plugging. Two fish with visual-olfactory occlusion returned--one from each downstream release point. These also were taken after September 20, and not inspected. Of the total 176 fish that returned to Spring Creek, 95 percent did not have occluded olfactory structures, whereas 8 returning fish, or the remaining 5 percent, had received the olfactory blocking treatment.

Elapsed times between release and return were recorded for individual fish observed before September 20. These were in the control and visual occlusion categories only and are shown in table 2. The mean return time for visually blocked fish was 7 days compared to 5 days for control fish. No visually occluded fish returned in less than 4 days but ten control fish had appeared within 3 days.

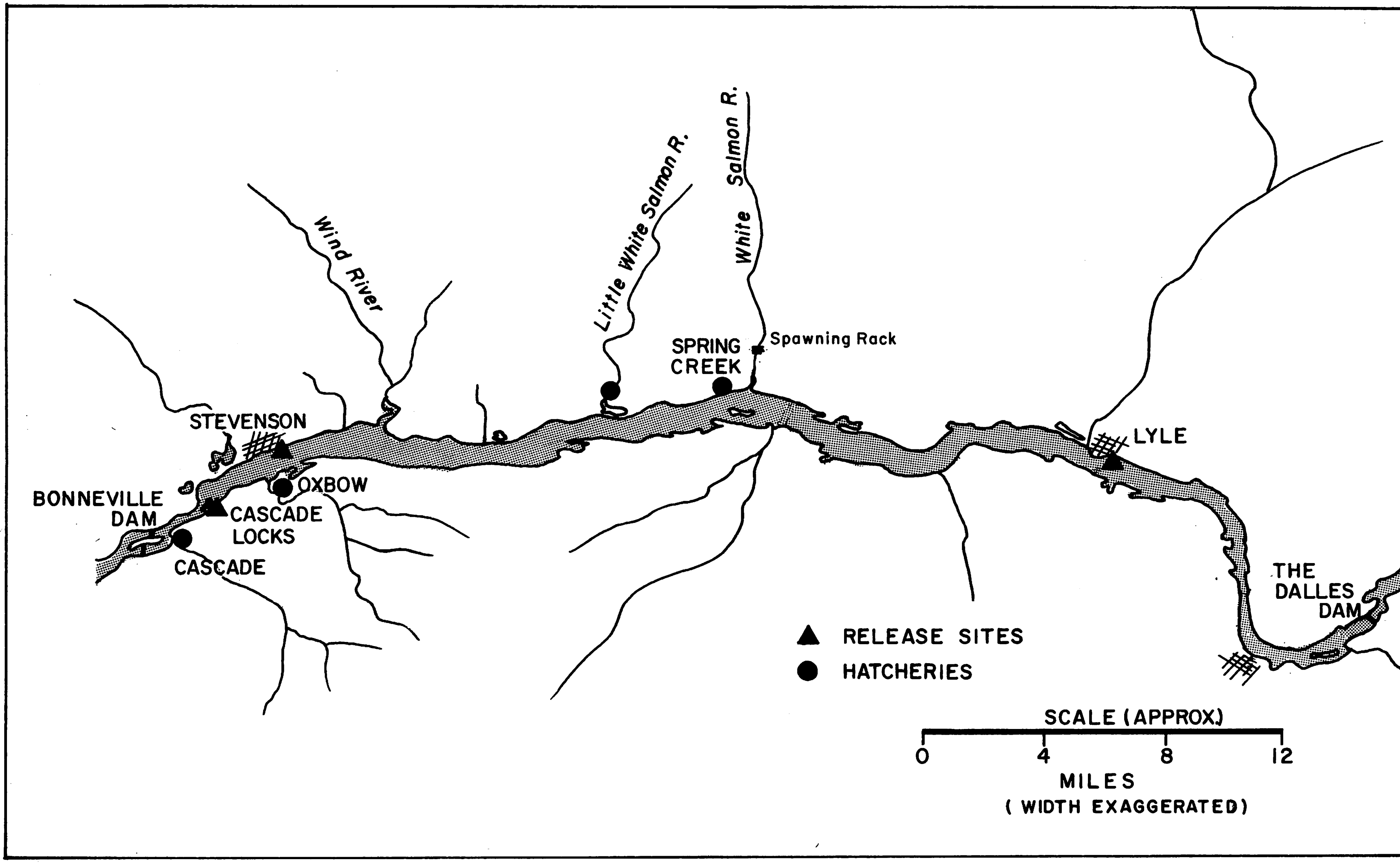

Figure 2.--Area of Columbia River where homing experiment was conducted. Fish were trucked from Spring Creek to release points. Other hatcheries are shown in relation to release points.

Table 1.--Summary of total tag recoveries from all fish released.

Release point	Recovery point	Control fish	EXPERIMENTAL TREATMENT Visual occl.	Olfact. occl.	Vis-Olf. occl.	Total
		96 rel.	96 rel.	75 rel.	74 rel.	
Downstream No. Shore (Stevenson)	Spr. Cr.	48	22	5	1	76
	Oxbow	9	11	1		21
	Cascade	2	2	6		10
	Little Wh.Salm.	5	1			6
	Big White Salmon	1	7			8
	All other spawn. activ. 1/		2	1		3
	Non-spawn. 2/	1		8	7	16
Total recovered		66	45	21	8	140
		96 rel.	96 rel.	77 rel.	76 rel.	
Downstream So. Shore (Cascade Locks)	Spr. Cr.	46	24	1	1	72
	Oxbow	23	14	3		40
	Cascade	4	4	15	1	24
	Little Wh.Salm.		2			2
	Big White Salmon	1	4			5
	All other spawning 1/		4	1		5
	Non-spawn 2/			8	8	16
Total recovered		74	52	28	10	164
		49 rel.	49 rel.	41 rel.	41 rel.	
Upstream No. Shore (Lyle)	Spr. Cr.	18	10			28
	Oxbow					0
	Cascade			1		1
	Little Wh.Salm.					0
	Big White Salmon	1	3			4
	All other spawning 1/	2	1			3
	Non-spawn 2/	1	2	2	3	8
Total recovered		22	16	3	3	44
Grand totals		162	113	52	21	347

1/ artificial spawning activities; hatcheries and spawning rocks.
2/ includes fish taken by sport and commercial fishermen or found in river.

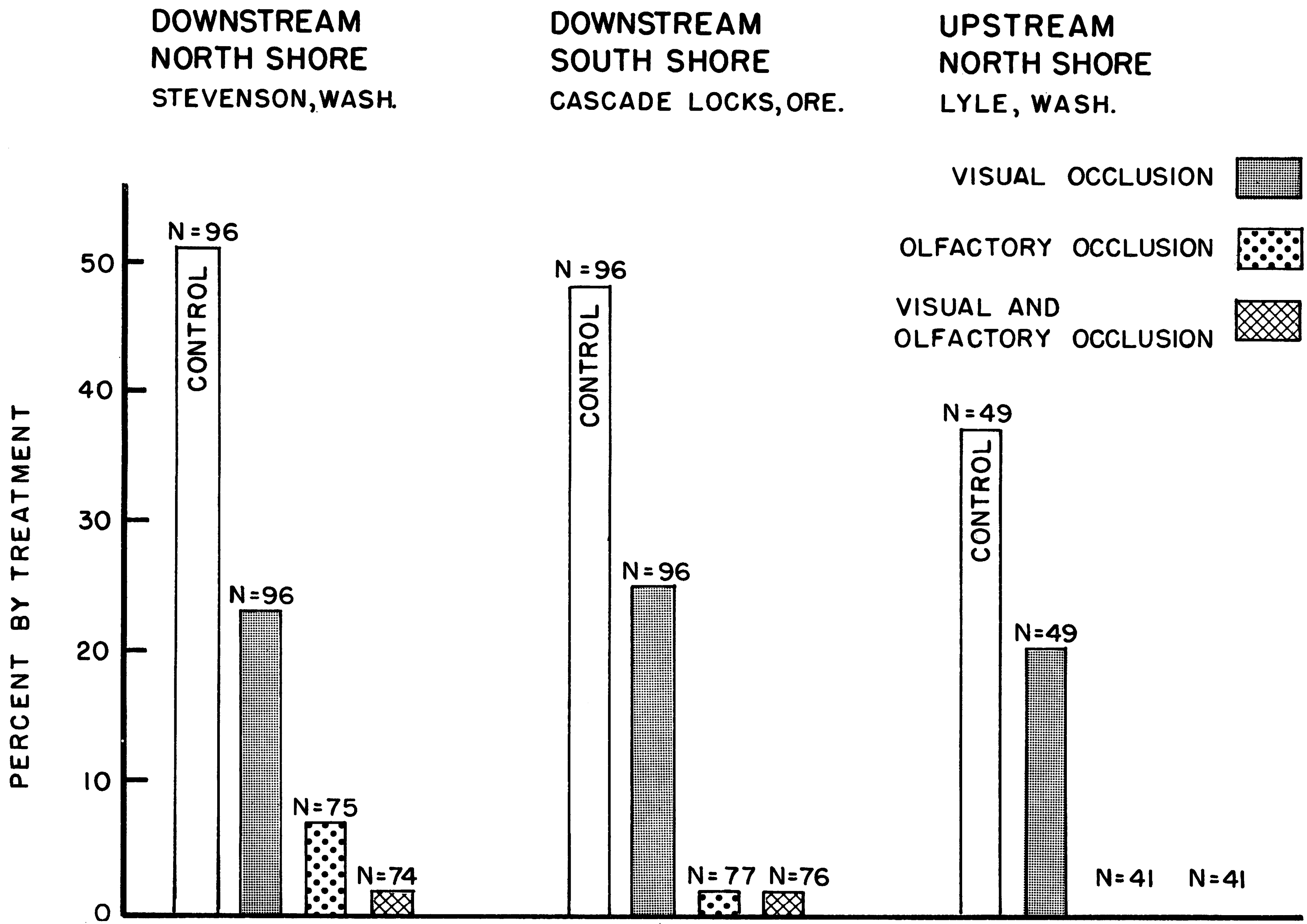

Figure 3.--Returns to Spring Creek by treatment category from each release point.

Table 2.--Days from release to return to Spring Creek--for individual fish observed between September 2 and September 21.

Treatment	RELEASE POINTS					
	Downstream North (Stevenson)		Downstream South (Cascade Locks)		Upstream North (Lyle)	
Control	Days to return	Number	Days to return	Number	Days to return	Number
	1		1	1	1	
	2		2	1	2	2
	3	4	3	2	3	
	4	6	4	4	4	
	5	2	5	5	5	1
	6	1	6	6	6	3
	7	2	7	3	7	
	8	2	8	1	8	
	9		9		9	
	10		10	1	10	
	11	1	11		11	
	12		12		12	1
	Mean Time = 5 days					
Visual occlusion	Days to return	Number	Days to return	Number	Days to return	Number
	1		1		1	
	2		2		2	
	3		3		3	
	4	2	4		4	
	5	1	5	1	5	
	6	2	6	1	6	2
	7	1	7	2	7	1
	8	2	8	3	8	
	9	1	9	1	9	
	10		10		10	1
	11		11	1	11	
	12		12		12	
	Mean Time = 7 days					
Olfactory occlusion	None returned					
Olfactory and visual occlusion	None returned					

Recoveries Away from Spring Creek

Of the 172 tags recovered from points other than Spring Creek, 132 or 77 percent were from hatcheries and spawn-taking operations, and 40, or the remaining 23 percent, were from points or sources not associated with spawn-taking (table 1). The effect of treatment on this distribution is shown in figure 4. In both the control and visual occlusion groups about 96 percent were recovered from spawn-taking operations. In the olfactory occlusion category, 61 percent of recoveries were from spawn-taking sources. Though fewer were recovered from the combination sensory treatment groups, the ratio was reversed from control and visual occlusion recoveries, with 95 percent coming from sources that were not related to spawn taking.

As shown in table 1, 121 of the 132 tags from spawn-taking other than Spring Creek were from within a 15-mile portion of the Columbia above Bonneville Dam. Of this number, 96 were from the Oxbow and Cascade hatcheries--each respectively about 1 mile above and 1 mile below the downstream south shore release site at Cascade Locks. Ninety-three percent of the tags recovered from the Oxbow hatchery were from the control and visual occlusion groups. At the Cascade hatchery, 34 percent of the recoveries were from these categories with the remaining 66 percent made up of olfactory occluded fish. These two hatcheries accounted for nearly all of the olfactory occlusion returns from spawn-taking sites other than Spring Creek. Four were from Oxbow, and twenty-two from Cascade. The remaining two were from the Kalama hatchery about 90 miles downstream from Bonneville Dam.

Relation of Recovery Site to Fish Size and Release Time

Whether control fish returned to Spring Creek or were recovered from another spawning site was related to individual size and the period of release during the 18-day treatment interval. This is shown in figure 5 for the total of all control fish released in the experiment. From releases during the total 18 days, the return to Spring Creek was greater for fish over 32 inches in length. In releases of the first 8 days, all recoveries of larger fish were at Spring Creek. Despite a decline in the total recoveries from release days 9 to 13 and 14 to 18, the majority of the larger fish were recovered from Spring Creek. Fish under 32 inches, however, did not return as specifically. Some were recovered at alternate sites from all release intervals. In the last period, days 14 to 18, the majority of recoveries were from sites other than Spring Creek.

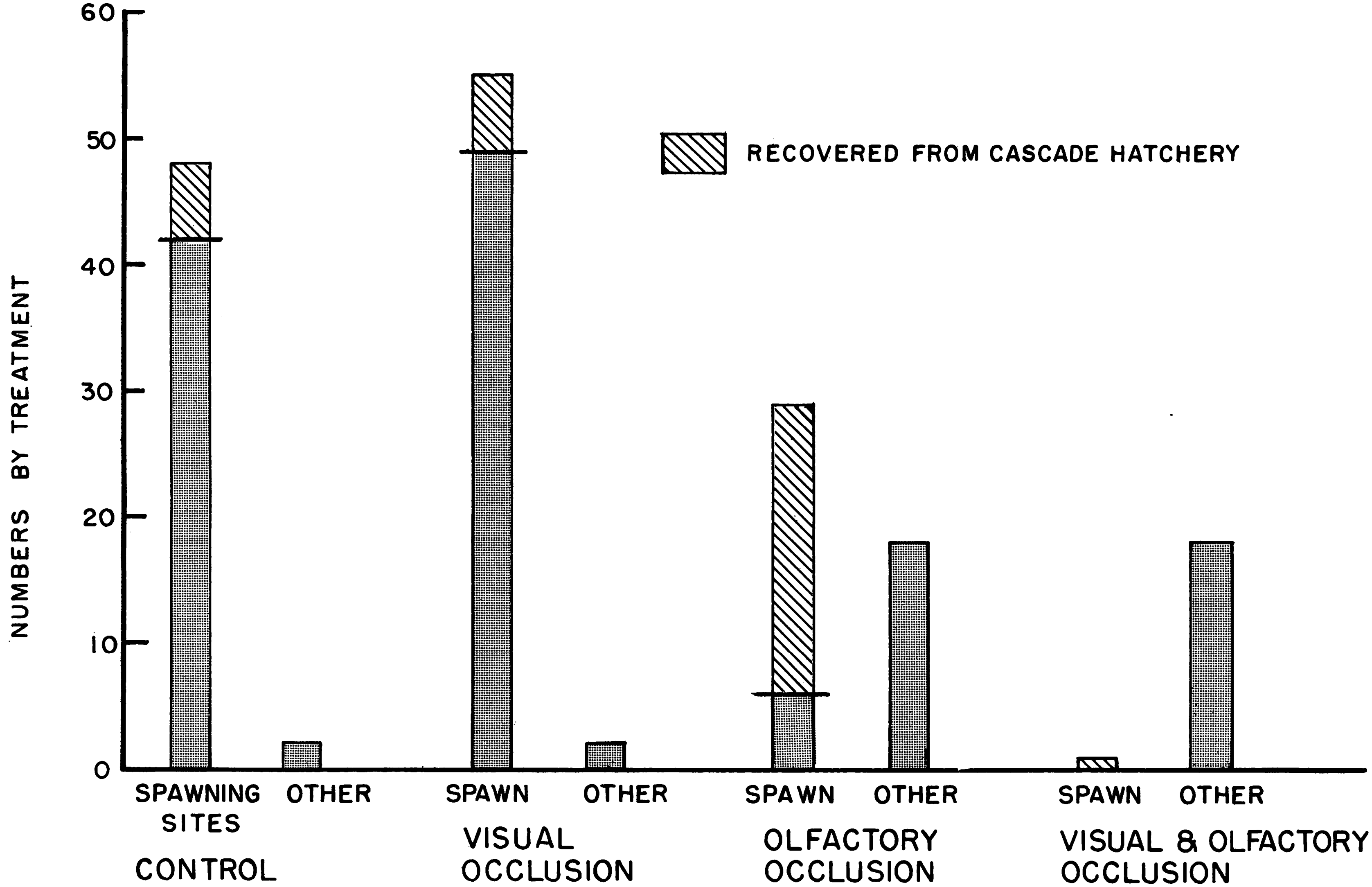

Figure 4.--Effect of treatment on type of recovery location for fish that did not return to Spring Creek. Recoveries from Cascade Hatchery show a disproportionate number of olfactory-occluded fish. Non-spawning recoveries were mostly from the commercial fishery below Bonneville.

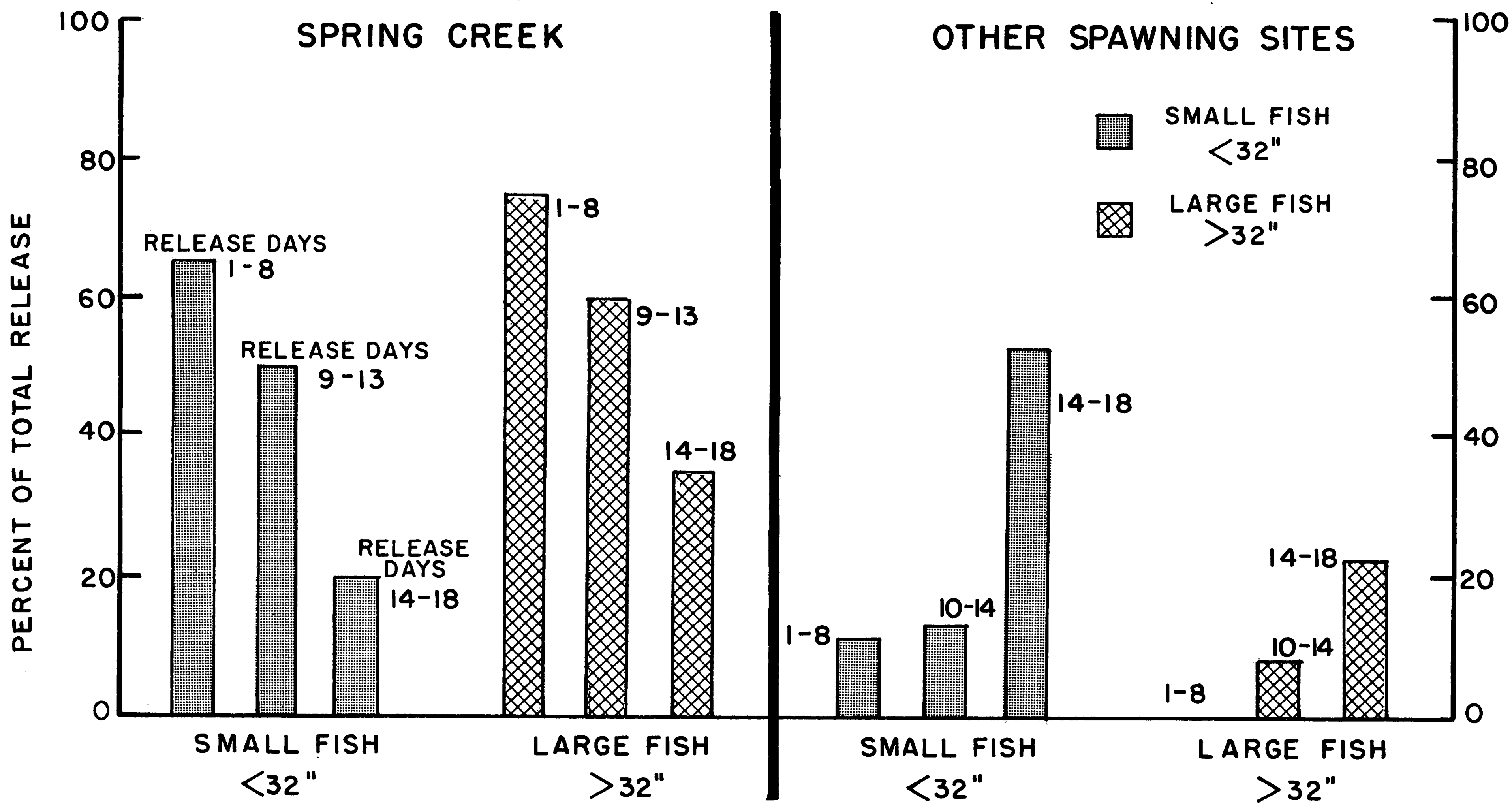

Figure 5.--Recovery pattern for untreated control fish. Fish size and period of release during the experiment affected whether fish returned to Spring Creek or were recovered at another spawning site.

DISCUSSION AND CONCLUSIONS

That final homing need not follow a fixed pathway was indicated by the numbers of control and visual occlusion fish returning from all release points. While such fish returning from upstream were proportionally fewer than from downstream releases, the absence of olfactory occlusion returnees from upriver suggests that some sort of odor recognition cued the unplugged fish back into Spring Creek. Perhaps as they moved down past the hatchery in a pattern of search, this cue was received. A search and return pattern from downstream is suggested by the proportionally larger but similar numbers of control and visual occlusion fish returning from each of the lower sites. Though the olfactory occlusion returnees may have reflected ineffective treatment, they also may have reflected the locations of the releases. All were from downstream, and six of the eight fish were from the north shore site on the same side of the river as Spring Creek.

The relatively constant ratios between control and visually occluded fish reappearing from each release suggest that sight played only a supporting role in the return. Though returning blinded fish were handicapped as shown by reduced numbers, delay, and a usually battered appearance on arrival, they did reappear in appreciable numbers. Sighted fish with blocked olfactory organs did not.

The primary directing role of olfaction in reattaining a spawning goal was indicated. However, the phasing appeared critical since time worked against the specificity of the response. Later in the experiment, when the fish were more mature, they increasingly appeared at the alternate spawning sites nearer to the release points. In part, this may have reflected diminishing physical capacities since smaller fish more often made such choices. Even in alternate spawning site choices, however, the importance of olfaction was apparent for when this sense was blocked, the fish evidently were less able to locate concentrations of potential spawners. In the one exception, where a significant number of olfactory occluded fish were recovered at the Cascade Hatchery, analysis indicated that this probably was related mostly to the location of this station with respect to the release points.

All the fish used in this test had once reached Spring Creek and the effect on return of this previous experience is not known. However, in view of the results, it seems highly probable that olfactory responses initially brought the fish into Spring Creek.

THRESHOLD OF PERCEPTION OF EUGENOL IN JUVENILE SOCKEYE SALMON

by

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September 1964

FISH-PASSAGE RESEARCH PROGRAM
U.S. Bureau of Commercial Fisheries
Seattle, Washington

INTRODUCTION

In order to evaluate the effect of environmental changes caused by high dams on the homing capabilities of salmon, a better understanding of the mechanisms of homing behavior is needed. Hasler and Wisby (1951) theorized that homing behavior of salmon during the later stages of migration is based on a response to the odor of the home stream. Field studies with both coho salmon (*Oncorhynchus kisutch*) (Wisby and Hasler, 1954) and chinook salmon (*O. tshawytscha*)1/ tend to support this theory. Hasler and Wisby suggested that it might be possible to condition young salmon to a synthetic odor substance and later decoy them to this odor when they return from the ocean to spawn. Such a procedure might have useful management applications in identifying and separating stocks of migrating adults. Prior to any investigation of this hypothesis, information is necessary concerning levels of sensitivity to odor compounds which might be used.

The chemical, eugenol, was selected for study since the minnow (*Phoximus laevis*) was found to detect its presence by means of the olfactory sense alone (Neurath, 1949). The threshold of perception of eugenol was determined for juvenile sockeye (*O. nerka*) in studies conducted at facilities of the Fish-Passage Research Program, Seattle, Washington. Results of this study are reported here.

MATERIALS AND METHODS

One hundred sockeye salmon obtained from the National Hatchery at Leavenworth, Washington, were held for 3 days before the beginning of the study in circular metal tanks, which were flushed with dechlorinated city water. During this period they were fed a diet of dry food pellets. The fish were 1 year old at the beginning of the study.

A modified form of the experimental setup used by Tarrant (1964) was employed (fig. 1). The 77-gallon plywood aquarium was flushed with dechlorinated city water at the rate of 117 gallons per hour. Water was introduced from the bottom

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1/ Groves, A. B., G. B. Collins, and P. S. Trefethen. Sensory factors in the homing of adult chinook salmon (*Oncorhynchus tshawytscha*). Manuscript in preparation.

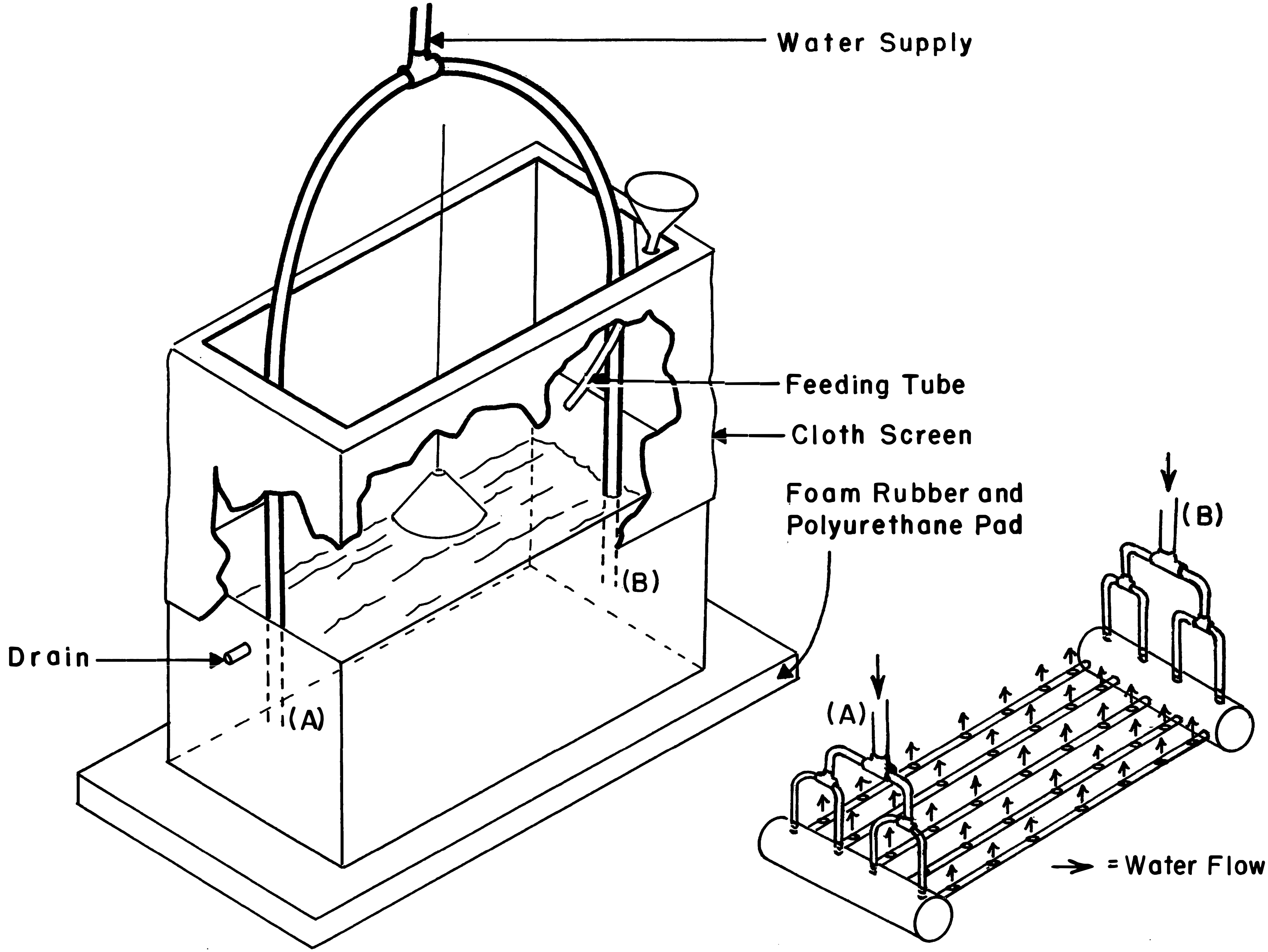

Figure 1.--Diagram of aquarium used for conditioning and testing juvenile sockeye salmon and plastic water diffuser for uniform dispersal of inflowing water. The water diffuser was located inside the aquarium.

of the aquarium through a plastic water diffuser (fig. 1) designed to disperse the water uniformly. Continuous illumination was provided by a 75-watt incandescent bulb placed 8 inches above the water surface, and undetected observation of the fish was possible from a darkened area above the light source.

Eugenol (only slightly soluble in water) was first dissolved in ethanol to facilitate uniform dispersal throughout the aquarium since ethanol mixes freely with both eugenol and water. Tests indicated that ethanol was not detected at the concentrations used in this study. Units of approximately 5 cc. of eugenol solution were injected through a serum stopper in the water supply line with a hypodermic syringe. A solution of methylene blue was injected periodically to determine the rate of mixing within the aquarium. Complete mixing occurred in an average time of 34 seconds after injection.

Forty seconds after the eugenol solution was injected, dry food pellets were dropped into the water through a tube above the aquarium (fig. 1). The fish fed actively at the water surface in the area where the food was introduced. After several associations the fish became conditioned to the chemical stimulus, which would then evoke a response prior to the introduction of food. The conditioned response consisted of increased activity followed by surface feeding behavior. Although the fish responded as a group, some individuals may have been cued by the movements of other fish.

The concentration of eugenol used in each test was calculated on the basis of the total volume of water in the aquarium. In order to insure complete flushing of the chemical from the aquarium, associations were limited to one a day. The fish were given 26 associations at a concentration of 2.50 p.p.m. followed by 34 associations at a concentration of 5.00 p.p.m. (The concentration was strengthened in order to hasten conditioning.) Concentrations were then attenuated in successive associations until the chemical stimulus no longer evoked a conditioned response. In this manner the threshold of perception of eugenol was determined. Ethanol, equal in volume to the test solution, was injected as a control before several of the associations. The sequence of associations and controls was varied.

RESULTS

Within 36 associations the conditioned response was well established, and after the 60th association attenuation of the concentration was begun. The fish responded to all concentrations of eugenol greater than 2.20 p;p.m. in less than 40 seconds after injection. The time required to respond increased at lower concentrations (table 1), and reinforcement of the response was delayed proportionately. If no surface feeding behavior occurred within 180 seconds after injection of the chemical, food was introduced, and the response was considered negative. At concentrations below 0.20 p.p.m., 240 seconds were allowed before the introduction of the food.

All concentrations of 0.32 p.p.m. or greater evoked a positive response. The percentage of positive responses decreased rapidly at lower concentrations (table 2). At 0.20 p.p.m. conditioned responses were generally weaker and more delayed than at stronger concentrations. Responses at 0.18 p.p.m. were negative with the exception of one weak response occurring 200 seconds after injection of the chemical. In tests at 0.16 p.p.m. there were no positive responses or any indication that the chemical was detected.

The fish were again tested with a more concentrated solution of eugenol, which evoked a strong conditioned response. The concentration was attenuated to 0.16 p.p.m., and the fish failed to respond at this level. This procedure was repeated twice with identical results.

In 32 control tests with ethanol there were 30 negative responses (table 2). The two positive responses in the control tests occurred after a period in which tests were run at approximately the same time each day for several weeks. Greater care was then taken in varying the time of day at which the tests were run, and no further positive control responses occurred.

DISCUSSION

Concentrations of eugenol used in the study were calculated on the basis of the total volume of water in the aquarium. Since concentrated solutions were introduced with the water supply and were dispersed throughout the aquarium, there is some question whether the fish may have responded to a stronger solution than the calculated concentration. However, the time from injection of the chemical until the fish responded (table 1) always exceeded the average time required for complete mixing within the aquarium. At 0.18 p.p.m. the response time exceeded the time required for mixing by a factor of six.

Below 2.30 p.p.m. the response time increased in geometric progression as the concentration of the chemical was

Table 1.--Average response times of a group of 100 juvenile sockeye salmon[1] at different concentrations of eugenol.

Concentration[2] (p.p.m.)	Number of tests	Average time from injection of eugenol until conditioned response occurred (seconds)
4.50 - 5.00	2	35
3.50 - 4.00	2	35
2.50 - 3.00	5	35
2.00 - 2.40	9	44
1.50 - 1.90	6	48
1.00 - 1.40	5	60
0.50 - 0.90	9	81
0.36 - 0.40	13	113
0.30 - 0.34	11	115
0.24 - 0.28	18	114
0.18 - 0.22	16	128

1/ Prior to the tests the fish had received 60 associations of eugenol with food.

2/ Between 2.50 and 5.00 p.p.m. the concentration was reduced by 0.50 p.p.m. at each attenuation step. Between 0.50 and 2.40 p.p.m. the concentration was reduced by 0.10 p.p.m. at each attenuation step. Between 0.18 and 0.40 p.p.m. the concentration was reduced by 0.02 p.p.m. at each attenuation step.

Table 2.--Responses of a group of 100 juvenile sockeye salmon[1/] in tests with eugenol[2/] and control tests with ethanol[3/].

	Eugenol			Control		
Concentration[4/] (p.p.m.)	No. of tests	No. of positive responses	No. of negative responses	No. of tests	No. of positive responses	No. of negative responses
4.50 - 5.00	2	2	0	1	0	1
3.50 - 4.00	2	2	0	-	-	-
2.50 - 3.00	5	5	0	4	1	3
2.00 - 2.40	9	9	0	1	0	1
1.50 - 1.90	6	6	0	1	0	1
1.00 - 1.40	5	5	0	1	0	1
0.50 - 0.90	9	9	0	1	0	1
0.36 - 0.40	13	13	0	1	0	1
0.30 - 0.34	13	11	2	3	0	3
0.24 - 0.28	23	18	5	7	1	6
0.16 - 0.22	31	16	15	12	0	12

1/ Prior to the tests the fish had received 60 associations of eugenol with food.

2/ Eugenol in solution with ethanol was made up to a volume of approximately 5 cc. for each test.

3/ In each control test ethanol was equal in volume to the eugenol solution in the following test.

4/ Between 2.50 and 5.00 p.p.m. the concentration was reduced by 0.50 p.p.m. at each attenuation step. Between 0.50 and 2.40 p.p.m. the concentration was reduced by 0.10 p.p.m. at each attenuation step. Between 0.16 and 0.40 p.p.m. the concentration was reduced by 0.02 p.p.m. at each attenuation step.

decreased geometrically. It has long been held that, as a stimulus decreases geometrically, the intensity of the sensation decreases arithmetically (Fechner's Law). Perhaps then there is a relationship between the concentration of the chemical, the intensity of the sensation, and the time required for the fish to respond.

The results of the tests indicated that the perceptual threshold for eugenol in sockeye salmon of this age was no lower than 0.18 p.p.m. Neurath (1949) using conditioned responses found that the threshold for eugenol in Phoxinus laevis was 0.06 p.p.m. Juvenile sockeye have been found to react to natural food extracts at 0.01 p.p.m. (McBride et al., 1962), and young coho salmon were reported by Wisby[2/] to detect morpholine at 0.000001 p.p.m. In these two studies a conditioned response technique was not employed.

Although no direct evidence was obtained that juvenile sockeye detected eugenol by olfaction, Phoxinus laevis was able to detect this chemical by the olfactory sense alone (Neurath, 1949). It seems likely that the responses of salmon to eugenol may also be mediated through this sensory system.

In view of the relatively high threshold concentration at which juvenile sockeye were found to detect eugenol, the suitability of this chemical for testing the conditioning and decoying technique suggested by Hasler and Wisby is questionable. Conditioning young salmon to eugenol solution in hatchery raceways might be practical. In small streams the amount of chemical required to condition young sockeye would tend to make the cost of prolonged application prohibitive.

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AN ATTEMPT TO SEGREGATE A SPECIFIC RACE OF CHINOOK SALMON (*Oncorhynchus tshawytscha*) BASED UPON RESPONSE TO HOMESTREAM WATER

by

Charles R. Weaver

September 1964

FISH-PASSAGE RESEARCH PROGRAM
U.S. Bureau of Commercial Fisheries
Seattle, Washington

INTRODUCTION

The construction of dams on the Columbia River and its tributaries has often resulted in physical and environmental changes detrimental to specific races of anadromous fishes. Not infrequently, relocation or artificial propagation programs are required to perpetuate these races. One of the basic problems encountered in such programs is that of fish collection. In some instances, this is merely a matter of trapping the fish as they arrive at the dam, and in others, several races may be involved. Some of these races may be destined for unaffected spawning areas, whereas others may be bound for areas that would be cut off from production. Thus, the problem of segregation arises.

The specific race or races must be identified and collected while the others are permitted to continue upstream. Although time of arrival and external physical characteristics can be used to differentiate races in some instances, more precise methods may be necessary with the increasing complexity of the "fish vs. dam" problem. The object of this study was to investigate the possibility of segregating races of migrating salmon based upon response to homestream water. Chinook salmon (*Oncorhynchus tshawytscha*) were used in the study.

METHODS AND MATERIALS

Our approach was based upon the hypothesis that chinook salmon "home" upon some distinctive quality in the water imparted from their parent stream. If presented with a choice of flows of differing quality, they would select the one having the greater concentration of their homestream water. In these experiments, we examined the response of a specific race of chinook salmon to such a choice. The chinook race studied was the one destined for Spring Creek Hatchery, approximately 25 miles above Bonneville Dam.

Two types of experiments were planned. In the first type, fish ascending the Washington-shore fish ladder were routed through the laboratory where they were offered a choice of entering one of five channels. During control tests, the flow velocity and composition of the water in the channels were identical. Under the test condition, a relatively small quantity of Spring Creek water was introduced into one of the channels. Since the racial composition of these fish was unknown, the response of the Spring Creek fish to the choice condition had to be determined by a tag-recovery method. After making a choice, all fish were tagged and released; the response of the Spring Creek fish to the choice array was determined from the tagged fish arriving at the hatchery.

The second type of experiment utilized only Spring Creek fish and was conducted after the foregoing series was completed. In these tests, surplus male salmon were transported directly from Spring Creek Hatchery in tank trucks, subjected to the choice array, and then returned to the hatchery.

The experimental area of the laboratory consisted of a choice area, 11.5 feet wide by 31.5 feet long--terminating at the upstream end into five identical channels, each 27 inches wide and 20 feet long (fig. 1). Channel walls were thoroughly sealed to prevent seepage into or from adjoining areas. Each channel led to a 40-foot long holding pen. Water was introduced at the upstream end of the laboratory, passed through the holding area, and then flowed over weirs into each channel. The water was approximately 2.5 feet deep in the holding pens and 1.8 feet deep in the channels and choice area. Velocities in the channels and choice area were approximately 1 foot per second; flow in each channel was approximately 3.6 cubic feet per second.

Spring Creek water was transported from the hatchery in two 1,000-gallon tank trucks. Water from the trucks entered the laboratory by gravity flow through a 1½-inch plastic pipe. Flows were introduced into the test channel (either #3 or #4, fig. 1) through a length of perforated pipe extending across the width of the channel just above the water surface. The spray from the pipe was directed into the weir overfall to provide thorough mixing with the river water entering the channel (fig. 1). Spring Creek water was metered into the channel at a rate of 10 to 11 gallons per minute, which was the maximum sustained flow that could be maintained with two trucks in operation (one truck on the road while the other was discharging at the laboratory). At Spring Creek, the discharge from the hatchery is approximately 10 c.f.s. Assuming this water is thoroughly mixed with the Columbia River (90,000 c.f.s.) water when it reaches Bonneville Dam, the above metering rate would provide a concentration of Spring Creek water in the test channel over 65 times that in the other channels.

Fish were introduced into the system individually, each fish being allowed to select a channel and enter the holding pen before another one was released. The holding pens were covered with floating panels, and the surrounding area was darkened to keep the fish in a quiescent state until they were tagged at the end of the day. A drop screen at the downstream end of each holding pen prevented fish from falling back downstream into the channels. These screens were raised momentarily as the fish ascended from the channels to the holding pen (fig. 1).

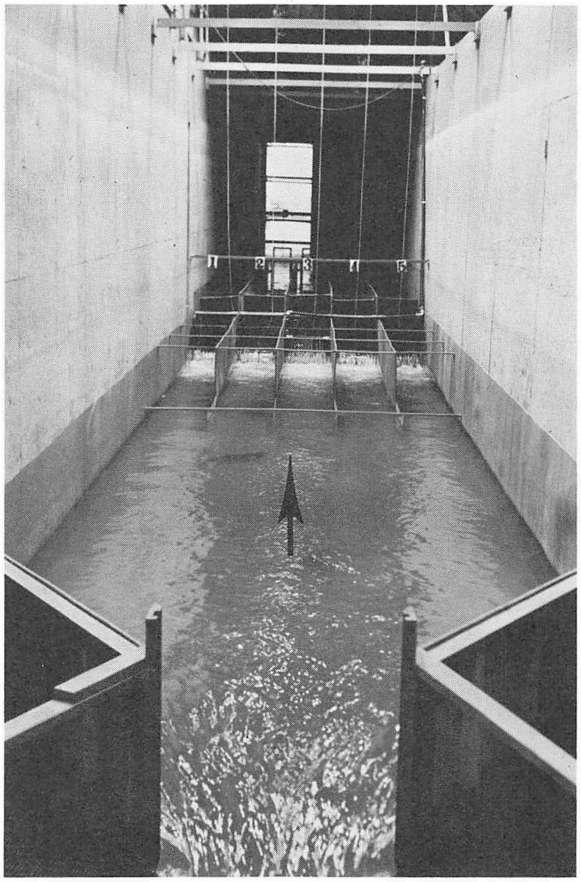

Figure 1.--View of choice area, channels, and holding pens (in darkened area) with Spring Creek water being introduced into channel #3. Note chinook salmon just below entrance to channel #2. Vertical ropes in background are used to operate drop screens at entrance to holding compartment.

Tagging was generally done with a four-man crew (fig. 2). One man crowded the fish from the holding pen into the tagging box; another held the fish while it was being tagged, and two men performed the tagging alternately. Fish were tagged with a small nylon-dart and vinyl-tubing tag approximately 2-3/4 inches long (fig. 3). The tag was inserted with a stainless steel applicator on the right side of the fish just below the dorsal fin; tag penetration was controlled by a set screw on the plastic handle holding the applicator.

Handling of fish during tagging was kept to a minimum by use of the tagging box and submersible holding trough. The tagging box was suspended from tracks and could be moved from pen to pen. In operation, the foam-padded trough was lowered to the bottom of the box, the holding pen gate was opened, and the fish was forced to swim into the box. The gate was then closed, and the trough holding the fish was raised out of the water by use of rope tackle and suspended from hooks while the fish was tagged. Trough and fish were then lowered back into the water, the upstream gate of the tagging box was opened, and the fish swam out and was free to leave the laboratory and continue its migration. Each fish was generally out of the water no more than 15 to 16 seconds.

RESULTS AND CONCLUSIONS

A total of 13 tests were conducted. Spring Creek water was added to channel #3 in four tests and to channel #4 in four tests. The remaining five tests were control trials in which no Spring Creek water was added. A total of 565 chinook were subjected to the choice condition and subsequently tagged and released. Approximately 25 percent (143) of the tags were recovered (table 1). The three major recovery sites were: (1) Spring Creek Hatchery, (2) the Indian fishery between Bonneville Dam and The Dalles, and (3) the Oxbow Hatchery (Fish Commission of Oregon). The most distant recoveries were the two fish recovered at Oxbow Dam on the Snake River. One of these had traveled the 450 miles in 23 days at an average rate of 19.6 miles per day. Another fish recovered at Rocky Reach Dam on the Columbia River, had traveled approximately 330 miles in 22 days--an average rate of 15 miles per day.

Of the 42 fish recovered at Spring Creek Hatchery, 17 were tagged during control tests, 15 were tagged during tests in which Spring Creek water was introduced into channel #3, and 10 were tagged when the test water was introduced into channel #4. A comparison of the response of the fish during test and control

Figure 2.--Tagging chinook salmon at the upstream end of the holding pen. Man in holding pen (extreme left) crowds fish into tagging trough after it is submersed in the box.

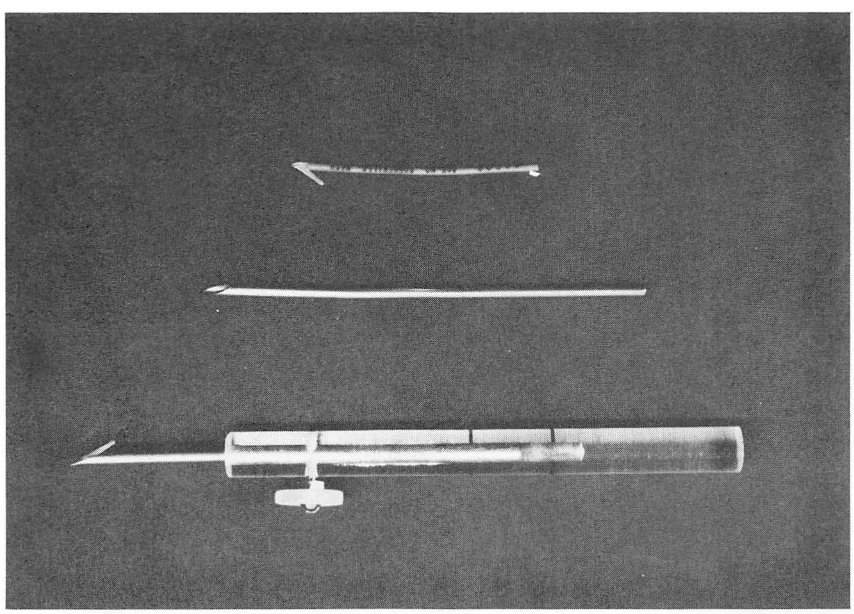

Figure 3.--Photograph of tagging equipment. From bottom to top are: Dart tag, applicator, and the two inserted into the plastic handle. Distance between the two marks on the plastic handle is 1 inch.

Table 1.--Daily record of chinook salmon tagged from August 31 to September 12 at Bonneville Dam and number recovered from each day's tagging.

Location of recovery	Aug. 31	September 1	2	3	4	5	6	7	8	9	10	11	12	Total
							Number tagged							
	27	23	30	47	35	48	53	42	49	49	34	65	63	565
							Number recovered							
Spring Creek Hatchery		2	3	2	5	1	5	5	3	3	3	3	7	42**
Indian Fishery*	1		1	2	1	3	3	3	6	4	2	1	6	33
Oxbow Hatchery		1	1	1	1	6	4	2	4	2	1	6	2	31
Priest Rapids Dam	2			2		1	2	1			1			9
Klickitat Hatchery-River			1		2		1			1	1	1	2	9
Little White Hatchery				3	1					1		1	1	7
Cascade Hatchery								1					3	4
McNary Dam					1	1								2
Oxbow Dam				1									1	2
Big White Salmon River			1											1
Deschutes River								1						1
Rocky Reach Dam							1							1
Total	3	3	7	11	11	12	16	13	13	11	8	12	22	142

* Between Bonneville Dam and The Dalles.

** Does not include one fish that was sighted but not recovered.

conditions (fig. 4) indicates that Spring Creek chinook salmon were not attracted to either channel #3 or #4 when Spring Creek water was introduced at the rate of 10 to 11 g.p.m.

The results of these tests do not necessarily discount the possibility that racial segregation can be accomplished at some point below the spawning grounds by attraction to homestream water. Several factors may have contributed to the inconclusive results of this exploratory study. Among these are the concentration of source water used, the effect of hauling and piping on the quality of the test water, and the specific origin of the test water. The water supply for Spring Creek Hatchery is a composite of at least five springs in the same general area. Water used in these experiments (with the exception of one test) was obtained only from the largest spring, which furnishes a significant proportion (possibly 50 percent) of the hatchery supply. This source was utilized because it afforded a convenient supply of gravity-fed water which would remain of uniform quality (unaffected by the odors which might be imparted by fish-handling procedures at the hatchery) throughout the series of experiments. Although it appears unlikely, it is possible that the homing quality of the Spring Creek water may be imparted by one or several of the smaller springs and thus was not contained in the water utilized in the experiments.

In the tests with surplus male salmon from Spring Creek Hatchery, an effort was made to compare the response of Spring Creek fish to (1) water taken from the entrance of the hatchery pond (mixture of all water sources) and to (2) water from the large spring utilized in the previous tests. These tests were, however, largely unsuccessful due to the reluctance of the fish to enter the choice area and channels after being transported from the hatchery. With the few fish tested, there did not seem to be an apparent difference in the response to the two water sources.

Summarizing, Spring Creek chinook salmon were not attracted to a supply of source water that was transported and discharged at dilute concentrations into a test area some 25 miles downstream of the spawning area. If additional studies of this design are proposed, special consideration should be given to techniques used and also to the effect of water temperature.

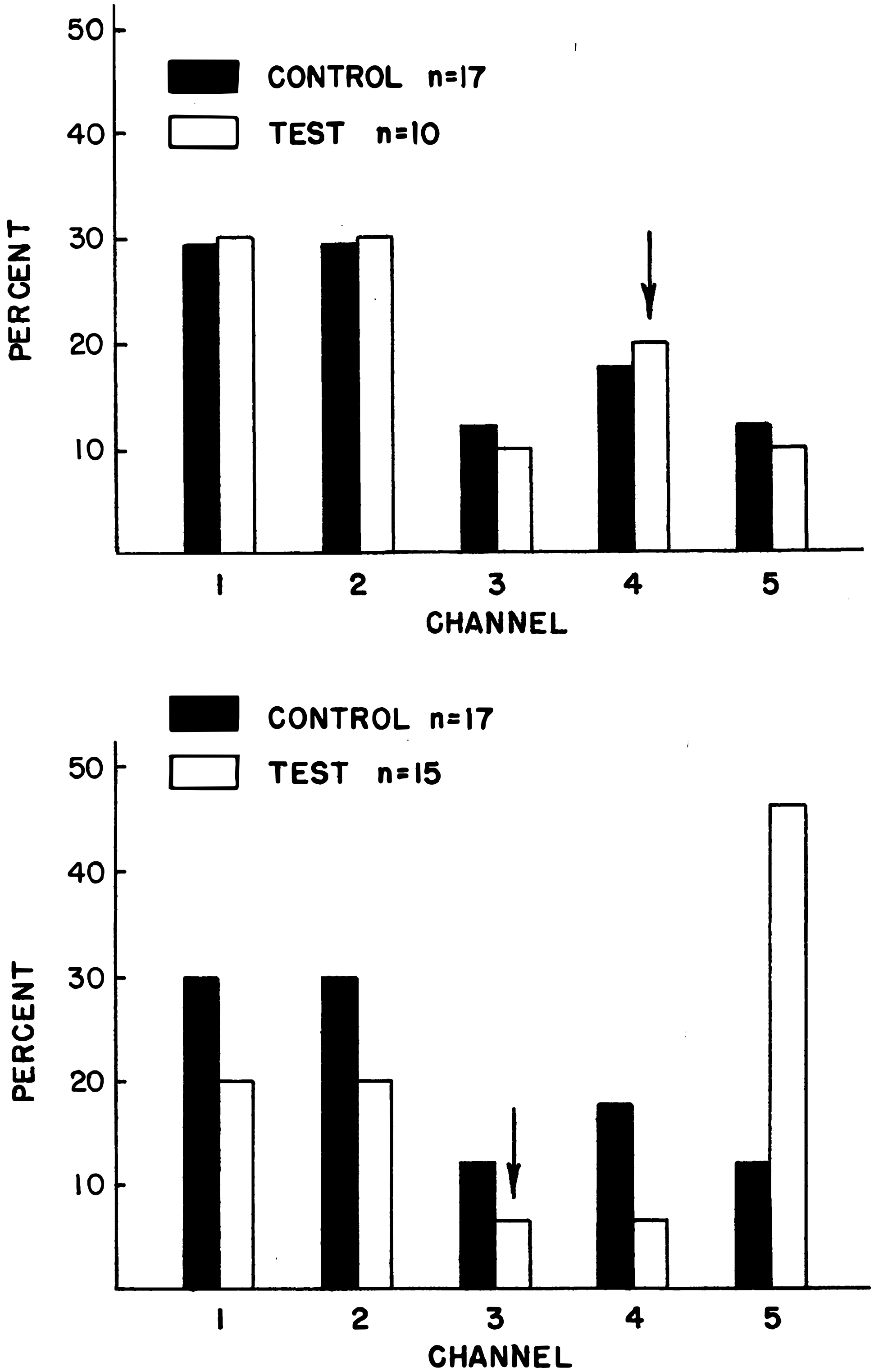

Figure 4.-- Results of tests at Bonneville on the response (proportions entering each channel) of Spring Creek chinook salmon to home stream water. Arrows indicate channel carrying added amounts of Spring Creek water under test conditions.

ACTIVITY CYCLES OF JUVENILE SALMON

by

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September 1964

FISH-PASSAGE RESEARCH PROGRAM
U.S. Bureau of Commercial Fisheries
Seattle, Washington

INTRODUCTION

A need for information concerning the cyclic activity of fishes becomes evident when attempting to interpret basic behavioral differences between juvenile salmon species. These behavioral variations should be considered when predicting or analyzing fish movement under various conditions; e.g., (1) through impoundments, (2) in the vicinity of dams, and (3) into traps and bypass systems.

Several methods of detecting and recording fish movement (activity) have been developed for use under laboratory conditions. Direct visual observation was probably the first method used. Spencer (1929, 1939) reported using a thread or string attachment between fish and recording device. The first electrical system was reported by Spoor (1941). He used a paddle, which when deflected by water currents caused by fish movement, made electrical contact providing a signal that could be recorded.

This paper reports a recently developed method of activity detection which provides a continuous graphic record for periods of a week or longer, and requires a minimum amount of attention by the operator. To test this system, a series of observations using three species of salmon fingerling--sockeye (*Oncorhynchus nerka*), silver (*O. kisutch*), and chinook (*O. tshawytscha*)--was undertaken at the Behavior Laboratory, Fish-Passage Research Program, Seattle, Washington.

MATERIALS AND METHODS

The activity detector was mounted in the fish chamber of a flow-through water system (fig. 1). The detecting device is a silicon crystal strain gage with a high degree of sensitivity and is available through commercial channels. The gage element, or sensor, was mounted on a 1-inch by 6-inch by .004-inch stainless steel paddle. The electrical resistance of the sensor changes when it is flexed or compressed. The gage element is in series with a 12-volt battery and a fixed resistor. The flexing and compressing of the paddle by water currents (caused by fish movement) changes the resistance of the gage and a voltage variation proportional to this change is produced across the fixed resistor. A capacitor blocks the fixed battery potential and passes the current variations as an alternating current signal to the recorder. A resistor across the recorder input provides a ground return for the recorder preventing pickup of external signals.

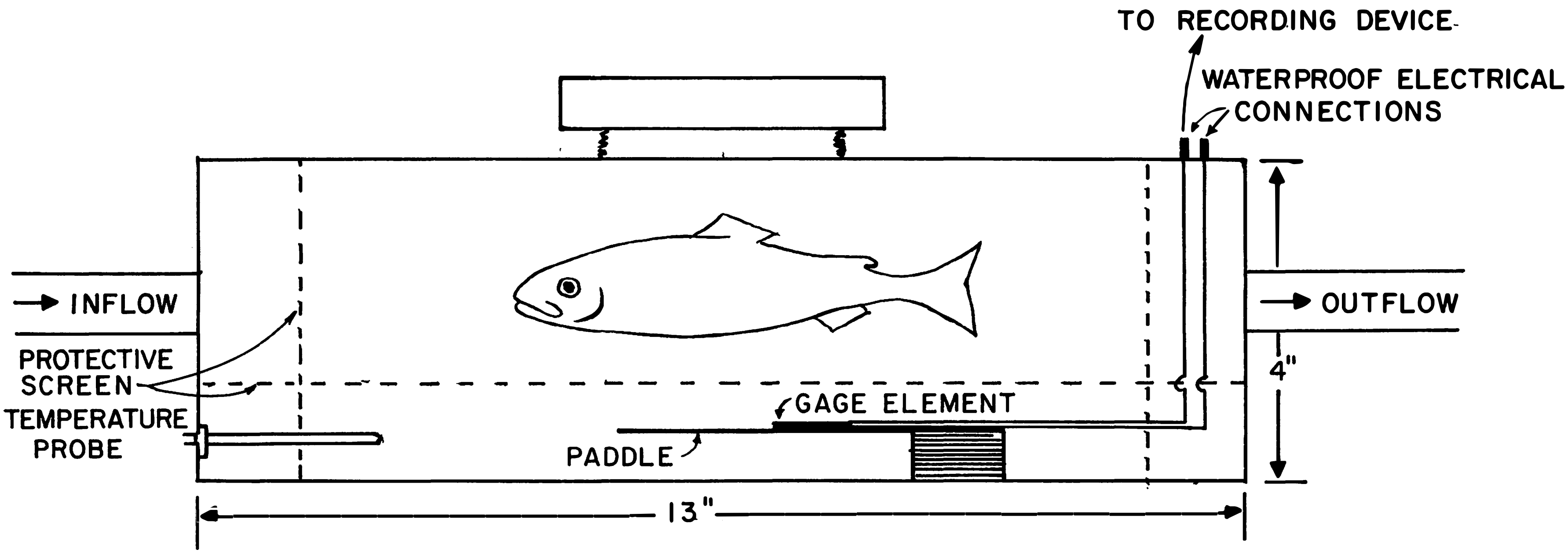

Figure 1.--Fish chamber of flow-through water system used in conjunction with activity detector.

The electrical signal is amplified and recorded at a position remote from the fish chamber. The amplifier used was a Sanborn Model 64-300A, and the recorder, a Sanborn Model 60-1300 Twin Viso. The gear drive mechanism was altered to provide a 6-inch per hour chart speed.

The fish used were selected randomly from populations maintained in an outside holding facility. The fish were assumed to be responsive to the daily fluctuations of the outside holding environment.

Prior to being placed into the chamber the fish were anesthetized with MS-222. After placing the anesthetized fish into the chamber, the water flow was adjusted and the chamber covered to provide a constant darkened condition. Precise temperature control allowed the chamber water temperature to be held constant at the level of the outside holding temperature. The chamber was also isolated from vibration and shock. After all adjustments were made the associated detection equipment was put into operation. The two-channel amplifier and recorder allowed the observations to be made as replicates using two chambers and detectors.

RESULTS AND DISCUSSION

Of 27 sockeye tested 24 or 90 percent demonstrated a period of evening activity (fig. 2). Observation periods ranged from 24 to 192 hours (1 to 8 days). 70 percent of the juvenile sockeye tested for longer than one 24-hour period repeated their period of activity one or more times. The replication occurred even though the observations were made under darkened conditions.

Szymanski (1914, 1919), a pioneer in the field of measuring general activity, concludes that activity rhythms of various animals tend to fall into two general classes. One group includes those organisms that demonstrate one active period in a 24-hour period, which he designates as monophasic. Animals exhibiting more than one active period, alternating with periods of rest, he terms polyphasic. The juvenile salmon used in these observations generally demonstrated activity of a monophasic nature.

Johnson (1926), by prolonged observations, has demonstrated that an innate monophasic rhythm persisted even in total darkness. Other experiments have demonstrated the persistence of activity patterns over considerable periods of time and under constant light and temperature conditions.

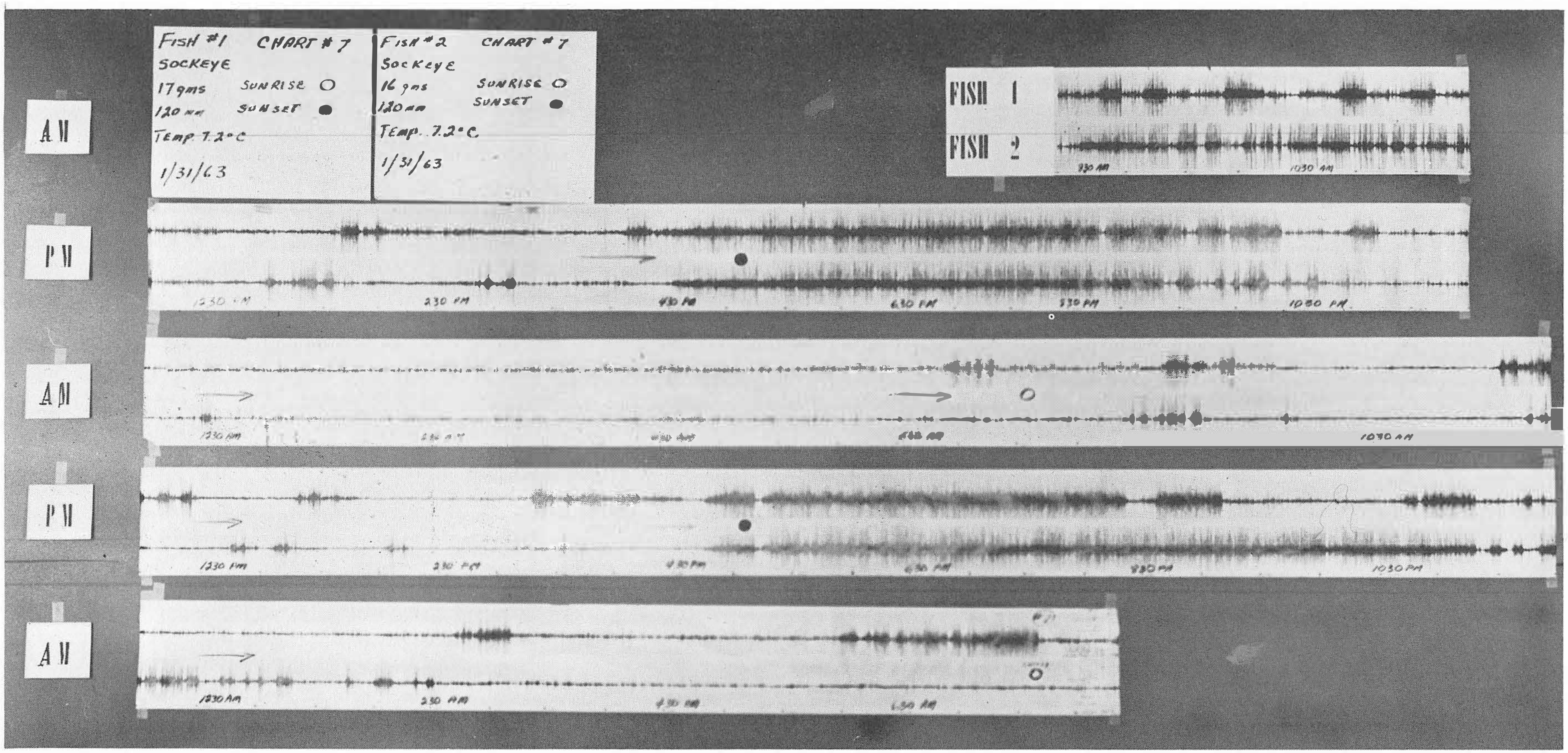

Figure 2.--Strip chart showing cyclic activity pattern of two juvenile sockeye salmon held in paired but separate chambers.

Twenty-four silver salmon were tested and 78 percent of these fish demonstrated approximately the same pattern as the sockeye, but greater individual variation. The 18 chinook tested exhibited activity of a sporadic nature with the active periods more concentrated during the late evening and early morning hours (fig. 3).

The data collected to date indicate that there are activity cycles within the behavioral patterns of juvenile salmon. The information also indicates, to some degree, that there are species differences.

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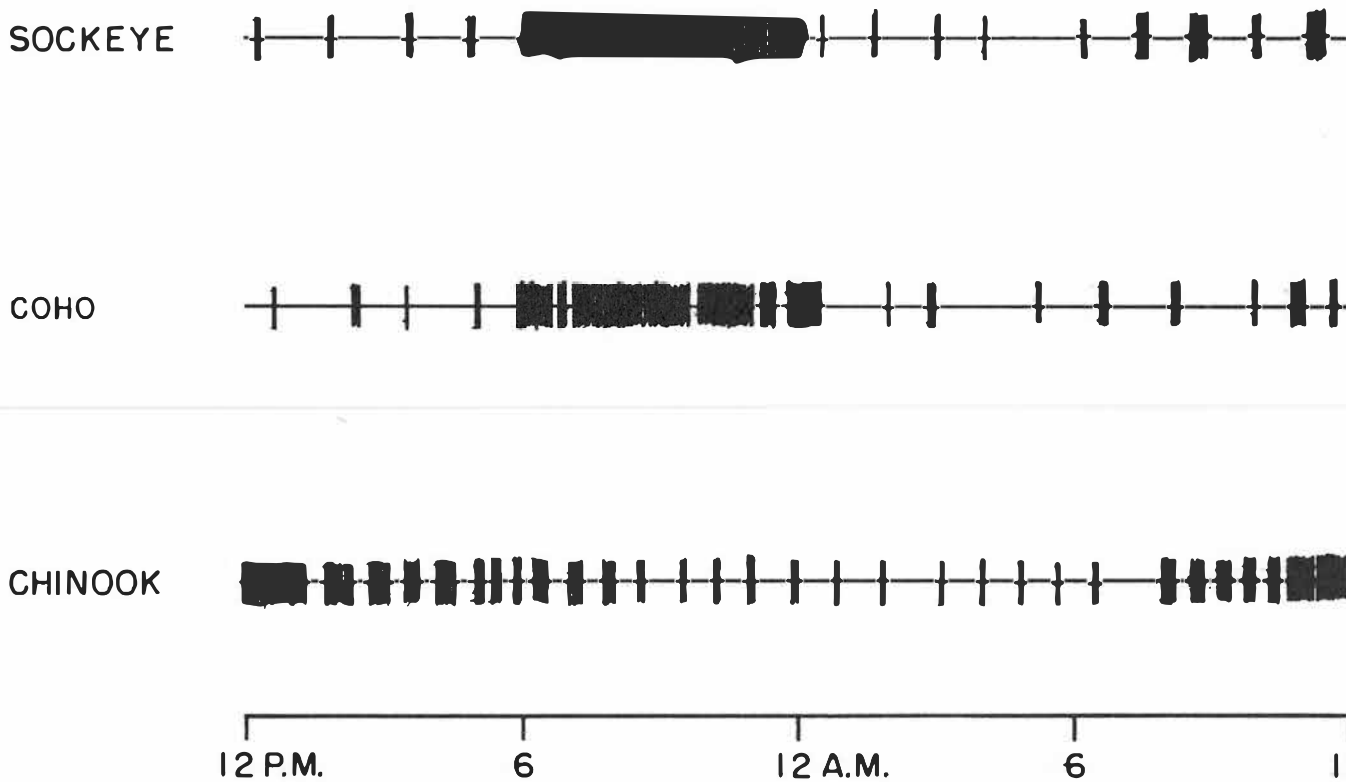

Figure 3.--Diagrammatic representation of strip chart recordings of three species of juvenile salmon, denoting time and pattern of concentrated activity.

EFFECTS OF WATER TEMPERATURE ON SWIMMING PERFORMANCE OF FINGERLING SOCKEYE SALMON (SUMMARY)

by

Alan B. Groves

October 1964

FISH-PASSAGE RESEARCH PROGRAM
U.S. Bureau of Commercial Fisheries
Seattle, Washington

SUMMARY

Relationships between water temperature and fingerling swimming ability may directly influence the effectiveness of diversion or guiding measures that are dependant upon the swimming ability of fish. The purpose of the following experiment was to assess the effects of water temperature on maximal swimming abilities of fingerling sockeye. The tests, conducted in the Behavior Laboratory of the Fish-Passage Research Program, consisted of swimming performance trials of individual fish acclimated to various temperature levels.

The swimming test device was a 50-foot U-shaped metal channel, 3 inches wide and 5 inches deep, through which 2 to 3 inches of water was circulated. Velocity in the channel was adjusted by varying the slope.

Fish tested were fingerling sockeye salmon (*Oncorhynchus nerka*) from the National Hatchery at Leavenworth, Washington. Their average length was 120 millimeters.

Groups of approximately 100 fish each were acclimated respectively to temperature levels of 35°, 41°, 50°, 59°, and 68° F. When tested, the fish had been acclimated for a minimum of 1 month in excess of the periods prescribed by Brett (1952). During trials, water temperature in the channel was regulated to the level of the acclimated test group. Short term, absolute capacities were assessed in the testing. The basic measure was the total distance that a fish could gain against a water velocity which forced a maximal effort. Performances of individual fish were measured in all instances.

Fifty fish from each acclimation group were tested at the same water velocity. For fish at each thermal level, the average distance gained against a 4-foot-per-second velocity in the channel is shown in figure 1. The curve indicates that temperature incrementally affected swimming performances up to 59° F. Above this level, performances declined. This is in agreement with a similar thermal effect noted by Brett (1958) in measuring cruising speeds of juvenile sockeye salmon.

In terms of distance gained, the largest performance difference shows over the 6 degrees between 35° and 41° F. The difference demonstrated over this interval equalled that shown over the 18 degrees between 41° and 59° F. The smallest performance difference was indicated between 50° and 59° F. To relate this to a louver, for example, the distance a fish guided across the structure could be contingent on the water temperature in relation to the swimming effort required.

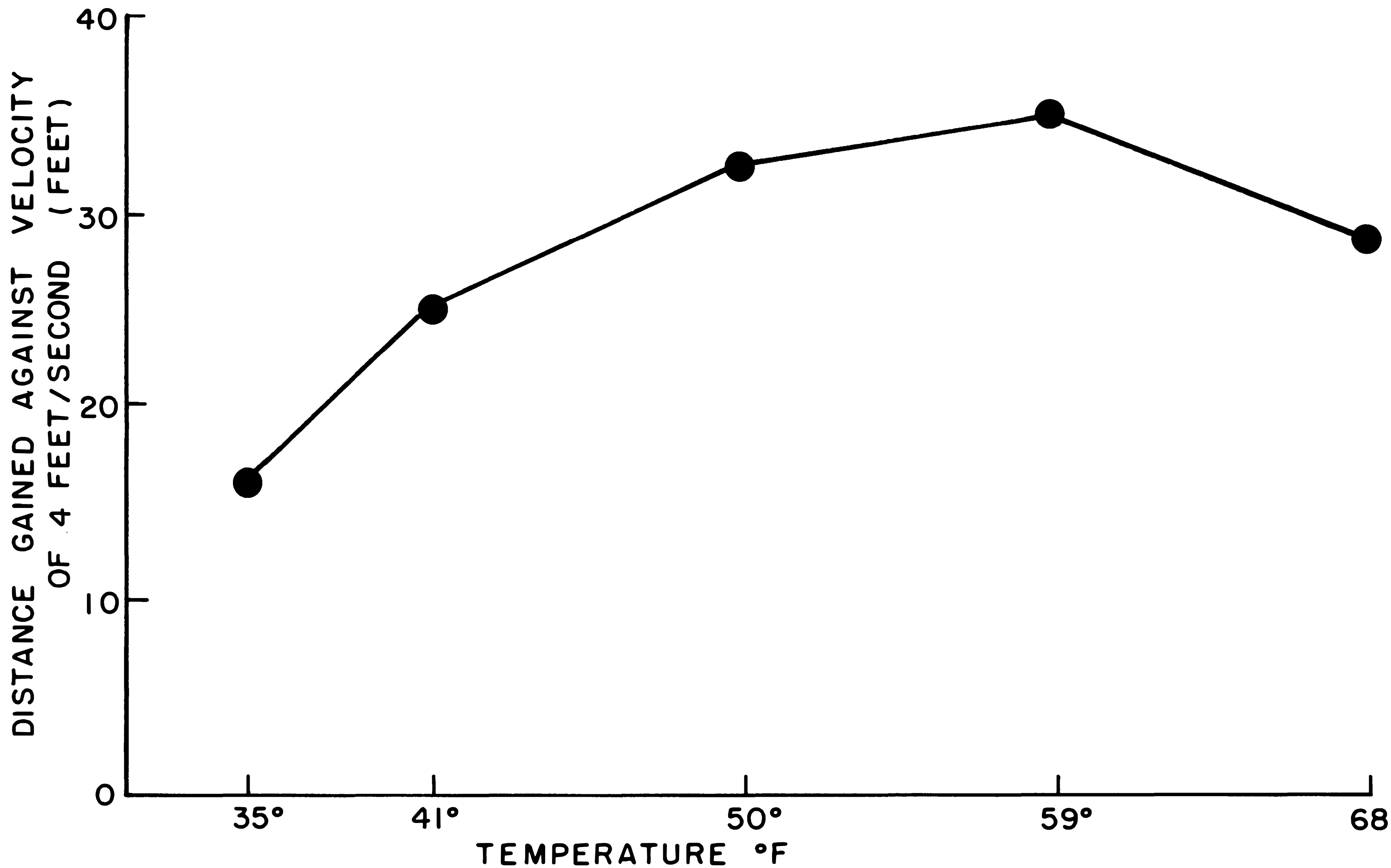

Figure 1.--Effect of water temperature on maximal swimming efforts of fingerling sockeye salmon. Points represent the averages of individual swimming trials of 50 fish acclimated and tested at the indicated temperature levels.

These results, from one species and size range, suggest that the efficiencies of fingerling diversion devices could be appreciably affected by water temperature, especially if the fish must make a strong swimming effort to negotiate a particular distance.

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EFFECTS OF WATER TEMPERATURE ON SWIMMING PERFORMANCE OF ADULT CHINOOK SALMON (SUMMARY)

by

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October 1964

FISH-PASSAGE RESEARCH PROGRAM
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Seattle, Washington

INTRODUCTION

It is known that swimming performance abilities of adult salmon may vary during migration. Paulik and DeLacy (1958) noted a decline in swimming capacities of sockeye measured at successive upstream points in the Columbia River which they related to effects of sexual maturity. Fishway swimming tests at Bonneville (Collins et al 1963) suggested that at this period in their migration salmon could climb extremely high ladders without becoming exhausted. In the Bonneville tests the performance ability was rated on the basis of the willingness or volitional tendency of fish to ascend the test fishways--a paced type of performance. The measures of Paulik and DeLacy were different. They evaluated the maximal or absolute abilities of fish by forcing them to swim against strong water velocities.

While such measures each apply to aspects of fish passage performance, additional information is needed to relate them. For example, do the absolute capacity measures apply directly as an index of fish passage capabilities? How may the results from the apparently tireless volitional performances observed in the tests at Bonneville be extrapolated to situations where sexual maturity is a factor? Also, how may water temperature variation pertain to these performance abilities?

To attempt to examine such relationships, tests of adult chinook swimming performances have been conducted on the lower Columbia River near Bonneville Dam. This has entailed measurements of absolute and volitional swimming performances of spring-run and fall-run fish at varied water temperatures.

MATERIALS AND METHODS

The Facility

The testing facility, installed at North Bonneville, Washington, was a self-contained recirculating hydraulic system designed especially to examine effects of water temperature variation on adult salmon swimming abilities. Actual testing components were a straight velocity flume, the same one used by Paulik and DeLacy, where absolute performances were measured (figs. 1 and 2); and a circular four-pool endless fishway, where volitional performances were evaluated (figs. 3 and 4). Each testing component could be supplied with water recirculated from either of two reservoirs maintained at different temperature levels. These also supplied the tanks in which the fish were held. The volume of each water mass was about 12,000 gallons.

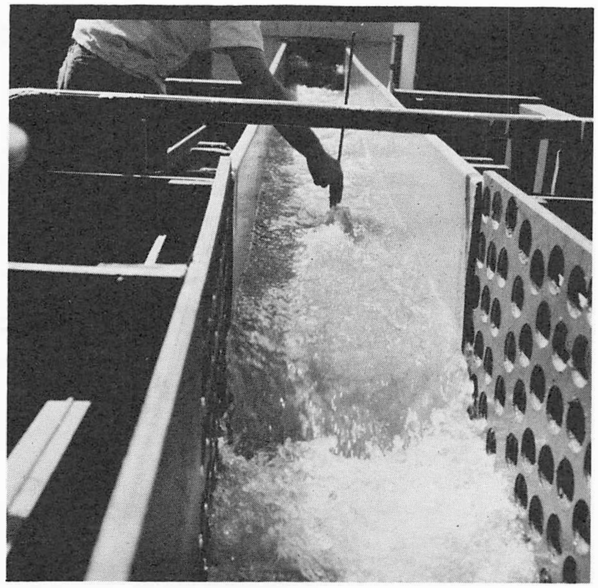

Figure 1.--View of swimming flume from the lower end. The channel is 20 feet long and 18 inches wide with a U-shaped bottom. In all testing the velocity was 7 feet per second.

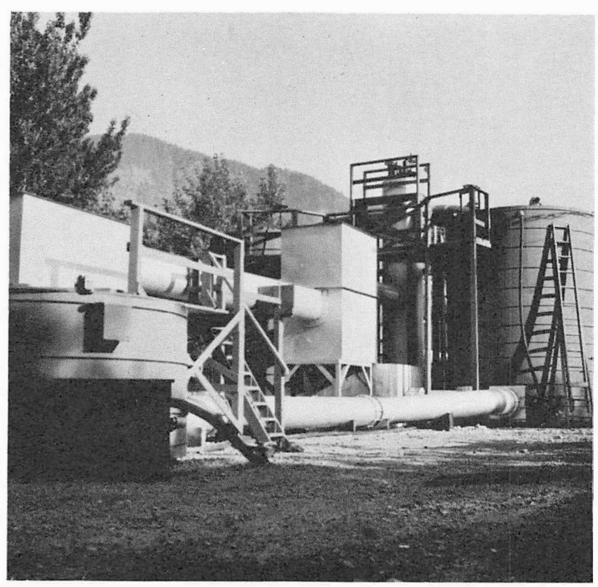

Figure 2.--Side view of test flume and related structures. One of two controlled temperature reservoirs stands at far right.

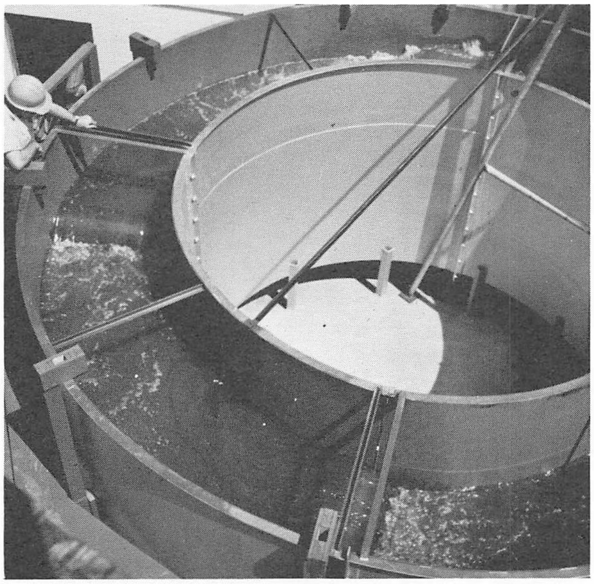

Figure 3.--Overhead view of the endless fishway swimming apparatus. Recirculating water enters at the upper right and drops counterclockwise over weirs that equally divide the 3-foot-wide circular channel into four pools, each 4 feet deep. The lowest pool, from which the water passes out, is 2 feet below the crest of the flow introduction weir.

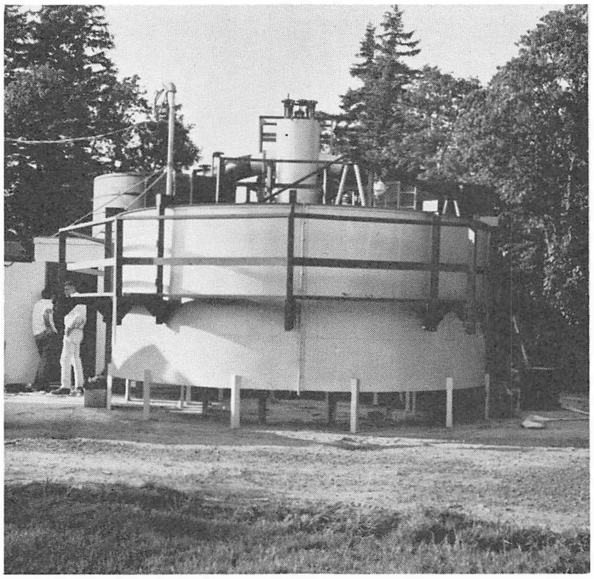

Figure 4.--Side view of circular endless fishway. Tower in center rear is standpipe used to provide constant head for water drawn from either reservoir at right and left.

Test Fish

Fish tested were spring- and fall-run chinook salmon obtained at Bonneville Dam, and a sample of male fall-run chinook from the Spring Creek hatchery upriver from Bonneville. After testing, the river fish were released at Stevenson, and the Spring Creek fish were returned to the hatchery.

Procedure

A test series consisted of successive performance trials of separate fish groups held and tested in changing or changed water temperature. During the same interval, control observations were made by conducting similar performance tests with fish held at constant temperatures--maintained equivalent to those temperatures at locations from which the groups were obtained.

Spring tests.--From April 28 through May 10, 1964, a total of 54 adult spring-run chinook, all presumably immature fish, were tested in two separate series. For each series, absolute and volitional performances were observed at the prevailing river temperature (52° F.) and in rising temperatures (52° to 64° F.).

Fall tests.--From September 14 through 28, 1963, a total of 52 fall-run chinook were tested. These fish were tested in two categories. The first tests were of fish from the river at Bonneville. While these showed secondary sexual characteristics they were not in spawning condition. Absolute and volitional performances of these fish were measured at the river temperature (69° F.) and in declining temperatures (69° F. to 52° F.).

The second series tested fish from the Spring Creek hatchery. These were all males considered surplus to the hatchery operation and mostly in spawning condition. Performances were measured at the hatchery water temperature (46° F.) and at the river temperature (69° F.).

Performance Measures

Absolute performance ratings were based on the maximal time that fish could be forced to make headway or maintain themselves against a fixed velocity. Volitional performances were scored on the number of endless fishway circuits completed in relation to the number of voluntary weir ascents. This latter value expressed performances resulting in direct passage through the fishway. The volitional performance measure differed from volitional activity, an index of general responsiveness which was determined from the total count of weir ascents, a value not necessarily related to passage through the fishway.

RESULTS AND DISCUSSION

Sample sizes were relatively small because of the capacity limits of the test facilities and the restricted seasonal interval that test fish are available. However, a summary (fig. 5) of the results offers certain tentative suggestions.

The performance capacities as measured did not appear to be related. High performances in one measurement were not necessarily reflected in another. The separation of these capacities also was indicated by the differing effects of temperature on the measured performances.

A decline in absolute performances probably related to maturity was indicated in these tests. The values for presumably immature spring-run fish were notably higher than those for the maturing fall fish. Such maturity effects were not shown, however, for fishway-type performance since the volitional performances were highest for fall fish at river temperatures with the next highest values noted for the sexually ripe hatchery fish tested at river temperatures.

Volitional activity measures indicated a pattern of responsiveness consistent with many observations of seasonal variation in behavior of chinook salmon. The spring-run fish were less active than the fall-run fish which in turn were highly active when they were in spawning condition. This did not appear to be related to temperature.

The observed effect of lowered water temperature on the volitional performance of fall-run river fish is of interest in view of thoughts of supplying cooler water to fishways in some areas where river temperatures are high. As indicated, an initial effect may be to lower the rate of progressive upstream passage.

While more field data is needed to confirm these indicated performance patterns and investigate the nature and range of temperature effects, a tentative conclusion appears to be that maturity may have less effect on fishway-type performance than water temperature changes. The limited information further suggests that mature chinook would not experience difficulty in negotiating standard fishways at routinely prevailing river temperatures.

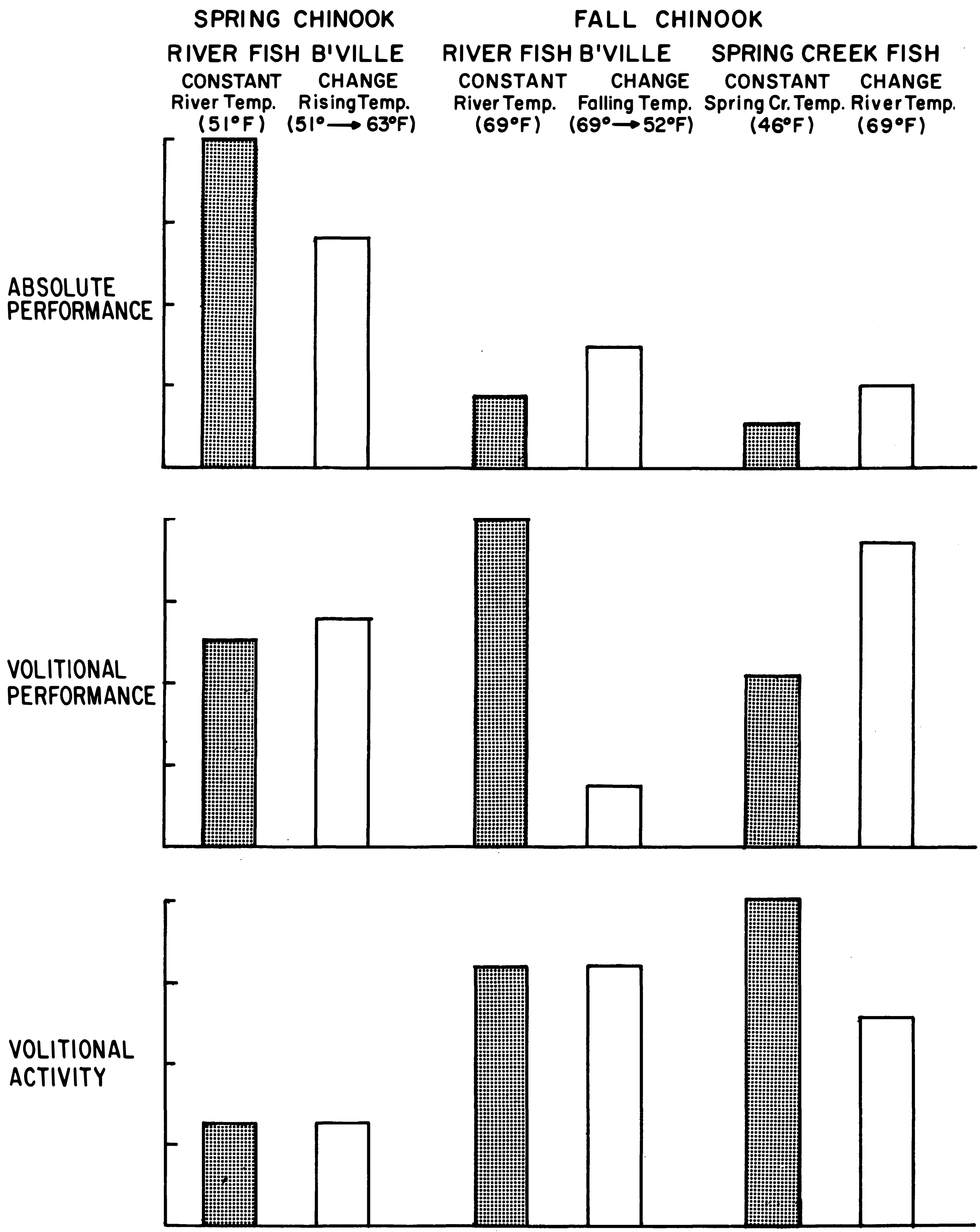

Figure 5.--Effects of water temperature on swimming performance of adult chinook. Bars depict the total measured performance in each test category. Obtained values are adjusted and shown on a common scale.

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TEMPERATURE - HOLDING EXPERIMENT AT OXBOW DAM

(SUMMARY)

by

Alan B. Groves

October 1964

FISH-PASSAGE RESEARCH PROGRAM
U. S. Bureau of Commercial Fisheries
Seattle, Washington

INTRODUCTION

Above-average losses of unspawned adult chinook salmon at the Oxbow Dam holding pond on the Snake River have occurred coincidentally with higher water temperatures in two of the past three operating seasons. In the season when lower water temperatures prevailed, fewer losses occurred. These events suggested that the lower temperatures benefited these fish.

In the fall of 1964 an experiment planned to investigate such benefits was attempted at the Oxbow hatchery. Adult migrants trapped at the dam were held in water cooled by mechanical refrigeration. The results are summarized in this report.

EQUIPMENT AND PROCEDURE

Facility

Fish were held at the hatchery in one of the 100-by 6-foot concrete raceways filled to about 3 feet in depth. The water volume was 1, 800 cubic feet, or about 13, 500 gallons. To offset solar heating effects, the raceway was shaded. Cooling was by circulating the water through a 30-ton-capacity commercial water chiller. In addition, two smaller 7. 5-ton-capacity chillers were installed as standby units. Water temperatures were held about a daily mean of 55° F. Daily variation was within ± 2° F. New water was supplied to flush the raceway at the maximal rate consistent with the cooling capacity of the 30-ton chiller. At the beginning of the test, 50 gallons per minute passed through the system. As river temperatures declined, this rate was increased to about 80 gallons per minute (Table 1). Mechanical aeration raised the dissolved oxygen content of the water from (about 7 parts per million), the river level, to about 9 parts per million within the raceway. To reduce the accumulation of particulate matter a filter was installed, but after a brief usage, this became inoperable because of clogging by algal material. Despite this, no appreciable buildup of suspended matter was evident. In order to avoid stagnation effects in the raceway, all equipment pumps were operated to circulate the water from the upper to the lower end. Total pumping capacity was about 700 gallons per minute. This in addition to the flushing flow of new water gave a total raceway flow generally above 750 gallons per minute.

Water Qualities

Water qualities were measured for monitoring purposes rather than detailed study, (Table 2). Since maximum accuracy was not a planned requirement, the following methods applicable for simplified usage in the field were employed.

Table 1.--Summary of flushing rate, prophylactic treatment, introduction of fish and mortalities in holding experiment at Oxbox Dam Hatchery.

Date 1964	Flushing Rate	Temperature Controlled Raceway: Malachite Green Treatment 1/	Number In 2/ M	Number In 2/ F	Mortality M	Mortality F	Cumulative Total Living	Hatchery Pond: Number In M	Number In F	Mortality M	Mortality F
Sept.	G.p.m.										
1	50	-	-	-	-	-	-	-	-	-	-
2	50	-	-	-	-	-	-	-	-	-	-
3	50	-	-	-	-	-	-	2	2	-	-
10	50	-	-	-	-	-	-	-	-	-	-
11	50	+	2	2	-	-	4	2	2	-	-
12	50	-	-	-	-	-	4	3	1	1	1
13	50	+	-	2	-	-	6	4	1	-	-
14	50	-	1	-	-	-	7	3	5	-	-
15	50	+	1	-	-	-	8	2	5	-	1
16	50	-	1	1	-	-	10	4	6	-	-
17	50	+	3	1	-	-	14	11	8	-	-
18	50	-	3	1	-	-	18	8	13	-	-
19	50	+	1	3	-	-	22	11	13	1	-
20	65	-	2	4	-	-	28	22	13	-	-
21	65	+	5	5	-	-	38	27	29	-	1
22	65	-	8	7	-	-	53	23	27	-	1
23	65	+	1	2	-	-	56	14	12	-	-
24	65	-	4	1	-	-	61	8	15	-	1
25	65	+	3	2	-	-	66	9	8	-	1
26	65	-	-	5	-	-	71	19	11	-	-
27	65	+	2	1	-	-	74	17	6	-	-
28	65	-	3	-	-	-	77	2	4	-	-
29	65	+	2	1	-	-	80	5	4	-	-
30	65	-	-	-	-	-	78 3/	3	4	-	1
Oct.											
1	80	+	4	1	-	2	81	5	7	1	-
2	80	-	-	-	1	2	78	2	3	-	-
3	80	+	-	4	4	22	56	4	4	-	-
4	80	-	-	-	3	6	47	6	3	-	-
5	80	-	-	-	4	5	38	0	0	-	-
6	80	-	-	-	6	-	32	1	1	-	2
7	80	-	-	-	3	4	25	4	-	-	-
8	80	-	-	-	5	2	18	-	-	-	-
9	80	-	-	-	1	4	13	-	-	-	-
10	80	-	-	-	-	-	13	-	-	-	-
11	80	-	-	-	-	1	12	-	-	-	-
12	80	-	-	-	-	-	12	-	-	-	-
13	80	-	-	-	-	1	11	-	-	-	-
14	80	-	-	-	-	-	11	-	-	-	-
15	80	-	-	-	-	-	11 4/	-	-	-	-
Totals:			46	43	27	49	(89)				

1/ Treatment level 1:2,000,000.
2/ Sexes identified only by superficial appearance when put into raceway.
3/ Two fish jumped out.
4/ Determined to be 4 females and 7 males (10-28-64).

Table 2.--Summary of water quality data for adult chinook holding experiment at Oxbow Dam Hatchery. Quality measurements were made from water within the experimental holding raceway and from supply water pumped from the river.

Date 1964	Temperature		Oxygen (O_2)		Ammonia (NH_3)		Carbon Dioxide (CO_2)		pH		Copper (Cu^{++})		Resistivity	
	R[1]	S[2]	R[1]	S[2]	R[1]	S[2]	R[1]	S[2]	R[1]	S[2]		S[2]	R[1]	S[2]
Sept.	°F	°F	Ppm	Ppm	Ppm	Ppm	Ppm	Ppm			Ppm	Ppm	Ohms/cm³	
1	56	70	8	7	-	-	8	8	8	8	.05	.05	2800	2800
2	55	70	8	-	.25	.25	7	-	8	8	.05	.05	2800	2800
3	55	70	10	-	.15	.07	-	-	-	-	-	-	-	-
10	55	68	9	-	.25	.25	5	-	8	-	.05	.05	2700	-
11	55	69	9	-	.25	-	6	7	8	-	.05	-	2900	2900
12	54	69	7	-	.20	-	8	-	8	-	.00	-	3000	-
13	54	69	8	7	.25	.25	7	7	8	8	.00	-	3000	-
14	55	69	9	-	.20	-	6	-	8	-	.00	-	2800	-
15	55	68	9	-	.25	-	6	-	8	-	.00	-	2900	-
16	54	70	9	-	.25	-	7	-	8	-	.00	-	2900	-
17	54	68	9	-	.25	-	7	-	8	-	.00	-	2900	-
18	54	67	9	-	.25	-	6	-	8	-	.00	-	3000	-
19	55	68	10	7	.25	.20	6	7	8	8	.00	.00	2900	2700
20	55	66	10	-	.25	-	6	-	8	-	.00	-	2900	-
21	55	66	10	-	-	-	-	-	8	-	.00	-	-	-
22	55	64	10	-	-	-	-	-	8	-	.00	-	-	-
23	55	65	9	-	-	-	-	-	8	-	.00	-	-	-
24	55	64	9	-	.15	-	6	-	8	-	.00	-	2700	-
25	55	65	9	-	-	-	-	-	8	-	.00	-	-	-
26	55	65	9	-	-	-	-	-	8	-	.00	-	-	-
27	55	64	9	-	-	-	-	-	8	-	.00	-	-	-
28	55	65	9	-	-	-	-	-	8	-	.00	-	-	-
29	55	64	9	-	-	-	-	-	8	-	.00	-	-	-
30	55	63	9	-	.25	-	-	-	8	-	.00	-	-	-
Oct.														
1	55	63	9	-	-	-	-	-	8	-	.00	-	2600	-
2	55	63	9	-	.15	-	-	-	8	-	.00	-	-	-
3	55	63	9	-	.15	-	7	-	8	-	.00	-	-	-
4	58[3]	63	9	-	.15	-	7	-	8	-	.00	-	-	-
5	59[3]	63	9	-	.15	-	7	-	-	-	.00	-	-	-
6	59[3]	63	9	6	.15	.10	7	7	8	8	.00	.00	2600	2700
7	55	63	9	7	.15	.15	7	7	8	8	.00	.00	2800	2900
8	55	63	9	7	.15	.15	7	7	8	8	.00	.00	2800	2700
9	55	63	9	7	.15	.15	7	7	8	8	.00	.00	2800	2800
10	55	-	10	7	.15	.20	6	7	8	8	.00	.00	2850	2790
11	55	-	-	-	-	-	-	-	-	-	-	-	-	-
12	55	-	10	8	.10	.10	7	6	8	8	.00	.00	2500	2500
13	55	-	9	-	.15	.15	7	-	8	8	.00	.00	2750	2800
14	55	-	9	9	.15	.15	7	7	8	8	.00	.00	2850	2900
15	55	-	-	-	-	-	-	-	-	-	-	-	-	-

1/ Raceway.
2/ Snake River.
3/ Elevated temperature due to temporary use of standby chillers.

1. Temperatures were measured with a standard mercury thermometer and an electronic thermometer and were monitored by a conventional 7-day thermograph. Measuring accuracy was within 0.2° F.

2. Oxygen measurements were made with a field kit manufactured by the Hach Chemical Co. The method was a modification of the standard Winkler titration and was accurate within 0.5 part per million of dissolved oxygen.

3. Ammonia determinations were by the direct Nesslerization method. Readings were made with a W. A. Taylor Color Comparator, using a color standard slide which covered a range from 0.0 to 1.0 part per million of ammonia nitrogen. Presumed accuracy averaged within 0.1 part per million over the range of the standards.

4. Carbon dioxide was checked with a Hach Chemical Co. field kit, whereby free carbon dioxide was assayed by titration with sodium hydroxide. Stated accuracy was within 1.0 part per million.

5. pH determinations were made with a Hach field kit, employing colorimetric indicator reactions compared against color standards. This was accurate within 0.5 pH unit from pH 4.0 to pH 10.0.

6. Copper or cupric ion was monitored, since some of the operating equipment had components of metallic copper or copper alloy in contact with the raceway water. The method consisted of colorimetric assay by use of a dithiocarbamate reagent. Readings were compared against a W. A. Taylor color standard slide ranging from 0.0 to 1.0 part per million. Presumed accuracy was within 0.05 part per million.

7. Electrical resistivity was monitored with a standard industrial-type resistance cell. Accuracy was within 100 ohms per cubic centimeter of water. The purpose of this measurement was to indicate undue changes in ionized dissolved solids in the raceway water.

Fish

The fish sample was obtained from the regular hatchery operation at Oxbow Dam. This operation is based on the adult chinook salmon trapped at the dam and transported to the hatchery for holding. As the fish were placed into the pond, each fourth or fifth fish was selected to be put into the experimental facility. This included males and females. It was planned to continue this representative apportionment until about 100 fish were in the raceway. To avoid extra handling and possible surface injuries, it was decided not to mark the fish. Due to the limited numbers in this particular run, a control group was not used.

In the hatchery procedure, while fish were being introduced into the holding pond, malachite green was administered daily to give a routine 1-hour treatment at a concentration of 1 part per million. To approximate similar prophylactic measures for the experimental fish, it was decided to treat this group with malachite green. Because the relative flushing rate of the raceway was less than that of the pond, the treatment was modified so that the dye concentration in the water was 1 part in 2 million and was administered only on alternate days as fish were introduced.

RESULTS AND DISCUSSION

Fish were first placed into the raceway on September 11, 1964. In the following 20 days, a total of 80 fish were introduced. In the morning of the 21st day, October 1, two dead fish were removed from the raceway. All other fish appeared normal. Later that same day, five new fish were added. The next day, October 2, three fish were found dead. The remaining fish appeared responsive and seemed normal. Early in the morning of October 3, prior to the usual inspection, four additional new fish were placed into the raceway. A little later, nine dead fish were discovered. That afternoon, the malachite green treatment was applied. Within 2 hours, several more fish were dead, others dying, and most of the group appeared lethargic. As an effort to improve presumed poor conditions, new water was flushed through the raceway which raised the temperature 2^{o} to 4^{o} F. Following this, the fish seemed more active. By the end of the day, however, 26 dead fish had been removed. No more fish were added to the raceway, and the malachite green treatments were discontinued. Mortalities continued to occur over the next 11 days. Of a total of 89 fish introduced, 76 died and 2 were lost in accidents, leaving 11 surviving fish (Fig. 1).

On October 4, pathologists from the Oregon Fish Commission visited the site and examined some of the fish which had died recently. No external or internal evidence of disease was present, and subsequent laboratory tests did not indicate any disease pathogens. However, some field observations made at that time noted an atypical appearance of the gills of the fish, which suggested possible effects from some external environmental factor.

Field measurements of water qualities did not indicate changes or deteriorations which would directly account for the heavy mortalities. Ammonia levels, as measured in the raceway, did not exceed the values observed for the inlet water from the Sanke River, which has been reported to contain up to 0. 96 part per million of ammonia nitrogen (Robeck et al, 1954). While the analytical method may have lacked highly quantitative accuracy at the indicated low level of ammonia content, previous use of the technique in other applications had demonstrated its sensitivity to relative changes in ammonia up to 1 part per million. Ellis (1948) states that detrimental effects on fish may be expected if ammonia levels are at 2. 5 parts per million or higher. More recent work (McKee and Wolf, 1963) reports that toxic effects have been noted at lower ammonia concentrations in connection with low dissolved oxygen, a condition not observed in this experiment.

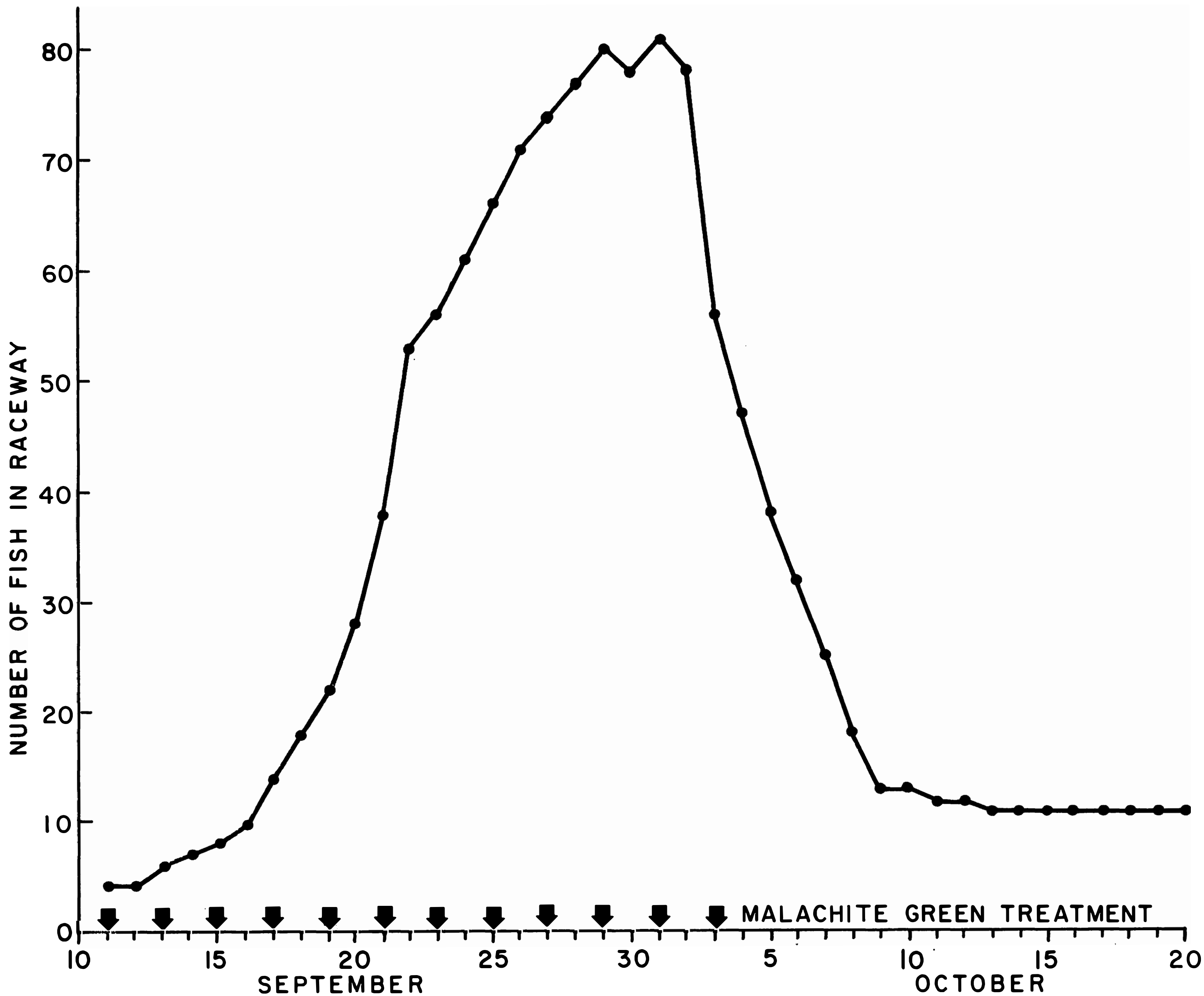

Figure 1.--Number of adult chinook in the experimental raceway holding facility at Oxbow Dam Hatchery.

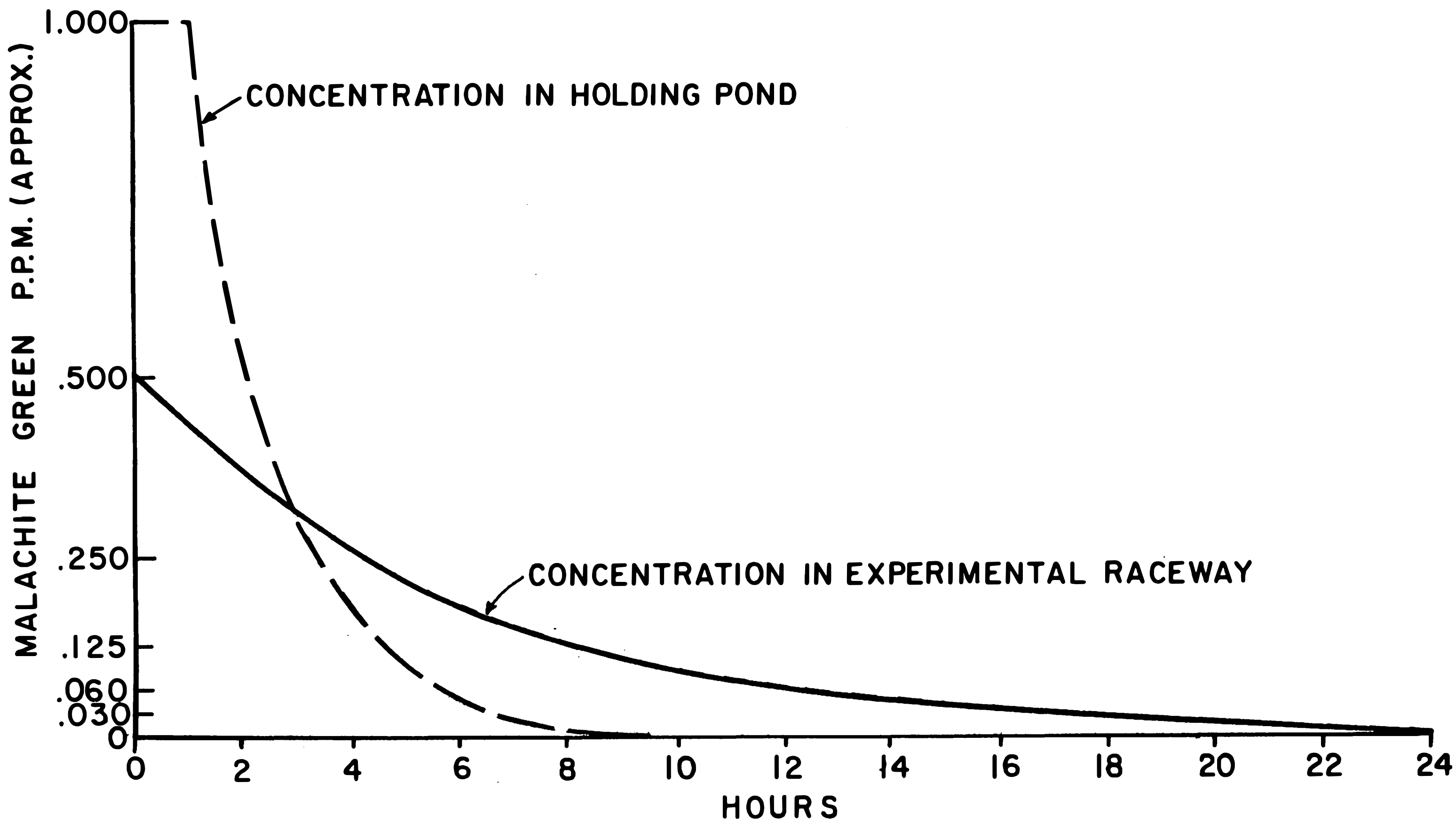

Figure 2.--Relative exposure of fish to malachite green dye in the hatchery pond and the experimental raceway. Points on dilution curves indicate time of flushing by one volume of water, based on estimated flows. Concentration values indicate relative levels and are not calculated.

To check the possibility of an accumulative toxic effect due to undetected low-level presence of copper and zinc, some laboratory tests of the raceway water were made using a Perkin-Elmer atomic absorbtion spectrophotometer. Neither metal was detected at a sensitivity level of 1 part per billion.

The malachite green treatment of the raceway, though planned to approximate the treatment administered to the hatchery pond, may have resulted in a greater exposure of the experimental fish to the dye. Even though the raceway treatments were half as frequent and the initial concentration less than that used in the pond, relative differences in flushing rates apparently subjected the experimental fish to longer exposure periods at higher concentrations. This is indicated by Figure 2. Estimating 20 water renewals in 24 hours for the hatchery pond, the malachite green could have been cleared from the pond in 9 hours. In the raceway, estimating six renewals in 24 hours, some malachite green still could have been present after 24 hours. Though the dilution curves in Figure 2 were not calculated to show the exact dye concentrations, the relative values for the treatments indicate that for other than the first 2-1/2 hours after application, the experimental fish were exposed to higher concentrations for longer periods. No direct evidence shows that this manner of exposure to malachite green caused the fish loss, but it should be considered, particularly since the gills of the fish seemed to have been affected by some unidentified factor. An apparent scarcity of specific information on the toxicology of the dye suggest that controlled testing is needed to define malachite green effects on adult salmon.

Although the loss of fish prevented a measure of benefits to be gained from lowered temperature, support for the initial premise is indicated by the evident successful holding this season in the hatchery pond. Water temperatures have been consistently lower than they were last year, and relatively fewer mortalities have occurred.

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RESPONSES OF JUVENILE CHINOOK SALMON TO PRESSURE CHANGES

by

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INTRODUCTION

Recent sampling of the gatewells at Ice Harbor Dam indicates that large numbers of juvenile salmon enter these areas of the dam during their downstream migration. It is suspected that response to pressure may be an important factor in the movement of fish into these areas as they are swept past the gatewell openings at a depth of 90 feet. In order to explore this possibility, the effects of pressure changes on vertical movement of juvenile chinook salmon (Oncorhynchus tshawytscha) were investigated.

MATERIALS AND METHODS

A hydraulic chamber (fig. 1) equipped with viewing ports and capable of withstanding pressures up to 275 pounds per square inch was employed. With this device, which has a capacity of 350 gallons of water, groups of fish could be subjected to sudden or gradual pressure changes so that behavior patterns could be observed. Fish distribution was determined by counting the fish in the lower half of the chamber and relating this to the total number of fish used in the test. Twenty counts were made to obtain the average distribution at each pressure level.

In tests of the effect of rapid pressure changes, pressure was increased from 0 to 40 pounds (equivalent to water pressure at a depth of 92 feet) in 2 minutes, and fish distribution was determined before and after the pressure increase. In tests of the effect of gradual pressure changes, pressure was increased from 0 to 7 pounds (equivalent to 16 feet) in 1-pound increments and from 0 to 40 pounds in 5-pound increments. Each of these tests lasted about an hour, and distribution was determined after each pressure increase.

Tests were also conducted to determine the rate of adaptation of juvenile chinook salmon at different pressure levels. Pressure was increased to the desired level, held there for approximately 24 hours, and then decreased slowly. Distribution was determined after each pressure change.

RESULTS AND DISCUSSION

Two tests in which the pressure was increased rapidly from 0 to 40 pounds resulted in an average change in vertical distribution of 28 percent--a rather pronounced change in view of the limited space within which the fish were able to move.

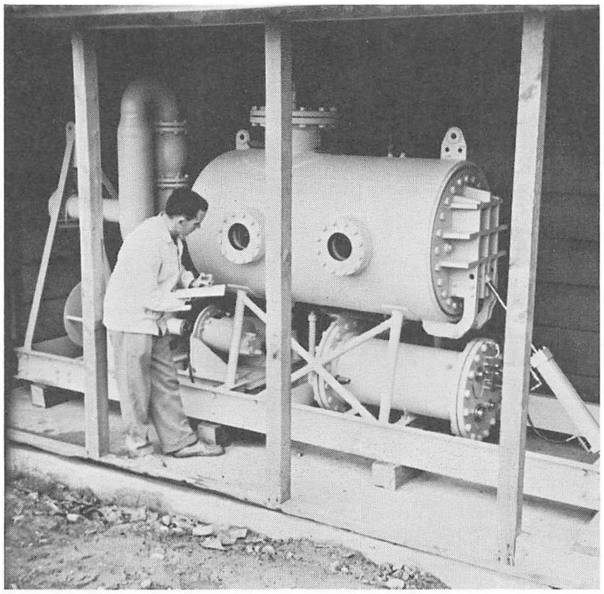

Figure 1.--Hydraulic chamber for studying the effect of pressure changes on fish.

Since the depth of the water was only 3 feet, any tendency toward upward movement was immediately checked at the roof of the chamber, resulting in exploratory movement in other directions.

Three tests were then conducted in which the pressure was increased slowly from 0 to 40 pounds in 5-pound increments (fig. 2). This also resulted in an average change in vertical distribution of 28 percent. Thus the change in vertical distribution for a 40-pound pressure increase was the same whether accomplished in 2 minutes or an hour. In these three tests the most pronounced distribution change occurred within the first 5 pounds of pressure increase.

Three tests were then run in which pressure was increased from 0 to 7 pounds in 1-pound increments (fig. 2) resulting in an average change in vertical distribution of 20 percent. In these tests a 1-pound pressure increase caused a noticeable change in fish distribution indicating pronounced sensitivity to the effects of pressure changes. Pressure sensitivity may be due to loss of hydrostatic equilibrium as determined by visual cues or due to proprioceptors within the body sensitive to contraction and expansion of the swimbladder.

Results of three adaptation tests are shown in figure 3. As pressure increased, more fish were found near the top of the chamber. When the pressure was decreased after approximately 24 hours, distribution returned to that of previous adaptation. However, this distribution now occurred at a higher pressure level. Fish held at 5 pounds (12 feet) had adapted to between 2 and 3 pounds of pressure. Fish held at 15 pounds (35 feet) and 40 pounds had adapted to between 6 and 7 pounds of pressure. This would seem to imply that rate of adaptation is a function of pressure only within certain limits. As the pressure was decreased further, the fish became extremely active, began to give off gas bubbles, and moved to the bottom of the chamber.

Results of these tests seem to indicate a high degree of sensitivity to pressure changes and a strong tendency to compensate for vertical displacement. These factors, together with a slow rate of adaptation, might well account for movement of fish into the gateslot wells as they are swept down past the openings.

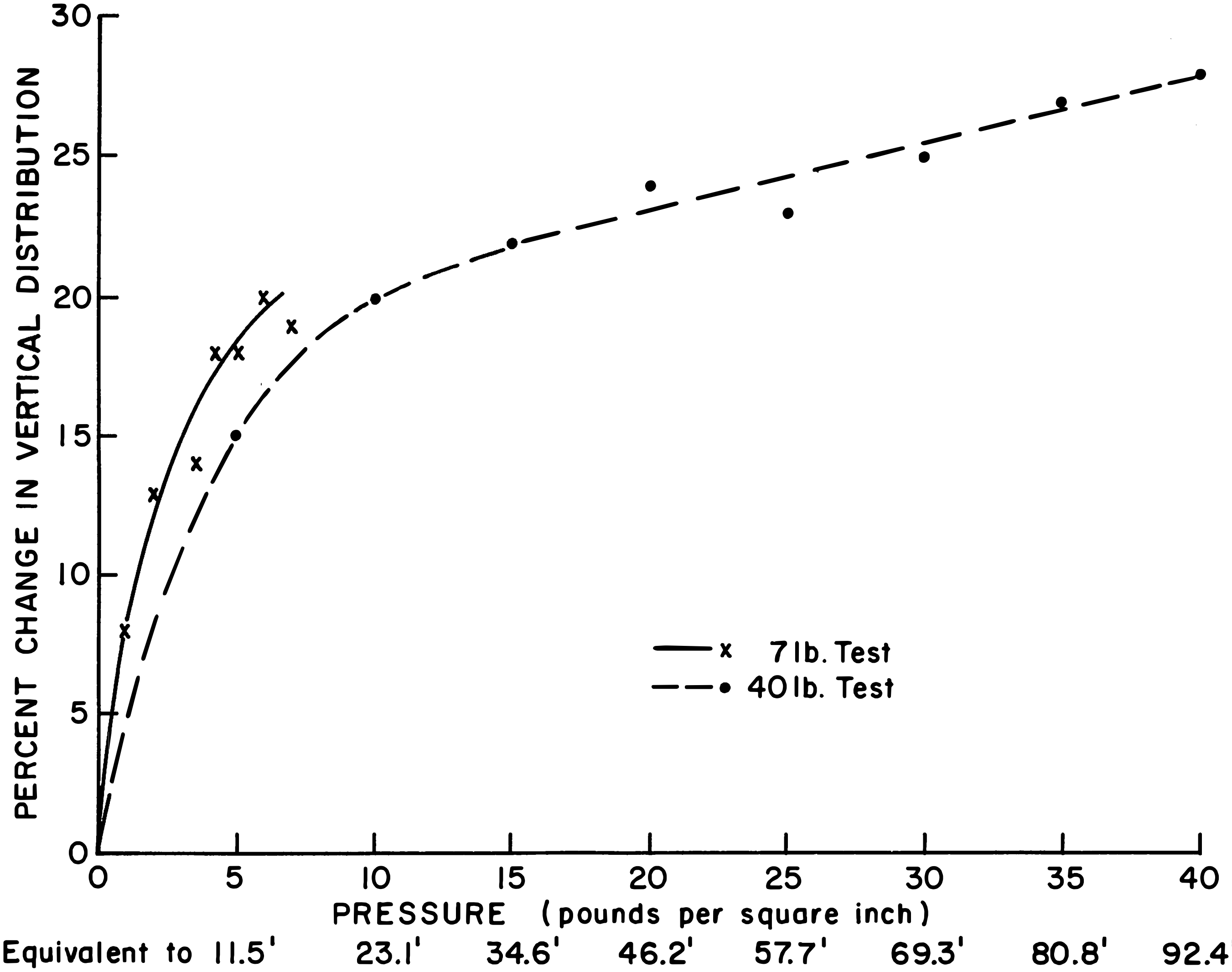

Figure 2.--Relation between vertical distribution of juvenile chinook salmon and pressure change in 3 feet of water. Each curve is based on an average of three tests, each of which lasted approximately 1 hour.

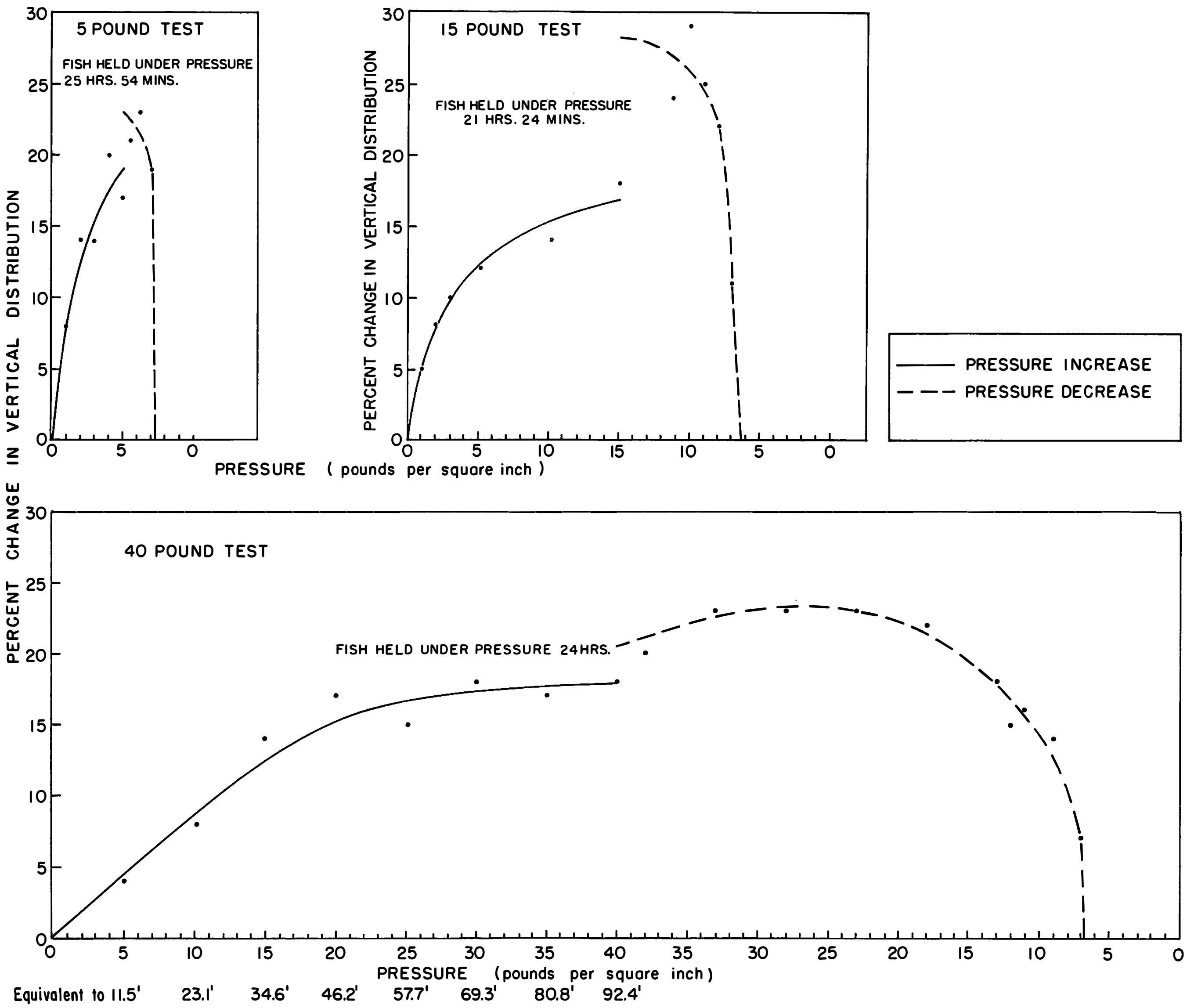

Figure 3.--Adaptation of juvenile chinook salmon to three different pressure levels. For the 5-pound test, the periods of pressure increase and decrease were approximately 1 hour each. For the 15-pound test, these periods were 1 hour and 36 minutes each. The 40-pound test pressure was reached in approximately 3 hours; the decrease required 2 hours, 12 minutes.

DISEASE

A STUDY TO CONTROL THE DISEASES INFLUENCING THE SURVIVAL OF ADULT CHINOOK SALMON IN THE COLUMBIA RIVER BASIN

by

Oregon Fish Commission

for

FISH-PASSAGE RESEARCH PROGRAM
U.S. Bureau of Commercial Fisheries
Seattle, Washington

October 1964

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A STUDY TO CONTROL THE DISEASES INFLUENCING THE SURVIVAL OF ADULT CHINOOK SALMON IN THE COLUMBIA RIVER BASIN

INTRODUCTION

This study was initiated in April 1962 to develop methods for the detection, prevention, and control of infectious diseases in adult chinook salmon. Three major research categories were to be studied: (1) determine if an immune response could be induced in fish by their exposure to antigens; (2) test certain drugs to determine their usefulness in the control of fish diseases; and (3) screen a selected group of chemicals to observe their effects on external infections caused by microbial agents. Additionally, pathogens affecting adult salmon were to be identified, a stock culture collection of these pathogens was to be maintained, and insofar as possible, physiological studies of the organisms involved were to be carried out. Within these categories, investigations have been conducted as follows:

IMMUNE RESPONSE

INTRODUCTION

The lack of extensive information on the immunological character of poikilothermic animals has been emphasized by Hildemann (1962). 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 (Hildemann, 1962), and the 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, as in most instances of the intraperitoneally-injected bacterial vaccines, 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 shows the production of hemagglutinating antibodies by sablefish at temperatures as low 5-7° C.

Duff (1942) was able to develop resistance to furunculosis disease in cutthroat trout by feeding an oral vaccine containing the causative agent *A. salmonicida.* It has also been recently shown that newly-hatched fry of Amazonian discus fishes 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, or indeed, the release of antibodies into the circulation (if the situation in fish is found to be analogous to that in frogs) (Elek, Rees and Gowing, 1962) 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 determine what concentrations can be expected under these conditions, and the relationship of these antibodies to actual protection for the fish. A study was undertaken to investigate active and passive immunization of rainbow trout, spring chinook and coho salmon against A. salmonicida.

MATERIALS AND METHODS

Experimental Animals

Agglutinating antibodies were prepared in 2-, 3-, and 4-year-old adult rainbow brood trout. The fish were obtained from the Oregon Game Commission Roaring River Hatchery and were held in circular tanks having a total water volume of 1,070 liters per tank, fed from spring water which maintained an average temperature of 12° C. These fish were fed Clark's "New Age" brood-fish feed at 30 grams per fish per week.

Juvenile coho used for passive immunization were kept in stock ponds at 13° C. and fed Oregon pellets until needed for experimentation. Water temperature was slowly raised to 17° C. over 3 days after the fish had been transferred to 16-liter tanks in the laboratory. These fish and those employed in the oral immunization experiment were obtained from Oregon Fish Commission (OFC) Sandy Hatchery and were of the 1961 brood. Their average weight was 19.3 gm. The production feeding of oral vaccine involved 1962-brood juvenile coho at the OFC Siletz Hatchery.

Adult spring chinook used in the experiment reported here were obtained from Eagle Creek National Hatchery.

Serum Preparation

Blood was removed from the fish by severing the tail. Blood samples from each fish were kept separate and were immediately placed at 12° C. for clotting. After about one hour all samples were placed at 2° C. for complete clot retraction. Rainbow serum samples held for passive immunization on coho were frozen. Other serum samples were stored at 2° C.

Agglutination

Agglutinating antigens consisted of washed suspensions from 24-hour (at 18° C.), modified furunculosis medium (MF) broth culture (0.25 gm 1-tyrosine, 5 gm yeast extract, 2.5 gm NaCl, and 10 gm tryptone). The cells were centrifuged, washed twice, then resuspended in cold 0.85% NaCl and the pH adjusted to 7.0. Fresh antigens were prepared before each experiment.

Agglutination was carried out according to the method outlined by Kolmer, Spaulding and Robinson, (1951) for brucella antigens (4 hours incubation at 52° C. followed by overnight at 4° C.

A. salmonicida stock cultures used in antigen preparation included 5000H

(Snake River) 1/, 5006Z (Siletz Hatchery), 5007W (Willamette Hatchery), 5010B (Big Creek Hatchery), 5012T (Trask Hatchery), 5016K (Klaskanine Hatchery), and ATCC 14174.

Experimental Infection

Juvenile coho involved in passive immunization were experimentally infected using a combination of two methods which had experimentally proved to be effective and measurable in our laboratory. First, immediately after injection of the serum, and while the fish were still anesthetized, (1:17,500 MS-222 Tricaine methanesulfonate) 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 in MF broth 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 MF broth 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 one hour with aeration. The same procedure was repeated again at 48 hours. All fish dying during this experiment were autopsied to determine the specific cause of death.

Fish which had received the oral vaccine in the laboratory were experimentally infected by introducing a 50 ml MF broth culture (24 hours at 18° C.) of A. salmonicida 5000H into the water supply daily through the first 15 days of the experiment.

Juvenile coho at the Siletz Hatchery experienced a natural infection of furunculosis. Had the infection not occurred, fish would have been removed to the laboratory for challenge.

Vaccine Preparation

Organisms used in antigen production were inoculated onto bottle slant cultures and incubated for 72 hours at 18° C. After incubation the growth was scraped from the agar with a glass rod and suspended in aqueous 0.85% NaCl at pH 7.0. Formalin was then added to 0.2% and the suspension placed at 25° C. for one hour. The suspensions were then placed, and permanently stored at 2-4° C.

For injected vaccines, the final cell concentration was adjusted to 3×10^{10} cells per ml. The vaccine was mixed 2:1 with Freund's (complete) adjuvant (FA) prior to inoculation, and injected intra-abdominally. A. salmonicida 5000H was employed in all experiments which involved intra-abdominal inoculations.

Vaccine for use orally in the laboratory immunization experiments was prepared in the same manner as the injectable vaccine, and was incorporated into Oregon pellets by addition of the preparation to the thawed food. The mixture was then repelleted with a modified home food grinder, and refrozen. This oral vaccine preparation contained A. salmonicida 5000H.

1/ Parenthesis indicate location where culture was isolated. All are from OFC hatcheries except the ATCC strain, and the Snake River culture which was obtained from the Idaho Fish and Game Commission hatchery at Oxbow Dam.

Vaccine for incorporation into Oregon pellets used in the production experiment at the Siletz Hatchery was prepared the same as the oral vaccine used in the laboratory experiment, except that incubation of the formalin preparation at 25° C. was omitted. A. salmonicida 5006Z, 5012T, and 5016K were employed in the ratio of 3:1:1 as antigens for this experiment.

Active Immunization

Organisms for antigen production were inoculated onto slant cultures of 5000H incubated for 72 hours at 18° C. After incubation the growth was scraped from the agar with a glass rod and suspended in 0.85% NaCl at pH 7.0. Formalin was then added to 0.2% and the suspension placed at 25° C. for one hour. The suspensions were then placed, and permanently stored at 2-4° C. The final vaccine cell concentration was 3×10^{10} cells per milliliter. The vaccine was mixed 2:1 with FA prior to inoculation, and injected intraperitoneally. Two-year-old rainbow received three injections, the second injection occurring one week after the initial inoculation, the third after an additional five weeks. Control fish in this group received either aqueous 0.85% NaCl or 0.85% NaCl plus FA. An injection of 2 ml vaccine was made initially, thereafter, each fish receiving 1 ml each time. Blood was removed from these fish four weeks after the final injection.

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

Spring Chinook Immunization

Information relative to spring chinook immunization was obtained on only 3 fish, since most of the test fish died prior to what was considered a minimum stimulatory period. These survivors were in a group which received a single 9-ml injection of the same vaccine used for intraperitoneal inoculation of the adult rainbows. Controls received the same amount of a saline-FA preparation.

Blood was removed eight weeks after the single injection and assayed for agglutinating antibodies.

Passive Immunization

Antiserum and control serum used for injection of juvenile coho consisted of pooled 20-fish samples (filter sterilized) from the same rainbow that were tested for agglutinating antibodies.

Three groups of coho were involved in this experiment: (1) 37 negative controls which received no serum; (2) 36 controls which received 0.5 ml undiluted sterile serum from control rainbows; and (3) 40 fish which received 0.5 ml undiluted sterile anti-5000H serum from vacinated rainbow. Each group was divided approximately in half and placed in two tanks (16 liter water volume) on a controlled water-temperature table. The experimental water temperature was held at 17° C. Serum was injected intraperitoneally 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.

Oral Immunization

Four groups of juvenile coho were involved in the laboratory experiments: (1) the first group received a total of 406×10^9 cells per fish over a 98-day period (4.14×10^9 cells/fish/day); (2) the second received a total of 122×10^9 cells per fish over a 46-day feeding period (2.65×10^9 cells/fish/day); (3) a third received a total of 42.3×10^9 cells per fish over a period of 22 days (1.9×10^9 cells/fish/day); and (4) a fourth consisted of control fish. One group of controls had remained in the laboratory for 98 days and a second had remained in the laboratory for 46 days. All fish had been held in the laboratory for at least 46 days. Vaccination temperature was held at 13° C. throughout the experiments.

During field studies at Siletz Hatchery, 1962-brood juvenile coho received a total of 22×10^{13} cells over a period of 81 days. There were 72,000 vaccine fish and 72,000 control fish fed during this period giving a total of 3.82×10^7 cells/fish/day. The average high temperature during this period was 16.6° C. with a maximum high of 20.5° C. The average low temperature was 13.4° C. with a minimum of 11.1° C. The total overall average was 15° C. for the 24-hour day.

RESULTS

Test for Agglutinins in Actively Immunized Rainbow

Uniformly high levels of agglutinating antibodies were attained in the 2-, 3-, and 4-year-old adult rainbow trout (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 reflects the residual of previous non-artificial stimulation.

Table 1. Agglutination of *A. 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 A. salmonicida 5000H by Immune and Control Serum of 3- and 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

Test for Agglutinins in Actively Immunized Adult Spring Chinook

As shown in Table 3, antibody formation was detected among the fish tested within eight weeks following the single vaccine injection. Loss of additional test animals through death hampered this experiment, and although the results obtained clearly show a difference between the control and vaccine fish, the relative differences one could expect among larger groups must be extrapolated conservatively.

Table 3. Agglutination of A. 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

Specificity of Anti-5000H Serum from Rainbows

An attempt to detect gross serological differences between strains of A. salmonicida was made on five additional isolates from OFC hatcheries and an ATCC strain. The homogeneity of the strains is apparent in Table 4.

Passive Immunization of Juvenile Coho

The protective value of immune rainbow serum is shown by the hourly mortality in Figure 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. It can be clearly seen that the administration of antiserum prior to infection not only delays the onset of furunculosis disease, but also supresses the normal mortality dynamics. The cumulative mortality is shown in Figure 2. It can be seen that the antiserum-treated fish follow a similar, though delayed curve, 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.

Table 4. 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	-	-	-	-	-	-	-	-	-

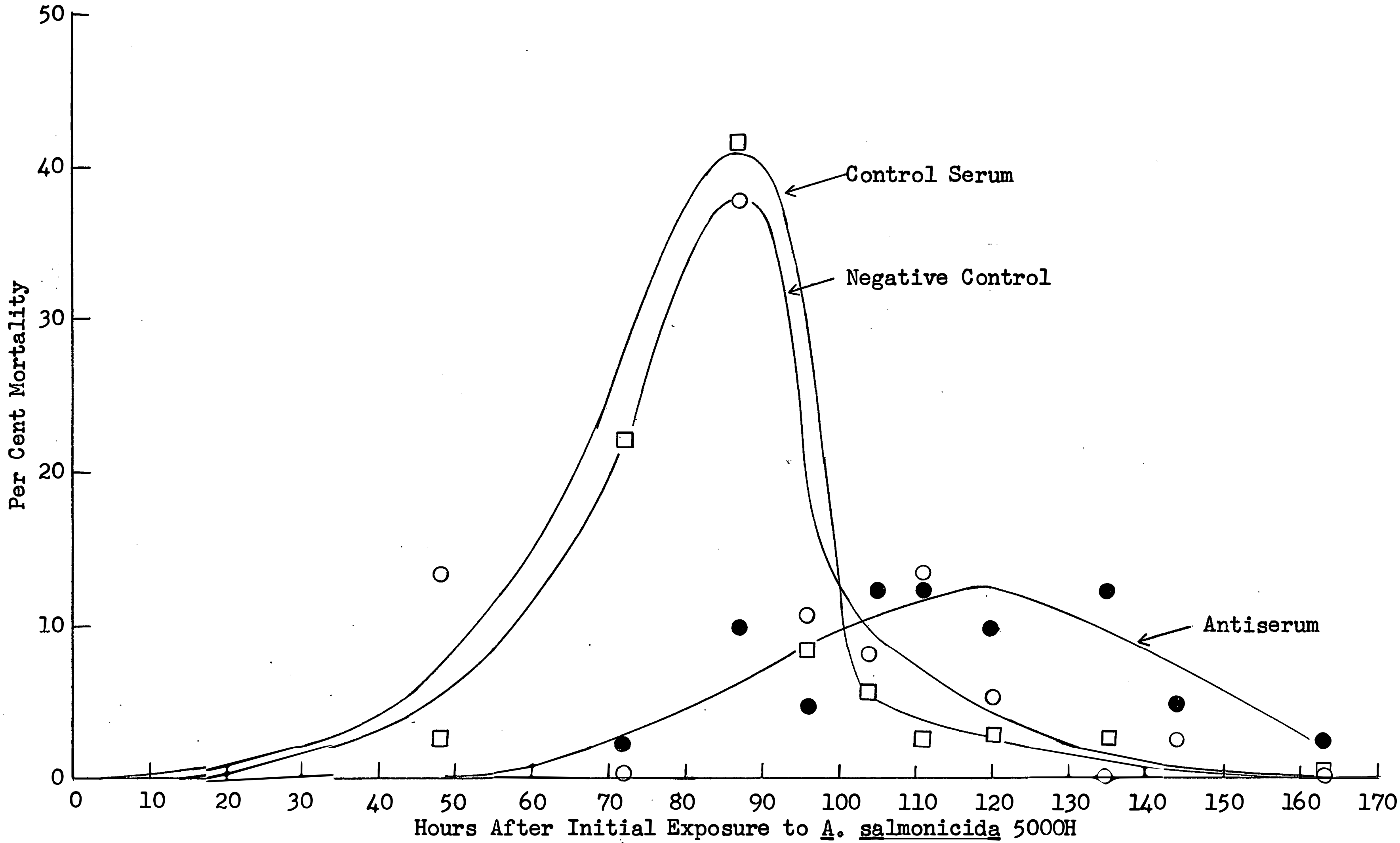

Figure 1. Hourly Per Cent Mortality of Negative Control, Serum Control, and Antiserum-Treated Fish.

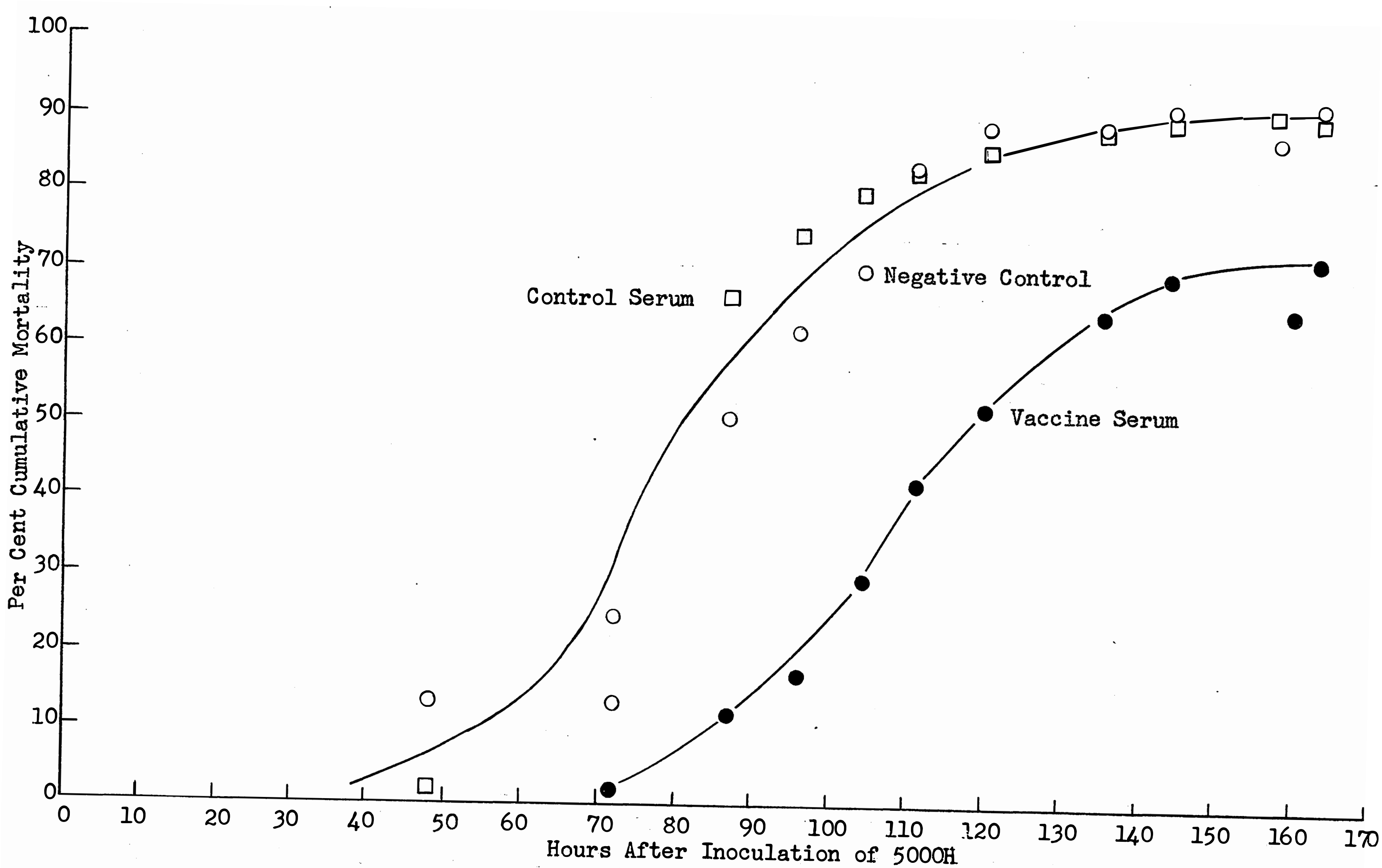

Figure 2. Cumulative Mortality of Juvenile Coho Infected with A. salmonicida 5000H After Receiving No Serum, Control Serum, and Antiserum From Adult Rainbows.

Oral Immunization Experiments

Although the mortality data on juvenile coho treated in the laboratory presented in Tables 5 and 6 would seem to suggest that some protection was conferred on the fish fed through 98 days, this was not true since when this group was analyzed statistically by X^2, the computed value (1.22 with one degree of freedom) was not significant at the 5% level.

No protection was observed among fish involved in the production experiment at the Siletz Hatchery. 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.

These fish were also tested for agglutinating antibodies. None were detected.

DISCUSSION

Without exception, all of the vaccinated rainbow trout showed a relatively high level of antibody production. Though the history of the 3- and 4-year-old fish included their involvement in several furunculosis epidemics during both the time that they were being raised and during their life as brood stock in holding ponds, no control showed antibodies comparable to even the more limited production found in two of the vaccinated fish. 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 not only included organisms from various OFC hatcheries, but also an ATCC strain, 14174. All showed high agglutination titers with the anti-5000H serum making it unlikely that agglutinins against another A. salmonicida was 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 the experiments, and over 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.

It is well known that although agglutinins may be present at high levels, it does not necessarily indicate the existence of "protecting", of "immunizing" antibodies. The co-existence, and stimulation of both these factors remained to be shown by passive protection experimentation. It was seen in this experiment 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 immunizing these juveniles passively 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 reproducible measurement of the disease progression resulting in a uniform mortality curve. 1/

1/ See section "The Pathogenicity of a Phage-Sensitive Strain of Aeromonas liquefaciens", following.

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

Sample	No. Fish	0-6	7	8	9	10	11	12	13	14	15	16	17
		Per Cent Cumulative Mortality Days after Initial Infection Attempt											
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 A. salmonicida 5000H Vaccine.

Sample	Fish	0-6	7	8	9	10	11	12	13	14	15	16	17
		Per Cent Cumulative Mortality Days after Initial Infection Attempt											
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.

Passive protection in fish is not surprising, since it had 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 large numbers of juvenile fish is obvious, since it would benefit the fish for so short a time. Its best application appears to be in the treatment of adult salmon after their return to the holding facilities. Since this is during the final phase of the salmon's life cycle, no consideration need be made for continuing protection, nor long-range effects as in the case of juveniles. In addition, the salmon are always immediately available during this period, and are usually present in numbers which could be treated practically. They are usually held, at most, from 3-4 months prior to spawning, encountering most disease problems either during the latter part of this period, or during high temperature phases of the holding pond water, or conditions of a similar predictable nature. With these factors in mind, a system of passive immunization or protection could be created, and as in the case of other animals, not just one, but several diseases could be combatted simultaneously with proper serum preparations. It is also logical to predict that other animals may be utilized as a source of immune serum as happens with other animals. Information from this study suggests that serum may possibly be prepared in the adult salmon themselves, perhaps in sufficient quantities to aid succeeding generations which return later to the holding ponds.

These results are limited, however, and do not show the high antibody titers found among fish which received a larger number of vaccine injections. Production of antibodies is apparently slow among all cold-water fishes, and this disadvantage is helped neither by the fact that these fish have a limited life expectancy, nor by the degenerative physiological state in which they exist. However, even though these fish may not be good suppliers of antiserum, they may be able to produce sufficient antibodies to protect themselves until the eggs or sperm can be harvested.

Although intra-abdominal inoculation of 2-, 3-, and 4-year-old adult 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.

Statistical analysis of mortality is supported in its non-significance revelation 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. Several circumstances could explain the lack of stimulatory effect by the oral vaccine, including insufficient time to allow antibody formation, lack of optimal temperatures, presence of vehicle inhibitors, suboptimal 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" substances 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. Active oral immunization of juvenile coho were unsuccessful both in laboratory and field attempts, however, these failures should not preclude additional studies on this method.

DRUG TESTS

INTRODUCTION

The objectives of the following experiments were to: (1) determine the ability of the sulfonamides, Sulmet (sulfamethazine), Gantrisin (sulfasoxizole), Bactrovet (sulfadimethoxine), and S.E.Z. (sulfaethoxypyradiazine) to produce blood or tissue levels in fish greater than 4 mg per 100 ml blood (mg%); (2) develop a suitable method for administering the sulfas; and (3) conduct antibiotic sensitivity tests on two organisms, A. salmonicida and A. liquefaciens.

Previous in vitro sensitivity tests indicated that Sulmet, Gantrisin, Bactrovet and S.E.Z. were effective in controlling the growth of A. salmonicida. These preliminary experiments, mostly involved with development and standardization of methodology have been omitted from this report in the interest of continuity and brevity.

The most extensive sulfonamide experiments were conducted with Bactrovet as the previous experiments on juvenile salmon indicated that this sulfa provided sustained blood sulfa levels without toxic effects.

In vitro antibiotic sensitivity tests were conducted on two strains of fish pathogens.

MATERIALS AND METHODS

Adult Experiments

As the adult salmon is presumably non-feeding when returning to the parent stream, two basic sulfa application techniques were exploited: oral forced-feeding and intramuscular injections.

Bactrovet was administered first to spring chinook from the Eagle Creek National Hatchery, then to coho from the Sandy Hatchery followed by steelhead and spring chinook from the Marion Forks Hatchery. The oral forced-feeding was investigated first because this technique seemed the simplest as it required no sterile equipment. Two forms were fed; tablets, and two injectable solutions. One solution was the 10% commercial preparation and the other a 25% laboratory preparation. The other sulfas fed orally were injectable 25% commercial S.E.Z. solution, Sulmet tablets, and Gantrisin tablets. These were fed only to spring chinook from Marion Forks. Intramuscular injections of Bactrovet were given to spring chinook, coho and steelhead.

Crystalin Bactrovet was dissolved in DMSO (Dimethyl sulfoxide) in the hopes of producing a 25% injectable solution.

Accessory Experiments on Juvenile Coho

As an adjunct to the adult salmon experiments, a test was conducted on juvenile coho to determine if sulfa levels could be analyzed from some part of the fish other than the blood with no sacrifice of accuracy. The fish in these experiments were fed Oregon pellets containing Sulmet. Blood was taken from the fish, the blood analyzed for sulfa content, and the remaining carcasses of each sample homogenized in a blender. The homogenized fish were then centrifuged and the supernatant body fluids analyzed. A similar experiment, utilizing a new veterinary quadrasulfa (sodiam sulfathiazole, sulfamerazine, sulfamethazine, and sulfaquinoxaline) was conducted on juvenile coho. The quadrasulfa was incorporated in Oregon pellets and fed at three concentrations: 2.5, 5, and 10 grams of sulfa per 100 pounds of fish.

In Vitro Antibiotic Experiments

In vitro sensitivity tests were conducted on Sandy River strains of *A. salmonicida* and *A. liquifaciens* to determine which antimicrobial agents might be best utilized for controlling furunculosis and possibly other bacterial and mycotic infections of adult salmon. *In vivo* tests are being formulated. Laison with several pharmaceutical supply firms is being maintained with the hope that as new drugs become available, those with special promise for fish disease control will be furnished for testing.

RESULTS

Adult Experiments

The forced-fed Bactrovet tablets produced only slight blood levels in the steelhead (Table 7 and Figure 3) but none in the chinook or coho. The injectable Bactrovet solutions administered orally to these fish produced only short-term, low blood levels (Tables 8 and 9 and Figure 3) in all species.

The most persistent blood sulfa levels from a single force-feeding were achieved with Sulmet and Gantrisin tablets fed to spring chinook (Table 9 and Figure 4). The water-soluble injectable S.E.Z. and Bactrovet solutions force fed to these fish produced blood sulfa levels, but of lower concentrations (Table 9 and Figure 4).

The commercial 10% intramuscular injections given to chinook, coho, and steelhead produced satisfactory blood sulfa levels (Tables 7 and 10 and Figure 5) but caused hemmorrhagic abscesses at the injection site.

The Bactrovet dissolved in DMSO precipitates out of solution of contact with body fluids thereby producing no sulfa levels in the fish.

Table 7. Blood Sulfa Levels in Mg-Per Cent[1/] of Adult Steelhead Force Fed Bactrovet Tablets and Capsules and Given Intramuscular Injections of Bactrovet at a Water Temperature of 54° F.

Drug Form	Rate of Mg Sulfa Per Lb of Fish	Hours After Treatment			
		24	48	72	96
Control	0	0	0	0	0
Tablets	120	0.06	1.60	0.80	1.50
Capsules	120	0.03	1.30	0.30	0
26% Lab Prep I.M. Injection	9.3	14.50	11.20	10.70	7.30

1/ Mg sulfa per 100 ml blood.

Table 8. Blood Sulfa Levels in Mg-Per Cent of Adult Coho Orally Force Fed Injectable Forms of Bactrovet at a Water Temperature of 54° F.

Drug Form	Rate in Mg Sulfa Per Lb of Fish	Hours After Treatment			
		24	48	72	96
10% Commercial Solution	100	3.60	0	--	--
25% Lab Prep Solution	100	1.90	0	--	--

Table 9. Blood Sulfa Levels in Mg-Per Cent of Adult Spring Chinook Orally Force Fed Sulfonimides at a Water Temperature of 54° F.

Drug	Rate in Mg Sulfa Per Lb of Fish	Hours After Treatment				
		24	48	72	96	120
Sulmet Tablets	100	0.60	4.00	4.50	4.60	4.50
Gantrisin Tablets	100	2.60	5.40	6.40	6.00	4.50
Bactrovet 30% Lab Prep. Sol.	200	1.80	1.60	1.40	0.60	--
S.E.Z. 25% Commercial Sol.	100	0.80	0.15	0.14	0	--
S.E.Z. 25% Commercial Sol.	200	4.00	2.60	1.70	1.20	--
Control	0	0	0	0	0	0

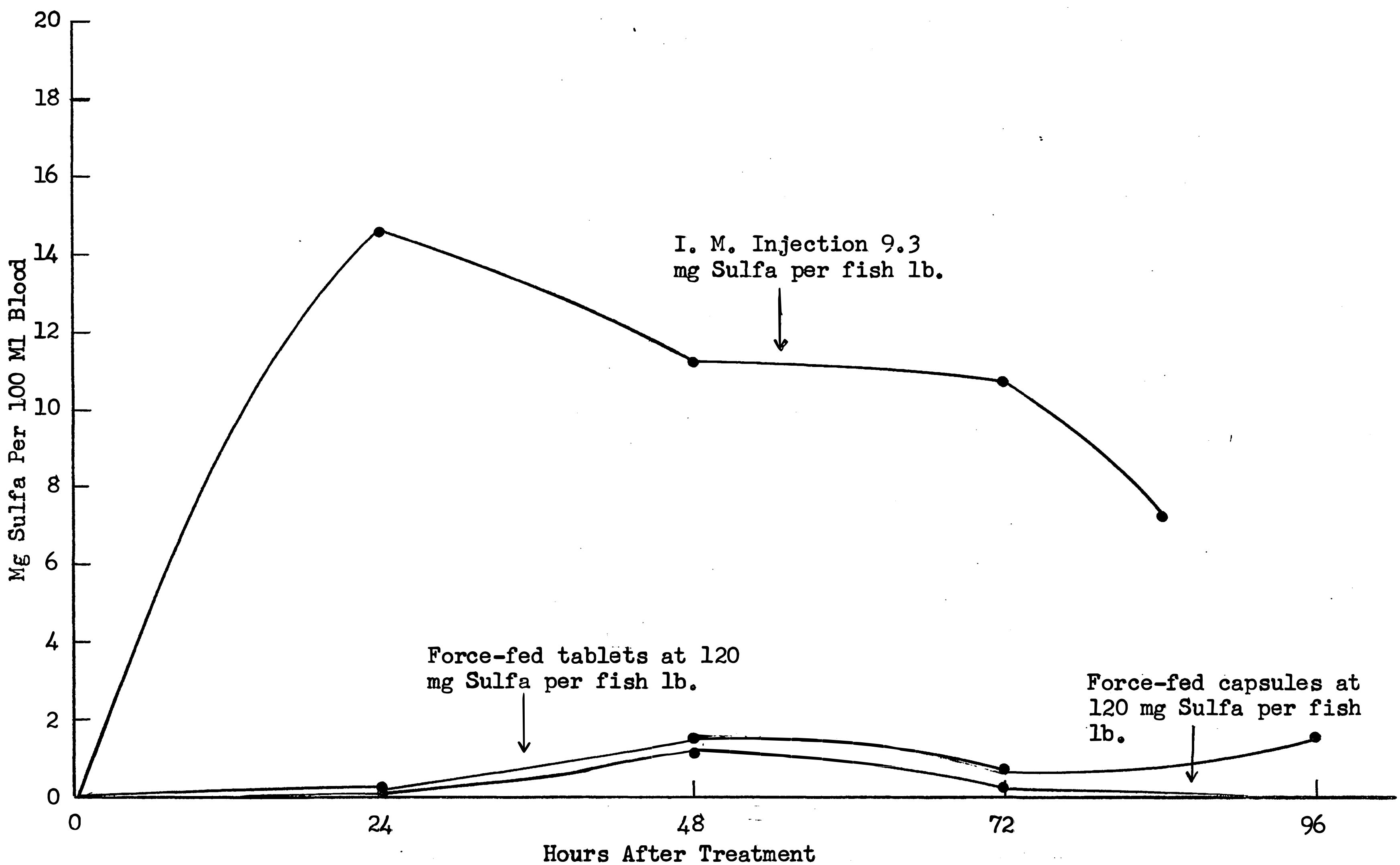

Figure 3. Blood Sulfa Levels of Adult Steelhead Given Intramuscular Injections and Force-fed Bactrovet Tablets and Capsules.

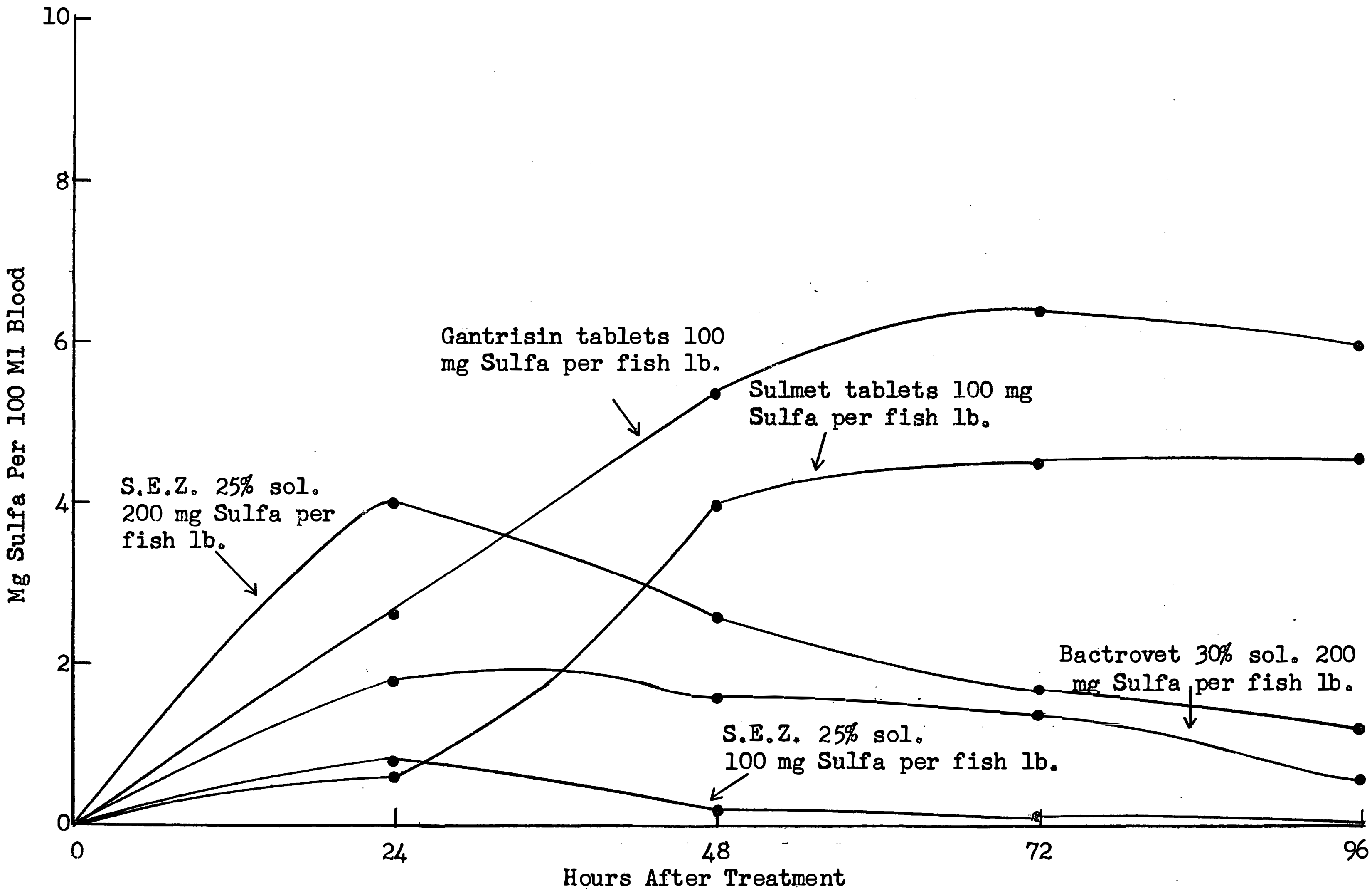

Figure 4. Blood Sulfa Levels of Adult Spring Chinook Force-Fed Sulfonamides.

Table 10. Blood Sulfa Levels in Mg-Per Cent of Adult Chinook and Coho Given Intramuscular Injections of Bactrovet at a Water Temperature of 54° F.

Drug	Rate in Mg Sulfa Per Lb of Fish	Hours After Treatment 12	24	48	72	96	120	144
Chinook								
10% Commercial Sol.	70	9.2	9.7	9.0	7.8 1/	12.8	12.0	9.3
10% Commercial Sol.	70	12.3	12.2	11.2	8.0 2/	15.1	16.3	13.5
Coho								
10% Commercial Sol.	100	--	12.8	8.0	6.2	--	3.5	--
25% Lab. Prep. Sol.	100	--	11.3	8.4	6.3	--	3.5	--
50% Lab. Prep. Sol.	100	--	18.8	15.8	13.0	--	4.5	--
Control	0	0	0	0	0	0	0	0

1/ Booster shot of 35 mg sulfa per lb of fish.
2/ Booster shot of 70 mg sulfa per lb of fish.

Accessory Experiments on Juvenile Coho

The resulting blood and tissue levels obtained from this experiment were comparable (Table 11), indicating that if a fish is not large enough to furnish adequate blood for analysis, that tissue analysis method might be employed.

The coho receiving the quadrasulfa produced blood levels proportional to the amount of sulfa received (Table 12). No toxic effects were observed in any lot.

In Vitro Antibiotic Experiments

The resulting sensitivity values of the antibiotic tests are listed in Table 13. Indications are that for this particular strain of A. salmonicida, only bacitracin would be non-effective. By contrast, only 9 of the antibiotics tested were effective in controlling the growth of A. liquifaciens.

CONCLUSIONS

Adult Experiments

Bactrovet force fed in any form to adults does not produce satisfactory blood sulfa levels. Consequently, this sulfa would have to be employed in the form of intramuscular injections. These injections produce high sulfa levels but the resulting hemorrhagic abscesses make this route undesirable. However, as these abscesses apparently do not hinder the fish's mobility or metabolism this method might be used in any emergency when no other course is effective. The sulfas, Sulmet and Gantrisin show the best possibilities as these seem to be absorbed by the fish when administered orally.

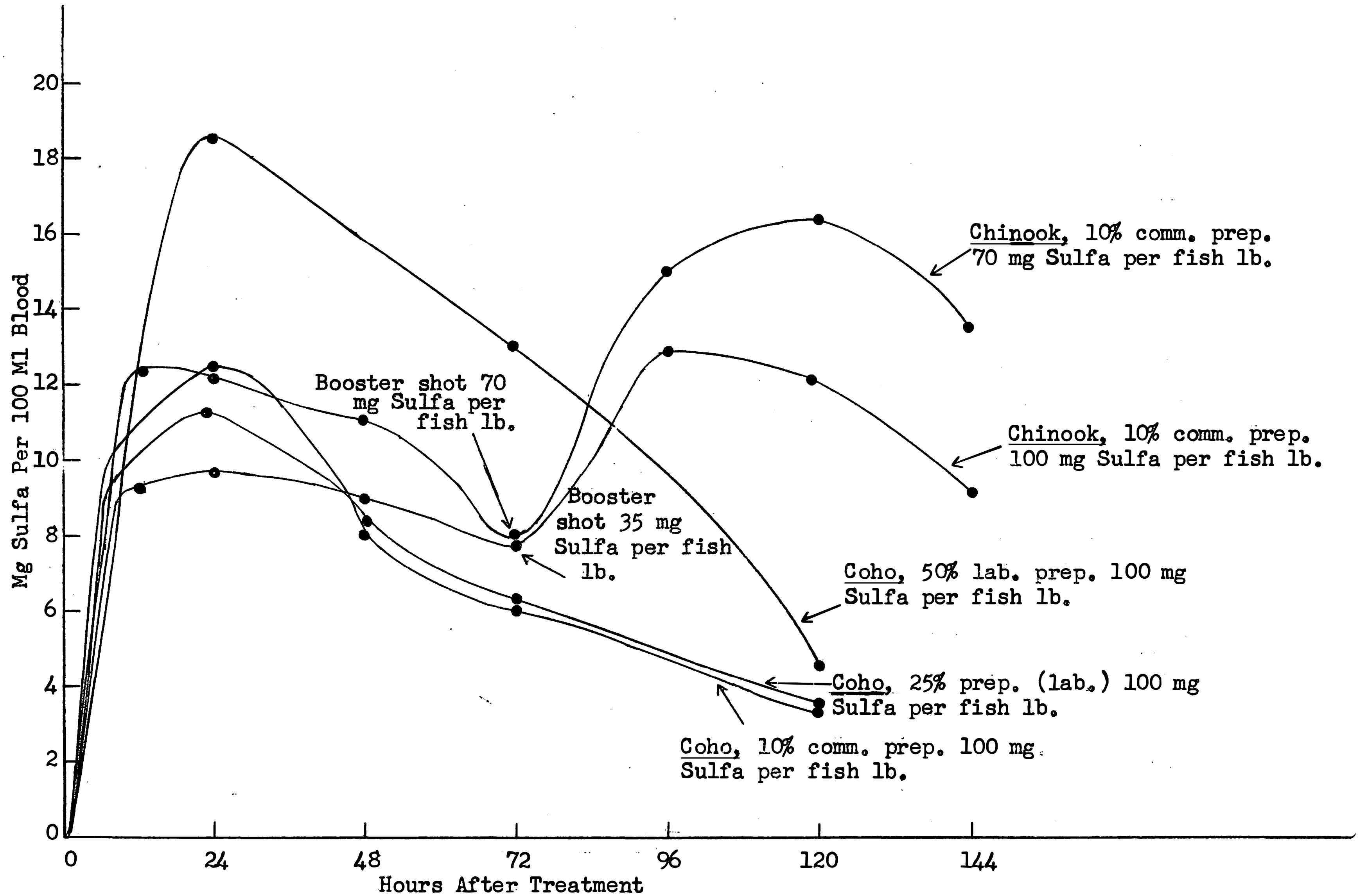

Figure 5. Blood Sulfa Levels of Adult Coho and Chinook Given Intramuscular Injections of Bactrovet.

Table 11. Comparison in Mg-Per Cent Between Blood Sulfa Levels and Tissue Sulfa Levels of Juvenile Coho Fed Oregon Pellets Containing 5 Gms and 2 Gms of Sulmet per 100 Pounds of Fish.

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

1/ Feeding rate--5 gm Sulmet per 100 pounds fish.
2/ Feeding rate--2 gm Sulmet per 100 Pounds fish.

Table 12. Tissue Levels in Mg-Per Cent of Juvenile Coho Fed Quadrasulfa (S-4) at a Water Temperature of 54° F.

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

Table 13. Sensitivity of a Sandy River Strain of A. salmonicida and A. liquifaciens to Fourteen Antimicrobial Agents.

Antimicrobial Agent	Organisms	
	A. Salmonicida	A. Liquifaciens
Furacin	S	R
Furadantin	S	R
Tetracycline	S	S
Colymycin	S	S
Carbomycin	MS	MS
Penicillin	MS	R
Polymyxim-B	S	S
Terramycin	S	S
Neomycin	S	MS
Chlorotetracycline	S	S
Erythomycin	S	MS
Aureomycin	S	S
Bacitracin	R	R
Dihydrostreptomycin	S	R

S - Sensitive
MS - Moderately sensitive
R - Resistant

Accessory Juvenile Coho Experiment

These tissue sulfa level experiments indicate that if a fish is too small to furnish adequate blood for analysis, the fish may be homogenized and the body fluids analyzed for sulfa levels with comparable accuracy. This technique might well be applied to fry or fish of similar size.

The quadrasulfa (S-4) produces satisfactory sulfa blood levels in the coho when fed at 5 and 10 gm of sulfa per 100 pounds of fish. No toxic effects were noted in the coho of 50 fish per pound, but might cause different results in fish of other species. Therefore, experimentation is recommended on each species of fish for which this drug might be used.

Antibiotic Experiments

The *in vitro* sensitivity tests indicate that on the strain of *A. salmonicida*, only Bacitracin was not effective in controlling growth, while in the case of *A. liquifaciens*, 5 antimicrobial agents were non-effective. These tests are not conclusive. *In vivo* tests may produce quite different results.

EXTERNAL INFECTIONS CONTROL

I. Topical Application of Malachite Green for Control of Common Fungus Infections in Adult Spring Chinook Salmon

INTRODUCTION

During adult chinook studies in 1962 at OFC holding ponds at Dexter Dam on the Middle Willamette River, the usual problem with external fungus infection was experienced. Treatment of the water supply with 1 ppm malachite green was not effective in curing this malady once it had become well established. Experience with 12 experimental fish suggested that topical application of a strong solution of malachite green to fungus-infected areas might prove effective in combatting the disease. Although these fish were concurrently involved in another experiment, and were being handled frequently, the original lesions were free of fungus and advanced healing was evident at the end of 6 weeks following one topical application of malachite green per week. In a few instances there was complete healing. Some benefit was noted at the OFC Sandy Hatchery after only one topical treatment of fungus-infected areas of fish upon their arrival at the holding ponds.

In 1963 an attempt was made to duplicate these earlier results in a more refined study. Fish were chosen which exhibited advanced fungus infections on the head, commonly called "sore heads". These were treated by various methods expected to accomplish results similar to those found earlier.

MATERIALS AND METHODS

Adult female chinook salmon were obtained from Eagle Creek National Hatchery. "Zinc-free" malachite green regularly used in OFC hatcheries was made up as a 10% aqueous solution (weight/volume) and applied directly to the lesions with a paint brush. Fish were anesthetized (1:17,500 MS 222) before treating and photographing.

RESULTS

Fish number 15 was treated for the first time on July 18, 1963 (Figure 6). Figure 7 shows the same fish one week after treatment, and while not as evident photographically as visually, the mycelial mat was reduced. Healing of the area had not yet begun. Figure 8 shows the same fish 3 weeks after the first treatment. The area above the eye clearly shows tissue repair. Close examination of the dark tissue projection at the back of the lesion also revealed regeneration around its tip. Figure 9 was taken 4 weeks after initiation of treatment. By this time the area above and just behind the eye was almost healed. Reexamination of the photographs reveals many other, more subtle spots of tissue repair.

Fish number 29 as shown in Figure 10 had an extensive fungus lesion on the right side of the head. Figure 11 shows the same fish 13 days after the first treatment. By comparison of the two photographs it can be seen that the mycelial mat was gone from the lesion, and the area just above the eye showed slight tissue repair at this time. The forward part of the lesion also exhibited healing. This fish did not survive past this treatment. The cause of death was diagnosed as furunculosis.

Fish number 17 (Figures 12 and 13), had an extensive fungus infested lesion covering the greater portion of the head. Figure 12 shows the fish before treat-

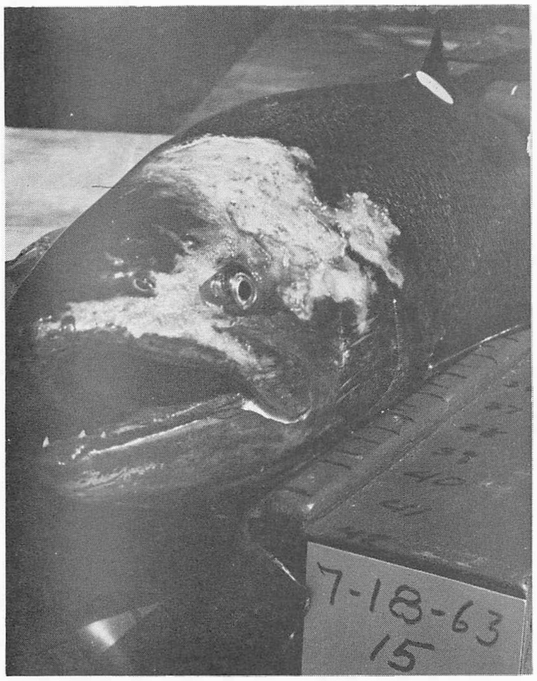

Figure 6.--Female Chinook, Before Topical Malachite Green Application.

Figure 7.--Same as Figure 6, One Week After Topical Application of 10% Aqueous Malachite Green.

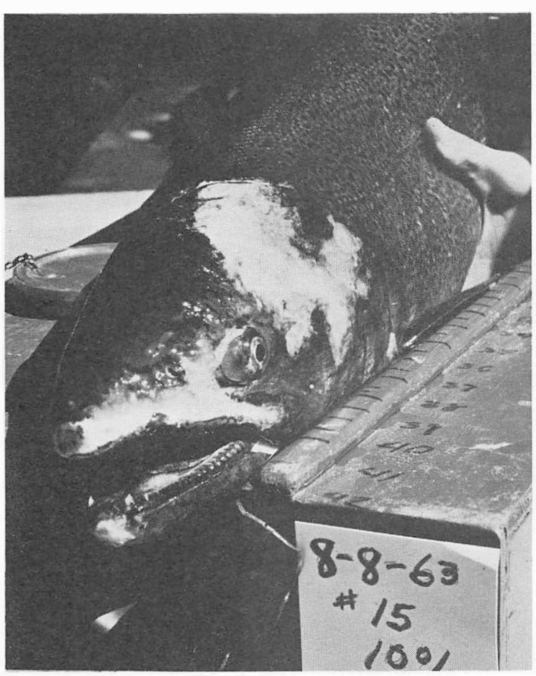

Figure 8.--Same as Figure 6, Three Weeks After Weekly Topical Application of 10% Aqueous Malachite Green.

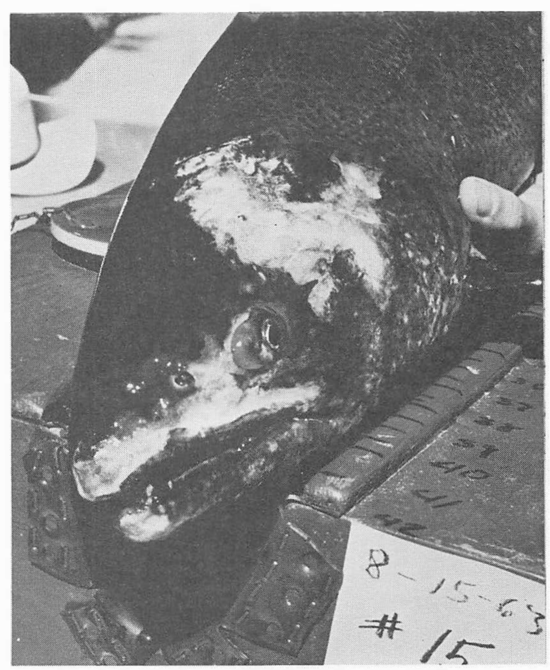

Figure 9.--Same as Figure 6, Four Weeks After Weekly Topical Application of 10% Aqueous Malachite Green.

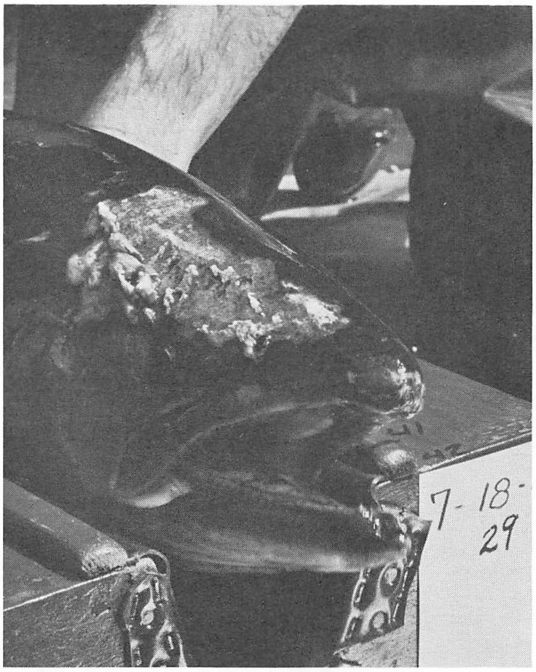

Figure 10.--Female Chinook, Before Treatment with Malachite Green.

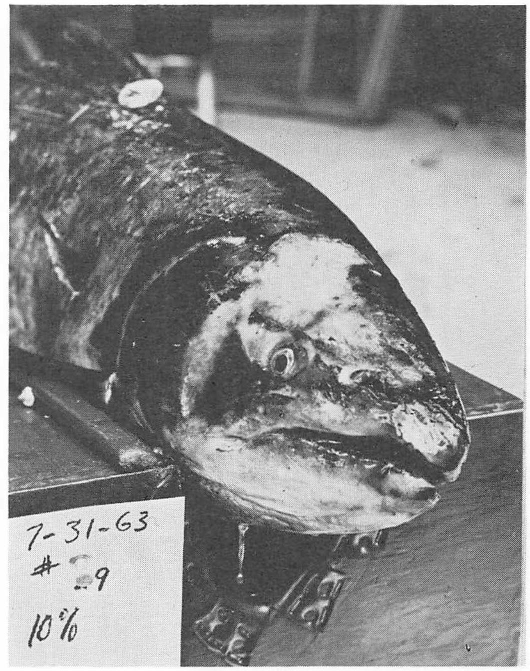

Figure 11.--Same as Figure 10, Thirteen Days After Weekly Topical Application of 10% Aqueous Malachite Green.

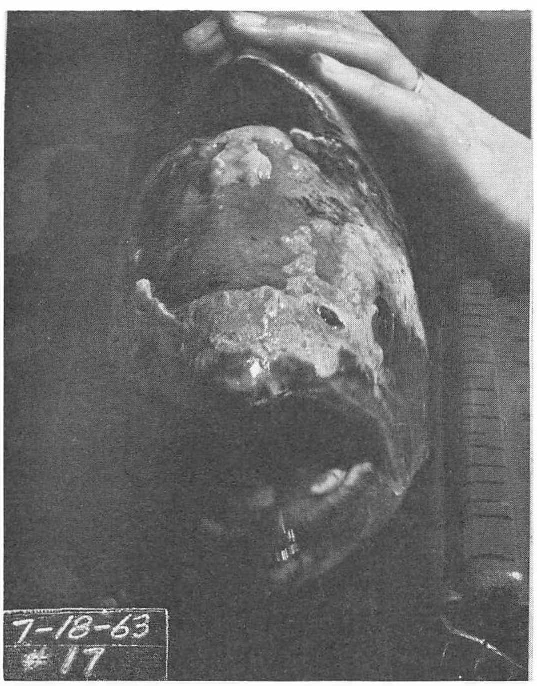

Figure 12.--Female Chinook, Before Treatment with Malachite Green.

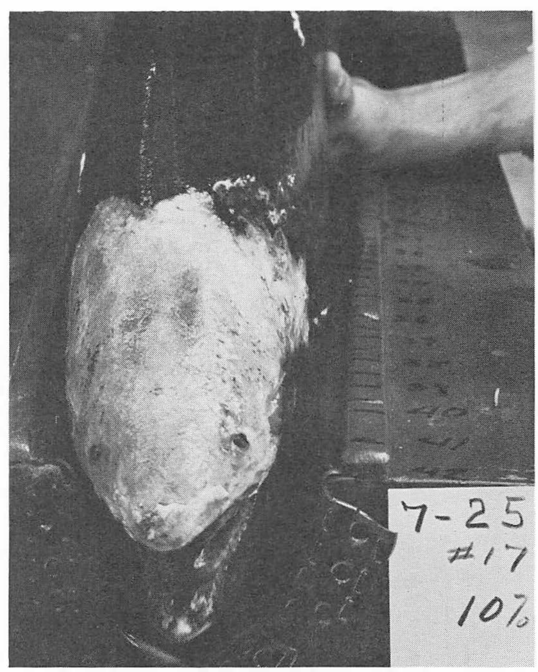

Figure 13.--Same as Figure 12, One Week After Topical Application of 10% Aqueous Malachite Green.

ment and Figure 13 shows the fish one week after treatment. By comparison most of the fungus mat had become less extensive during this short period, however, healing could not be recorded since the animal did not survive until the next examination. The cause of death was diagnosed as a secondary *A. liquefaciens* infection.

DISCUSSION

It is evident that an extensively "fungused" lesion on adult chinook salmon can be cleared of the infection by topical application of malachite green with subsequent regeneration of the damaged tissues. Topical application affords an opportunity to subject the fungused area, with safety to the fish, to a concentrated solution of fungicide, and to rid the area of the infectious agent. Once the lesion is free of the agent, healing will follow with relative rapidity.

Treatment of each fungused fish in this manner, each week, on an operational basis is in many cases impractical. However, if situations were to arise in which a small number of females were on hand, or in which the fish were being frequently handled (as experimental animals or ripeness tests during spawning periods) one could easily justify the topical application procedure.

CONCLUSIONS

Concentrated malachite green, when applied directly to a fungused lesion clears the lesion of fungus and weekly treatments promote healing of the area. No fungus tested resisted this treatment.

II. Water Treatment for Control of Common Fish Diseases: Chemical Toxicity Studies

INTRODUCTION

This investigation was undertaken to establish total immersion toxicity levels of malachite green, ethyl mercury phosphate (lignasan) and pyridylmercuric acetate (P.M.A.) for chinook and coho salmon. Each has been employed in water treatment procedures for the control of fish diseases. The primary objective of this investigation was to establish toxicity levels for adult salmon, but juvenile studies were conducted during the winter when adults were not available.

MATERIALS AND METHODS

All toxicity studies were conducted by subjecting the fish to the chemical for one hour. The fish were observed for 72 hours after the treatment period and the number of deaths noted at 24-hour intervals. All studies on adult chinook were conducted at Dexter holding ponds, in water temperatures of 13-14° C. The test on coho adults (jacks) were performed at Sandy Hatchery, in water temperatures of 6-10° C. All experiments on juveniles were conducted at Clackamas with water temperatures maintained at 15° C. Chinook yearlings were obtained from Willamette Hatchery, and the coho came from Sandy Hatchery. The juveniles of both species were subjected to identical experimental conditions, therefore the toxicity for each species is plotted on the same graph for comparison.

RESULTS

Adult Experiments

Figure 14 shows malachite green is toxic for adult chinook at concentrations greater than 5 ppm with an LD_{50} between 8-9 ppm; LD_{100} was experienced at 10 ppm. An LD_{50} is the dosage or concentration at which 50% death can be expected. Lignasan (ethyl-mercury phosphate) was toxic for chinook at concentrations beyond 2 ppm (Figure 15). The LD_{50} is between 2-3 ppm and LD_{100} occurs at 4 ppm. PMA (pyridylmercuric acetate) is toxic for chinook at concentrations above 10 ppm (Figure 16). The LD_{50} is between 20-25 ppm, while the LD_{100} occurs at 35 ppm. Figure 14 shows that malachite green is toxic at concentrations over 2 ppm. The LD_{50} is between 3-4 ppm and the concentration at which LD_{100} occurs is 5 ppm. In Figure 15 it can be seen that Lignasan is toxic at over 2 ppm. The LD_{50} is between 4-5 ppm, with an LD_{100} of 6 ppm. PMA (Figure 16) was toxic at concentrations over 14 ppm, with an LD_{50} between 16-18 ppm. LD_{100} was observed at 30 ppm.

Juvenile Experiments

Figure 17 shows the toxic effect of malachite green begins at a concentration above 1 ppm for juvenile coho and above 2 ppm for juvenile chinook. The LD_{50} for coho is approximately 3 ppm and 4.5 for chinook. LD_{100} for both species was reached at a concentration of 6 ppm. Lignasan (Figure 18) was toxic at concentrations greater than 2.5 ppm for chinook and 3.0 ppm for coho. The LD_{50} is approximately 4 ppm for both species, while LD_{100} was reached at a concentration of 4.5 ppm for chinook but not until 6 ppm for coho. Figure 19 shows the toxic effect of PMA on chinook begins at concentrations over 8 ppm and on coho at

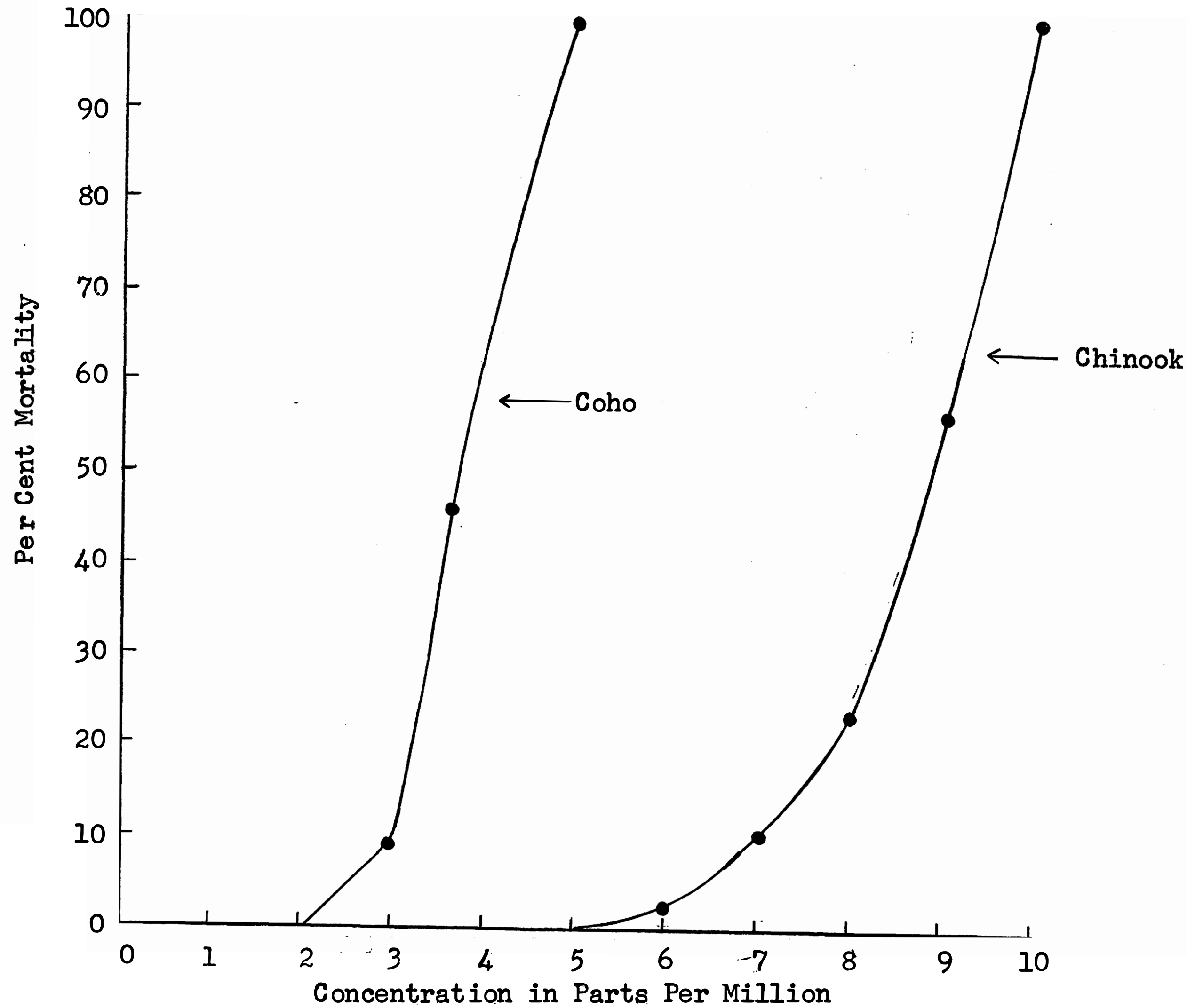

Figure 14. Toxicity of Malachite Green to Adult Chinook and Coho.

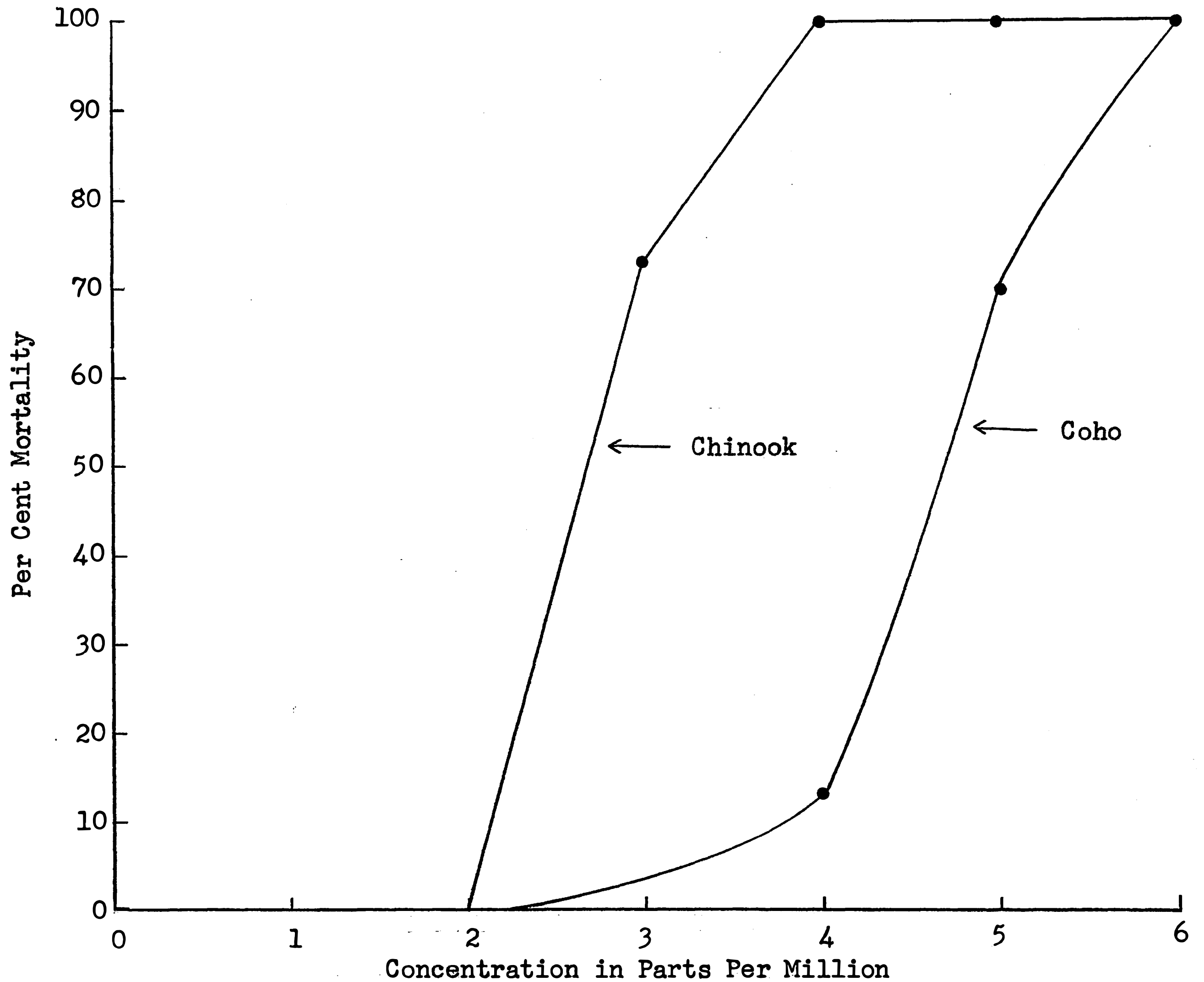

Figure 15. Toxicity of Lignasan to Adult Chinook and Coho.

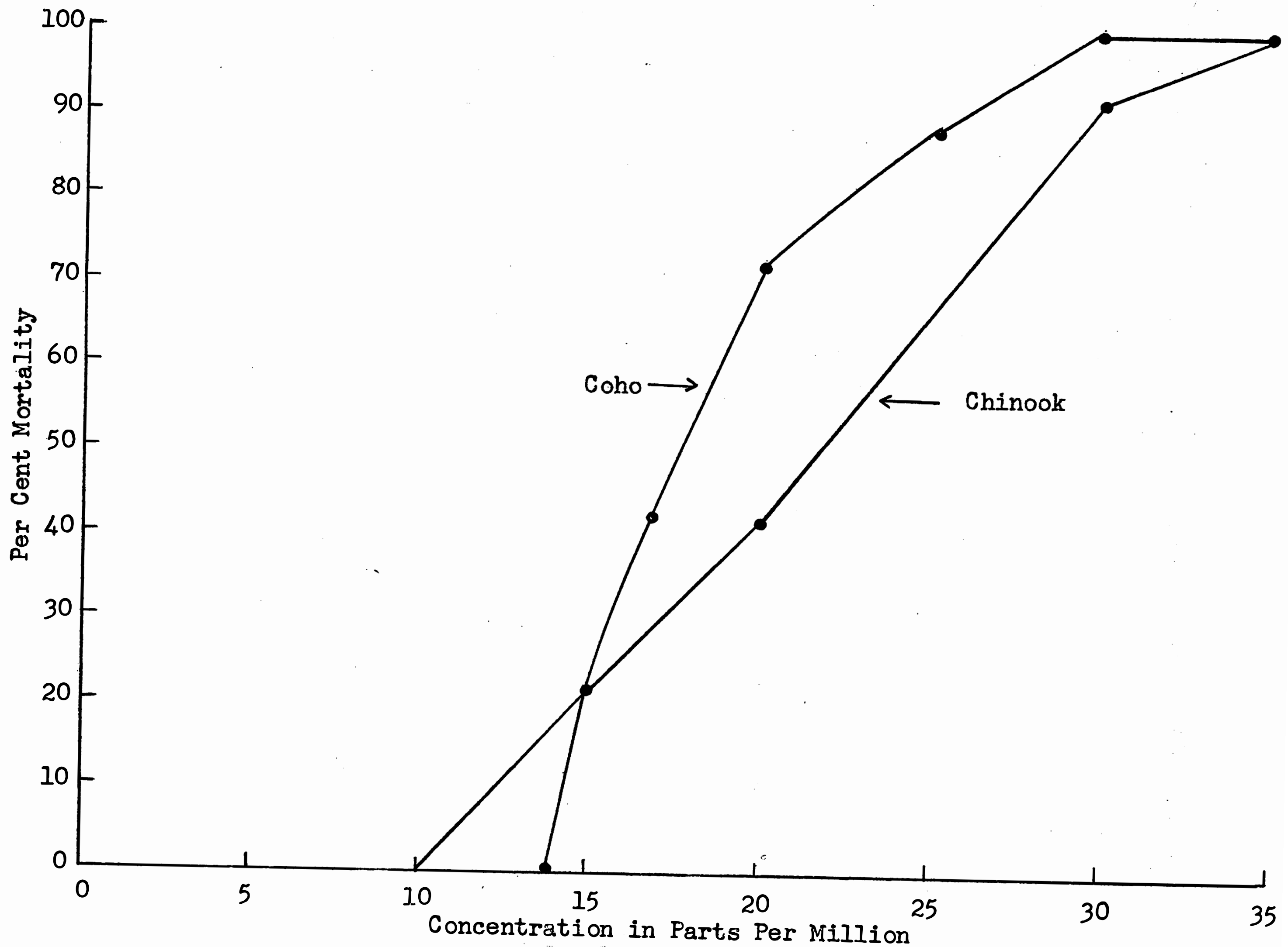

Figure 16. Toxicity of P.M.A. to Adult Chinook and Coho.

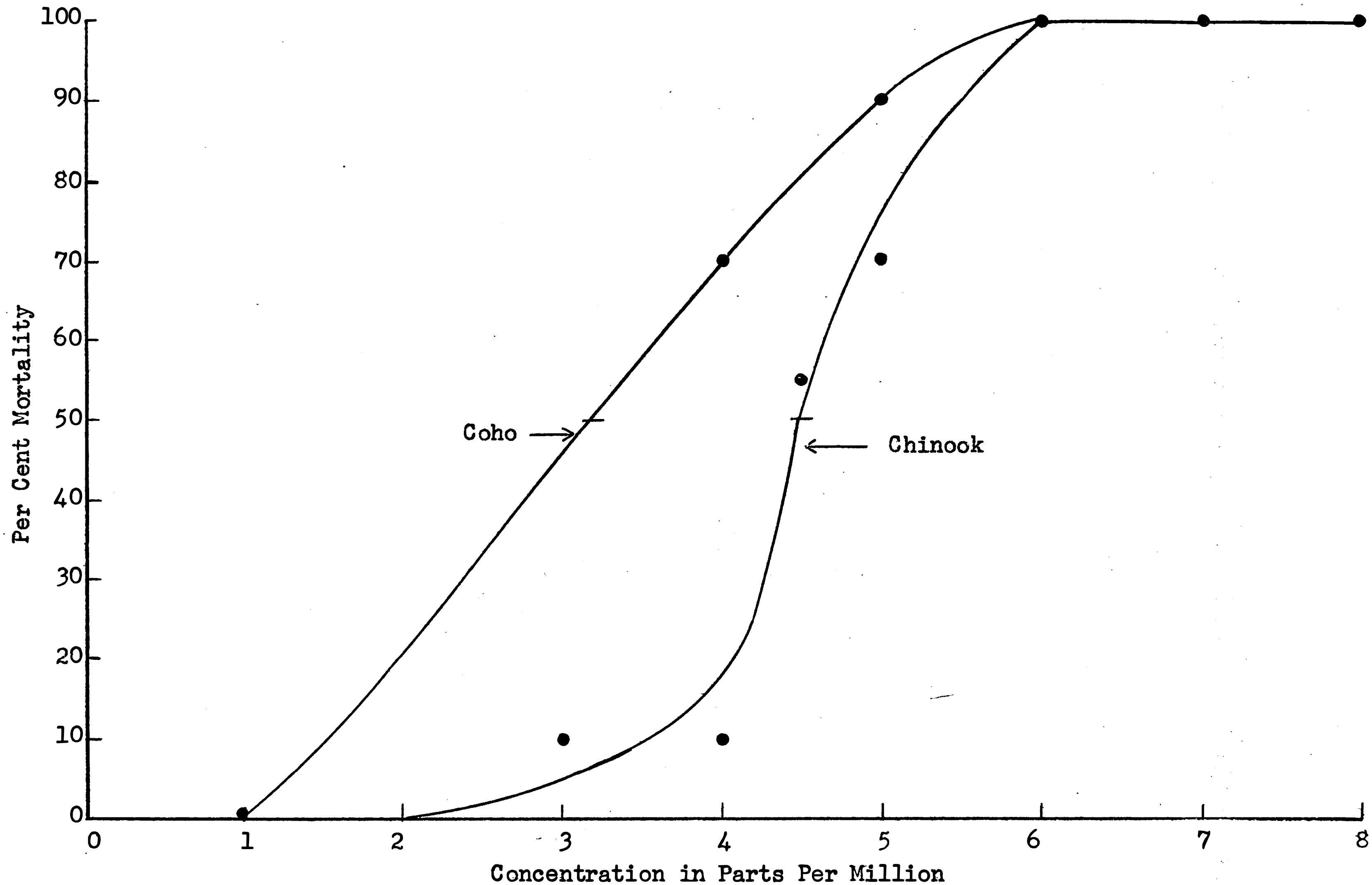

Figure 17. Toxicity of Malachite Green to Yearling Chinook and Coho.

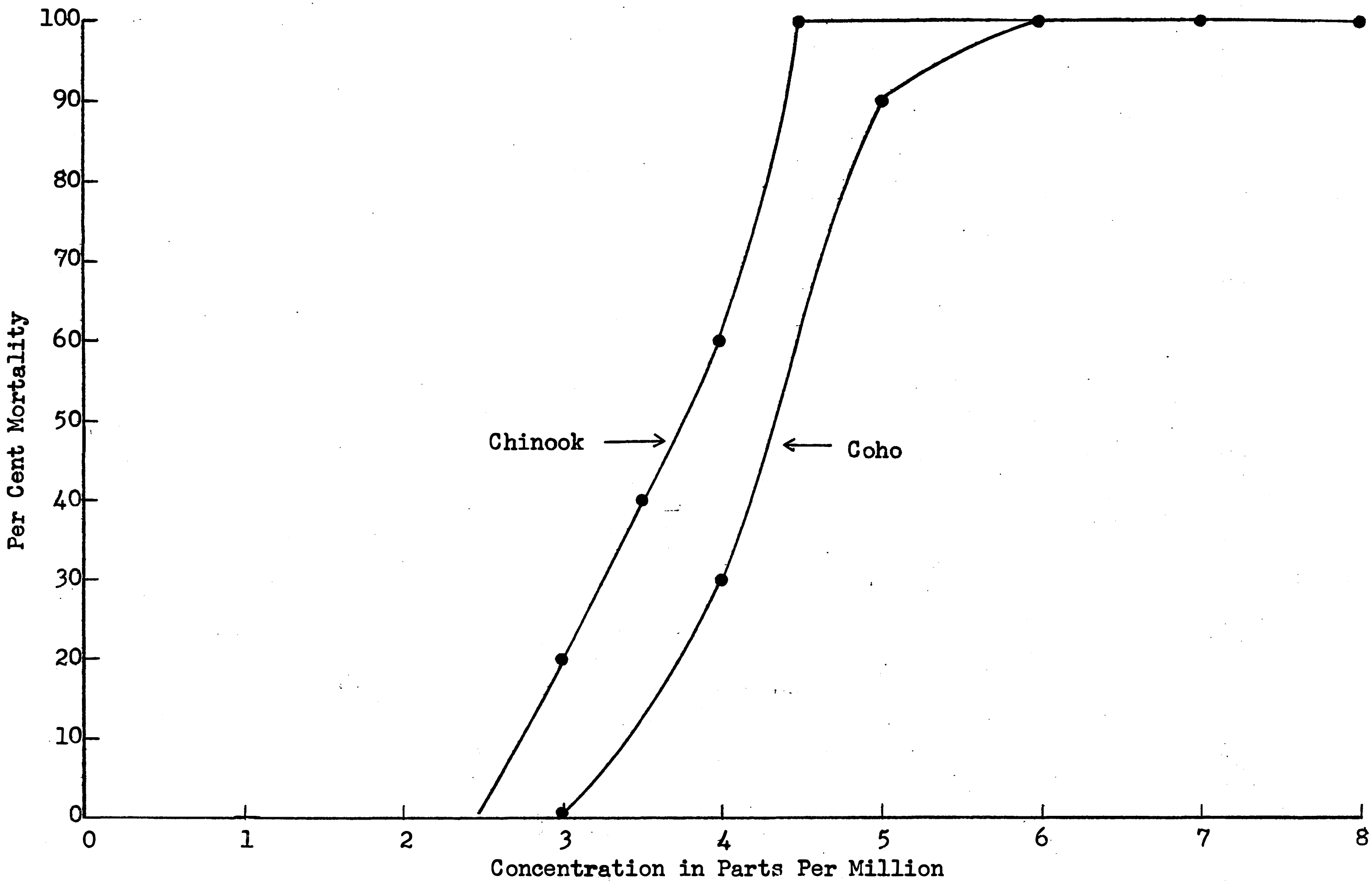

Figure 18. Toxicity of Lignasan to Yearling Chinook and Coho.

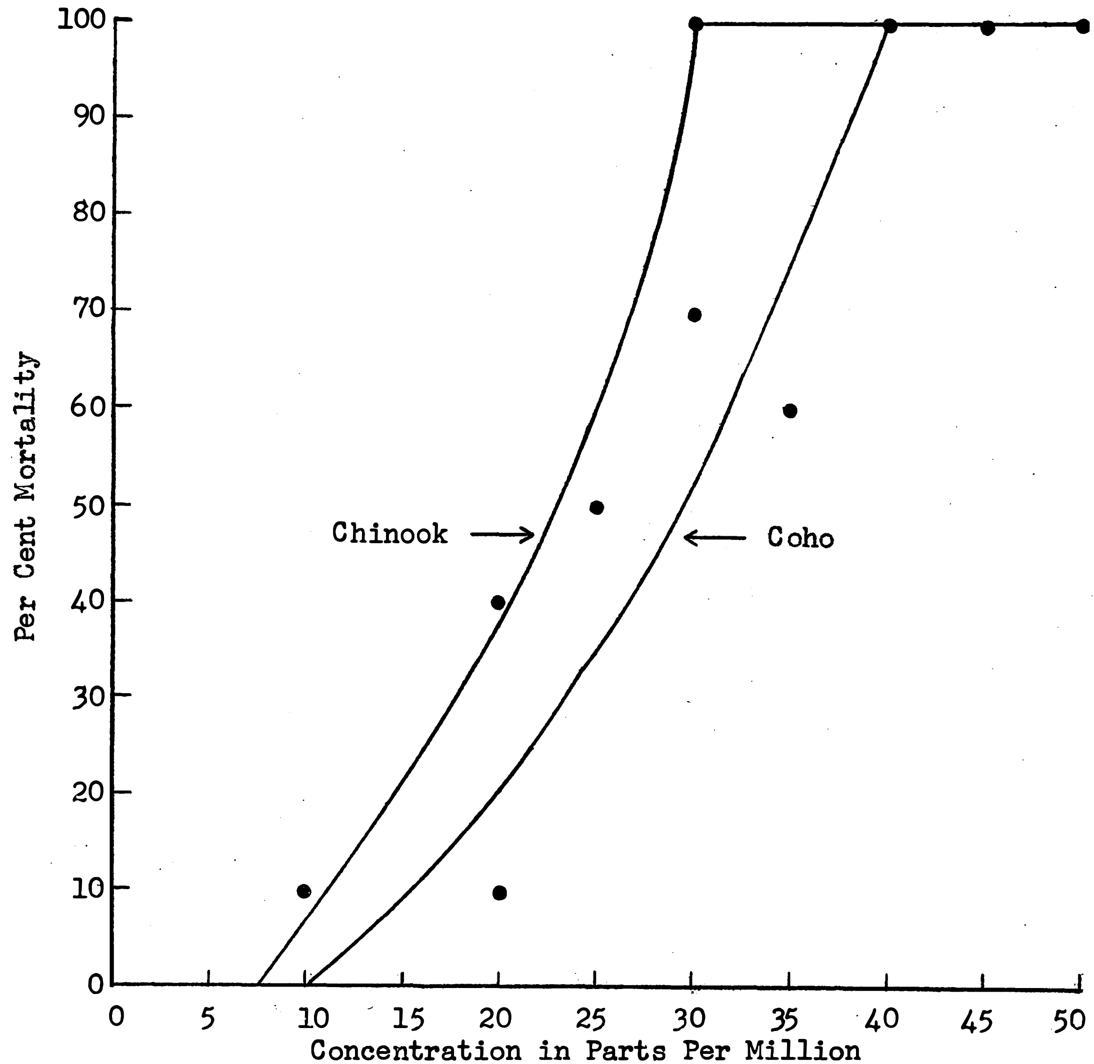

Figure 19. Toxicity of P.M.A. to Yearling Chinook and Coho.

greater than 10 ppm. The LD_{50} for chinook is approximately 23 ppm and for coho approximately 30 ppm. The LD_{100} for chinook was 30 ppm, and for coho, 40 ppm.

CONCLUSIONS

A study of these results indicates that there are differences in the ability of coho and chinook salmon to withstand the toxic effect of the chemicals tested. However, such comparison should not be made as the experiments were carried out at two different sites, at different times, and at different water temperatures. Variations in experimental results caused by water quality differences was clearly shown by two experiments, not reported here, in which the water was slightly turbid. In the turbid water the toxic level of malachite green for adult coho dropped to less than 1 ppm, as compared with greater than 2 ppm in non-turbid water at the same site. Thus under adverse water conditions possibly all water treatment chemicals should be used with great caution.

The experiments with juvenile salmon can be compared because identical experimental conditions were imposed on both species. It can be seen that chinook yearlings are more resistant to the toxicity of malachite green than coho. The reverse is true with Lignasan where coho yearlings are slightly more resistant than chinook. The experiments with PMA show coho are again more resistant to the chemical's toxic properties.

The variations in experimental results caused by water quality indicates that in order to establish accurate guidelines for use with various waters, experiments should be performed at each hatchery or holding facility involved.

MISCELLANEOUS

I. Area Survey Reports

The Middle Fork of the Willamette River and the Snake River were selected as study areas, since a comprehensive knowledge of the two groups of fish (viz. spring chinook in the Willamette and fall chinook in the Snake) would allow a comparison with information gained prior to this study. Periodic disease surveys were made in these areas and laboratory and other information gathered in Special Reports as follows:

Udall Disease Investigations, Hatchery Examinations of Adult Chinook, Summer 1963

Udall Disease Investigations, Hatchery Examination of Adult Chinook, Oxbow Hatchery, September-October 1963

Udall Disease Investigations, Hatchery Examinations of Adult Chinook, Dexter Holding Ponds, September-October 1963

In review, information contained in these reports leads us to the following conclusions:

1. While Chondrococcus columnaris was isolated from between 30-80% of the dead fish examined, it was not considered a primary cause of death. In many cases, columnaris lesions appeared to be in an advanced state of healing.

2. Secondary invaders, viz., A. liquefaciens and other opportunists of the Pseudomonad type, appeared to be a principle cause of death. In most cases of death of this type, there appeared to be a correlation between abrasions and other external injuries and generalized infection. Deaths due both to primary pathogens, i.e., A. salmonicida, and to secondary invaders appear to be enhanced by high holding pond temperature.

3. Malachite green treatment of holding pond water is beneficial as an antifungal agent and as a general preventative in disease transmission.

4. Parasites of the myxosporidian group, identified as Ceratomyxa sp., contribute to a marked degree to deaths in the study areas. (While not a part of those surveys summarized here, findings from autopsies of fish from other areas of the state bear out the same conclusion). If Ceratomyxa sp. are not a primary cause of death, they are important in establishing a route of entry for secondary invaders. They were, in several instances, encountered in sufficient numbers in fish examined to be considered generally debilitating.

Additional reports are being prepared for the current season's operations, 1964.

II. A Synthetic Medium for the Growth of *Aeromonas salmonicida*

INTRODUCTION

Since a well-defined study of pathogenicity, drug resistance, susceptibility, or the physiology of an organism requires a chemically-defined, or synthetic medium, it was thought advisable to undertake the development of such a medium for *A. salmonicida*, a fish pathogen of importance in this study.

MATERIALS AND METHODS

Cultures included *A. salmonicida* 5000H isolated from Snake River adult fall chinook and *A. salmonicida* 5-14174 (ATCC 14174) obtained from American Type Culture Collection were used as test organisms.

Chemicals: All amino acids, vitamins, and nucleic acids were obtained from Calbiochem, California. All other chemicals were reagent grade chemicals.

RESULTS

Through culturing of 5000H and 5-14174 on various media lacking in all but one compound the following medium was found to adequately support the growth of these two organisms:

NH_4Cl	2.0	gm/liter
$MgSo_4 \cdot 7\ H_2O$	0.1	" "
KH_2Po_4	1.5	" "
NA_2HPO_4	3.5	" "
Glucose	50.0	" "
Arginine	100	mg/liter
Methionine	100	" "
Aspartic Acid	100	" "
Leucine	100	" "
Glutamic Acid	100	" "
Histidine	100	" "
Serine	100	" "
Glycine	100	" "
Tyrosine	100	" "
Tryptophan	100	" "
Phenylalanine	100	" "
Lysine	100	" "
Alanine	100	" "
Pyridoxal Phosphate	0.0006	gm/liter
Pantothenate	0.001	" "
Riboflavin	0.001	" "
Niacin	0.002	" "
Para Amino Benzoic Acid	0.002	" "
Biotin	0.000002	gm/liter
Folic Acid	0.00002	" "
Uracil	0.02	" "
Adenine	0.02	" "

Distilled water	1000 ml/liter
For solid medium add:	
Agar	15 gm/liter
pH	7.0
Autoclave	15 minutes at 15 lbs.

DISCUSSION

It was found that most of the amino acids could be individually deleted from the medium without completely inhibiting *A. salmonicida* growth. Growth was, however, retarded. A similar effect was noted when individual vitamins were deleted. Removal of tyrosine, tryptophan, and histidine had little effect on the growth.

CONCLUSIONS

A. salmonicida has the ability to form most of the biosynthetic intermediates necessary for its growth (either completely from simpler components, or by alteration of already-supplied amino acids), however in most instances these are produced in minimal amounts not sufficient to support more than limited growth.

III. The Pathogenicity of a Phage-sensitive Strain of *Aeromonas liquefaciens*

INTRODUCTION

A gram negative rod-shaped bacterium was isolated from the kidneys of over 70% of the adult fall chinook mortalities occurring at the Oxbow Dam Hatchery on the Snake River in 1962. The frequency of its occurrence in culture from these mortalities prompted its identification. It was later classified as *A. liquefaciens*.

Work was undertaken to determine if *A. liquefaciens* is a pathogen, and also if it can be considered an important cause of adult chinook mortalities.

With the isolation of *A. liquefaciens* from the mortalities, a phage was also isolated. The indicator strain was designated 2035H as a stock culture number and the phage numbered 2035H Ø. This strain proved useful in determining the pathogenicity of *A. liquefaciens*.

MATERIALS AND METHODS

Cultures

Cultures were isolated and maintained on Meuller-Hinton (Difco) medium and transferred weekly. Incubation was at room temperature. Liquid cultures were maintained in a broth containing 11.5 gm/liter Bacto Casitone (Difco), 50 gm/liter brain-heart infusion (Difco), and 1.5 gm/liter soluble starch, (pH is adjusted to 7.0).

Cells of 2035H used for injection were centrifuged from liquid culture and washed twice with sterile saline. Cells were injected as a saline suspension. Cells for feeding experiments were harvested by centrifugation, and the packed cells added to the thawed food and mixed. The mixture was then refrozen until fed.

Fish

Juvenile spring chinook from Willamette Hatchery and juvenile coho from the Sandy River Hatchery were used as experimental fish. They were weighed and distributed 10 fish per group.

Autopsy and Reisolation of Test Organism

The phage indicator strain was used in all experiments of infectivity. When a mortality occurred, cultures were made from the kidney onto Meuller-Hinton agar, and the resulting growth tested for phage sensitivity by the agar overlay method (1). This proved to be a rapid and concise method for identification of the isolated organism. This phage was found to be specific for the indicator strain 2035H. This was shown by absence of plaque formation when 2035H Ø was tested against 20 other isolates of *A. liquefaciens* from the same area.

Feeding Experiments

Oregon pellets were thawed, and the cells from an 18-hour broth culture, plus other test ingredients were added, (such as ground glass). 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.

RESULTS

Table 14 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; 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 determine if the organism could enter by way of an abrasion of the skin. The slime layer was first scraped away from the side of a fish and the skin lightly abraded with fine sand paper. This area was "painted" with a 5-hour culture. 1/ Figure 20 presents the results of this experiment. The first mortalities occurred at 65 hours after infection and the rate was nearly constant until 81.2% of the test fish were dead (140 hours after infection). Four control fish also died between 135 hours and 140 hours, however, the test bacterium could not be isolated from them.

It is apparent that a fatal infection with A. liquefaciens can be produced by abrasion or some similar trauma. In this laboratory fatal infections caused by A. salmonicida could be produced in this same manner. The method consistently was found to give uniform and reproducible measurement of infectivity (9).

Ground Glass Feeding Experiments

Wolfe and Dunbar (12) attempted to establish a route of entry through the intestine for the agent of kidney disease by using ground glass in the food, plus the bacterium. A similar experiment was designed using four lots of fish. One lot received the bacterium incorporated into the diet. The second lot received the bacterium plus 5% (by weight) ground glass in the diet. Corresponding control groups received the normal diet only, and diet plus ground glass but no bacteria. The rationale behind this was: if Ceratomyxa sp. infection of the intestinal tract had provided the route of entry of the bacterium under natural conditions, then the ground glass in the diet should experimentally reproduce a similar intestinal lesion and fatal systemic infection.

Slide preparations were made from organs of the mortalities at the Snake River for Ceratomyxa sp. identification. In the limited number of slides, Ceratomyxa sp. was determined as being present. It is known, from the work of Wood (13) at Washington hatcheries on the Columbia River, the incidence was quite high during the fall of 1962. This information promoted a search for some method whereby an infection with the isolate from the Snake River could be established by way of the intestinal route.

Table 15 gives the results of this experiment. It can be seen that the group receiving the ground glass plus the bacterial suspension contracted a fatal infection. The fish which received the bacterial suspension only in the food did not contract the infection. The group that received the ground glass but no bacteria

1/ In the modified Meuller-Hinton broth A. liquefaciens grows very rapidly and a moderately turbid culture could be grown in this time.

Table 14. Experimental Routes of Infection for A. liquefaciens.

Route of Inoculation	Per Cent Mortality End of 24 hr.	Number of Organisms per ml Injected or Infinial Concentration
Intraperitoneally	100	100 x 10^7
Subcutaneously	100	100 x 10^7
Feeding 1/	0	10.9 x 10^7
Control (SP)	0	0
Control (Sub Q)	0	0
Control (Feeding)	0	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 cultured.

incurred some mortalities, however, the infection was not caused by the test organism 2035H susceptibility. Again, the phage sensitivity of 2035 H was of practical value.

DISCUSSION

The bacterium which is discussed here was identified as a member of the Aeromonas genus by the Presumptive Identification Chart Published by Bullock (2). The bacterium was then classified as use of Bergy's Manual of Determinative Bacteriology and by use of published results of Omers (3, 4, 7, 8, 10, and 11). Several isolates of this same bacterium were all classified as A. liquefaciens and later compared to other members of the Aeromonas genus obtained from the American Type Culture Collection.

From the evidence which has been presented it can be seen that this bacterium causes death of juvenile salmon if it is interperitoneally or intramuscularly injected. When the organism is fed to fish in a food containing ground glass, the infection can be established, however, no infection occurred by general water contact alone in these experiments. The results point to the conclusion that A. liquefaciens lacks power of invasiveness and to establish an infection. It must be introduced into the tissue by some other means.

These conclusions are further supported by field observations of a group of adult spring chinook salmon during the summer of 1963. Again, Ceratomyxa sp. infection was observed in the majority of these fish from which A. liquefaciens was isolated.

CONCLUSIONS

A. liquefaciens is able to establish a fatal infection in fish if given a portal of entry.

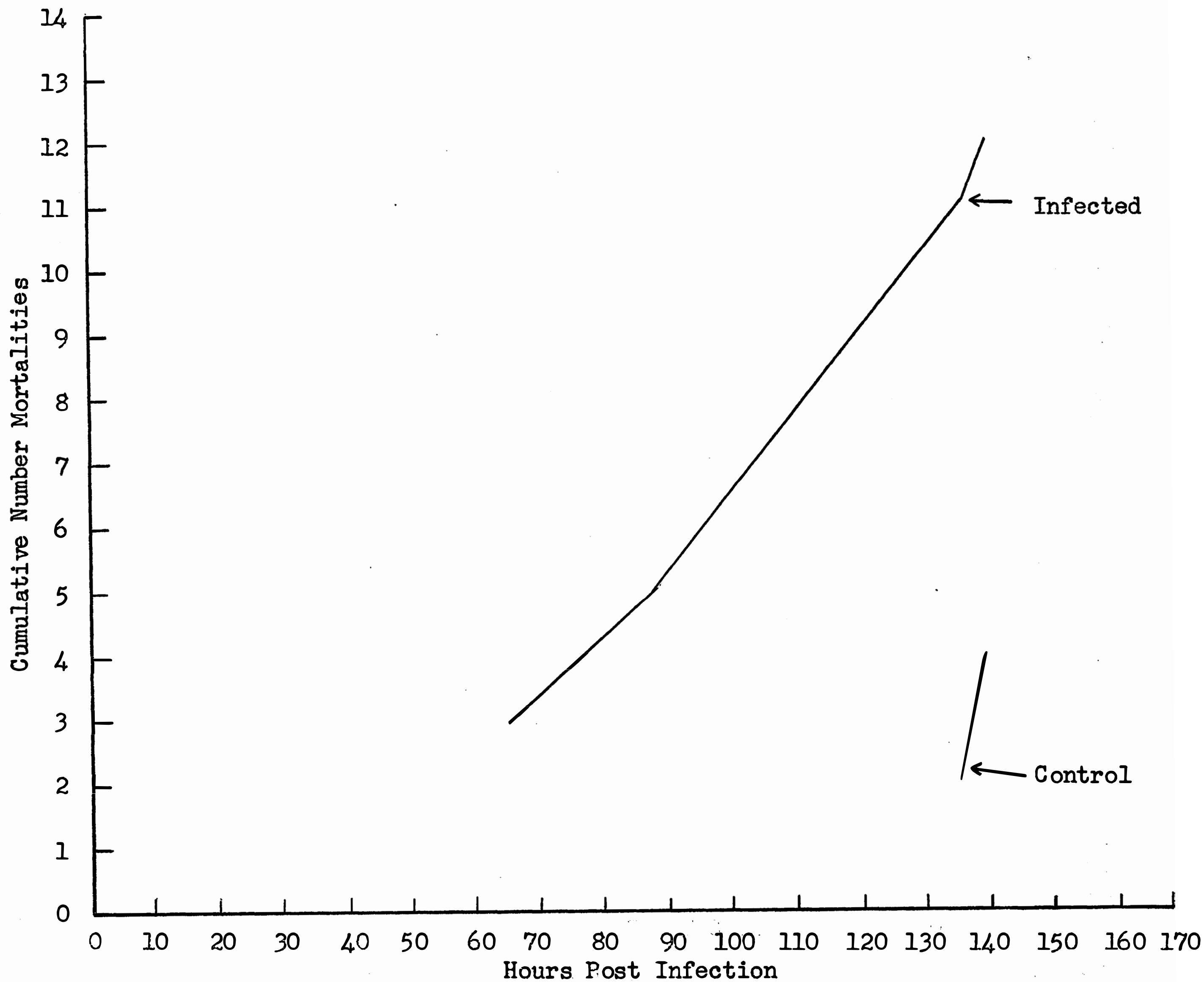

Figure 20. Cumulative Mortalities of Abraded Juvenile Coho After Infection with <u>A. liquefaciens</u> 2035H at 20° C.

Table 15. Thirty-Day Feeding 1/ Experiment Using the Isolated Bacterium from the Snake River.

Diet	Number of Mortalities 2/ Diagnosed as 2035H Infection
Control (Oregon Pellet)	0
Oregon Pellet plus Bacterium	0
Oregon Pellet plus Ground Glass	0
Oregon Pellet plus Ground Glass and Bacterium	5

1/ Rate of 1.5% of body weight per day.
2/ The organism used in this experiment was a phage sensitive strain, therefore the isolates from the mortalities were tested for the sensitivity to the phage.

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