EFFECTS OF TEMPERATURE UPON YOUNG CHINOOK SALMON

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

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Approved by Lauren R. Donaldson

Department Fisheries

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We have carefully read the thesis entitled Effects of Temperature Upon Young Chinook Salmon submitted by Allyn Henry Seymour in partial fulfillment of the requirements of the Degree of Doctor of Philosophy and recommend its acceptance. In support of this recommendation we present the following joint statement of evaluation to be filed with the thesis.

Mr. Seymour has studied the effects of water temperature upon the early development of the chinook salmon. He has approached the problem first by evaluating the effects of different temperatures held constant throughout the period of investigation. A second series of experiments were run by rearing the eggs for varying lengths of time at city water temperatures and then changing them to various constant temperatures for the remainder of the time. A third series was reared at variable temperatures. In this last series, eggs were obtained from the Skagit and Green Rivers in Western Washington, the Entiat River in Eastern Washington and from the Sacramento River in California in order to evaluate racial differences in temperature effects. Control lots were run in all experiments.

Through these experiments Mr. Seymour has compared the different methods of measuring temperature effects and has demonstrated a difference between races in the effect of temperature on rate of development as well as the effects of temperature on duration of hatching period, mortality rate, abnormalities, growth and meristic characters.

Mr. Seymour has demonstrated his ability to conduct original experimental work and to analyze and evaluate the results. Both his experimental design and execution are excellent and his thesis is worthy of acceptance as a Doctoral dissertation.

THESIS READING COMMITTEE:

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Date Aug. 3 1956

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The experiments were conducted under the guidance of Dr. L. R. Donaldson, chairman of the Supervisory and Reading Committees. Errors and omissions in the manuscript have been corrected from suggestions made by Dr. R. Van Cleve and Dr. A. C. DeLacy, members of the Reading Committee. Valuable aid as to techniques and methods was received from Dr. A. D. Welander. Calculation of the threshold temperature and its confidence interval was suggested by Dr. D. G. Chapman and Mr. C. O. Junge.

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I. INTRODUCTION

To the fish living in the Columbia River and other Pacific Coast streams, changes are occurring in the environment because of the impoundment of water for hydroelectric power, the increase in pollution, and the diversion of river water for irrigation. In the future the use of river water to cool nuclear reactors also may change the environment for river inhabitants.

One of the environmental factors that is changed is water temperature, a factor to which fish, a poikilothermic animal, respond readily. Usually the change is to warmer water and temperatures that are not favorable for the survival of salmon. An exception is the Shasta Dam on the Sacramento River where the withdrawal of water from the storage reservoir is from a level that is below the thermocline. As a consequence, river temperatures below the dam during late summer are now as much as 20°F lower than formerly (Cope, 1949 and 1952) and salmon and trout production has increased (Moffett, 1949; Smith, 1950).

The changes that are occurring in the Columbia River and in other Pacific Coast streams may subject salmon eggs and young to unfavorable water temperatures, a condition which also could occur from the early or late arrival of the parent fish upon the spawning ground, or from abnormal weather conditions. These are some of the reasons for accumulating more information on the influence of temperature upon salmon eggs and young. Specifically, the objectives of these experiments were to measure the effects of temperature upon chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), in regard to:

(1) the rate of embryological development

(2) mortality

(3) occurrence of morphological abnormalities

(4) growth rate

(5) the determination of the number of vertebrae, dorsal fin rays, and anal fin rays.

The period of observation was from the egg to the fingerling stage.

Objective (5) above was included for the purpose of making a contribution to the limited information now available on this subject for the Pacific salmon and because of the current interest in the use of meristic characters as one of the means of identifying the racial origin of salmon now being caught in the off-shore waters of the North Pacific. After Heincke's investigations in 1898 meristic characters have been used frequently to identify races (see p. 96). Heincke identified groups of herring as races when the difference in the counts of vertebrae between groups was statistically significant.

Variation in the number of vertebrae between individual fish is a consequence of both genetical and environmental factors. In field samples neither genotypical nor phenotypical variation can be determined because the past history of the individual is not known and therefore the vertebral count of the parents and the environmental factors influencing vertebral formation are not known. However, in laboratory experiments such as these, some estimate of genotypical and phenotypical variation can be made and this information is important to interpretation of results of racial studies based on field samples.

The chinook salmon were selected for experimentation because they are economically important, are reared extensively and are available. In the Columbia River and other rivers of the west coast of the United States that are most affected by dams, pollution and water diversions, this species is often the most important. Over 30 million fry and fingerlings were reared and released by the Washington State Department of Fisheries in 1953. In addition, this species is readily available for experimental use, either from the Fisheries Center, University of Washington, or from nearby hatcheries of the Washington State Department of Fisheries.

The chinook salmon, also known as king, spring, quinnat, tyee or blackmouth, is the largest of the Pacific Coast salmon and usually spawns in the large rivers. Spawning occurs as early as August in Alaska and as late as December in California, the extremes of its geographical distribution. The eggs are deposited in a nest in the gravel of the stream bed that has been dug by the female, are fertilized by the male, and then are covered with gravel. While in the gravel, the eggs hatch. The young fry* work downward into the stream bed, as they are negatively phototropic. When most of the yolk has been used, the fry emerge from the gravel and soon start to seek their own food. The seaward migration may begin immediately, but often is delayed until a few months after feeding, and occasionally as much as a year. The chinook salmon live in the sea until maturity, which is usually at an age of 3½ or 4½ years, but may vary from 2½ to 6½ years. At maturity the

*"Fry" is the stage which follows hatching and during which the yolk sac is absorbed; when feeding begins, the fry become "fingerlings."

chinook salmon, weighing 10 to 50 pounds, returns to its natal stream to spawn and die (Clemens and Wilby, 1946).

Although the difference in spawning time from Alaska to California may be as great as four or five months, spawning occurs when stream temperatures are falling. Jordan and Everman (1896) write, "...it spawns in August and early September when the water has reached a temperature of about 54°F." Temperatures observed at the time that chinook salmon are spawning in some streams of British Columbia, Washington, Oregon, and California are recorded in Table 1. The water temperature during incubation of the egg is descending, with minimum temperature occurring shortly before or after hatching, and is rising during the late fry and early feeding stages. The water temperature cycle in the hatchery at the University of Washington as shown in Figure 1 is typical of the annual water pattern of streams in which chinook salmon spawn in the Puget Sound region. However, many streams in other areas of the Pacific Coast have minimum water temperatures of 32° or 33°F.

TABLE 1

Temperatures of Some Pacific Coast Rivers at the Time of Spawning of the Chinook Salmon

River	Month	Temperature, °F, Arrival	Spawning	Reference
Nechako (B.C.)	September	59-61	58-55	Hourston, 1953
Quesnel (B.C.)	late Aug.	60	50	Jackson, 1953
North Thompson (B.C.)	early Sept.	60		" "
South Thompson (B.C.)	mid Sept.	62	58-55	" "
Columbia (Wash.)	Oct.-Nov.		62-50	Olson-Foster, 1956
Skagit "	September		52	See page 16
Entiat "	October		52	" " 17
Green "	October		50	" " 17
Toutle "	October		58-42	Burner, 1951*
Willamette (Ore.)	September		64-43	Matson, 1948
Sacramento (Cal.)	October		54-52	Pelnar, 1953

*Also includes a record of temperatures for other tributaries of the Columbia River

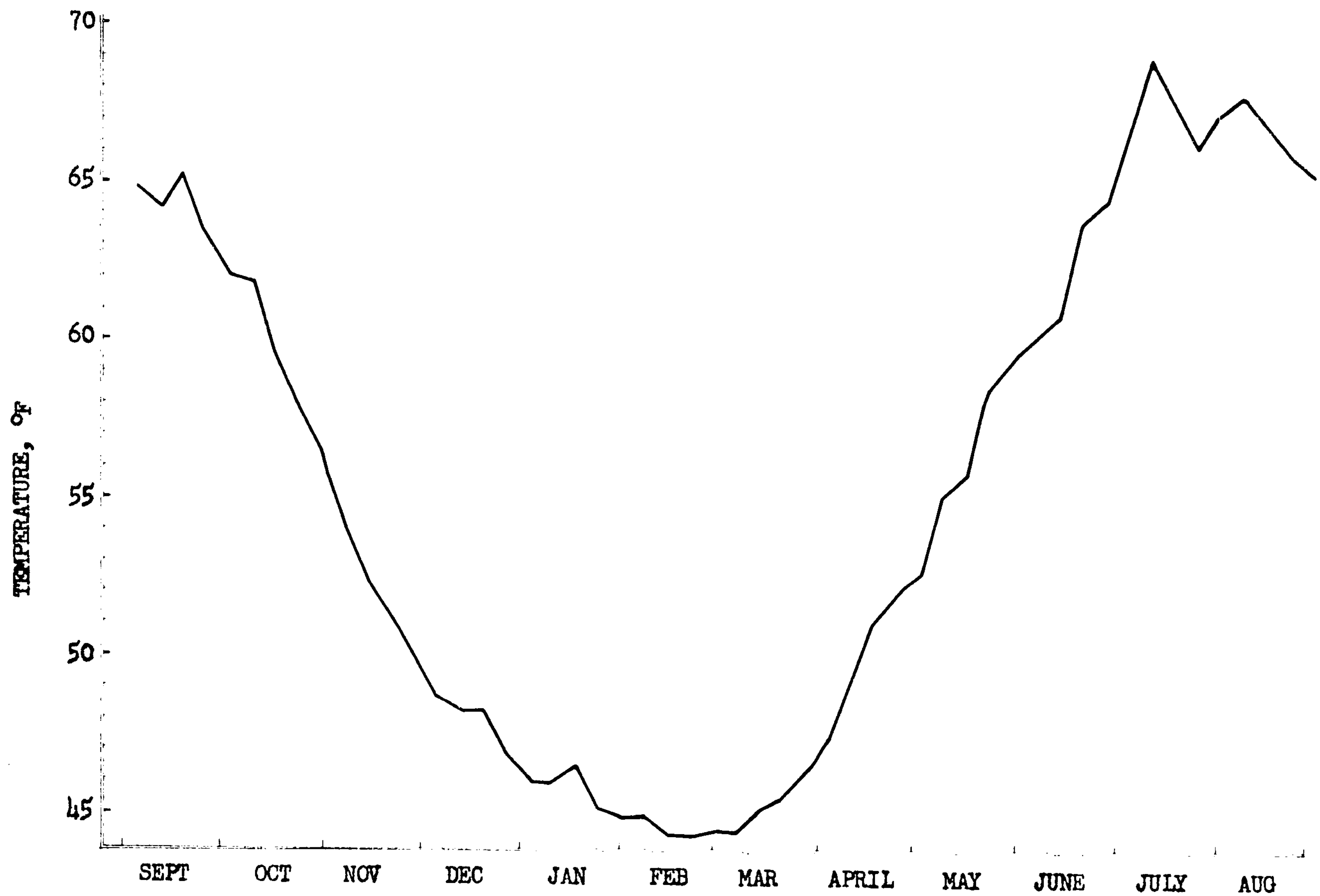

Figure 1. Average Water Temperature by **Weeks at** the **University of Washington Hatchery, November 1950 to July 1953**

II. PLAN OF EXPERIMENTS

The effects of temperature upon young chinook salmon have been observed in three experiments in three successive years--1951-52, 1952-53, and 1953-54. These experiments differed in four principal aspects: (1) water temperature pattern, (2) source of water, (3) racial stock, and (4) number of pairs of parents (Table 2).

Two of the many possible temperature patterns were considered; temperatures were either maintained at a constant level or were altered throughout the experiment in a manner similar to that which occurs under natural conditions. Ideally, the experimental temperatures should be those to which the fish are exposed in nature, for it is possible that normal development of eggs and fry is adjusted to the natural water temperature pattern. The natural water temperatures at the time of the deposition of eggs in the gravel are declining and continue to decline during the incubation period. Hatching occurs about the time that the water temperatures reach their lowest level in the annual temperature cycle, but emergence of the fry from the gravel does not occur until water temperatures are increasing. However, in an experiment in which the temperature is not maintained at a constant level, the average temperature may not be related to the observed effect if the character being observed is influenced by temperature for only part of the observational period. Because the critical periods for the chinook salmon were not known, temperatures of the lots for the first two experiments were constant; for the third experiment temperatures were altered throughout the experiment in a manner somewhat similar to the temperatures that occur

TABLE 2

Summary of Factors Related to Experiments I, II and III

Experiment	Date	Stock	Water Supply	Temp. Pattern	Temperature Range, °F Exper. Lots	Temperature Range, °F Control Lots*	No. of Spawn. Pairs	No. of Exper. Lots	Ave. No. of Eggs in Each Lot
I	11/15/51 - 10/2/52	Green	City	Constant	34-65**	54-42-69	1	8	547
II	10/13/52 - 1/14/53	"	"	"	45-67#	60-47	1	8	518
III	9/11/53 - 3/11/54	Skagit	Well	Altered	45-65##	56-53	3	5	504
	10/8/53 - 5/8/54	Entiat	"	"	"	"	1	5	559
	10/30/53 - 5/8/54	Green	"	"	"	"	1	5	452
	10/30/53 - 5/8/54	Sacramento	"	"	"	"	4	5	586

*Tap water as received at the hatchery

**34, 40, 45, 50, 55, 60, $62\frac{1}{2}$, 65°F

#45, 50, 55, $57\frac{1}{2}$, 60, $72\frac{1}{2}$, 65, $67\frac{1}{2}$°F

##45, 50, 55, 60, 65°F

under natural conditions.

Experiment I

In the first experiment eight lots were reared at constant temperatures in water from the city main, and all eggs were taken from one pair of chinook salmon of the Green River race. The experiment was initiated November 15, 1951, and concluded October 2, 1952.

A pair of mature chinook salmon from the Green River Hatchery of the Washington State Department of Fisheries, twenty-five miles southeast of Seattle, was transported to the University of Washington in a live-tank and then spawned. The pair used for spawning arrived during the last week of the 1951 run of chinook salmon to the Green River Hatchery, which is located at Soos Creek. At this time the daily temperature of Soos Creek varied from 42° to 46°F. After being spawned, the pair were photographed (Fig. 2) and size measurements and counts of meristic characters were made (Table 3).

The eggs from this pair were divided into eight experimental and four control lots averaging about 560 per lot. One of the eight experimental lots was placed in each of the following constant temperatures: 34°, 40°, 45°, 50°, 55°, 60°, $62\frac{1}{2}$° and 65°F. A maximum temperature of 65°F was chosen because in an earlier trial experiment all eggs at a constant temperature of 73°F and 67°F had died. The control lots were reared at the water temperature as received from the city main, which was 55°F at the beginning of the experiment and 41°F, the low for the year, at time of hatching. Graphs of the temperature history of the lots of the first experiment are shown in Figure 3.

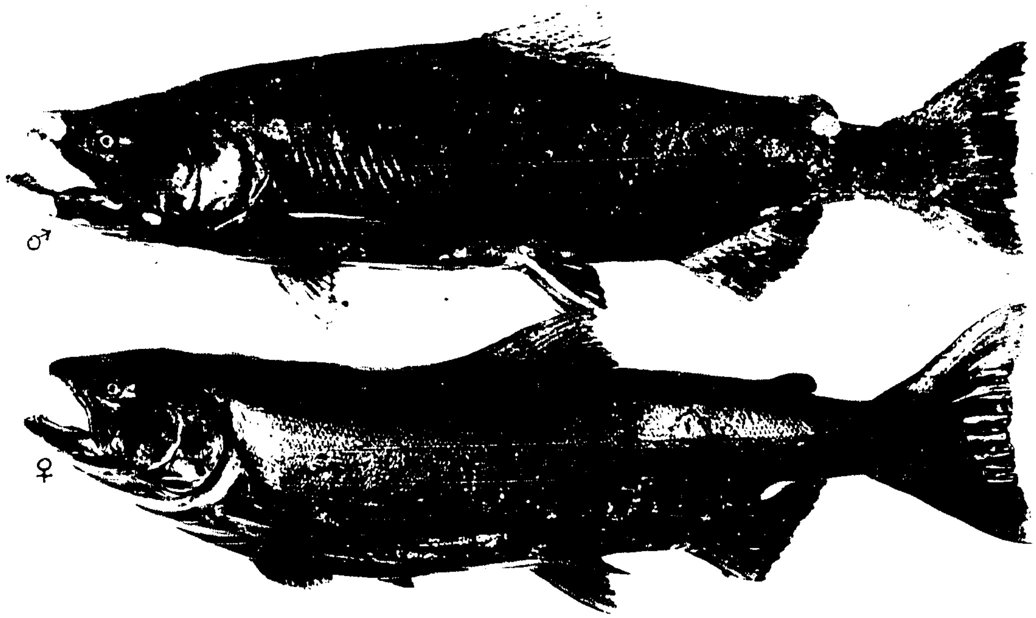

Figure 2. Chinook Salmon, Parents of the Experiment I Egg Lots

TABLE 3

Measurement Data of the Parents of the Lots for Experiments I and II

	Experiment I		Experiment II	
	Male	Female	Male	Female
Weight (spawned), kg	8.83	8.54	8.87	9.14
Length (fork), cm	95.2	92.3	94.0	95.2
Vertebrae	67	68	67	66
Dorsal rays, all	13	14	13	13
Anal " "	17	18	18	18
Branchiostegal rays	15	17	14	16
Gill rakers, first arch	9 + 13	9 + 12	10 + 15	9 + 13
Pyloric caeca	155	-	-	-
Scales, lateral line	142	139	134	140
" above " "	-	30	-	-
" below " "	31	29	30	30
Eggs, number		7024		6864
" weight before water hardening, kg		3.18		2.83
" diameter after water hardening, cm		0.93		0.89
Age (scales)	3 +	4 +	3 +	3 +

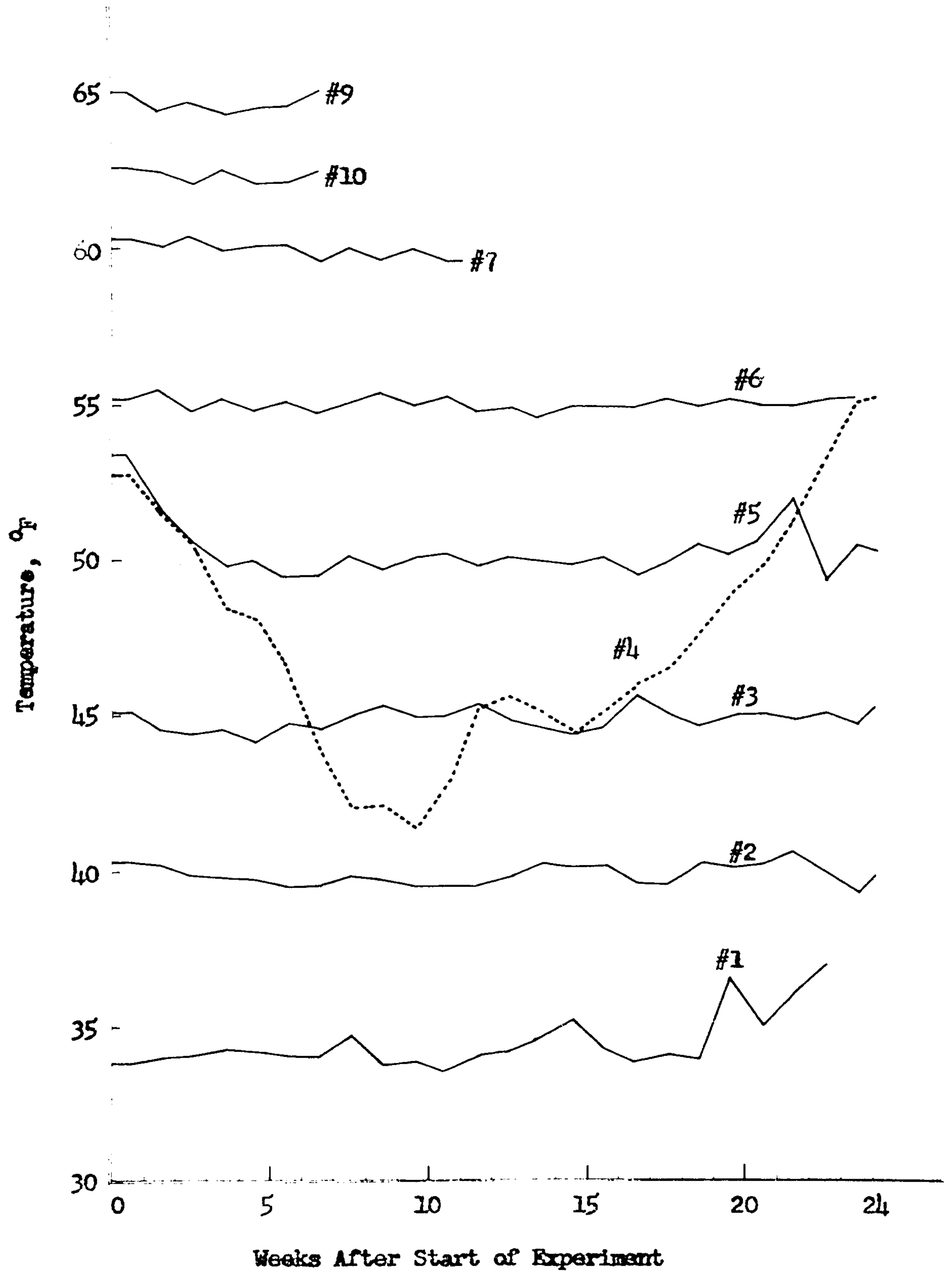

Figure 3. Lot Temperatures for Experiment I

In this experiment eight sublots of 100 eggs each were removed from the controls to either a higher or lower temperature. After $3\frac{1}{2}$ weeks at city water temperature three of the sublots were transferred to constant water temperatures of 60°, $62\frac{1}{2}$° and 65°F. Three other control sublots were transferred after 2, $2\frac{1}{2}$ and $3\frac{1}{2}$ weeks at city water temperature to a constant temperature of 34°F. Two additional sublots were held at 40° and 45°F for four weeks and were then moved to a constant temperature of 60°F.

Later, another section was added to the first experiment for the purpose of observing the effects of temperatures of 60°F and higher upon fingerlings. At these temperatures none of the original lots survived to feeding. To establish this part of the experiment, the control lots were pooled on May 1, 1952, and four random lots of 100 each were withdrawn. Of these four, one was left at the temperature of the city water and the others were transferred, after gradual tempering, to constant temperatures of 60°, 67° and 74°F.

Experiment II

The second experiment duplicated the first by the use of one pair of chinook salmon from the Green River race and by the incubation of the eggs in the same water supply and at constant temperatures.

The second experiment was started on October 13 with a pair from the early part of the 1952 run which was late in arriving at the Green River Hatchery because of warm weather (Fallert, 1952). The temperature of the water at the time the fish were taken for spawning was 52°F. Size measurements and counts of meristic characters of the two parents for the 1952 experiment are given in Table 3.

Constant temperatures in the eight experimental lots were 45°, 50°, 55°, 57½°, 60°, 62½°, 65° and 67½°F. These temperatures differ from the first experiment by the omission of the 34° and 40° lots and the addition of the 57½° and 67½°F lots. As in 1951 there were four control lots, but since Experiment II started a month earlier than Experiment I, the incubation temperature of the controls was higher in 1952. The city water temperature at the beginning of the experiment was 61°F and at hatching was 55°F, the average incubation temperature for the control lots being 11 degrees higher than in 1951. The temperature history of the Experiment II lots is shown in Figure 4.

Experiment II was concluded unexpectedly on December 28 by an accident that caused a great increase in mortalities in all lots. The cause of death was a high pH of the water supply created by a change of charcoal in the filter system. The details of this accident have been reported by Seymour and Donaldson (1953). At the time of the accident the lot at lowest temperature had just completed hatching. The experiment was concluded at this time, as there was 100 per cent mortality in some lots and injury of unknown extent to those that did survive.

Experiment III

The third experiment was basically different from Experiments I and II. The temperatures were changing rather than constant; the water was from a new source, a well that had been drilled to provide water of lower temperature for the hatchery; eggs from four races were used; and the eggs for one race were from one or more pairs of parents. On September 11,

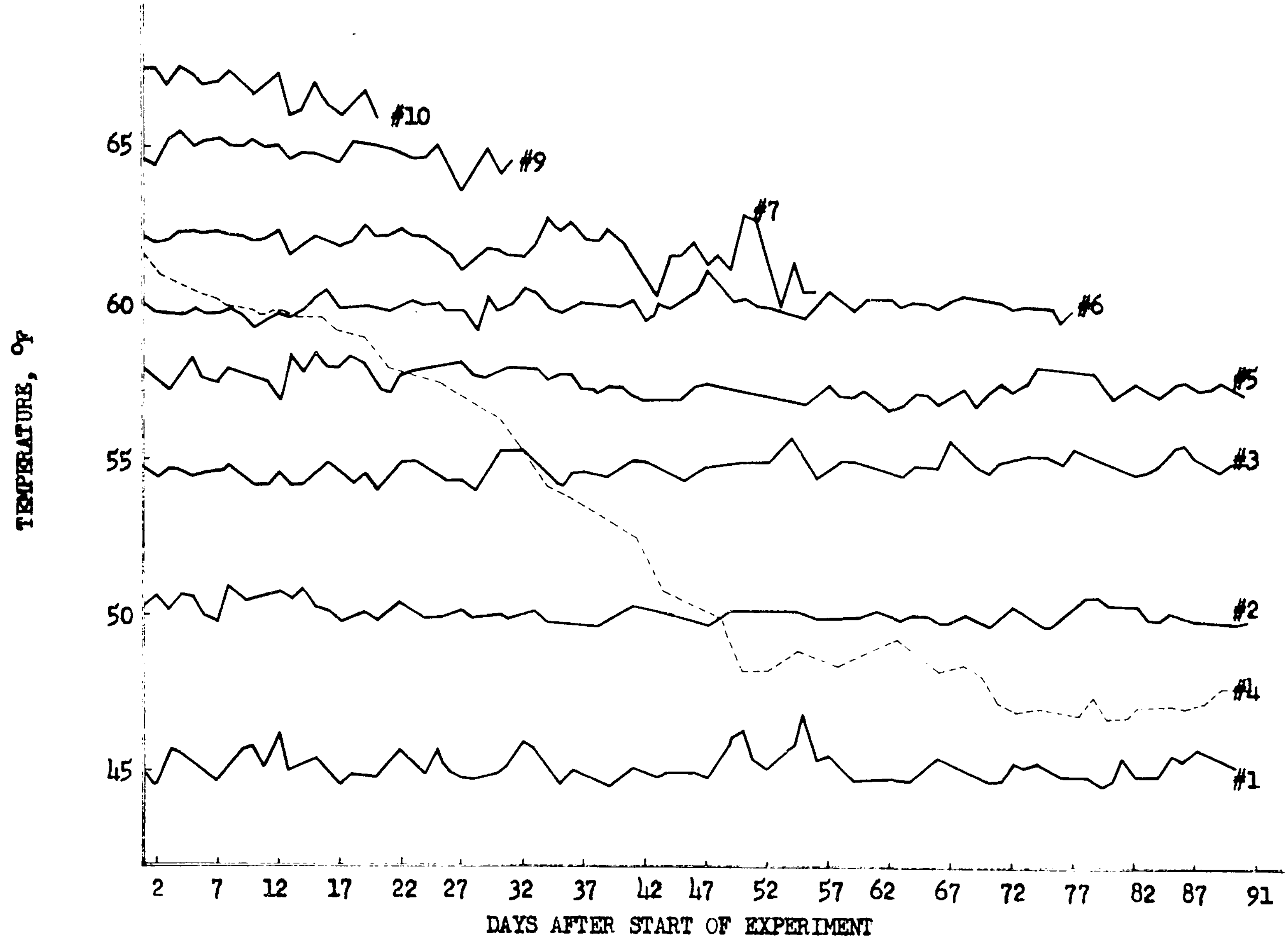

Figure 4. Observed Temperatures by Days for Lots of Experiment II

1953, the experiment was started and was continued until May 8, 1954.

For the purpose of evaluating racial differences caused by temperature, especially the rate of development and meristic characters, eggs were obtained from four rivers: the Skagit, in northwestern Washington; the Entiat, a tributary to the Columbia River in eastern Washington; the Sacramento, in California; and the Green, near Seattle (from which eggs were obtained for the two earlier experiments). Unsuccessful efforts were made to obtain eggs from Alaska, first from the Naknek River, Bristol Bay, and later from Alexander Creek near Anchorage. It was considered desirable to use Bristol Bay fish as representative of the races of chinook salmon near the northern limit of their distribution. Because the eggs from Alaska were not available, eggs from the early Skagit River run were obtained.

The eggs from the Skagit River chinook salmon were taken on September 11. The parents, three females and two males, were gaffed from the Marblemount spawning grounds. The water temperature at time of egg-taking was 52°F. After fertilization and water-hardening the eggs were combined into one lot, placed in a large thermos jug and taken to Seattle. Water temperature in the jug upon arrival in Seattle four hours later was 59°F. One-half of the eggs were then forwarded to Mr. Burrows at the U. S. Fish and Wildlife Fish Cultural Laboratory at Entiat, Washington, and the remaining half divided equally into five experimental and two control lots of about 500 eggs each.

The eggs from Entiat River were obtained on October 8 from a single chinook female about 15 pounds in weight. After fertilization the eggs were water-hardened for three hours and were then placed in the same type of thermos jug as had been used for the Skagit River eggs. The Entiat River water temperature was 52°F at this time. The jug was pre-cooled with crushed ice and, after being filled with eggs and water, was covered with insulating material. Upon arrival of the jug in Seattle four hours later, the water temperature inside was 49°F. The eggs were divided equally into six lots of about 560 each.

The eggs of the chinook salmon from the Sacramento and Green Rivers were taken October 30. The Sacramento eggs were spawned in the morning at the Coleman Station and flown to Seattle the same day. One-half of these eggs was taken to the Fish Cultural Laboratory at Entiat. The eggs were shipped from California in a special container with ice, and upon arrival in Seattle the temperature about the eggs was 38°F. On the day of spawning the temperature of the Sacramento River at the place from which the salmon were taken varied from 52° to 54°F. Four pairs of chinook salmon were used and the eggs were mixed before division into experimental and control lots of about 580 each. The fish in the Sacramento River on October 30 were the first of the fall run of chinook salmon; the peak of the season's run was expected later.

The Green River eggs were taken from one female and fertilized by one male. The eggs were transported to the University in a thermos jug in which they were retained until the Sacramento eggs arrived. The temperature in the jug increased during this period from 52° to 59°F. Both groups of eggs were handled in the same manner after being removed from the shipping containers. The average number of Green River eggs per lot was about 450.

The temperature pattern chosen was that which closely resembled the pattern expected in nature. The average temperature of the city water in the hatchery of the School of Fisheries from November 1950 to July 1953 was the pattern (Fig. 1), but the actual temperatures were at a lower level for some lots.

For the five experimental lots the starting temperatures were 45°, 50°, 55°, 60° and 65°F. After the start of the experiment the water was cooled one degree every five days to a temperature of 34°F. The eggs were held at that temperature for twenty days, after which the temperature was increased at the rate of one degree every five days. The temperature history of the Experiment III lots is shown in Figure 5.

The water source for the third experiment was from a well, whereas city water was used in Experiments I and II. This was of importance in two respects. First, the temperature of the control lot which was reared in the tap water was practically constant, with a range of only two degrees, from 56° to 54°F. Secondly, this water carried a high organic load which resulted in the very rapid accumulation of slime molds, algae, and protozoans on the eggs, in the tanks and troughs, and in the coils of the cooling system. This necessitated more handling of the eggs than was desirable and interfered with the flow of water in the refrigerated tanks.

By using ultraviolet light an effort was made to control the slime molds, algae and other organisms growing on the bottom and sides of the troughs and tanks. Three ultraviolet lights were placed across the head of a trough without fish, a few inches above the water. The water depth was four inches. After three weeks the mass of organisms beneath the lamps was only slightly less than in other parts of the trough; therefore the use of the ultraviolet light was discontinued.

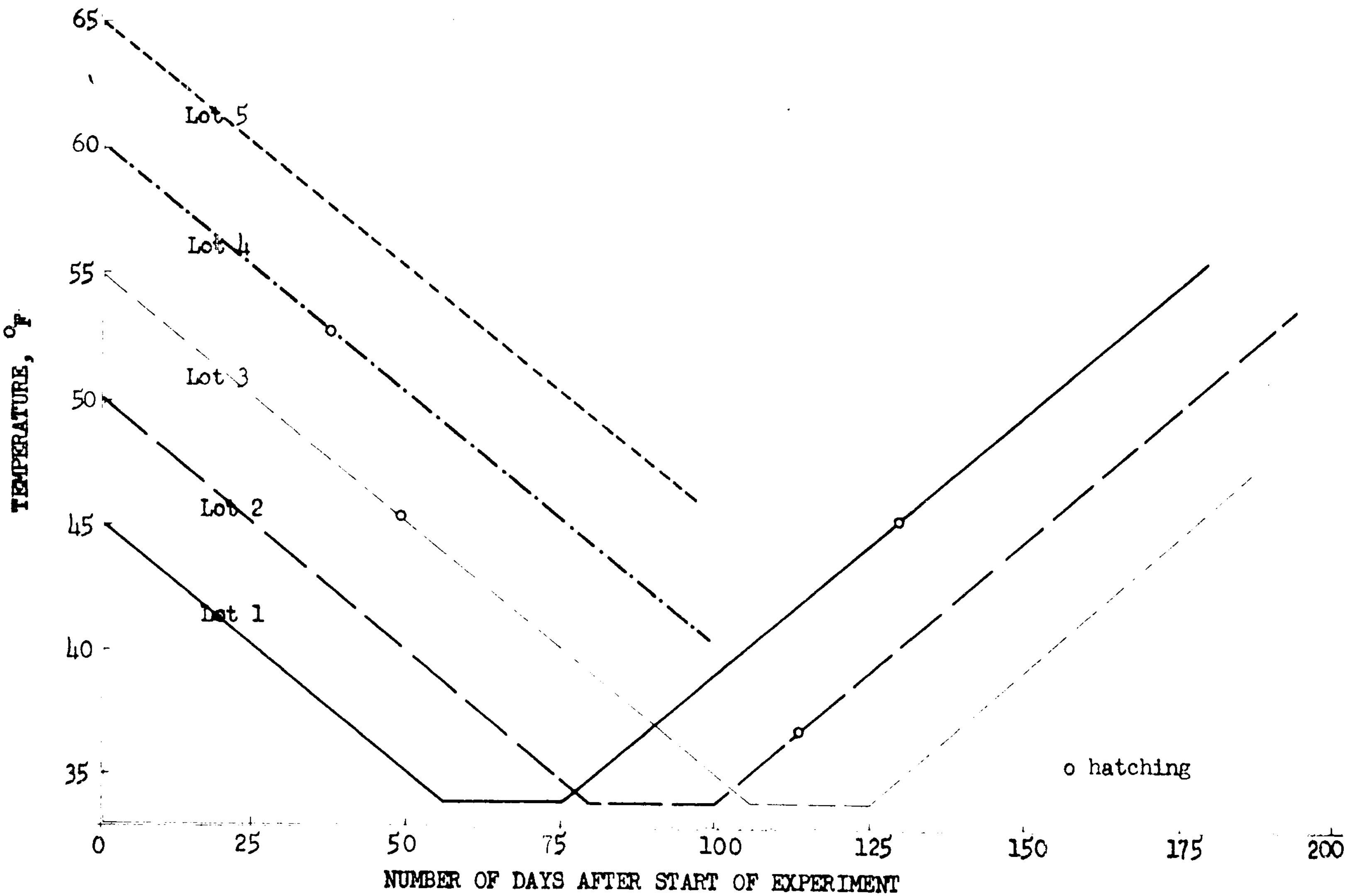

Figure 5. Predicted Temperatures from the Beginning of the Experiment to the Fingerling Stage for the Lots of Experiment III

Mortality in all lots of Experiment III in the late fry and fingerling stages was unusually high, and although the specific reason was not found, the cause was believed to be either directly or indirectly associated with the well water. Mortalities were especially high in the Green River eggs and, moreover, they occurred earlier than in the other lots, indicating that other factors associated with the condition of the eggs themselves were contributing to the mortalities.

Two lots, Sk 6 and E 6, which were reared inadvertently under abnormal conditions are not included in the discussion of results obtained from the other lots. At the beginning of the experiment these two lots were in troughs containing well water in the main hatchery and were not transferred to the controlled-temperature hatchery until November 14. On October 29 the dissolved oxygen in the water flowing into the trough with Lots Sk 6 and E 6 was found to be only 3.0 ppm. This condition was corrected the following day. However, by that time Lot Sk 6 had been in the low-oxygen water for 48 days and Lot E 6 for 21 days.

The significance of low oxygen tension was not appreciated until the data were analyzed some time after the conclusion of the experiment. Then it was found that in the two lots affected the incubation period was 18 per cent longer, the increase in the average number of vertebrae was as much as 2.6, and the increase in abnormal vertebrae was about 10 per cent.

III. MATERIALS

The hatchery and equipment of the School of Fisheries in Room 124, Fisheries Center, were used for these experiments (Fig. 6). The room is provided with six tanks and four troughs with tap water and heated tap water supplied to each. Refrigerated tap water is supplied to four of the tanks.

The tanks are made of baked enamel with a Thermo-pane glass front and are 60 inches long, 23 inches high and 12 inches deep. Protection from temperature change was provided by two inches of cork on the back, bottom and sides, an insulated lid on top, and the Thermo-pane front. The concrete troughs are 15 feet long, 12 inches wide and 8 inches deep.

Water. For the first two experiments the supply water was from the city main. To remove chlorine gas that is added occasionally to the city water, all water for hatchery use was passed through a charcoal filter.

In Experiment III well water replaced city water. The temperature of the well water was 53° to 56°F, a favorable temperature for chinook salmon. Analyses of both the city and well water is given in Table 4.

For the low-temperature tanks the tap water was cooled in a Temprite Instantaneous Cooler and temperatures down to freezing were available. Heated water was provided by running water through a coil in a steam jacket. Intermediate temperatures were obtained by mixing warm and cool water, using a shower type valve, either Powers model 34504 size C-20 or Powers model HVE.

Trays. A tray was designed so that the eggs could be kept in order and examined individually, if necessary, with a minimum of handling.

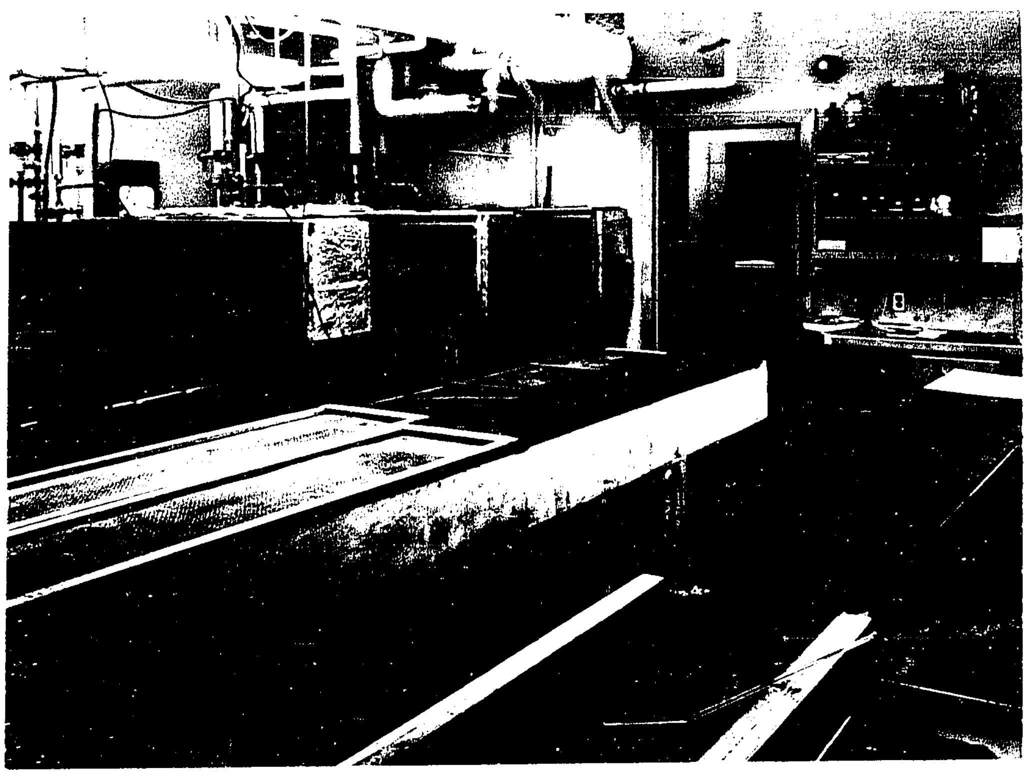

Figure 6. The Controlled-temperature Hatchery Room, Fisheries Center, University of Washington

TABLE 4

Mineral Analysis of City and Well Water

Date	City Water* 1/7/52	City Water* 1/5/53	City Water** 1/29/54	Well Water** 1/29/54
		Parts per Million		
Total Solids	42.	44.	45.	137.
Silica (SiO_2)	2.6	11.		
Iron (Fe)	0.08	0.04	4.	25.
Aluminum (Al)	0.2	0.01		
Calcium (Ca)	6.8	8.8	7.	12.
Magnesium (Mg)	0.7	1.	2.	15.
Potassium (K)		0.3		
Sodium (Na)		1.88		
Bicarbonate (HCO_3)	25.	29.	60.	117.
Sulphate (SO_4)	1.9	2.9		
Chloride (Cl)	1.2	1.06		
Total Hardness (as $CaCO_3$)	19.8	26.1	23.	87.
Alkalinity to Phenolphthalein	0.	0.		
" " Methyl Orange	20.	24.		
pH	7.2	7.4	7.6	7.4

* Analysis by Seattle Water Department
** " " Applied Fisheries Laboratory

The tray consisted of glass rods supported in a 10- by 12-inch wooden frame. The rods were spaced so that the eggs were supported, but the larvae, upon hatching, dropped through. One tray held about 600 chinook salmon eggs, 20 rows of 30 eggs each.

Damboards. Since in the third experiment there were as many as four different lots in the same tank, it was necessary to divide the tank into compartments and to have the damboard between compartments impassable to the fry and fingerlings. The compartments were made tight by wedging a damboard against a half-inch sponge rubber gasket on the sides and bottom of the tank. The damboard was made of two plates, one inch apart, which were perforated at the top on the upstream side and at the bottom on the downstream side. Successive damboards differed in height by one-half an inch, with the higher damboard nearer to the head of the tank. Building and arranging the damboards in this manner created circulation of water throughout each compartment.

Thermographs. Tank and trough temperatures were constantly recorded on a circular chart, a thermogram, by means of a seven-day Bristol Recorder. Temperatures were checked daily with a calibrated mercury thermometer which could be read to 0.1°F, and an adjustment was made to the recording pen if the pen was in error.

IV. METHODS

Egg-taking. In Experiment I the eggs were taken by incision and were fertilized in a pan without water. After fertilization the eggs were divided into equal lots and placed in pans in the tank in which they were to be reared. During the next two hours the eggs were water-hardened and tempered with the temperature gradually changing from that of the eggs at time of fertilization to that of the rearing temperature, the greatest change being 14 degrees Fahrenheit. The eggs were then placed on the glass rod trays. In Experiments II and III the eggs were water-hardened for two hours at the site of egg-taking and then were transported to the School of Fisheries. Immediately after arrival at the School they were divided into lots and tempered for two to four hours to the rearing temperatures.

Handling. Eggs and fry were moved only when absolutely necessary. The tanks were cleaned with a siphon and the dead eggs and young were removed without disturbing the remaining eggs or fry. Except for the removal of the eight sublots in Experiment I, the eggs and fry of Experiments I and II were undisturbed. As mentioned above, this was not true in Experiment III. Accumulation of organic debris and mud made it necessary to agitate the eggs gently about once a week to prevent smothering. Also, some egg lots were transferred from one tank to another during the eyed egg stage.

Mortality records. Mortality was calculated from the number of eggs and young removed after the first day. The removals on the first day were mostly infertile eggs. Dead eggs were removed daily, but since death of an egg is not necessarily immediately apparent, mortality values for any one day during the egg stage may actually be greater than shown in Tables 9 and 11. This source of error is not present after hatching.

The cumulative mortality was calculated by weeks for each lot. The losses were separated into two categories; one category was natural mortality, the other removals and accidental deaths. The cumulative mortality took into account losses from both categories and was computed for each seven-day period. The cumulative mortality for week n was the sum of three items: (1), the cumulative mortality for week n-1; (2), the sum of the daily natural mortalities for week n; and (3), the natural mortality that would have occurred in week n among those removed for samples or killed accidentally. Item (3) was calculated by multiplying the natural mortality rate for week n by the sum of the number of individuals that were removed for samples or had died accidentally during week n and the number of individuals that would have survived to the end of week n-1, having previously been removed for samples or having died accidentally.

The per cent cumulative mortality was equal to 100 times the cumulative mortality divided by the number of eggs at the start of the experiment.

Mortality for all stages was calculated in this manner. Mortality to the 50 per cent hatching stage did not include fry mortalities for those weeks in which there were both egg and fry mortalities.

Estimation of the time to hatching. Hatching was defined as the time when both the head and tail were free of the shell. At both high and low temperatures only the heads of many larvae would break through the shell and the larvae eventually would die. Such larvae were considered egg mortalities and were not counted as being hatched.

The time to hatching was arbitrarily selected as the time when 50 per cent of the eggs had hatched--the median of the hatching period--and could not be determined until completion of hatching. Other choices would have been the time to the first hatch, the last hatch, the mean of the hatching period, or the mode of the hatching period. At normal temperatures the choice would have made little difference, since the hatching period is short. At low temperatures the hatching period of some lots was more than one month, with a period of no hatching intervening between the extreme values; therefore the time to hatching of either the first or the last egg was considered an inappropriate estimate of the time to hatching.

The hatch of eggs was counted daily at 11 a.m. From the daily hatching records the time to 50 per cent hatch was calculated to the nearest 0.1 of a day, the last significant figure being determined by linear interpolation. By this method of estimating time of hatching the error is relatively greater in those lots with the shortest hatching time, i.e., those at highest temperatures.

Estimations of average temperature. The temperatures could not be controlled perfectly and therefore it was necessary to measure the temperature fluctuations in order to have a reliable estimate of the average temperature for the period of observation. The temperatures were recorded on thermograms from which the average temperatures were calculated. Since the thermometers attached to the mixing valves were calibrated in

Fahrenheit units, the thermograms were calibrated in the same units.

The average temperature was calculated with the accuracy allowable under the conditions of the experiment. The reliability of the thermometer, the accuracy of the thermograms, the temperature changes at various positions on the egg tray, and the methods of calculation all influence the accuracy of the estimates of average temperatures. The thermometer used to make the daily temperature readings and to correct the thermograms was accurate to 0.1°F; the thermograms could be read to the nearest 0.2°F; and the greatest temperature difference between various positions on the egg tray was 0.2°F. A graphical method and an arithmetical method were used to estimate average temperatures, but the error in either method was not measured. However, the total error from all sources in the calculated average temperatures is believed to be less than 0.5°F.

Estimates of average temperatures derived by means of calculating areas under the thermogram or as derived arithmetically from daily averages over short intervals are more accurate than averages from daily maximum and minimum temperatures. For Experiment I average temperatures were estimated by computing the area enclosed by the thermogram. From the area, which was computed with a planimeter, the average temperature for each seven-day period was read directly from a table. In the table the areas enclosed by circles made by various constant temperatures are listed. For constant or nearly constant temperatures the planimeter values are approximately correct, but for fluctuating temperatures there is an error because the areas of the circles do not change linearly with temperature. The planimeter method was chosen for the first year's experiment, as the temperatures were nearly constant, especially during incubation.

For Experiment III daily average temperatures were determined arithmetically from temperatures during short intervals in which there was either no change of temperature or a constant change in temperature. To determine the daily average, temperatures during each interval, weighted by the number of hours of each interval, were averaged. For example, if the temperature during the first twelve hours of the day was constant at 50°F, then steadily rose during the next three and a half hours to 56°F, then declined steadily to 52°F during the following two hours, after which the temperature remained constant for the rest of the day, the daily average temperature was calculated to be

$$\frac{(12x50) + (3.5x53) + (2x54) + (6.5x52)}{24} \text{ or } 51.4^{\circ}F.$$

To examine the difference in average temperatures that would occur from the two methods described, eight thermograms were selected in six of which the temperature variation was average (smooth thermogram) and in two of which it was extreme (irregular thermogram). Temperatures calculated from the planimeter measurements averaged 0.3 per cent less than temperatures calculated arithmetically and the error was the same for both smooth and irregular thermograms. Although similar results were obtained by the two methods, the method of arithmetically averaging daily temperatures, which is more tedious than the planimeter method, was used to calculate average temperatures for the Experiment II data.

There is no practical way of estimating the variance of the temperatures that were recorded in Experiments I and II. The variance was probably not great, as temperatures were usually maintained within one degree Fahrenheit of the desired temperature except on occasions when the refrigerated water supply was reduced or cut off. This occurred when ice or organic material accumulated in the coils of the cooling unit and when there was a power failure to the refrigeration unit. The shutting off of the refrigerated water was most frequent during Experiment III and occurred four times during the experiment to the tank receiving 34°F water. An estimate of the variance of the daily average temperatures could be made, but this would not indicate temperature shock, as usually the deviations from the temperature pattern were of only a few hours' duration and changed the daily average relatively little. Although the temperature shock effect from daily temperature changes is not known, a comparison of the daily maximum-minimum temperatures of the experimental lots with maximum-minimum temperatures in the Green River (Ellis, 1953) and the Sacramento River (Wallich, 1901) shows that the experimental lots were not exposed to any greater daily temperature changes than occur in nature (Table 5).

Preservation. Before being preserved, most specimens were placed in urethane. They were then measured, weighed and radiographed. The preserving fluid was 4 per cent formaldehyde with 0.7 per cent NaCl.

Radiographs. Counts of vertebrae and fin rays were made from radiographs and from stained specimens. All the fry and fingerlings that were selected for counting were radiographed, but the radiographs were not readable for some of the fry with skeletons that had not yet ossified. These fry were then stained and the vertebrae and fin rays in many could be counted.

TABLE 5

A Comparison of the Daily Temperature Range for the Green and Sacramento Rivers and for Experiments II and III

	Green Oct. 1952- May 1953	Sacramento Sept. 1898- Feb. 1899*	Exp. II Oct. 1952- Jan. 1953	Exp. III Sept. 1953- May 1954
Temp. Range, °F	Number of Days		Number of Tank Days	
0.5	2	1	661	902
1.5	11	2	120	220
2.5	68	12	19	72
3.5	30	27	10	40
4.5	55	14	3	19
5.5	18	14	1	18
6.5	35	10	1	5
7.5	13	1		5
8.5	8	1		7
9.5	2			2
10.5	1			
11.5				2
12.5		2		2
13.5		1		3
14.5				
15.5		3		2
16.5		1		
17.5		1		
18.5				1
19.5		2		1
20.5				

* Only four observations Nov. 17 - Feb. 18

For radiographs of small fish soft radiation of high intensity is needed. Sharper and clearer radiographs can be obtained from an X-ray diffraction unit than from a diagnostic type of tube because the beam of X-rays is smaller and there is less secondary radiation from the window (Bonham and Bayliff, 1953). A water-cooled Machlett O-2 X-ray Diffraction Unit with a copper target and a beryllium window was used in these experiments.

Kodalith Ortho Type 2 film produced good results and, being insensitive to red light, was convenient to use. A sheet of film placed in a black, light-proof envelope was positioned beneath the X-ray tube and the fish arranged on a sheet of cellophane resting on top of the film envelope. The fish were blotted dry and covered with a sheet of cellophane to reduce further drying which sometimes resulted in movement of the fish during exposure.

A typical exposure for 30 two-inch fish on a sheet of 5- by 7-inch film with the window 24 inches from the film and the unit operating at 50 PKV (peak kilovolts), 12 MA (milliamperes) and full wave rectification was 4½ minutes. The film was developed for 40-50 seconds in Dektol diluted with two parts of water. For larger fish (five-inch) Type M X-ray film was used in order to shorten exposure time. With the tube at 32 inches and operating at 50 PKV and 12 MA, exposure time was 25 seconds and development time 4 minutes.

Satisfactory radiographs have been made of chinook salmon fry as small as 36 mm (fork length). These fish were reared at 40°F for 10 months and did not increase in length after hatching, whereas younger 38 mm fry in the yolk-sac stage did not give satisfactory radiographs.

Ossification appeared to be a function of both size and age. Fingerlings were used for radiographs when available. For those fry that did not give a readable radiograph the staining technique was also tried.

Staining. The technique used was that of Hollister (1934) for clearing and dyeing of fish for bone study, with the modifications of Tåning (1944). Fry preserved in 4 per cent formaldehyde were washed in water, bleached with hydrogen peroxide, washed, placed in 2 per cent potassium hydroxide and then stained with alizarin (alizarin sodium sulphonate). Bleaching was accelerated by exposure of the fish in the hydrogen peroxide solution to ultraviolet light. From the stain the fry were transferred to glycerin for clearing. Vertebrae and fin rays of the glycerin-preserved specimens were counted with a dissecting microscope at a magnification of 7 X.

The ossified structures stained very distinctly. Of the two methods the staining method was more effective for young fry, although all the possibilities for the radiographic method, such as voltage and amperage changes and types of photographic paper, were not explored. Accurate counts were easily made of the specimens of fingerling size prepared by either method, but the radiographic method was preferred because the radiographs provided an orderly, permanent record that was available for rechecking and because the method was faster.

V. ENVIRONMENTAL CONDITIONS

The experiment was designed to measure the effect of one variable, temperature. Other factors were assumed to be either of no effect or of equal effect in all lots.

Oxygen. Analysis was done by the basic Winkler Method as outlined by Ellis, Westfall and Ellis (1948).

On five occasions--January 10, January 25, May 26, October 1, and December 29, 1952--oxygen determinations were made of the city water in all ten tanks and troughs with samples taken from the intake, the outlet, the surface and the bottom. In all samples the dissolved oxygen was greater than 70 per cent of the saturation level and in most cases greater than 90 per cent. There were no significant differences in oxygen values of samples from the intake or the outlet, the surface or the bottom (Table 6).

The well water used in Experiment III was practically devoid of oxygen as it entered the reservoir tank at the Fisheries Center, the value for dissolved oxygen in parts per million being 0.20 or approximately 2 per cent of saturation. After spilling into the reservoir tank through wire mesh screens, the oxygen increased to 3.4 ppm.

In the controlled-temperature hatchery the water was jetted into the tanks and troughs, which further increased the free oxygen in the water. Values ranged from 7.4 to 10.9 ppm and the per cent saturation, from 68 to 80. However, two lots in the main hatchery were in water of low oxygen content, 3 ppm, on October 28. The tap water to the troughs holding these two lots entered from the bottom without mixing with the

TABLE 6

Oxygen Content of Water In Tanks and Troughs

Tank or Trough	City Water 1/10/52 ppm	%Sat	1/25/52 ppm	%Sat	5/26/52 ppm	%Sat	10/1/52 ppm	%Sat	12/29/52 ppm	%Sat	Well Water 10/28/53 ppm	%Sat	11/18/53 ppm	%Sat
1, inlet														
outlet	12.39	94			12.31	89	11.28	84			10.90	80	11.20	79
2, inlet														
outlet	13.02	99			10.38	83	11.39	89			8.65	68		
3, inlet														
outlet	12.49	103			10.59	87	10.15	83	9.71	92	8.95	77		
4, inlet			12.46	100										
outlet	12.62	101	12.30	99	9.31	96	8.49	89			8.30	75		
5, inlet			11.45	103										
outlet	12.06	106	11.37	102	10.43	92	8.44	72			8.00	75		
6, inlet			11.59	107										
outlet	11.67	110	11.69	108	10.07	95	8.34	83			7.45	75		
7, inlet	10.42	105	10.60	105										
outlet	10.27	103	10.57	104	9.73	98	6.58	68						
8, inlet	12.71	102	12.55	101										
outlet	12.58	101	12.50	100	9.81	99	9.15	96	10.77	91	7.85	93		
9, inlet														
outlet					9.48	103	9.45	97			7.90	84	7.75	79
10, inlet	11.29	116												
outlet	11.17	115			8.92	103	9.18	96			7.55	78		
#3, main hatchery											2.95	27	9.20	87

air. To correct this condition overhead jets were installed.

Dissolved oxygen levels of 3 ppm or lower are hazardous or lethal to fish in lakes or streams and 5 ppm or more should be present for favorable conditions (Ellis et al., 1948). Low oxygen levels also influence the development of fish eggs, as Johansen and Krogh (1914) have shown that levels below 50 per cent saturation delay the development of plaice eggs.

Because the well water used during the third experiment was rich in organic material, the biological oxygen demand (BOD) of the water also was determined. Three 250-ml samples of well water incubated five days in a hatchery trough at the well water temperature of 55°F had net oxygen losses of 0.9, 0.6 and 0.7 ppm. A fourth sample treated in a similar manner, except that the bottle was deliberately loaded with organic material growing on the bottom of the trough, had no free oxygen after five days. Although there was a positive BOD, the decrease in oxygen from inlet to outlet was no greater than the measurement error, 0.2 ppm or less.

Water flow. Water flows were determined empirically for each tank and averaged 1.2 gallons per minute with a range of $\frac{1}{2}$ to $1\frac{1}{2}$ gallons per minute. The flow to the cold water tanks was limited by the capacity of the refrigeration unit but satisfactory oxygen levels were maintained as indicated above.

pH. The hydrogen ion concentration was determined with a Beckman Model H2 Glass Electrode pH Meter. Values were in the range of 7.5 to 7.8 except on one occasion when the charcoal in the filter system was replaced. This resulted in a great increase in the pH value of the water, a great mortality and the conclusion of Experiment II as described on page 14. Acidity in excess of pH 4.1 or alkalinity greater than pH 9.5 are immediately lethal to brook trout (Creaser, 1930).

Light. The hatchery room for the temperature experiments is an inside windowless room. All lots were exposed equally to the flucrescent lights in the room. During incubation and throughout the fry stage the lights were on about two hours a day during the daily routine of taking temperatures, removing dead eggs and young, *etc.* In the feeding stage lights were on about 10 hours each day. McHugh (1954a) has shown some evidence that visible light during embryonic development of the grunion, *Leuresthes tenuis*, influences the number of vertebrae. Mean vertebral number is relatively low in bright light, intermediate in subdued light, and high in darkness. In nature salmon eggs are in darkness, being buried in the gravel of the stream bottom.

VI. DISCUSSION AND RESULTS

Rate of Embryological Development

General expressions. (a) Historical. The rate of development of poikilothermic animals varies directly with temperature. An expression of the relationship between temperature and rate of development has been sought by many in the hope of classifying physiological processes according to the size of their coefficients. It was hoped that the size of the coefficients would reveal, by comparison, the chemical or physical processes which are the foundation of the biological reactions.

Mathematical expressions proposed to describe the relationship of temperature to speed of development may be classified as either theoretical or empirical. In the first category are van't Hoff's Q_{10}, Arrhenius' μ, and Thompson's x or Q_1, all of which are basically the same equation. The three equations are compared in Table 7 and are shown to be of like form after logarithmic transformation.

In the Arrhenius equation temperature is expressed in absolute units and in reciprocal form, but Bêlehrádek (1935) pointed out that the reciprocal of the absolute temperature is practically a linear function of temperature on the centigrade scale between the limits of 0° and 40°C. Therefore, the expressions of Arrhenius and van't Hoff are virtually equivalent; both imply that a proportional increase in speed of development produced by a given difference in temperature is constant throughout the temperature range at which an animal may develop. If μ fits any particular set of empirical data, Q_{10} should fit equally well and *vice versa* (Andrewartha and Birch, 1954); what is true of Q_{10} is also true of x or Q_1.

TABLE 7

A Comparison of the Arrhenius, Van't Hoff and Thompson Temperature Coefficients

	Arrhenius	Van't Hoff	D. W. Thompson
Coefficient	μ	Q_{10}	x(also δ_1)
Temperature symbol	x	x	t
Temperature units	Kelvin	Centigrade	Centigrade
Temperature difference	$x_1 - x_2$	$x_1 - x_2$	n
Rate Symbol	y	y	v
Original equation	$\frac{y_2}{y_1} \; e^{2u(\frac{1}{x_1} - \frac{1}{x_2})}$	$Q_{10} = (\frac{y_1}{y_2})^{\frac{10}{x_1 - x_2}}$	$\frac{V_{t+n}}{V_t} = x^n$
Transformed equation	$\mu = \frac{4.6(\log y_2 - \log y_1)}{\frac{1}{x_1} - \frac{1}{x_2}}$	$\log Q_{10} = 10(\frac{\log y_1 - \log y_2}{x_1 - x_2})$	$\log x = \frac{\log V_{t+n} - \log V_t}{n}$

Empirical equations that have been used to express the relationship of temperature to speed of development include the hyperbola, catenary and logistic. The widely used temperature summation rule is the equation of an equilateral hyperbola,

$$yx = k$$

$$\text{or } y(x-a) = k,$$

where $\underline{y}$ is time of development, $\underline{x}$ is temperature, $\underline{a}$ is threshold temperature and $\underline{k}$ the temperature summation constant.

This rule states that the product of time by temperature above the threshold is constant regardless of incubation temperature, and that the reciprocal curve, $\frac{1}{y} = kx$, is a straight line. Usually the observed reciprocal values fall on a straight line only in the median portion of the temperature range of development, and often the temperature-time curve has an exponential form, the reciprocal curve being s-shaped. For this reason Davidson (1944) believed that the temperature-summation theory is an unsatisfactory representation of the facts and that its use should be discontinued.

In 1926 Bêlehrádek proposed the formula

$$y = \frac{a}{x^b} \quad \text{or} \quad y = \frac{k*}{x^b}$$

as a better method than μ or Q_{10} for describing rate of development. The temperature summation rule is of the same general form but with $b = 1$. When it was necessary to introduce biological zero into the formula, the equation became $y = \frac{k}{(x-a)^b}$; but since Bêlehrádek measured temperatures in degrees centigrade and biological zero was practically 0°C for the

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*$\underline{k}$ will be substituted for the $\underline{a}$ used by Bêlehrádek in order to be consistent with symbols used above.

animals studied, "it was not necessary to complicate the formula by a fifth factor" (Bêlehrádek, 1929).

By logarithmic transformation Bêlehrádek's equation becomes $\log y = \log k - b \log x$, which implies that when the logarithm of time is plotted against the logarithm of temperature the values lie in a straight line. Bêlehrádek found this to be true for conduction in the sciatic nerve of the frog, locomotion of *Paramecium*, and embryological development of the Mediterranean flour moth. Also, values of *b* were found to be more constant than μ or Q_{10}.

The data used by Bêlehrádek for the Mediterranean flour moth were from a paper by Janisch, who used the same data to demonstrate that the time-temperature relationship can best be expressed by a catenary curve,

$$y = \frac{m}{2}\left(a^{x}+a^{-x}\right).$$

In this equation *y* represents the time required for development at the given temperature *x* in degrees centigrade; *m* is the time for development at the optimum temperature; *a* is a constant.

Janisch (1925) believed that the catenary fitted the observed data throughout the temperature range of development and was later supported in this view by Uvarov (1931). However, in a later experiment on the rate of embryological development of the same moth, *Ephestia kuhniella*, Voute in 1936 obtained results that were considerably different from those obtained by Janisch. For points at temperatures above the peak Voute believed that the catenary does not fit (Davidson, 1944).

A form of the logistic curve was found by Davidson to be a better fit to the flour moth data than either the catenary or Bêlehrádek's modification of the hyperbola. Davidson observed that often the temperature-time curve was of the exponential form and the reciprocal was similar to a form of the logistic curve developed by Pearl and Reed (1920). This formula,

$$\frac{1}{y} = \frac{L^*}{1+e^{a-bx}},$$

has been successfully applied by Davidson to describe the relationship between temperature and rate of development at constant temperatures for six species of insects. In this formula $1/y$ is velocity, that is, reciprocal of the time required to complete development at a given temperature $\underline{x}$; L, $\underline{a}$ and $\underline{b}$ are constants. L is the parameter representing the distance between the upper and lower asymptotes of the logistic curve and can be calculated from the following formula,

$$L = \frac{2P_1P_2P_3-P_2^2(P_1+P_3)}{P_1P_3-P_2^2},$$

where P_1, P_2 and P_3 are values for $1/y$ on the curve at three equally spaced temperatures on the abscissa.

Replacing $1/y$ with P, the original equation can be transformed to the equation of a straight line, $\log_e \frac{L-P}{P} = a-bx$, and the constants $\underline{a}$ and $\underline{b}$ can be calculated by the method of "least squares." In essence this equation states that for a given set of data to be expressed by the logistic curve, a plot of the logarithm of L-P/P and temperature should be points on a straight line.

(b) Fish. The early history of the search for a satisfactory law relating temperature to speed of development was centered around the temperature summation rule, although it was not identified as such.

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*In the original equation Davidson used the symbol K, but to avoid confusion with k in the temperature summation rule, the K in Davidson's equation will be replaced with L.

Reaumer in 1735 suggested that the sums of the daily temperatures were related to the maturation of plants. Bonnet with chicks and deCandolle with plants were others who early recognized the dependence of development upon temperature, but it was a century later before quantitative observations of the effect of temperature upon development of fish eggs were made (Thompson, 1952). Hayes (1949) reviewed these early observations as follows:

> Davy in 1856 and Coste in 1858 gave some fragmentary figures showing that warmed water speeds up the development of salmon eggs. Probably the first modern work was done in 1859 by Stephen H. Ainsworth who experimented with eggs of the brook trout, Salvelinus fontinalis, in a little spring fed fish pond near West Bloomfield, New York. His table showing the incubation periods of eggs at various temperatures was published by Norris in 1868....Seth Green (1870) stated that "trout eggs will hatch in 50 days at a mean water temperature of 50°F and for each degree colder or warmer five days more or less will be required, the difference, however, increasing the farther we recede from 50 degrees."

Wallich in 1901 suggested a thermal unit system and Apstein in 1909, a temperature unit called "Tagesgrade," day degrees, both being expressions of the temperature summation rule. By Wallich's definition a temperature unit meant one degree above 32°F for a period of 24 hours. For the chinook salmon from the Sacramento River reared at average temperatures of 43° to 50°F the number of thermal units to hatching was constant at about 900.

The "Tagesgrade" is the product of temperature in centigrade units and days, but differs from Wallich's idea of thermal units in that the threshold temperature was reckoned from the lowest point at which development could take place, rather than from the freezing point of water.

Reibisch (1902) calculated the threshold temperature from observations of Dannevig (1896) upon the influence of temperature on the development of the eggs of the plaice and the cod. The data for incubation temperatures and days to hatching were combined in pairs to form equations of the type $(t_1-x)n_1 = (t_2-x)n_2$, in which t is temperature of incubation, x is threshold temperature and n is number of days to hatching. This equation was solved for x and the average value for all the paired temperatures was considered to be the threshold temperature for the species. For the plaice the average was -2.4°C and the range -1.2° to -4.0°C; for the cod the average was -3.0°C and the range -1.2° to -13.2°C. Using the average values, Reibisch's calculations were constant and for this reason he concluded that the theory of temperature summation with the proper temperature threshold was valid.

Johansen and Krogh (1914) took exception to the idea of Reibisch that a certain amount of heat or energy from outside of the egg was necessary for development.

> When the eggs have the same temperature as their surroundings, they get no supply of heat from outside.... The energy which is undoubtedly necessary for the development, is derived in the case of fish eggs, as in all other eggs, from the chemical processes involved in the metabolism of the eggs....The temperature must be looked upon as a factor which will have a certain influence upon the velocity of the chemical reactions and other processes involved in the development. The theoretical problem is to obtain a quantitative measurement of this influence and to express it in such terms that a comparison with regular chemical reactions becomes possible.

Using Dannevig's data, Johansen and Krogh found Q_{10} also to be unsatisfactory but believed the temperature-development relationship was linear when temperature and the reciprocal of time to hatching were the variables

This means that the change in rate of development is proportional to the change in temperature, and the equation that expresses this relationship is the temperature summation rule in its reciprocal form, $\frac{1}{y} = \frac{x}{k}$, in which *y* is time, *x* is temperature and *k* is the temperature summation constant.*

A variation of the general form of the Bělehrádek formula, $y = \frac{k}{x^b}$, was used by Price (1940) to describe the rate of development of the whitefish, *Coregonus clupeaformis* (Mitchill). For the Lake Erie whitefish spawning begins in late November when decreasing water temperatures approach 6°C and the four-month incubation period is at temperatures only slightly above freezing. Price found that the rate of development was different above and below 6°C and proposed a two-part equation to describe this condition. His equation is of the form

$$T = \frac{M}{A_1^t} \left.\begin{matrix} 0^{\circ} \\ 6^{\circ}C \end{matrix}\right\} ; \quad T = \frac{M}{A_2^t} \left.\begin{matrix} 6^{\circ} \\ 12^{\circ}C \end{matrix}\right\} .$$

where T is time of development and *t* is temperature. Values of A_1 averaged 1.13 and of A_2, 1.19.

For the Salmonidae the history of experiments in which there are some data relative to the rate of development is summarized in Table 8. These experiments were reviewed for formulae expressing the temperature-development relationship and if none was given, the curve of temperature, *x*, and reciprocal of time, 1/y, was plotted from the data. This curve was arbitrarily selected as it was simple to plot and as likely as any to have a linear relationship. The slopes of these curves increase with

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*Nomenclature varies with authors but for consistency in this report translation to these standard terms will be made where necessary.

TABLE 8

Summary of Temperature-Rate of Development Experiments with Salmonidae Eggs

Investigator	Date	Species	Range of Temp.	Temp. Pattern	Source of Data	Fit of the curve 1/y = x/k to the relationship of temperature to rate of development
Ainsworth	1868	brook	37-54°F	x**	h#	Poor; for 50°F, 1/y is high
Green	1870	trout				No data; incubation period is 50 days at 50°F
Wallich	1901	chinook	42-51°F	x	h	Good; suggested temperature unit system
Kawajiri	1927	masou	6-16°C	c***	e##	Good; high mortalities; non-circulating water
Kawajiri	1928	rainbow	7-12°C	c,x	e	Fair
Gray	1928	brown	3-12°C	x	h	Poor; for temp. above 5°C, 1/y is high
Belding, et al.	1932	A. Salmon	33-42°F	x	h	Very poor; points widely scattered
Embody	1934	brown	2-11°C	c,x	e,h	Poor; similar to the results of Gray (1928)
Embody	1934	brook	2-14°C	c,x	e,h	Fair; above 5°C, 1/y values are high
Embody	"	rainbow	3-16°C	c,x	e,h	Good;
Embody	"	lake	2-10°C	c,x	e,h	Fair; concave to abscissa
Merriman	1935	cutthroat	6-11°C	c	e	Good; only 3 points
Rucker	1937	sockeye	8-14°C	c	e	Good; only 3 points
Foster	1949	rainbow	43-53°F	x	e	Fair; (x-ray experiment)
Donaldson	1950	sockeye	55-32°F	x	e	No data; eggs moved to 32°F at various stages
Donaldson	1955	chinook	55-67°F	x	e	Fair; eggs moved to 53°F from high temperatures
Burrows	1956	chinook	35-60°F	c	e	Good; includes low temperature data

*brook trout, _Salvelinus fontinalis_; chinook, _Oncorhynchus tshawytscha_; masou, _Oncorhynchus masou_; rainbow, _Salmo gairdnerii_; brown trout, _Salmo trutta_; Atlantic salmon, _Salmo salar_; lake trout, _Cristivomer namaycush_; cutthroat, _Salmo clarkii_; sockeye, _Oncorhynchus nerka_.

**x-changing temperatures

***c-constant temperatures

#h-data from hatchery records

##e-experimental data

temperature and in general are slightly s-shaped when the experimental temperatures extend over the entire range of temperatures at which development is possible. In several experiments the relation of $1/y$ to $\underline{x}$ was not linear. Rockwell (1956) has plotted the rate of development curves for forty-one experiments with fish including the experiments listed in Table 8, except for the experiments of Foster (1949) and Burrows (1956).

In addition to the rate of development experiments listed in Table 8, other temperature experiments with chinook salmon have been carried on and include the following: Brett (1952) determined the upper and lower temperature <u>tolerance</u> for fingerlings of five species of Pacific salmon; Johnson and Brice (1953) made observations on the effect of water temperature during incubation on the <u>mortality</u> of chinook salmon; Donaldson (1955) reported on the <u>survival</u> of the early stages of the chinook salmon after varying exposures to upper lethal temperatures; Olson and Foster (1956) determined the temperature <u>tolerance</u> of eggs and young chinook salmon at temperatures above and below that of the Columbia River; Burrows' 1956 data are not published, but include in part observations on <u>mortality</u> and <u>rate of development</u> of chinook salmon eggs and fry at low temperatures.

Controlled temperature experiments with Pacific salmon other than the chinook include those of Kawajiri (1927a), Rucker (1937), Donaldson and Foster (1940), Donaldson (1950) and Rockwell (1956).

<u>Constant temperature experiments</u>. Other than Wallich's temperature summation system and a provisional velocity of development curve for Pacific salmon eggs by Rockwell (1956), the temperature and rate of development relationship for chinook salmon had not been determined. In the

search for an equation that would best describe this relationship estimates of μ, Q_{10} and x were made and the fit of the data to the temperature summation rule, the hyperbola and the logistic was tried.

The incubation temperatures and the number of days to hatching for Experiments I and II are presented in Table 9 and Figure 7. The data used in the search for an equation to describe the temperature and rate of development relationship were selected from lots reared at temperatures between 39.8° and 57.8°F. Both above and below this range mortality increased markedly (Fig. 20) and the rate of development curve flattened (Fig. 15). The rapid increase in mortality is interpreted to mean that the increase in deaths is due to temperature; the flattening of the rate of development curve could be interpreted to mean that the fast-growing individuals are killed first at high temperatures and the slow-growing individuals are killed first at low temperatures. To avoid the possibility of the influence of lethal temperatures upon rate of development the data were limited to those lots reared in the temperature range, 39.8° to 57.8°F. There were only four lots from each of Experiments I and II that were reared at constant temperatures in this range and therefore the data from the two experiments were combined.

In combining the results of Experiments I and II it is assumed that between broods of different years the rate of development is not statistically significant. Differences indicated by the rate of development trend lines for Experiments I, II and III in Figure 8 are not great.

The relatively greater deviation of the trend lines at high temperatures (Fig. 8) may be expected for two reasons. First, error in estimation of hatching time was slightly greater when the incubation period

TABLE 9

Water Temperature, Incubation Period and Per Cent Mortality of Green River Chinook Salmon Eggs in Experiments I and II

Temperature Pattern	Year	Lot	Temperature °F	°C	Days to 50% Hatch	Per Cent Mortality
Constant	1951	1	34.0	1.11	-	100
		2*	39.8	4.33	128.6	6
		3*	44.7	7.06	79.1	6
		5*	50.6	10.33	50.2	13
		6*	55.1	12.83	40.0	5
		7	60.2	15.67	34.0	22
		10	62.4	16.89	31.4	78
		9	64.6	18.11	28.[illegible]	99
Constant	1952	1*	45.2	7.33	[illegible]3.4	1
		2*	50.2	10.11	50.9	2
		3*	54.6	12.56	38.8	2
		5*	57.8	14.33	34.0	2
		6	59.8	15.44	32.1	35
		7	62.0	16.67	3[illegible].7	85
		9	64.8	18.22	-	100
		10	67.0	19.44	-	100
Changing; at city water temperature	1951	4	47.4	8.56	62.3	4
		8A	46.9	8.28	66.1	3
		8B	47.0	8.33	65.0	6
		8C	47.2	8.44	62.5	15
Changing; at city water temperature	1952	4	58.5	14.72	32.4	5
		8	59.0	15.00	32.7	2
		8A	"	"	"	3
		8B	"	"	"	4

* Selected for curve fitting because of low mortality

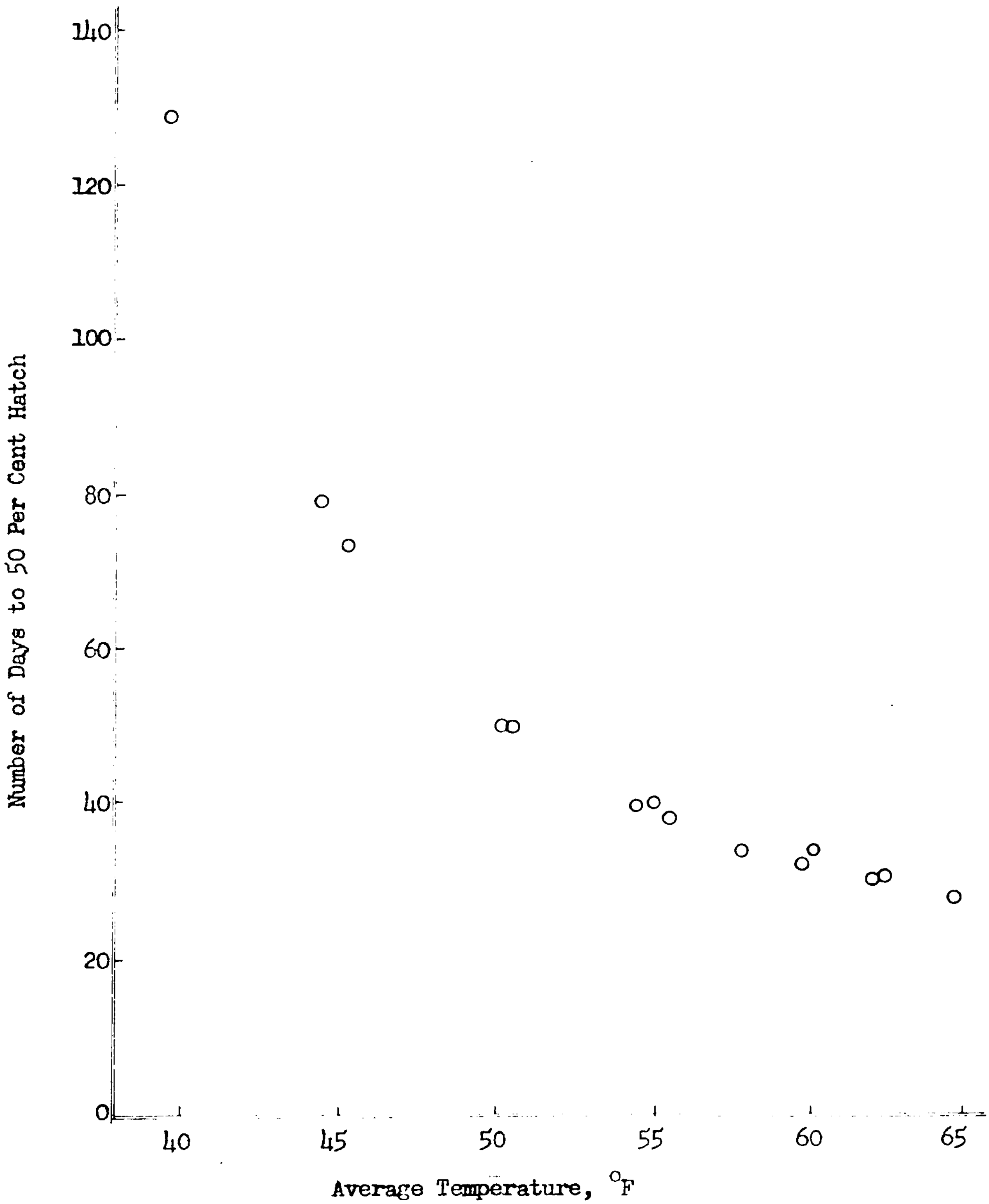

Figure 7. Average Temperature and Number of Days to 50 Per Cent Hatch for All Lots Reared at Constant Temperatures

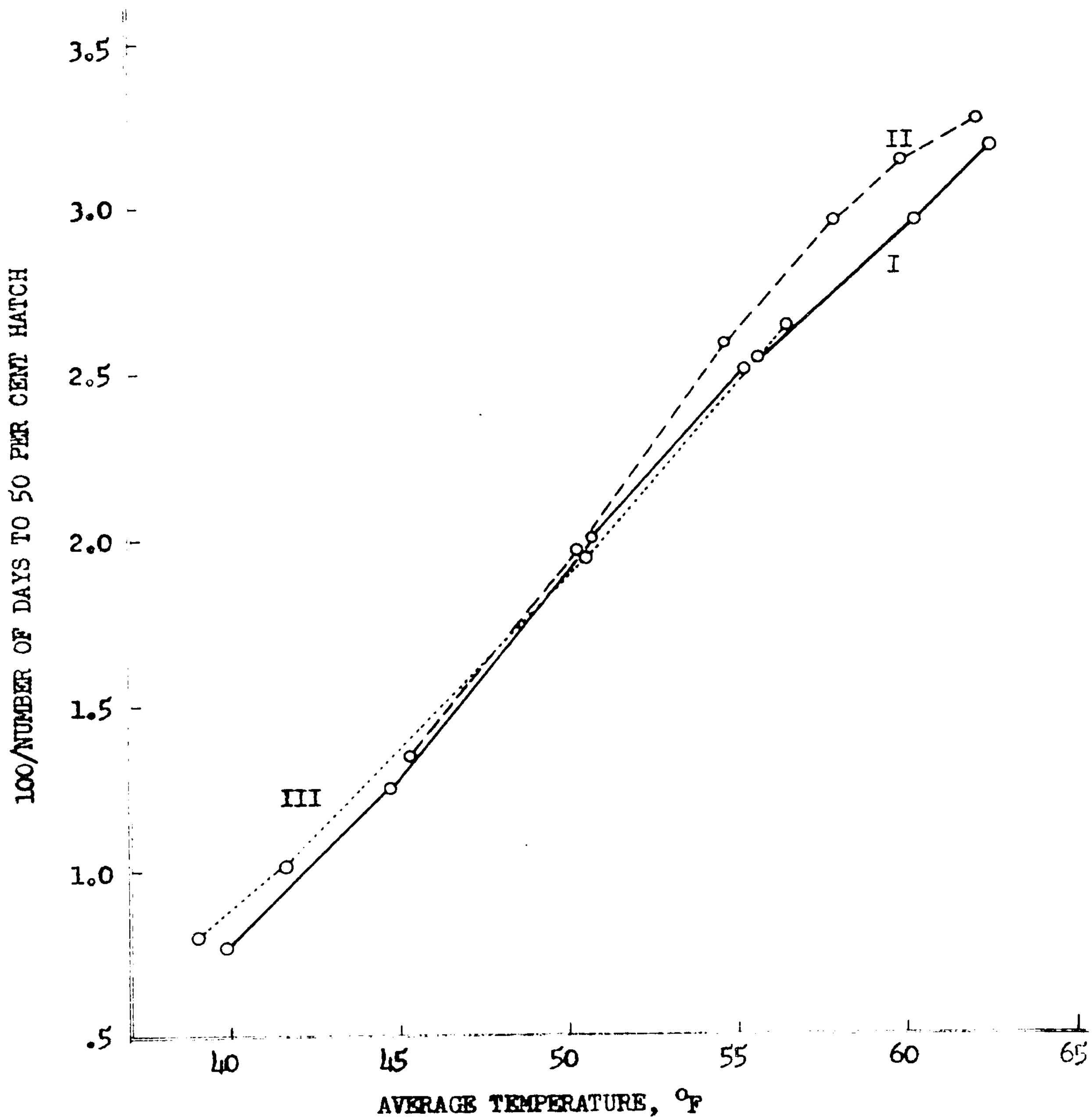

Figure 8. The Average Temperature and Rate of Development for Eggs of the Chinook Salmon from Green River for Experiments I, II and III

was short (page 27). Secondly, the ordinate of the rate of development curve is the reciprocal of the number of days to hatching, and the graphical representation of one day for a short incubation period, that is, high temperature, is greater than for one day of a long incubation period. For example, the difference in 100/y for y = 29 and y = 30 is 0.12; and for y = 100 and y = 101 is 0.01.

(a) Temperature coefficients. Using the eight lots from Table 9 that were selected for low mortality rates and combining them two at a time, 28 combinations were obtained for which μ, Q_{10} and x were calculated (Table 10). Omitting the values when the temperature difference is less than 0.3°C*, the range for μ was 12,000 to 29,500; for Q_{10}, 2.11 to 6.40; and for x, 1.08 to 1.20, respectively, clearly indicating that these coefficients are not constant for the relationship of temperature to the rate of development for chinook salmon. The average value for μ of 20,000 and for Q_{10} of 3.64 agrees with the statement by Hayes (1949) that "any Q_{10} value for salmon and trout can be converted to the corresponding value of Arrhenius' formula with negligible error (5 per cent) if multiplied by 5500."

Since the values of μ and Q_{10} are not constant, then the relationship between the logarithm of the speed of development and temperature is not linear (page 38). To investigate the shape of the curve expressing this relationship two graphs were made; for μ the variables were the reciprocal of temperature in Kelvin units and the logarithm of the

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*For slight changes in temperature the relationship of temperature to time of incubation is not accurate due to experimental error and occasionally may show a slight increase in incubation time with an increase in temperature.

TABLE 10

Temperature Coefficients and Threshold Temperatures for Chinook Salmon from Green River Reared at Constant Temperatures

Temp., °C*		Temperature Coefficients			Threshold Temperature, a*	
x_1	x_2	Q_{10}*	x*	u*	°F	°C
4.33	7.06	5.93	1.19	27700	32.0	0.00
"	7.33	6.40	1.20	29500	32.6	0.33
"	10.11	4.98	1.17	25400	33.0	0.56
"	10.33	4.83	1.17	24700	32.9	0.50
"	12.56	4.30	1.16	23200	33.4	0.78
"	12.83	3.96	1.15	21800	32.9	0.50
"	14.33	3.79	1.14	21300	33.3	0.72
7.06	7.33	(13.2)	(1.29)	(49800)	(38.2)	(3.44)
"	10.11	4.26	1.16	23200	34.8	1.56
"	10.33	4.05	1.15	22200	34.5	1.39
"	12.56	3.67	1.14	20900	35.2	1.78
"	12.83	3.27	1.13	18900	34.1	1.17
"	14.33	3.20	1.12	18700	34.8	1.56
7.33	10.11	3.79	1.14	20900	33.9	1.06
"	10.33	3.61	1.14	20000	33.5	0.83
"	12.56	3.42	1.13	19600	34.7	1.50
"	12.83	3.03	1.12	17600	33.4	0.78
"	14.33	3.02	1.12	17700	34.3	1.28
10.11	10.33	(1.93)	(1.07)	(9200)	(21.4)	(-5.89)
"	12.56	3.04	1.12	18000	36.1	2.28
"	12.83	2.42	1.09	14200	32.2	0.11
"	14.33	2.61	1.10	15500	34.9	1.61
10.33	12.56	3.18	1.12	19100	37.0	2.78
"	12.83	2.47	1.09	14600	32.9	0.50
"	14.33	2.65	1.10	15900	35.5	1.94
12.56	12.83	$y_2 - y_1$ is negative			-	-
"	14.33	2.11	1.08	12000	31.9	-0.06
12.83	14.33	2.98	1.12	18000	39.8	4.33
	Average	3.64	1.13	20000	34.1	1.17

*See following page

( ) $x_2 - x_1$ less than 0.3°C; estimates inaccurate in this range

TABLE 10, continued

x		y
°F	°C	days
39.8	4.33	128.6
44.7	7.06	79.1
45.2	7.33	73.4
50.2	10.11	50.9
50.6	10.33	50.2
54.6	12.56	38.8
55.1	12.83	40.0
57.8	14.33	34.0

x = temperature of incubation; for u temperature in degrees absolute = 273.18 °C

y = number of days to 50% hatch

a = threshold temperature

$$u = \frac{(4.6)\ \log(\frac{1}{y})_2 - \log(\frac{1}{y})_1}{\frac{1}{x_1} - \frac{1}{x_2}}$$

$$\log Q_{10} = \frac{(10)\ \log(\frac{1}{y})_2 - \log(\frac{1}{y})_1}{x_2 - x_1}$$

$$\log x = \frac{\log(\frac{1}{y})_2 - \log(\frac{1}{y})_1}{x_2 - x_1}$$

$$a = \frac{x_1 y_1 - x_2 y_2}{y_1 - y_2}$$

speed of development; for Q_{10} the variables were temperature in centigrade units and the logarithm of the speed of development. From inspection of Figure 9 it would appear that 8.5°C is a critical temperature. Values of μ and Q_{10} above and below this temperature are as follows:

Coefficient	μ	Q_{10}
4.0° - 8.5°C	28600	6.32
8.5° - 14.0°C	16100	2.69
4.0° - 14.0°C	21800	3.95

The values for x (or Q_1) in Table 10 may appear to be relatively constant, but x is one-tenth the log of Q_{10} and therefore no better a measure of the rate of development than Q_{10}. The reason for the more constant value of x is that the number is obtained from that part of the log table in which a large change in the logarithm corresponds with a relatively small change in the number. Thompson (1952) lists the x or Q_1 for a great variety of organisms and points out the constancy of values, from 1.08 to 1.20. However, in terms of Q_{10} the range of values for the same data is 2.2 to 6.2.

In conclusion, a single value for any one of the thermal coefficients, μ, Q_{10} or x, does not adequately describe the rate of development of the chinook salmon egg.

(b) Threshold temperature. The first step in fitting the chinook salmon data to either the temperature summation rule or the Bêlehrádek equation was to estimate the threshold temperature, also called "schwelle," biological zero or critical zero for growth. The threshold temperature is a factor in both of these equations and often has been disregarded when the incubation temperatures have been measured in centigrade units.

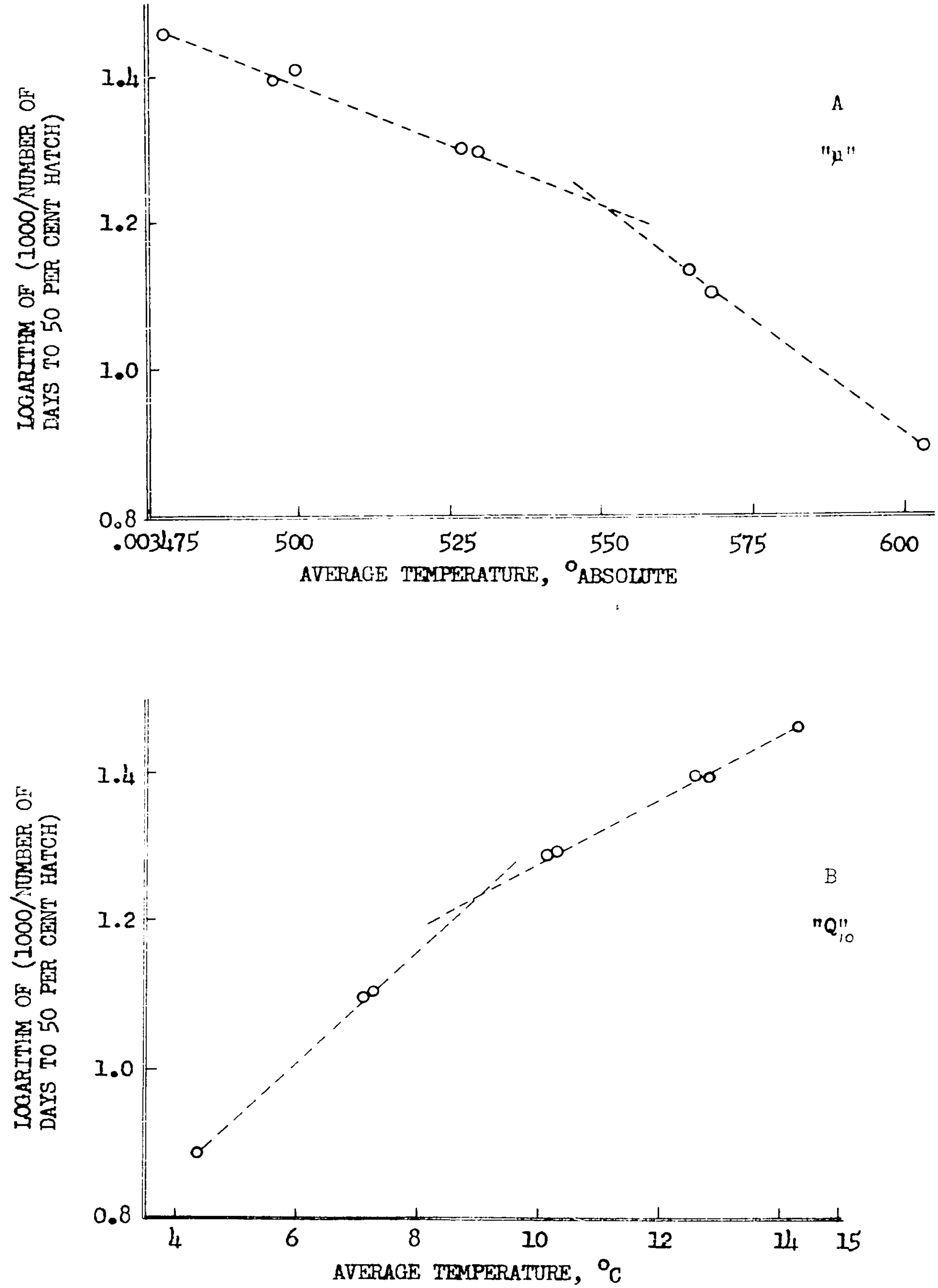

Figure 9. Relationship of Temperature to the Logarithm of 1000/Number of Days to Hatching. A, Temperature as the Reciprocal of the Absolute Temperature. B, Temperature in Degrees Centigrade

The threshold temperature of many animals is near 0°C and therefore the correction for this factor is not great.

Reibisch estimated the threshold temperature by a method already described (page 43); Johansen and Krogh (1914) extrapolated the rate-temperature curve to the $\underline{x}$ (temperature) axis and called the point of intersection the threshold temperature, but acknowledged that "it is not legitimate to assume that the curve of development remains straight beyond that part which has actually been investigated." Krogh (1914), Shelford (1927) and others have recognized the change in rate of development at high and low temperatures, but an estimate of threshold temperature other than by extrapolation or by the method of Reibisch has not been proposed.

Two other estimates of the threshold temperature can be derived from the temperature summation equation, $y(x-a) = k$, where $\underline{y}$ is the number of days to hatching at temperature $\underline{x}$, $\underline{a}$ is the threshold temperature and $\underline{k}$ is the temperature summation constant. In one method the number of temperature units to hatching is assumed to be the same for eggs incubated at one temperature as at any other temperature. This can be stated in equation form as follows:

$$y_1(x_1-a) = k$$

$$y_2(x_2-a) = k$$

$$y_1(x_1-a) = y_2(x_2-a)$$

$$\text{from which } a = \frac{x_1y_1-x_2y_2}{y_1-y_2}$$

Reibisch calculated the threshold temperature in essentially the same manner for paired observations and averaged the values to determine the threshold value for the species. For the chinook salmon the value calculated in this manner was 34.1°F (Table 10).

The general estimate of the threshold temperature for the species, using the same data, is the regression coefficient of $x_1y_1-x_2y_2$ on y_1-y_2 which was 32.8°F for the chinook salmon reared at constant temperatures. The value of the regression coefficient is a better estimate of threshold temperature than the average of the paired observations for two reasons: (1) the better fit to the data as shown in Figure 10, and (2) the smaller value for the coefficient of variation of k when 32.8°F rather than 34.1°F was used as the threshold temperature. The coefficient of variation, C, in per cent was 3.47 and 4.25, respectively.

A second method of estimating threshold temperature involves both a and k, whereas k was eliminated from the above equation. Using the same symbols, the temperature constant and threshold temperature are derived as follows:

$$y(x-a) = k$$

$$\frac{1}{y} = \frac{x-a}{k}$$

$$\frac{1}{y} = -\left(\frac{a}{k}\right)+\left(\frac{1}{k}\right)(x)$$

This equation is in standard form for the equation of a straight line, $y = a+bx$, and both $\left(\frac{1}{k}\right)$ and $-\left(\frac{a}{k}\right)$ can be determined. The factor $\frac{1}{k}$ is the regression coefficient of the rate of development, $\frac{1}{y}$, on temperature, x. By substitution in the standard form the threshold temperature, a, is found as follows:

$$-\frac{a}{k} = \left(\frac{\overline{1}}{y}\right)-\left(\frac{1}{k}\right)(\overline{x})$$

$$\frac{a}{k} = \frac{\overline{x}}{k} -\left(\frac{\overline{1}}{y}\right)$$

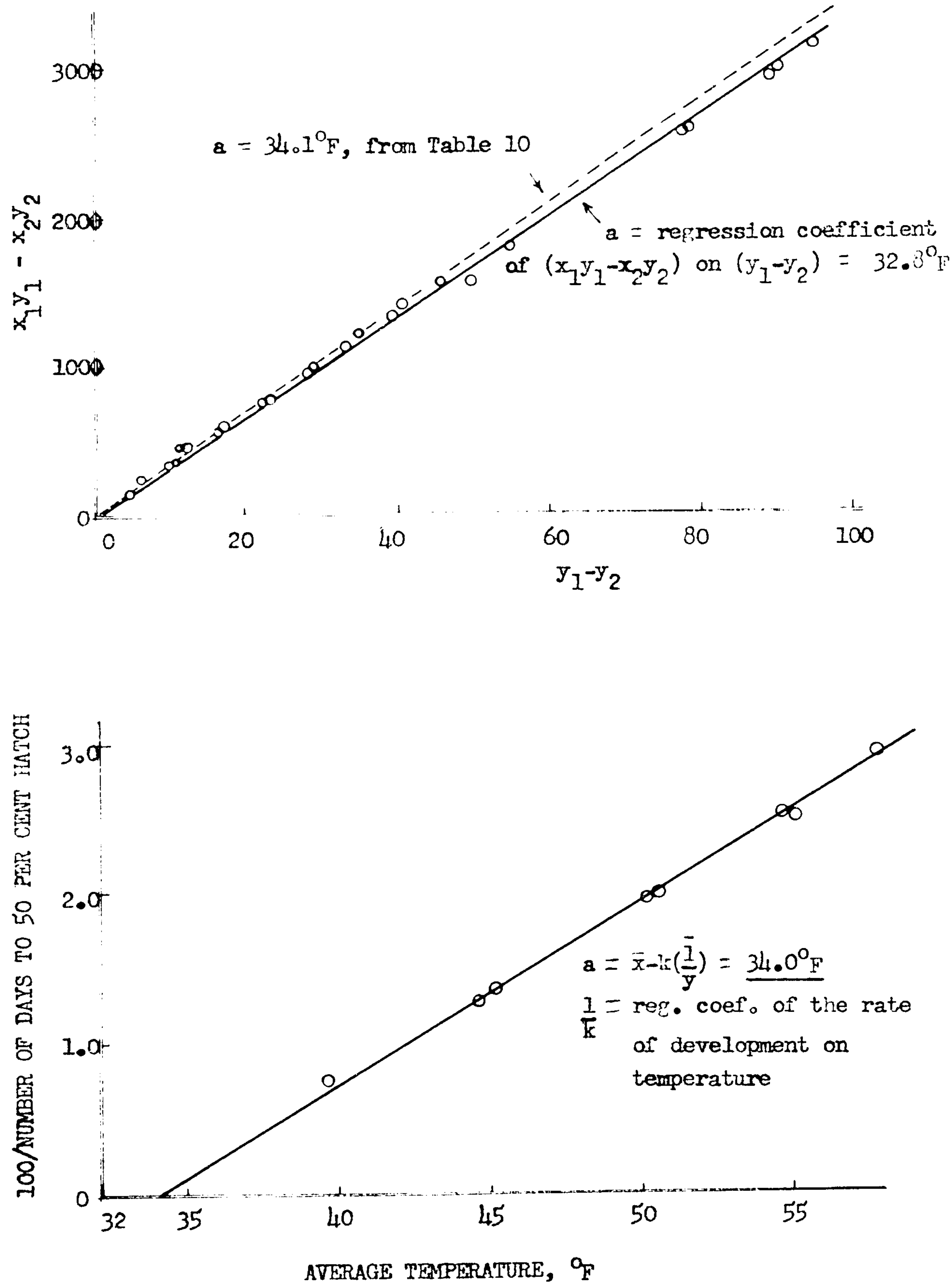

Figure 10. Three Estimates of the Threshold Temperature, a, for Eight Lots of Experiment I and II. A, a Determined from Values of x and y. B, a Determined from Values of x, y and k.

$$a = \bar{x} - k\left(\frac{\bar{1}}{y}\right)$$

By this method the threshold temperature for the chinook salmon was found to be 33.8°F. The linearity of the relationship of the rate of development to temperature is shown by the closeness of the points to the regression line in Figure 10.

There is a good fit of the data to both of these two new methods of estimating the threshold temperature. The latter method is preferred because the regression line is not required to pass through the origin and the variables $\bar{x}$ and $\frac{\bar{1}}{y}$ and the constant k are more easily determined.

The confidence limits for the threshold temperature derived by the second method can be determined by solving the following equation for a:

$$\left[\left(-\frac{\hat{a}}{\hat{k}}\right) + \left(\widehat{\frac{1}{k}}\right) a\right]^2 = t^2_{n-2}\, s_{\frac{1}{y}\cdot x} \left[\frac{1}{n} + \frac{(a - \bar{x})^2}{\sum_{i=1}^{n} (x_i - \bar{x})^2}\right]$$

estimate of $\frac{1}{k} = \frac{1}{k}$ as defined above

estimate of $\frac{a}{k} = \frac{a}{k}$ as defined above

t_{n-2} = 5 per cent point of Student's t for n-2 items

$s_{\frac{1}{y}} \cdot x$ = standard deviation from regression of the rate of development on temperature

$\bar{x}$ = mean of temperature observations

n = number of observations

The confidence interval for the threshold temperature is defined by the values of the two roots of the equation, and for the chinook salmon data these values were calculated to be 33.0° to 34.5°F.

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*Equation derived by D. G. Chapman, Mathematics Department, University of Washington.

In conclusion, the threshold temperature for Green River chinook salmon reared at constant temperatures in the range of 39.8° to 57.8°F is about 33.8°F; values of three different estimates were 32.8°, 33.8° and 34.1°F. The 95 per cent confidence interval was 33.0° to 34.6°F for the threshold temperature of 33.8°F.

(c) Temperature summation rule. The threshold temperature having been estimated, the process of curve fitting was resumed. The linearity of the regression of $x_1y_1-x_2y_2$ on y_1-y_2 and of $1/y$ on x in Figure 10 shows that the temperature summation rule is a good expression of the relationship between the rate of development and temperature for incubation of chinook salmon eggs from Green River in the temperature range of 39.8° to 57.8°F.

(d) Bêlehrádek equation. The fit of the chinook salmon data to the Bêlehrádek equation and to the logistic was also tried. The Belehradek equation by logarithmic transformation becomes the standard form of the equation of a straight line:

$$y = \frac{k}{x^b}$$

$$\log y = \log k - b(\log x)$$

The value of b, which is called a thermic coefficient, is the regression coefficient of log y on log x. For the chinook salmon b was -0.968 when corrected for a threshold temperature of 33.8°F and -1.12 for the uncorrected data (Fig. 11). The value of b for the embryonic development of *Salmo fario* as calculated by Bêlehrádek (1929) was 0.99; for *Oncorhynchus nerka* as calculated by Rucker (1937), 1.06.

In conclusion, since the temperature summation rule and the Bêlehrádek equation are identical when b = 1 (the chinook data show the value

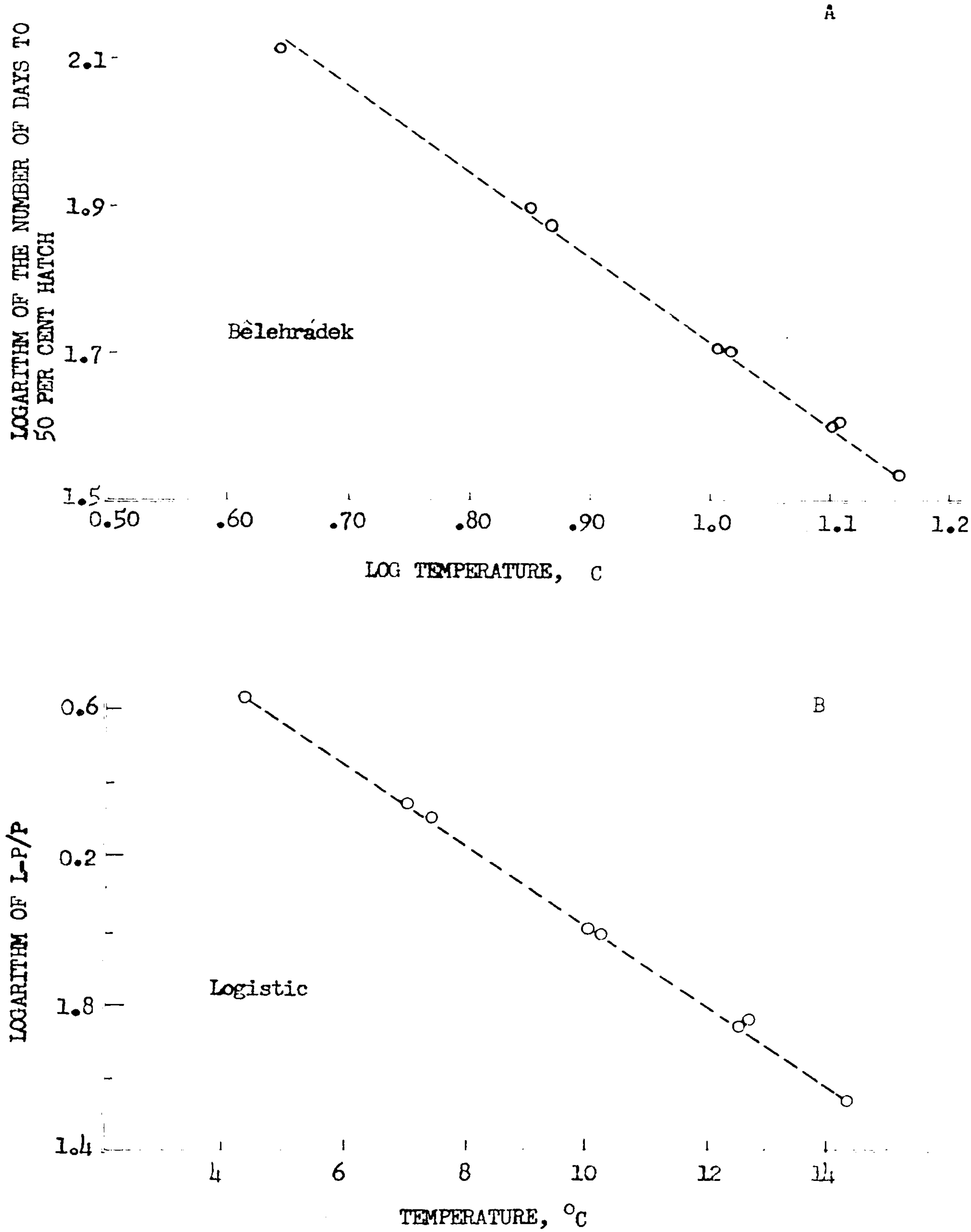

Figure 11. Relationship of the Bèlehrádek and Logistic Equations to the Rate of Development Data for Eight Lots of Experiments I and II. A. Relationship of the Logarithm of Temperature to the Logarithm of the Number of Days to 50 Per Cent Hatch. B, Relationship of Temperature to the Logarithm of L-P/P.

of *b* to be approximately 1), then the relationship of either (1) the speed of development to the temperature or (2) the logarithm of the number of days to hatching, to the logarithm of the temperature is linear for lots reared at constant temperature in the range from 39.8° to 57.8°F.

(e) Logistic curve. The logistic curve used by Davidson (1944) was of the form

$$\frac{1}{y} = \frac{L}{1+e^{a-bx}}$$

Replacing 1/y with P the equation is developed as follows:

$$P = \frac{L}{1+e^{a-bx}}$$

$$L = P+P(e^{a-bx})$$

$$\frac{L-P}{P} = e^{a-bx}$$

$$\log_e \frac{L-P}{P} = a-bx$$

The last form is again a form of the equation of a straight line for which *a* and *b* can be determined by standard methods. L can be estimated as described on page 42. The relationship of $\log \frac{L-P}{P}$ to *x* should be linear if the logistic equation describes the temperature development relationship; for the chinook salmon it appears to be so (Fig. 11). The values for the constants were 3.96 for L, 2.46 for *a*, and 0.242 for *b*, the equation for the curve being

$$\frac{100}{y} = \frac{3.96}{1+e^{2.46-0.242x}}$$

In Figure 12 the logistic curve closely fits the points that are determined by the relationship of temperature to the number of days to hatching; and the reciprocal of the logistic curve fits equally well to the points that are determined by the relationship of temperature to the

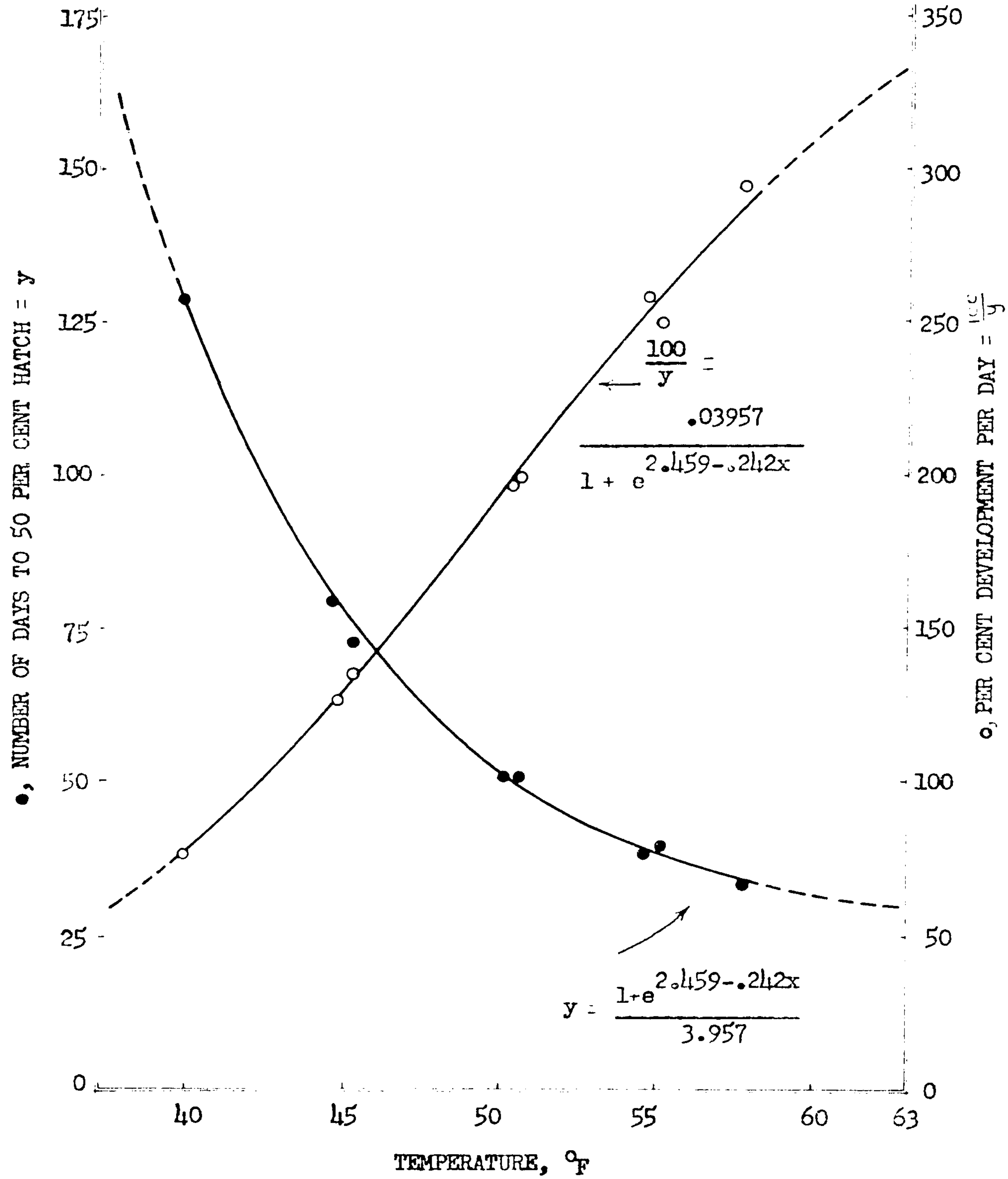

Figure 12. The Relationship of Temperature and the Number of Days to Hatching, to the Logistic Curve and Its Reciprocal for Eight Lots of Experiments I and II

speed of development (the reciprocal of the number of days to hatching). The relationship of temperature to speed of development was shown to be linear in Figure 10 and to fit the logistic in Figure 12, but this is explained by the fact that the temperature range of the lots selected for curve fitting lies in the region of the point of inflection of the logistic curve, a region where the logistic curve is nearly a straight line.

The apparent good fit of the logistic curve to the points in Figure 12 does not necessarily mean that the logistic expresses the theoretical relationship between temperature and rate of development. For three curves in which the deviations of the points from the curves were less than shown here for the chinook salmon, Browning (1952) tested the goodness of fit by the χ^2 test and found that the probability of the calculated curve describing the relationship of temperature to the rate of development was less than 0.0001.

In conclusion, for the temperature range of 39.8° to 57.8°F the data fit the logistic curve, but the fit is no better than either the temperature summation rule or the Bêlehrádek equation.

<u>Altered temperature experiments</u>. Experiment III differed from I and II in that eggs from four races* were used and the temperature pattern was changing rather than constant. The temperature history for each lot identified by the same symbol was similar, that is, the temperature history of the eggs of Skagit Lot 1, Entiat Lot 1, Green Lot 1 and Sacramento Lot 1 was similar. Water temperature, incubation period and per

*The chinook salmon from the Skagit, Entiat, Green and Sacramento Rivers are considered to be separate races.

cent hatch are given in Table 11.

(a) Incubation rate. The rate of development of the Sacramento eggs was fastest, with Green, Skagit and Entiat eggs following in the order given. To determine the rate of development for each race relative to the race from the Sacramento River, the number of days to hatching for each race was divided by the corresponding value for the Sacramento River race. This was done for each temperature lot by races and then the average values for the four races were determined. For the eggs from the salmon of the Sacramento, Green, Skagit and Entiat Rivers these values were 100, 97.2, 94.3 and 92.4, respectively. Actually, the average temperatures for similar temperature lots varied slightly between races and, for comparative purposes, the number of days to 50 per cent hatch was adjusted to a common temperature by linear interpolation (Table 12).

Evidence of differences in incubation rates of the four races was obtained from three sources: (1) inspection of Figure 13, which shows the number of days to hatching for each race at each temperature, (2) test of the significance of the difference in the number of days to hatching by Student's *t*, and (3) the consistent ranking of the races in regard to the number of days to hatching at various temperatures as shown in Figure 14.

The relationship of the number of days after the start of the experiment to the cumulative percentage hatched is presented in Figure 13 and shows that the Sacramento egg lots completed hatching before the Entiat egg lots began except in Lot 2. Lot 2 eggs were hatched at temperatures of 34° to 36°F and consequently the hatching period was extended. The 5-95 percentile deviation, $\frac{P_{95}-P_5}{2}$, of the days to 50 per cent hatch

TABLE 11

Water Temperature, Incubation Period and Per Cent Mortality of the Chinook Salmon Eggs of Experiment III

Stock	Lot	Temp., °F Start	Low	End	Ave.	No. of Days	Per Cent Mortality
Skagit River	1	45	34	45	38.8	132.0	4
	2	50	34	36	39.8	112.0	3
	3	55	44	44	49.4	56.0	2
	4	60	51	51	55.9	38.7	2
	5	65	59	59	61.3	31.9	40
	6*	55	54	55	54.7	46.7	8
Entiat River	1	45	34	46	38.8	133.0	6
	2	50	34	36	40.6	102.0	2
	3	55	42	42	48.4	62,2	2
	4	60	51	51	55.2	42.3	2
	5	65	58	58	61.3	33.5	11
	6*	55	54	55	55.2	46.4	9
Green River	1	45	34	44	39.0	125.0	42
	2	50	34	36	41.6	97.6	36
	3	55	45	45	50.2	51.8	63
	4	60	52	52	56.3	38.1	63
	5	65	63	63	64.0	--	100
	6	56	54	54	55.4	39.7	52
Sacramento River	1	45	34	43	39.0	124.0	2
	2	50	34	36	41.6	97.4	2
	3	55	44	44	50.5	48.6	1
	4	60	53	53	56.5	35.5	1
	5	65	60	60	62.2	28.4	24
	6	56	54	54	55.5	38.0	3

* Incubated in water of low oxygen content

TABLE 12

Observed and Adjusted Temperatures and Days to 50 Per Cent Hatch for Chinook Salmon Eggs Reared at Changing Temperatures, Experiment III

Lot	Stock	Observed Ave. Temp. ,°F	Observed Days to 50% Hatch	Adjusted Ave. Temp. ,°F	Adjusted Days to 50% Hatch	5-95 Percentile Deviation to 50% Hatch	Hatching Rate Relative to Sacramento
1	Sacramento	39.0	124.0	39.0	124.0	2.27	100.
	Green	39.0	125.0	"	125.0	4.31	99.2
	Skagit	38.8	132.0	"	128.0	1.85	96.9
	Entiat	38.8	133.0	"	130.0	2.12	95.4
2	Sacramento	41.6	97.4	41.6	97.4	11.00	100.
	Green	41.6	97.6	"	97.5	10.08	99.8
	Skagit	39.8	112.0	"	101.4	5.12	96.1
	Entiat	40.6	102.0	"	96.2	7.12	101.
3	Sacramento	50.5	48.6	50.5	48.6	2.75	100.
	Green	50.2	51.8	"	51.2	2.98	94.9
	Skagit	49.4	50.0	"	53.2	2.00	91.4
	Entiat	48.4	62.2	"	56.0	2.12	86.8
4	Sacramento	56.5	35.5	55.2	38.2	0.50	100.
	Green	56.3	38.1	"	40.3	1.16	94.8
	Skagit	55.9	38.7	"	40.5	2.72	94.3
	Entiat	55.2	42.3	"	42.3	1.23	90.3
5	Sacramento	62.2	28.4	61.3	29.6	1.60	100.
	Green	-	-	-	-		
	Skagit	61.3	31.9	"	31.9	2.66	92.8
	Entiat	61.3	33.5	"	33.5	2.83	88.4
6*	Sacramento	55.5	38.0				
	Green	55.4	39.7				
	Skagit**	54.7	46.7				
	Entiat**	55.2	46.4				

* "Controls", temperature range 56 - 53°F

**Incubated in water of low oxygen content

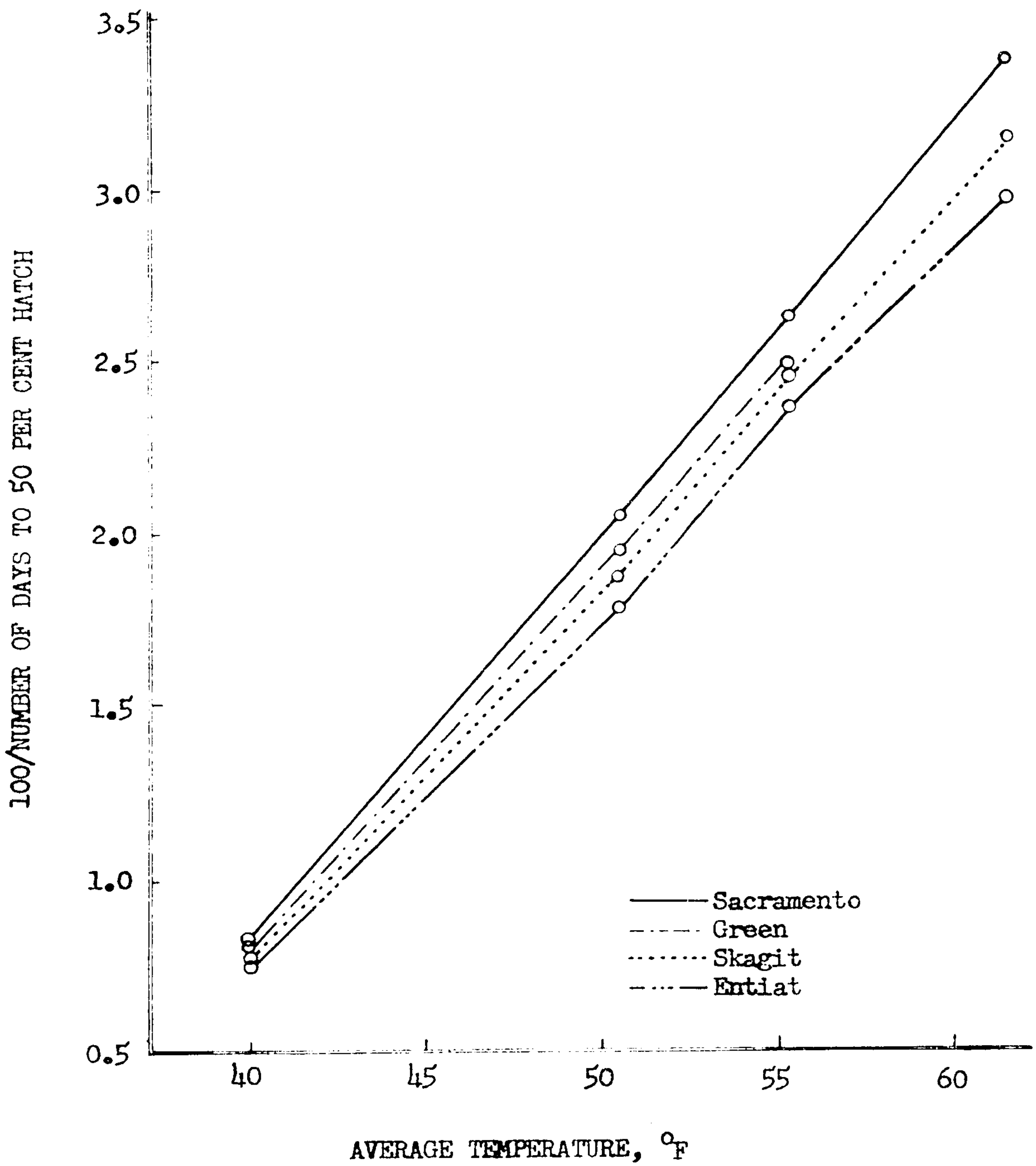

Figure 13. The Average Temperature and Rate of Development for the Eggs from Four Races of Chinook Salmon in Experiment III

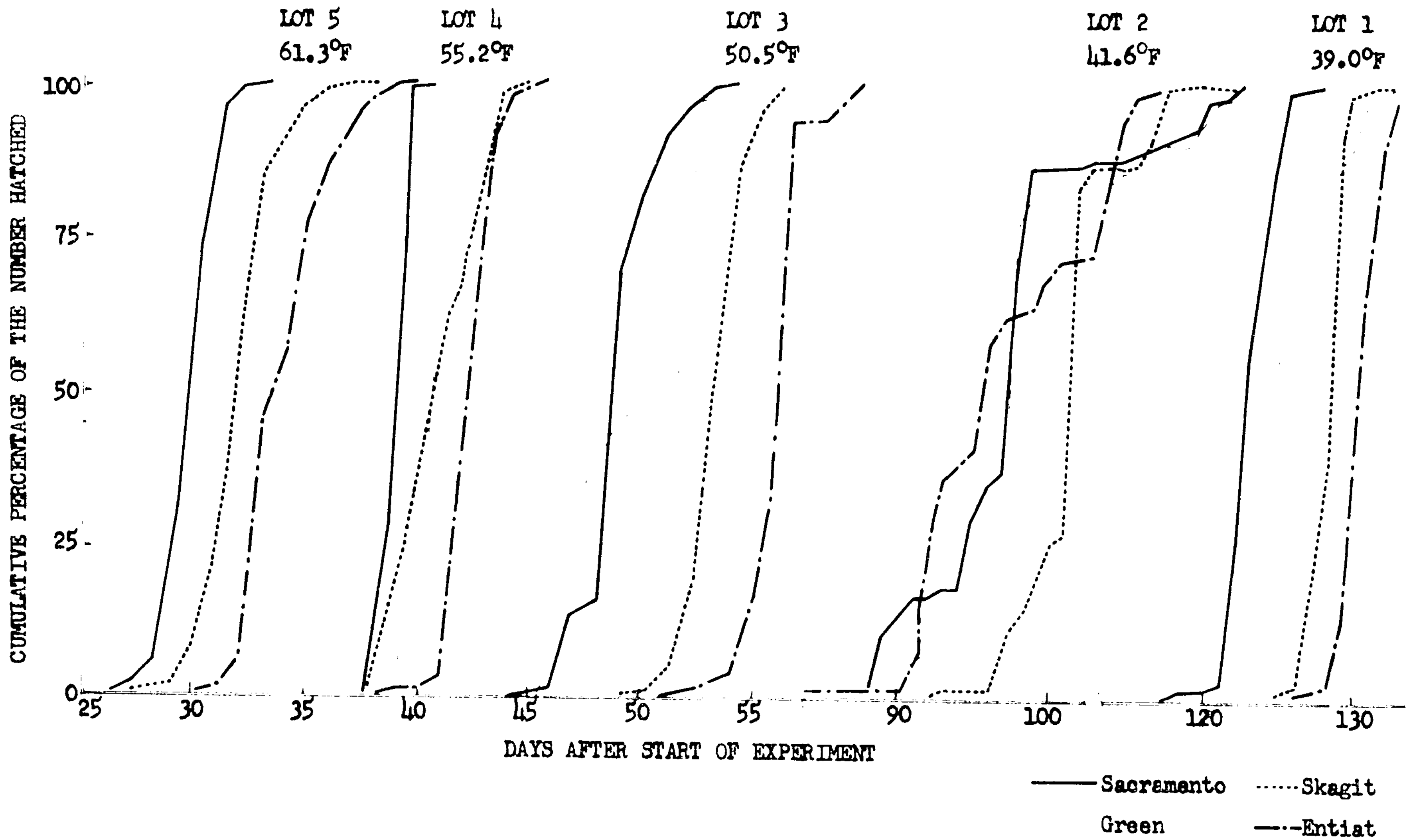

Figure 14. Cumulative Percentage of the Number of Eggs Hatched [illegible]

(Table 12) is three to four times greater for Lot 2 than for any of the other lots, and for this reason the 50 per cent hatching date is a less reliable estimate of hatching for Lot 2 than for lots with a shorter hatching period.

The difference in the number of days to hatching for each race and each lot was tested for significance by Student's *t*. For Lots 1, 3, 4 and 5 (see Table 5 for lot temperatures) the probability of the *t* values was less than .01, that is, the differences in mean hatching times between races at the same temperature were highly significant. The non-normal distribution of the number of days to hatching makes use of the *t* test questionable and prohibits its use for Lot 2 data.

When the four races are arranged in the order of the number of days to hatching, the order remains the same at average temperatures of 39.0°, 50.5°, 55.2° and 61.3°F. Lot 2 data were not included for reasons given above. The order, beginning with the race with the shortest time to hatching, is Sacramento, Green, Skagit and Entiat. These data are plotted in Figure 13. The probability of these values randomly aligning in this order is $(\frac{1}{4!})(\frac{1}{4!})(\frac{1}{3!})$, or one chance in 3456.

For the conditions of Experiment III, the differences in the rate of development between races are evident from Figures 13 and 14 even though some of the differences are not great. With larger samples from each race, that is, more spawning pairs, results different from those obtained here would be possible if it so happened that the salmon in these experiments were atypical representatives of their race.

In conclusion, the incubation rate of the Sacramento eggs in these experiments was about 8 per cent faster than the Entiat eggs; this difference is significant. The rate of development of the Green and Skagit eggs was intermediate to the Sacramento and Entiat eggs.

(b) Threshold temperature. The threshold temperature and confidence limits were calculated for the four races by the methods given on page 60 and are tabulated in Table 13. Although the same order for the races that prevailed for the rates of development is present for the threshold temperatures and confidence interval, the confidence intervals overlap widely and limit the significance that may be attached to the ordering effect.

The range of threshold temperatures, 31.1° to 32.6°F, for lots reared at changing temperatures is less than the value of 33.8°F for the threshold temperature of lots reared at constant temperatures in the range from 39.8° to 57.8°F. To investigate further the difference in threshold temperatures between lots reared at constant temperatures and at changing temperatures, the threshold temperature and confidence interval were calculated for lots reared at constant temperatures at all temperature levels. In Table 13 the results of these calculations show that the threshold temperatures of the lots reared at constant temperatures are higher and lie outside the range of lots reared at changing temperatures, but since the confidence intervals overlap, the differences may not be significant.

In conclusion, the threshold temperatures of the four stocks reared at changing temperatures range from 31.1° to 32.6°F. The range of comparable values for eggs from the Green River stock at constant temperatures is higher, 32.7° to 34.0°F, but the confidence intervals for the two groups overlap.

TABLE 13

Estimated Threshold Temperatures and Confidence Limits for Experiments I, II and III

Experiment	Race	Estimated Threshold Temperature*	Confidence Limits
III	Sacramento	32.6°F	31.2-33.9°F
"	Green	31.7°F	29.8-33.3°F
"	Skagit	31.6°F	29.8-33.2°F
"	Entiat	31.1°F	28.9-33.0°F
I & II	Green, Temp., 40-58°F	33.8°F	33.0-34.6°F
I	Green, All temps	32.7°F	30.4-34.5°F
II	Green, All temps	34.0°F	31.8-35.8°F

*Threshold temperature $= \bar{x}-k(\bar{\frac{1}{y}})$ where $\frac{1}{k}$ is the regression coefficient of the rate of development on temperature.

(c) Temperature summation constant, $\underline{k}$. Having determined the threshold temperature, $\underline{a}$, the temperature summation constant, $\underline{k}$, was estimated. Two estimates were made; one in which the threshold temperatures were the calculated values as determined in Table 13, and a second in which the threshold temperature was arbitrarily taken as 32°F.

The values of the temperature constant are given in Table 14. When the calculated values of the threshold temperature are used it is seen that the $\underline{k}$ values vary more between races but have a smaller standard error than when the threshold temperature of 32°F is used. For eggs from the Green River stock the average value of the temperature summation constant, when 32°F is the threshold temperature, is 932, which is equivalent to 932 temperature units as defined by Wallich (page 43). This is similar to his estimate of 900 temperature units to hatching for Sacramento River chinook salmon especially if allowance is made for the more rapid rate of development of the Sacramento fish (page 72).

In conclusion, the best estimate of the temperature summation constant, $\underline{k}$, is made when the threshold temperature is calculated from the equation, $a = \bar{x} - k(\frac{1}{y})$, where $1/k$ is the regression coefficient of the rate of development on temperature. However, if $\underline{a}$ is unknown, 32°F is a reasonable estimate of $\underline{a}$. Using the calculated estimates of $\underline{a}$, the values of the temperature summation constant up to the time of hatching for the eggs of Experiment III from the Sacramento, Green, Skagit and Entiat River chinook salmon were 860, 940, 950 and 1020, respectively.

(d) Incubation time of Experiment I sublots. Experiment I was basically a constant temperature experiment. However, there were five sublots of 100 eggs each in which the eggs were moved during the incubation

TABLE 14

Temperature Summation Constant, k

$(y)(x-a) = k$

Experiment	Stock	No. of Lots	Range Ave. Temp. °F	a*	k – s.e.	a	k – s.e.
I	Green	11	40-65	32.7	919– 5.8	32	960– 9.1
II	"	10	45-62	34.0	815– 5.7	"	897–10.3
I & II	"	8	40-58	33.8	828–10.2	"	939–17.7
III	"	5	39-56	31.7	944 - 9.4	"	922-12.1
"	Sacramento	6	39-62	32.6	855-15.4	"	887-14.1
"	Skagit	5	39-61	31.6	951-12.9	"	921-17.0
"	Entiat	5	39-61	31.1	1020-17.1	"	953-26.7
Average for Green River					877- 3.97		930- 6.64
Average for Experiment III					941- 6.11		921- 8.01

*Threshold temperatures from Table 10

period from medium to high water temperatures and three sublots in whic the move was from medium to low water temperatures (see Table 15). Although the eggs were transferred for the purpose of observing the effec upon meristic characters and upon the relation of relative change in temperature to per cent mortality, the results also show an effect upon the rate of development.

The rate of development at the average temperature of incubation was slower for the sublots moved to the high temperatures than for lots reared at the corresponding constant temperature. For sublots moved to the low temperatures there were no lots reared at the corresponding constant temperature for comparison, but the rate of development of the sublots was as fast or possibly faster than expected from the projectio of the rate of development curve (Fig. 15).

<u>All experiments combined.</u> An adequate expression of the rate of development for lots reared at constant temperatures in the optimum ran and for lots of the four stocks reared at changing temperatures has bee found. In an effort to find a general empirical equation to fit all th data, even though several complexities may have been introduced by combining lots--irrespective of race, temperature pattern, year or mortality--the temperature summation rule, the Bêlehrádek equation, and the logistic curve were fitted to the temperature-development relationship for fifty lots from the three experiments. This included all lots excel the two that were incubated in water of low oxygen content. The values for average temperatures and the number of days to hatching for these lots are to be found in Tables 9, 11 and 15.

The fit of the temperature summation rule in the reciprocal form t these data was tried by plotting the relationship of the average

TABLE 15

Water Temperature, Incubation Period and Per Cent Hatch of Experiment I Sublots

Lot	Temperature History	Temp. °F	Days to 50% Hatch	Per Cent Mortality
8C9	At city water temperature 55 to 48°F, for 25 days then to constant water temperature of 65°F	55.9	40.3	93
8C10	Same as 8C9 except to 62½ °F water	55.7	43.4	28
8C7	Same as 8C9 except to 60°F water	54.8	43.3	41
3A7	28 days at 45°F, then to 60°F	51.2	50.3	3
2A7	28 days at 40°F, then to 60°F	49.8	57.8	36
8C1	25 days at city water temperature, then to 34°F	37.1	144.0	3
8Ba2	18 days at city water temperature, then to 34°F	36.5	158.0	23
8Ba1	14 days at city water temperature, then to 34°F	36.1	172.0	42

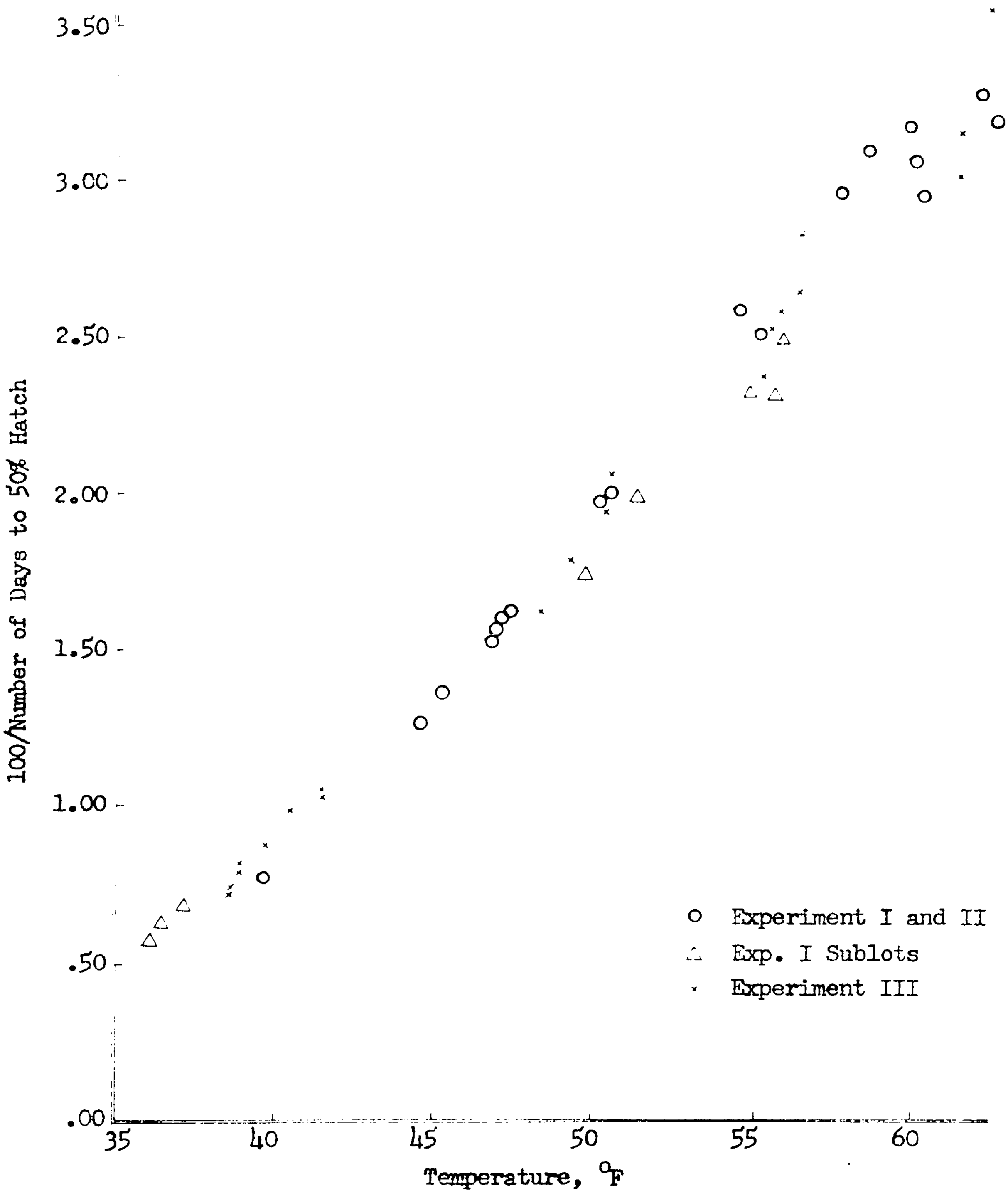

Figure 15. Rate of Development and Temperature for All Lots, All Experiments

temperature to the reciprocal of the number of days to hatching (Fig. 15). The relationship was not linear and therefore a curve that fitted the data more closely was sought.

The fit of the Bêlehrádek equation to these data was tested by plotting the relationship of the logarithm of the average temperature to the logarithm of the number of days to hatching (Fig. 16A). This relationship also deviated from linearity.

(a) Logistic curve. For the logistic curve the fit to these data was tested by plotting the relationship of temperature to the logarithm of L-P/P (page 42). As a trial run, the relationship of temperature to the log of L-P/P were plotted for eight lots that were approximately equally spaced throughout the temperature range at which chinook salmon eggs develop (Fig. 16B). Since this relationship was practically linear, the logistic curve was then fitted to the data for all lots from Experiments I, II and III (Fig. 17).

The constants for the logistic curve were determined by the methods described on pages 42 and 63. For L, values of P at 3°, 9° and 15°C were used. The calculated equation for the number of days to 50 per cent hatch is

$$y = \frac{1+e^{2.306-.2022x}}{.04404}$$

For this equation the standard error of estimate, $s_{y.x}$, which is an estimate of the fit of the calculated curve to the observed data, is 3.14. The reciprocal of y, times 100, is the per cent development per day for which the equation is

$$\frac{100}{y} = \frac{4.404}{1+e^{2.306-.2022x}}$$

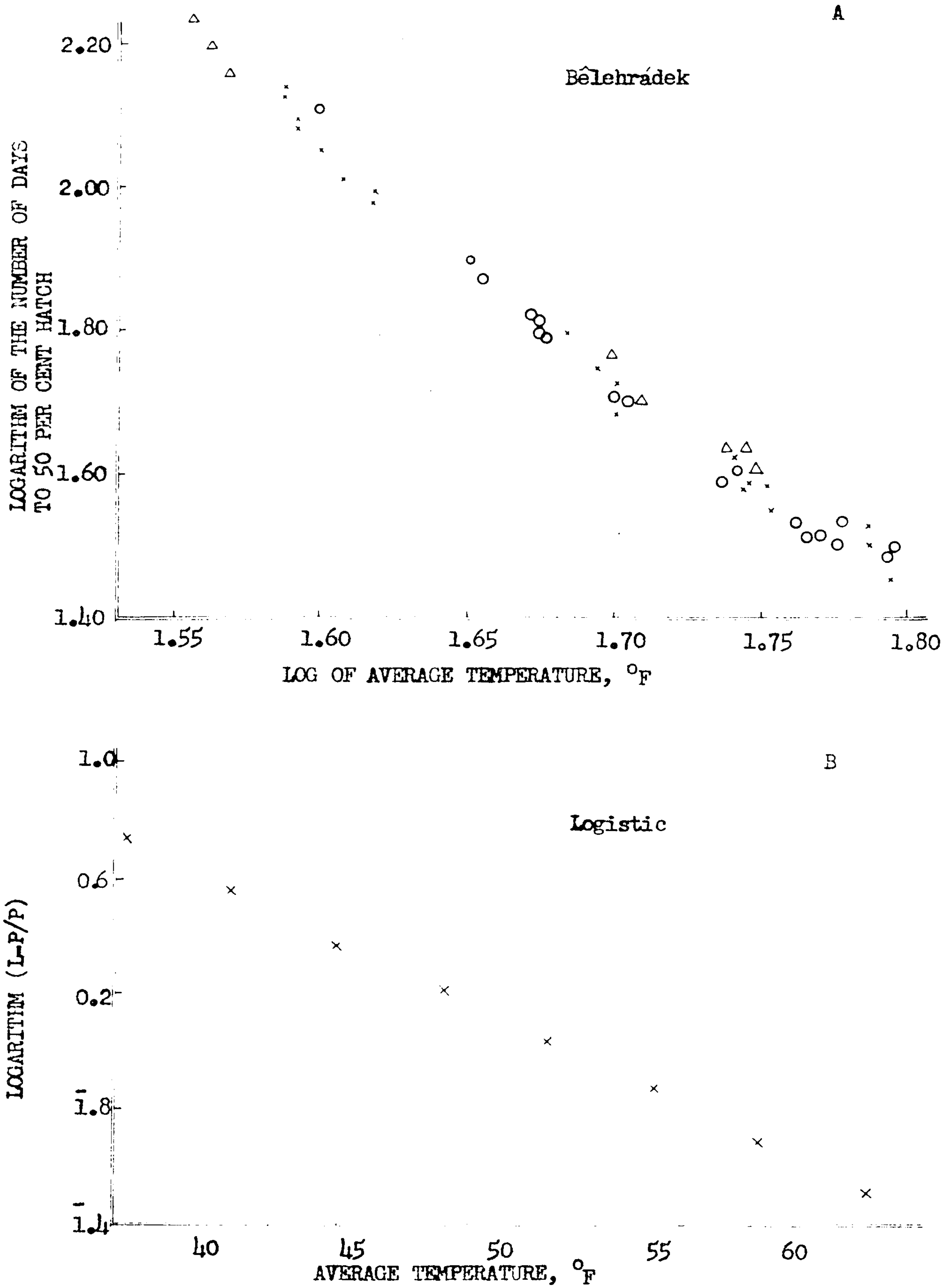

Figure 16. Relationship of the Bêlehrádek Equation and the Logistic Equation to the Rate of Development Data from all Lots. A, Relationship of the Logarithm of Temperature to the Logarithm of the Number of Days to Hatching. B, Relationship of Temperature to the Logarithm of L-P/P.

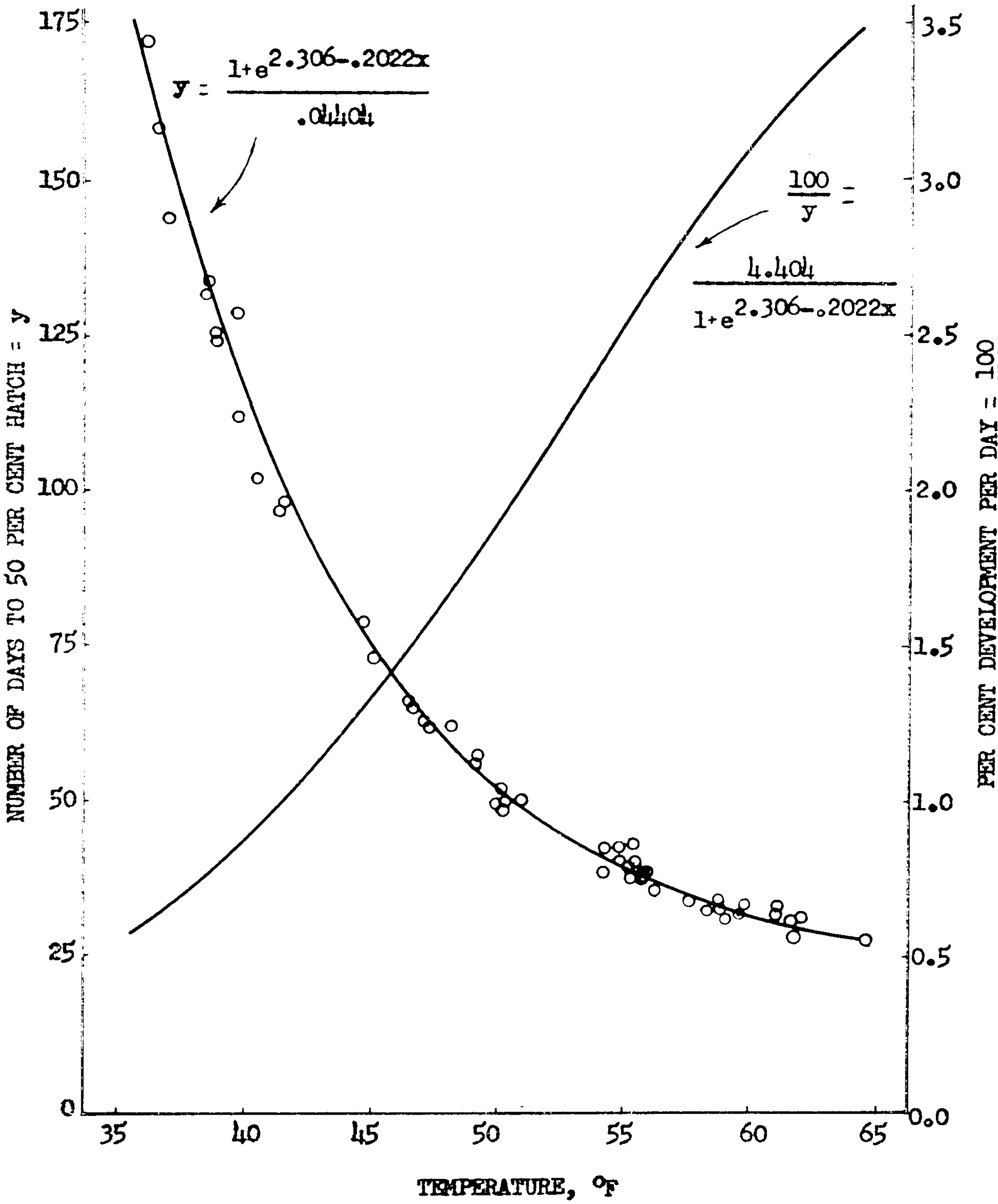

Figure 17. **The Relationship of Temperature and the Number of Days to Hatching to the Logistic Curve and Its Reciprocal, for All Lots**

In conclusion, when data from all lots in the three experiments are used, the equation of the logistic curve in the form

$$y = \frac{1+e^{2.306-.2022x}}{.04404},$$

where *y* is the number of days to hatching at temperature *x*, best describes the temperature-development relationship. The standard error of estimate for this curve is 3.14.

<u>(b) Duration of hatching period</u>. The duration of the hatching period was measured by the number of days between the hatching of the fifth percentile egg and the ninety-fifth percentile egg and was called the 5 to 95 percentile range for the hatching period of chinook salmon. By not using the first and last five per cent of the total range, the few very early or late hatching eggs that occasionally would occur were not included. The relationship of the temperature at time of hatching to the 5 to 95 percentile range for the hatching period is shown in Figure 18.

The duration of the hatching period might be expected to be influenced by the rate of development and thus to decline with increase in temperature, but this was not exactly true as shown in Figure 18. From 35° to 40°F the duration of the hatching period rapidly declined, but above 40° the length of the hatching period was short and without noticeable change with respect to temperature. The range of average temperatures for which the mortality of eggs and fry of the chinook salmon is a minimum, is also the range for which the duration of the hatching period is a minimum. It would appear that a short hatching period is associated with a high survival.

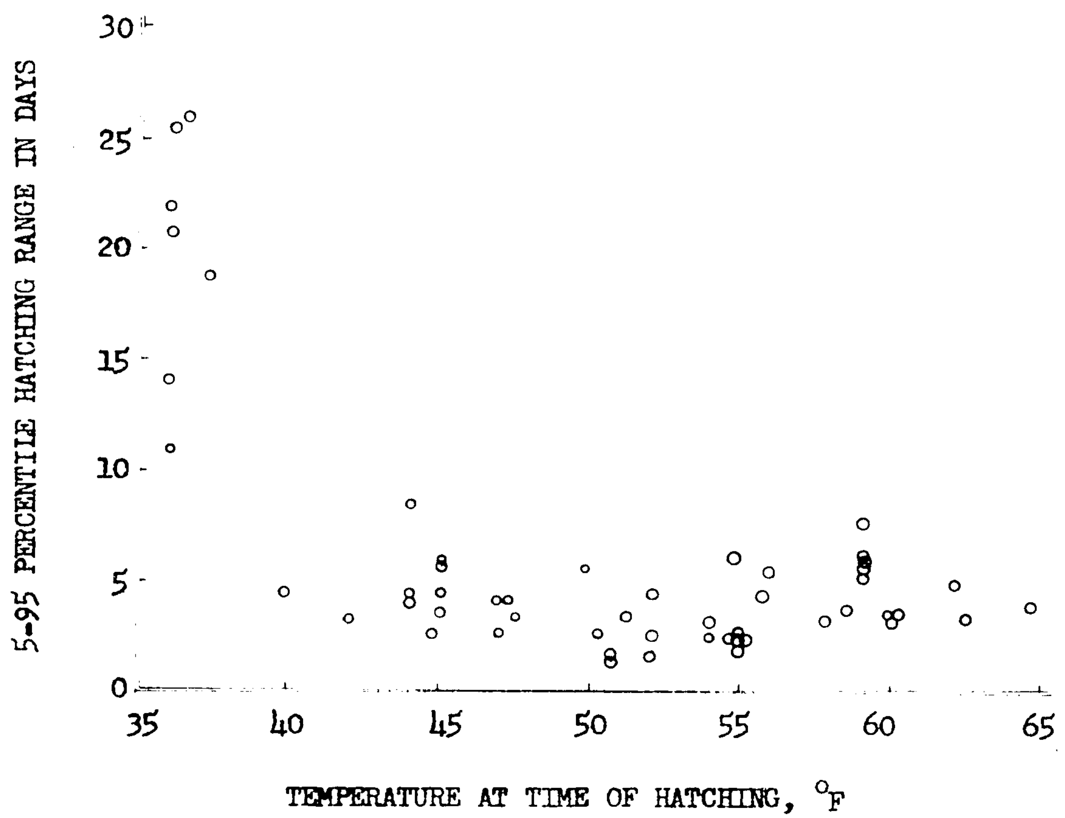

Figure 18. The 5 to 95 Percentile Range for the Hatching Period of Chinook Salmon

Mortalities

Lots reared at constant temperatures. The weekly cumulative mortalities of the lots reared at constant temperatures in Experiments I and II are shown in Figure 19. For lots reared at corresponding temperatures in both experiments--60°, 55°, 50°, 45°F--the graphs up to the tenth week (the end of Experiment II) are averages of the two experiments. By inspection of Figure 19 the lots can be classified into four groups as follows:

1. Lots in which mortality during incubation is 100 per cent, that is, no hatch. This includes the lots reared at average temperatures of 34°F and 65°F and higher.

2. Lots in which a few survive to hatching but die in the yolk-sac stage. In this category are the 62.5° and 60°F lots.

3. Lots in which the mortality to hatching is low but is followed by a high mortality during absorption of the yolk sac. After feeding has begun, mortality is again low. The 57.5° and 55°F lots are in this group.

4. Lots such as 50°, 45° and 40°F, in which mortality is low during incubation, yolk-sac and fingerling stages. This is the optimum temperature range in respect to mortality for chinook salmon reared at constant temperatures.

One explanation of the high mortality that occurred during the yolk-sac stage to the lots reared at 57.5° and 55°F is that the organization of the physiological processes is out of step. Hayes (1949) wrote that physiological processes have optimum temperatures which vary with the process. For example, bile formation is favored at high temperatures

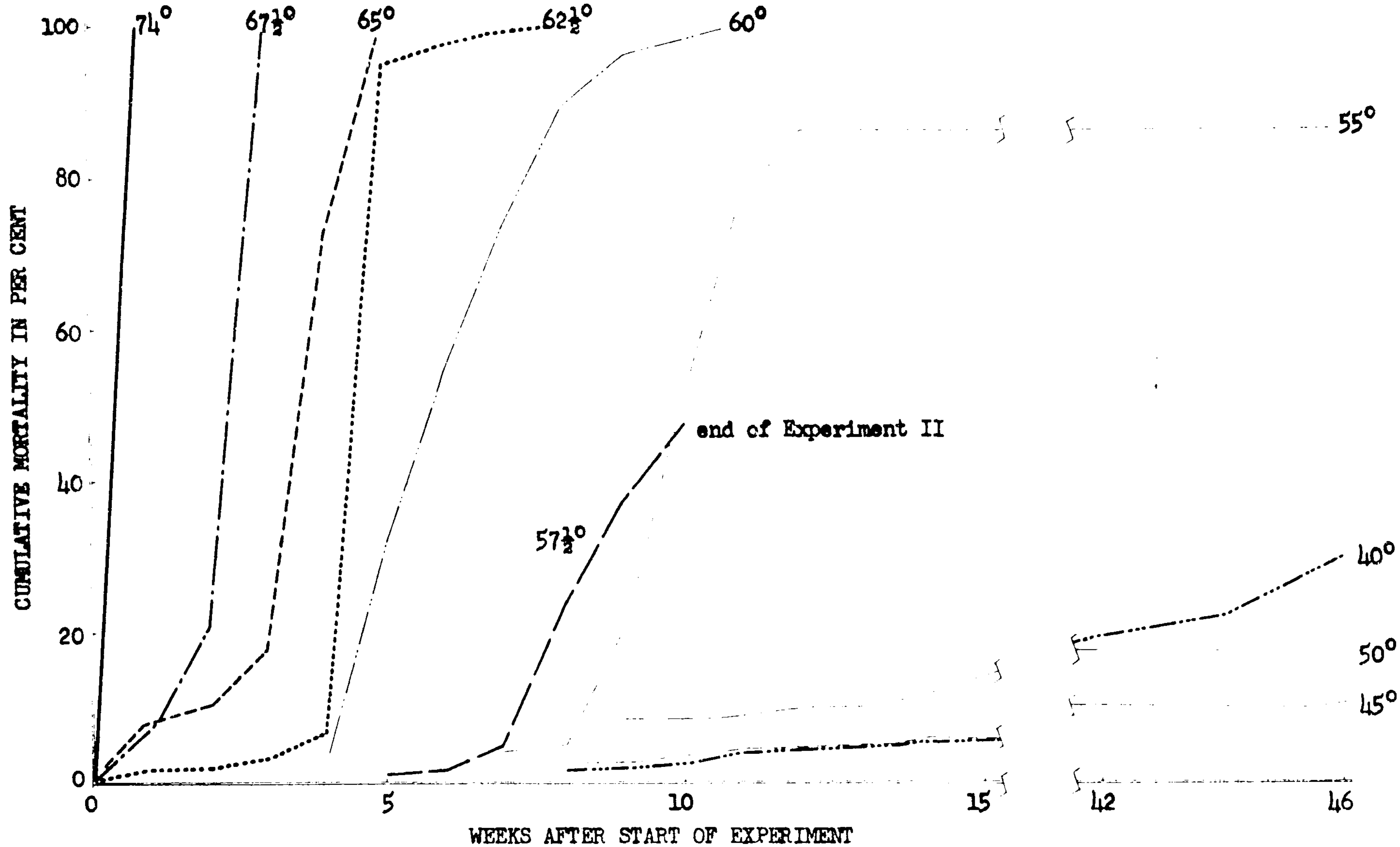

Figure 19. Cumulative Mortality in Per Cent by Weeks for Lots Reared at Constant Temperatures in Experiments I and II

and circulation in the yolk at low temperatures. Thus, exposure of the egg to an unfavorable temperature may not result in death until the yolk-sac stage.

The relationship of temperature to per cent mortality at time of hatching is shown in Figure 20. To supplement these observations, especially at low temperatures, Burrows' data for similar experiments with chinook salmon at the Entiat Hatchery are included (Table 16). The rapid increase in mortality at temperatures of 60°F and higher and at temperatures lower than 40°F are to be noted.

From Figure 20 an approximation of the "lethal temperature 50 per cent, LT_{50}," was made. This is the temperature at which 50 per cent of the individuals die from temperature effects. Taking into account the mortality not due to temperature, which was assumed to be the average mortality in the optimum range (4.6%), the LT_{50} was the temperature at the 55% mortality level. The curve of mortality in Figure 20 crossed the 55% level at two places, 36.5°F and 60.8°F, which are the estimates of LT_{50}.

Changing temperatures. The egg mortalities for the Sacramento, Green, Skagit and Entiat races are listed in Table 11. From inspection of the table several facts are evident.

First, the high mortality of the lots from the Green River stock was not due entirely to temperature. In Lot 5 the egg mortality was 100 per cent; for the five other lots, the mortality was at least ten times as great as the average mortality for the other three races; therefore it is evident that some of the mortalities to the Green River lots were from causes other than temperature. Also, at an average temperature of

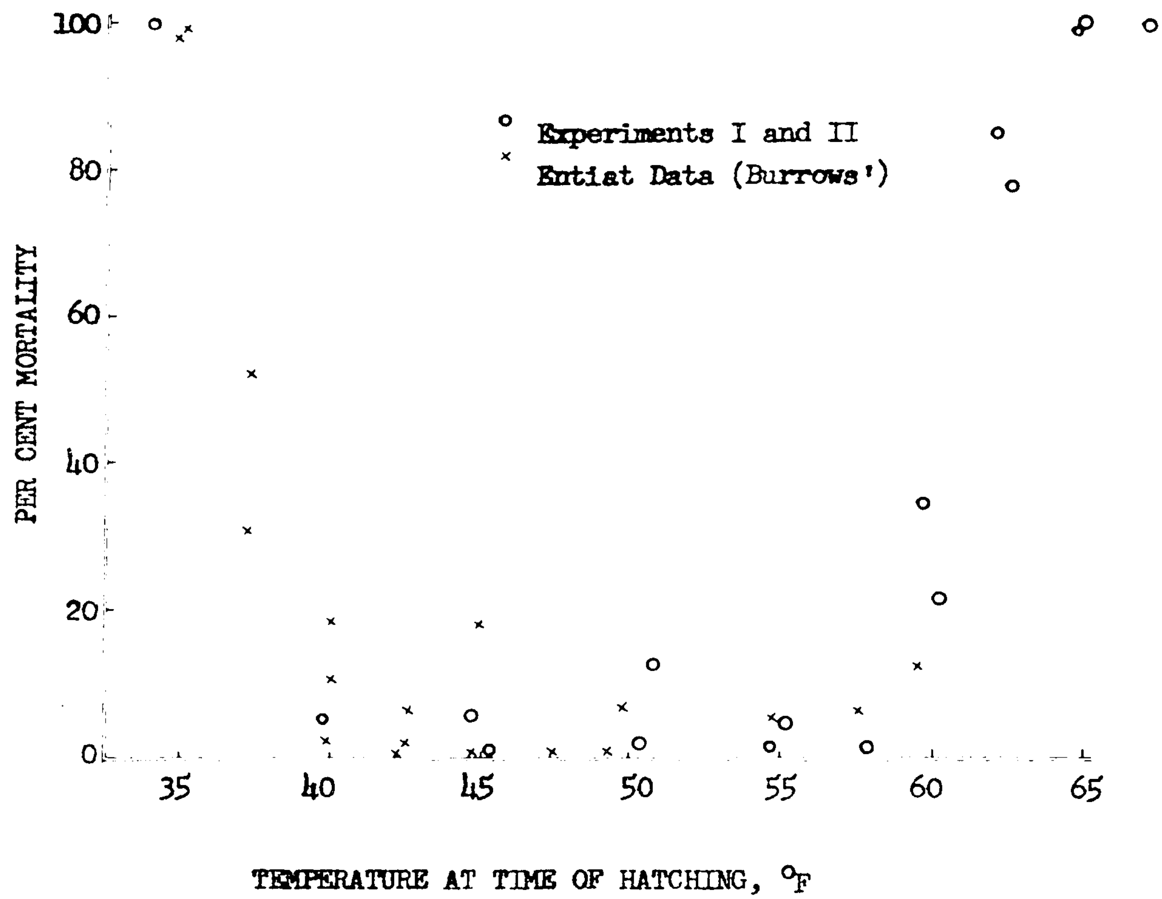

Figure 20. Temperature and Per Cent Mortality for Lots Reared at Constant Temperature

TABLE 16

Water Temperature, Incubation Period and Mortality of Chinook Salmon Eg. Reared at Constant Temperatures at the Entiat Hatchery (Burrows' Data)

Year	Brood Stock	Temp., °F	Days to 50% Hatch	Per Cent Mortality
1952-53	Entiat	49.78	52.19	7.1
		54.88	41.88	5.7
		57.53	36.69	6.1
		59.61	34.34	12.4
1953-54	Entiat	35.10	204.00	99.6
		37.35	157.54	52.6
		40.05	120.11	18.5
		42.64	92.38	6.1
		44.89	76.82	18.4
1953-54	Skagit	34.89	206.33	98.7
		37.29	160.29	30.9
		40.04	123.49	10.2
		42.54	94.00	2.1
		44.87	76.32	0.9
1955-56	Entiat	39.94	121.10	2.7
		42.40	94.69	1.3
		44.74	78.91	0.7
		47.38	63.40	0.6
		49.21	55.44	1.1

62°F, the highest average temperature for lots of Experiment III, the effect of temperature upon mortality is marked. From 55° to 62°F the increase is about 20 per cent and corresponds to a similar increase at corresponding temperatures for the lots reared at constant temperatures. Finally, there is no difference in the tolerance to low temperatures of Sacramento, Skagit or Entiat stocks as shown by egg mortalities. The minimum temperature was 34°F and some lots from all races were incubated at this temperature for twenty days, the descent and ascent from the minimum being one degree every five days.

These data are not adequate to define temperature tolerance. The tolerance of chinook salmon eggs to limited exposures at high temperatures has been investigated by Donaldson (1955). He found that the exposure time necessary to cause 10 per cent kill averaged 1½, 4 and 13 days at temperatures of 67°, 65° and 63°F, respectively. For fingerling chinook salmon Brett (1952) has determined the temperature tolerance.

Abnormalities

The term "abnormal fish" is difficult to define. In this report the definition is limited to individuals with morphological abnormalities that can be recognized visually. For the egg stage per cent mortality is a good measure of abnormality, since any egg that fails to hatch is abnormal, strictly speaking.

For the fry--the stage from hatching to feeding--mortalities were classified as to type by the terms used by Foster (1949) to identify abnormalities in the progeny of rainbow trout exposed to X-rays. These terms include the types of abnormalities that were found by Welander

(1948) to occur in the young of chinook salmon exposed to X-rays in the egg stage. Identification of abnormalities was made from preserved specimens.

In Table 17 the per cent and number of different types of abnormal fry in Experiment I are summarized. For the few individuals with two abnormalities, both types were recorded. The number of abnormalities increased at the extreme temperatures, but were principally abnormalities such as "developmentally deficient," "weak body structure" and "serous fluid" rather than the monster-like abnormalities of "spinal curvature," "distorted jaw" and "twinning."

Growth

Eggs from lots of Experiment I were weighed before and after water-hardening and near the mid-point of the incubation period; fry were weighed and measured once, just after hatching; and the fingerlings were weighed at two-week intervals from May until October. Results of the measurements of the eggs and fry are summarized in Table 18.

The rate of water absorption by the egg was measured by placing 30 eggs in a ruled trough and observing the total length of the row at five-minute intervals. After 35 minutes in water the eggs had reached maximum size. At the time of placing the eggs in the water the diameter was not determined, as the eggs were soft and somewhat irregular in shape. After absorption of water the eggs were firm and spherical and the average diameter of a sample of 30 eggs was 9.3 mm. The increase in weight during the water absorption period was 15.0 per cent as determined from a sample of 136 eggs that averaged 379 mg before and 436 mg after water-hardening.

TABLE 17

Abnormal Fry In Lots Reared at Constant Temperatures

Lot	2	3	4	5	6	7	10
Temp. °F	40	45	City Water	50	55	60	62_2
Number Hatched	368	352	501	440	480	404	120
Per Cent Abnormal	8	2	6	2	5	40	65
Number of Abnormalities by Type*							
D	5	3	10	1	2	53	32
W	16	3	7	0	2	45	10
S	3	1	2	1	0	32	30
C	5	0	5	0	8	19	0
L	1	0	3	0	4	11	6
J	0	0	1	0	0	0	0
E	1	1	1	0	0	3	5
T	0	0	2	3	6	1	0

* D, developmentally deficient; W, weak body structure; S, serous fluid; C, spinal curvature; L, shortened body; J, distorted jaw; E, defective eye; T, twinning

Table 18

Average Weights and Lengths of Eggs and Fry From Experiment I

Weights in milligrams and lengths in millimeters of formalin preserved specimens

	Lot	Temp.	n	Total Weight	Yolk Weight	Shell Weight	Fry Weight	Fork Weight
Eggs								
approximate	2	40	10	411 ± 4.9	313 ± 4.0	27.6 ± 1.14		
midpoint	3	45	20	412 ± 3.2	319 ± 2.2	27.0 ± .60		
of	4	47*	20	402 ± 3.4	331 ± 3.2	26.0 ± .44		
hatching	5	50	20	410 ± 3.5	335 ± 3.4	22.2 ± .57		
	6	55	20	402 ± 3.2	332 ± 2.6	24.1 ± .27		
	7	60	20	403 ± 4.1	344 ± 3.3	23.4 ± .33		
	8	47*	20	404 ± 3.8	332 ± 2.9	18.4 ± .55		
	10	62	20	411 ± 3.4	355 ± 1.8	18.3 ± .35		
	9	65	20	398 ± 2.8	351 ± 2.5	16.4 ± .32		
for all lots			170	405 ± 1.3	336 ± 1.4	21.7 ± .30		
Fry								
just after	2	40	10	346 ± 2.5	260 ± 2.9		80.0 ± 4.2	24.0 ± .40
hatching	3	45	10	340 ± 1.0	276 ± 2.4		58.5 ± 1.9	22.3 ± .13
	4	47*	20	334 ± 1.8	268 ± 1.5		63.3 ± 0.9	22.9 ± .12
	5	50	20	321 ± 2.5	266 ± 1.9		49.6 ± 0.9	21.8 ± .23
	6	55	20	337 ± 2.8	275 ± 2.7		59.2 ± 0.9	22.0 ± .08
for all lots			80	334 ± 1.1	270 ± 1.0		59.8 ± 1.1	22.5 ± .08

*Average of city water temperatures

For the samples withdrawn from the egg lots at the estimated mid-point of incubation the weight of the yolk as listed in Table 18 also includes the weight of the embryo. These values are not comparable from lot to lot because of two uncorrected errors; one is the error in estimating the mid-point of hatching, the other is the loss of weight due to dehydration of the egg during the weighing process. The error in estimating the mid-point of hatching ranged from minus 28 per cent to plus 16 per cent and was determined by subtracting the number of days to the mid-point of hatching from the number of days to the time when the sample was withdrawn, and dividing this value by the number of days to the mid-point of hatching. The loss of weight from dehydration for an egg before removal of the shell was at the rate of 18 mg per hour. While waiting to be weighed the eggs were subjected to dehydration for a period of a few minutes to one-half an hour, a loss in weight of perhaps 1 to 10 mg. However, the inverse relationship between temperature and shell weight is probably true even if consideration is given to these two errors.

The weights and lengths of the newly hatched fry decrease with temperature but in an irregular manner. The fry from the 40°F lot were definitely larger than the fry from the lots reared at higher temperatures, which agrees, in general, with Gray's observation. For *Salmo fario* Gray (1928) made the following statement, "When eggs are incubated at low temperatures the embryos at the moment of hatching are significantly larger than those hatching from eggs incubated at higher temperatures." This is also probably true for the chinook salmon, but it is to be remembered that the fish reared at lower temperatures are also older at the time of hatching, the age for the 40°F lot being 128 days as compared to 50 days for the 50°F lot.

During the fingerling stage lots were weighed and counted at approximately two-week intervals. Five lots survived to the fingerling stage, including the control lots which were reared at city water temperature. The fish were weighed as lots rather than individuals, as it is not feasible to weigh live fish of this size individually.

To investigate growth rates of fingerlings at constant temperatures above 55°F a control lot was subdivided on May 1, five weeks after the start of feeding, into four groups of 100 each. One group was retained at city water temperature and the other three were transferred to water temperatures of 60°, 67° and 74°F. Temperatures were raised at the rate of one degree per day from the city water temperature of 54°F on May 1 to the temperature selected for the lot.

Growth curves for the original lots and for the lots started on May 1 are shown in Figure 21. The difference between lots is obvious. From 40°F upward to 55°F the growth rates increase and from 60°F upward the growth rates decrease. The average weight at 46 weeks for the 40°, 45°, 50° and 55°F lots was 0.4, 3.3, 12.6 and 18.1 grams, respectively. For the lots started May 1 the average weights were 11.2 and 7.5 grams for the 60° and 67°F lots. The 74°F lot did not survive.

The maximum growth rate for fingerlings reared at constant temperatures occurs at about 55°F, but the fastest growth rate shown in Figure 21 is for the lot reared at city water temperatures. This was observed during the 30th to 32nd week at water temperatures of 60° to 63°F, but since it is reasonable to assume that there is a short lag in the response of growth to temperature, the optimum temperature for this lot is

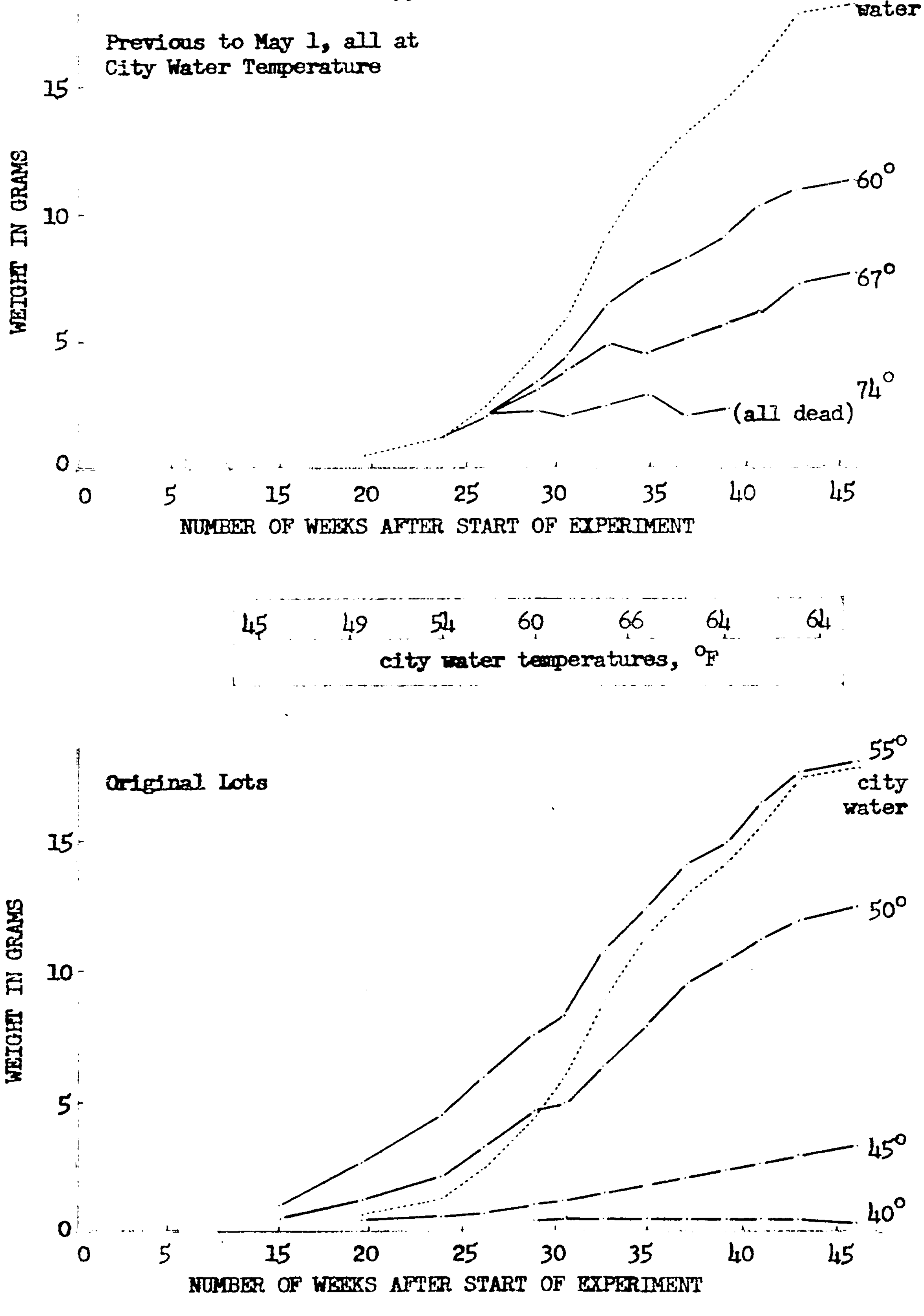

Figure 21. Weight Curves for Experiment I

likely to be during the 28th to 30th week at temperatures of 57° to 60°F.

The effect of mortality upon growth rate was slight for the lots that survived from the beginning of the experiments (November 15), as fingerling mortality was less than 5% except for Lot 2 which failed to feed. In the experiment started May 1 the 74°F lot died in 15 weeks and the mortality was greater than 80% in 22 weeks for the lot at 67°F.* The growth rates for the 74° and 67°F lots are less reliable for this reason.

In conclusion, the optimum temperature for fingerling growth is between 55° and 60°F. The growth rates by temperature lots decrease in regular order on either side of the optimum.

Meristic Characters

The classification of fishes especially as to species depends to a great extent upon the count of meristic characters. Also, the use of meristic characters, particularly vertebrae, became a widely accepted method for defining races after Heincke's investigations in 1898 on the races of herring.

Even before Heincke's investigations the geographical differences in vertebrae number within species had been associated with temperature. Gabriel (1944) wrote as follows:

> Following the early generalizations of Günther (1862) and Gill (1863) that the number of vertebrae is higher in genera of fishes inhabiting northern latitudes than in related fishes from tropical regions, Jordan (1891) prepared a "law" setting forth an inverse relationship between the vertebrae number of a species and the water temperature prevailing in its geographic range.

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*The upper lethal temperature limit for chinook fingerlings as stated by Brett (1952) is between 24 and 24.5°C (75°-76°F).

From Jordan's "law" and Heincke's work the idea developed that racial differences could result from the effect of temperature upon meristic characters.

A great many racial studies confirm Jordan's "law." A few of these are Hubbs (1925), Rounsefell and Dahlgren (1932), Tester (1938) and McHugh (1954b) on the herring; Schmidt (1930) on the cod; Weisel (1955) on the cyprinids; and Mottley (1937) on the trout. However, racial studies have two shortcomings when used to demonstrate the effect of temperature upon meristic characters. One is that temperatures during development are estimated, not known; and secondly, the counts of the meristic characters of the parents are unknown.

Laboratory experiments on meristic characters of fish are few. Tåning (1952) reviewed these experiments, which include Schmidt (1917, 1919, 1920 and 1921), Mottley (1934 and 1937), Tåning (1944, 1946 and 1950), Gabriel (1944), Heuts (1947 and 1949) and Dannevig (1950). To this list Marckmann (1954) and Lindsey (1954) should be added. Conclusions from these experiments are that either low oxygen or high CO_2 pressure increases the number of vertebrae; pH in the range 6.4 to 7.8, egg size, fry size, or early or late hatching have no effect on vertebrae number; and salinity and temperature modify both vertebrae and fin ray number.

Modification of vertebrae number by temperature has not been consistent in the laboratory experiments. Schmidt (1921), Tåning (1950) and Lindsey (1954) have shown that the lowest number of vertebrae occurs at intermediate temperatures while the results of experiments by Gabriel (1944) and Dannevig (1950) show an increase in number of vertebrae with decreasing temperature.

In the present experiments counts were made of vertebrae, dorsal rays and anal rays to investigate the variability associated with temperature that occurs in the meristic characters of the chinook salmon.

Vertebrae. The number of vertebrae was determined by counting the centra between the basioccipital and the urostyle.* The vertebrae as seen in a radiograph are shown in Figure 22. When abnormal vertebrae were encountered, the number was determined by counting the arch elements, but when both the centra and arch elements were in doubt, no count was made. In the caudal area the centrum was counted as one if separation was not complete.

The vertebrae counts of lots from Experiments I and II are recorded in Table 19 and shown graphically in Figure 23. The u-shaped curve of Experiment I shows that the number of vertebrae increases at both high and low temperatures and is similar to the findings of Schmidt (1921) and Tåning (1952) for the sea trout and Lindsey (1954) for the paradise fish. The data for Experiment II are limited to the high temperatures but substantiate the Experiment I data for those temperatures.

For Experiment III the record of vertebrae counts is tabulated in Table 20 and is shown graphically in Figure 24. These data do not show the same increase in the number of vertebrae at high and low temperatures as was seen in the Experiment I data; on the other hand, there is no decrease in vertebrae number at high temperatures such as was found by Gabriel (1944) and Dannevig (1950).

The temperatures given in Table 20 and Figure 24 are the average values during the incubation period but more properly should represent

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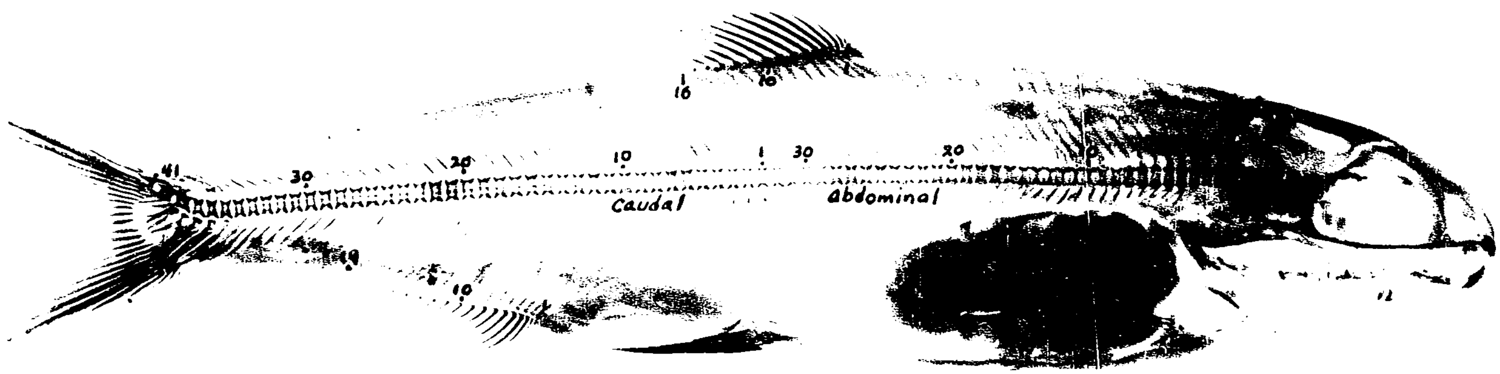
*Vladykov (1954) states: "Urostyle is the posterior terminal segment which follows the last undoubted centrum. In Salmonidae the urostyle remains non-ossified."



vertebrae, 73 (32 abdominal + 41 caudal*)

anal rays, 19

dorsal rays, 16

Figure 22. Radiograph of a Chinook Salmon Fingerling (x 3¼)

* The first caudal vertebra of a salmon is defined as the vertebra with a "sudden increase in length of the haemal spine." The first haemal spine is indeterminate as it is a minute process which gradually becomes longer on succeeding vertebrae (Clothier, 1950).

TABLE 19

Vertebral Counts of Green River Chinook Salmon Reared at Constant and City Water Temperatures

Exp.	Lot	Temp., °F	Number of Vertebrae 66	67	68	69	70	71	$\bar{x}$	n	$s_{\bar{x}}$
I	2	39.8			23	135	59	1	69.17	218	.0410
	3	44.7		1	109	84	2		68.44	196	.0377
	5	50.6		2	175	79			68.30	256	.0298
	6	55.1		1	59	44			68.41	104	.0504
	7	60.2		1	2	6	3	1	69.08	13	.288
	4	47.4*		7	196	75			68.24	278	.0291
	8	47.0*		12	249	103	1		68.25	365	.0268
II	3	54.6	7	90	72	6			67.44	175	.0476
	5	57.[illegible]	2	63	117	18			67.76	200	.0440
	4	58.5*	2	71	93	22			67.72	188	.0495

* City water

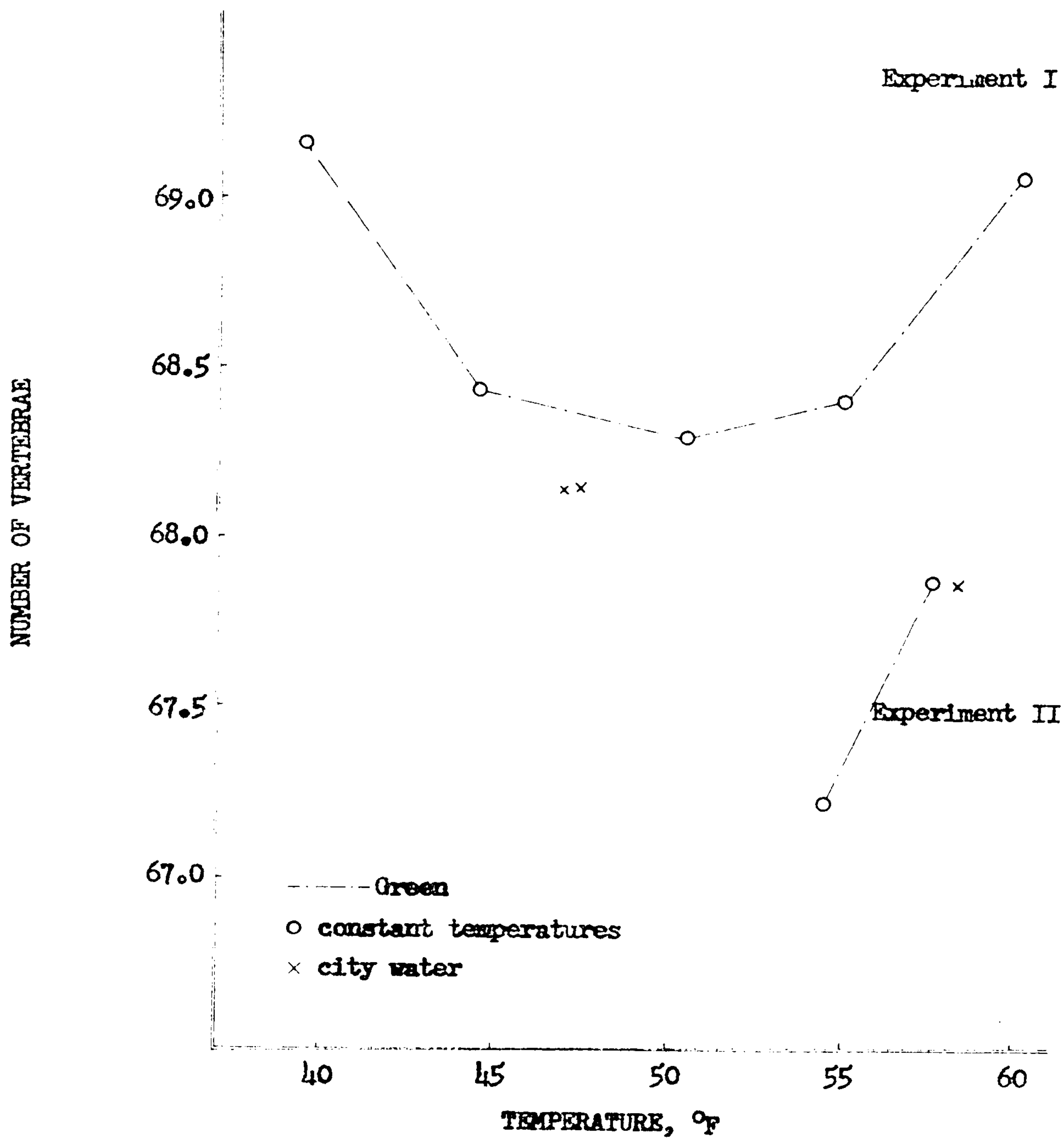


Figure 23. Average Number of Vertebrae and Temperature for Lots of Experiments I and II

TABLE 20

Vertebral Counts of Sacramento, Skagit, Green and Entiat River Chinook Salmon of Experiment III

Lot	Temp., °F	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	$\bar{x}$	n	$s_{\bar{x}}$
		Number of Vertebrae																		
Sa 1	39.0		5	16	44	18	1											65.93	84	.091
2	41.6	1	7	51	67	39	8	6	3									66.09	182	.089
4	56.5		1	23	117	98	13											66.39	252	.047
5	62.2			7	11	14	1	1										66.35	34	.163
6	55.5*		16	108	133	36	3											65.67	296	.046
Sk 4	55.9				1	25	104	81	13									68.36	224	.052
5	61.3				3	20	30	15	3									67.93	71	.109
6**	54.7*					5	6	18	33	24	15							70.09	101	.130
G 1	39.0						4	26	46	23								69.89	99	.081
2	41.6				2	1		5	9	4								69.43	21	.320
4	56.3				2			12	10	5	1							69.57	30	.233
6	55.4*			1	1	1	14	40	25	2								69.07	84	.104
E 1	38.8								10	39	29	4						71.33	82	.083
3	43.4								5	19	6							71.03	30	.355
4	55.2								3	149	247	29	3	1				71.73	432	.031
5	61.3								5	65	114	56	11	2	3	1		72.16	257	.064
6**	55.2								2	8	13	26	107	137	70	28	1	74.72	392	.066

* At constant temperature, 54-56 °F

** Water of low oxygen content during incubation

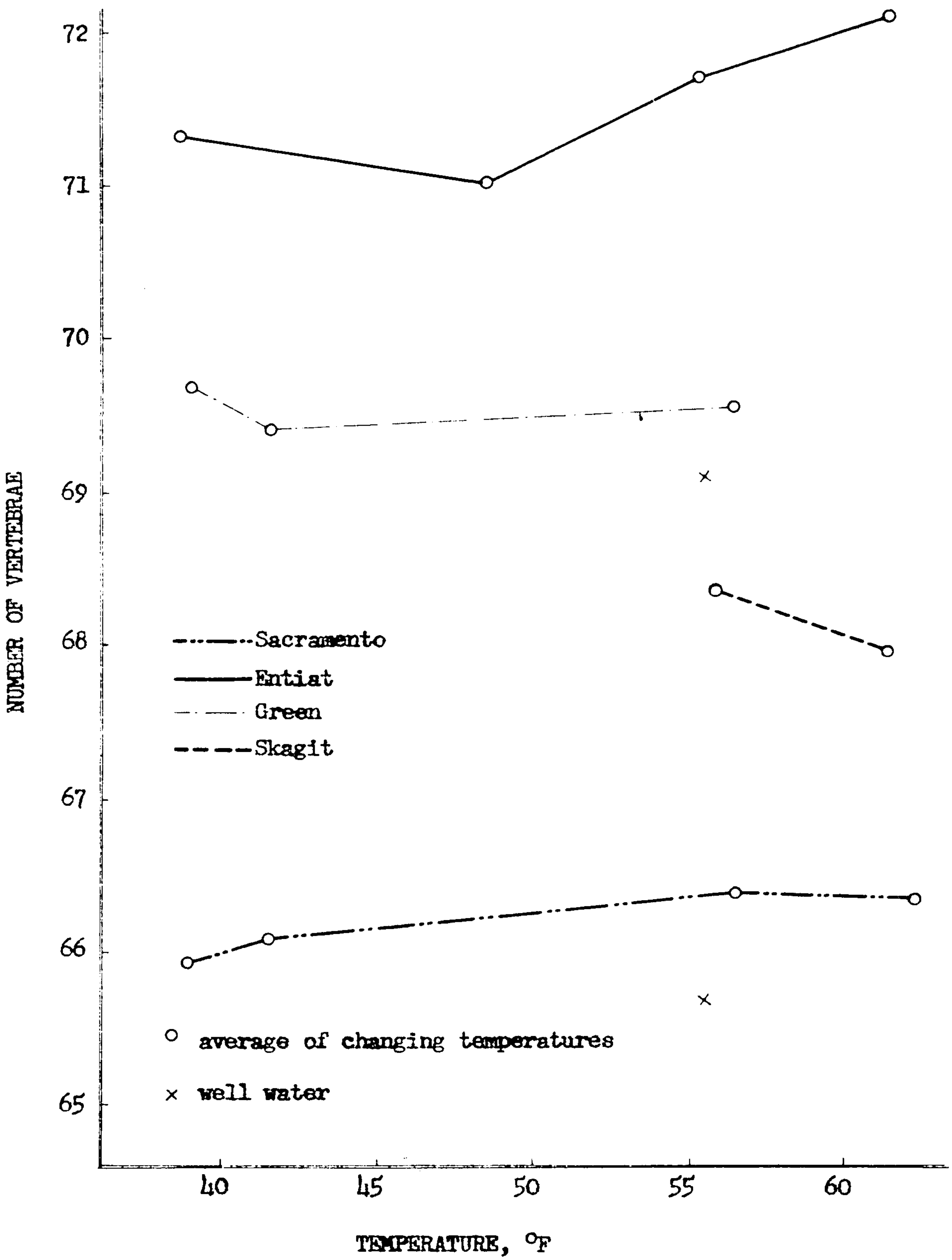


Figure 24. Average Number of Vertebrae and Temperatures for Lots of Experiment III

the temperatures at the time the number of vertebrae is determined. Tåning (1952) has shown that the plastic period for determination of the number of vertebrae in Salmo trutta trutta is from 145 to 165 D° (day degrees with temperature in degrees centigrade) for which the total incubation period is 400 D°. Using temperature values calculated on the basis that the plastic period for chinook salmon is the same as for Salmo trutta trutta, the curves expressing the relationship between temperature and the number of vertebrae in Experiment III were shifted to the right but changed only slightly in shape.

To provide information from which the plastic period for vertebrae formation in the chinook salmon could be established the eight sublots of Experiment I were transferred to water of either higher or lower temperatures at various times during embryological development. Fry from some of these lots survived to a size suitable for staining or radiographing but were too few to make accurate observations concerning the plastic period for vertebrae formation. However, in Experiment III the data from Lot E 6 suggest that the plastic period begins before the 21st day for eggs that hatch in 46 days. Lot E 6 was incubated in water of low oxygen content for the first 21 days, after which the oxygen level was normal. The number of vertebrae in this lot was greater by 2.6 than in any other Entiat lot at either higher or lower temperatures, and for this reason it is believed that the plastic period for the vertebrae of the chinook salmon of this lot began before the 21st day of incubation.

From the Experiment III data in Table 20 the great variability in number of vertebrae between stocks can be seen. The racial averages of the lots for all temperatures are about 66 for Sacramento, 68 for Skagit, 69 for Green and 72 for Entiat. By Ginsburg's (1938) definition the difference between the Sacramento and Entiat races is equivalent to a species difference since the overlap in the vertebral counts of the two races is less than ten per cent.

The number of vertebrae was generally greater than the 66 reported by Jordan and Evermann (1896) for the species. From the report by Foerster and Pritchard (1935) the average number of vertebrae for chinook salmon was calculated to be 69.10 ± 0.14, and from Townsend (1944) the average was 67.4. The range for Experiments I, II and III was 63 to 77 (for lots other than E 6).

There was a marked increase in the number of vertebrae in Lots Sk 6 and E 6 which were accidentally incubated in water of low oxygen content. These lots were not included in the average values for the Experiment III data because of the abnormal conditions. The average number of vertebrae for Sk 6 was 1.73 greater than for any other Skagit lots and for E 6 the increase was 2.62 over other Entiat lots. Since there were no other obvious differences between Lots E 6, Sk 6 and other lots, exposure to water of low oxygen content is assumed to have caused the increase in the number of vertebrae. There is substantiating evidence as to this conclusion from Tåning (1952), who found that low oxygen content during incubation increased the number of vertebrae. In the range from 58 to 98 per cent oxygen saturation the increase in vertebrae of the sea trout was about 0.1 for each 10 per cent decrease in oxygen saturation (see Fig. 7, *op. cit.*). The effect of higher or lower oxygen levels upon number of vertebrae is not known.

Under the conditions that existed in Experiment III genotypic variation in the number of vertebrae was greater than phenotypic variation.

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There was a marked increase in the number of vertebrae in Lots Sk 6 and E 6 which were accidentally incubated in water of low oxygen content. These lots were not included in the average values for the Experiment III data because of the abnormal conditions. The average number of vertebrae for Sk 6 was 1.73 greater than for any other Skagit lots and for E 6 the increase was 2.62 over other Entiat lots. Since there were no other obvious differences between Lots E 6, Sk 6 and other lots, exposure to water of low oxygen content is assumed to have caused the increase in the number of vertebrae. There is substantiating evidence as to this conclusion from Tåning (1952), who found that low oxygen content during incubation increased the number of vertebrae. In the range from 58 to 98 per cent oxygen saturation the increase in vertebrae of the sea trout was about 0.1 for each 10 per cent decrease in oxygen saturation (see Fig. 7, *op. cit.*). The effect of higher or lower oxygen levels upon number of vertebrae is not known.

Under the conditions that existed in Experiment III genotypic variation in the number of vertebrae was greater than phenotypic variation,

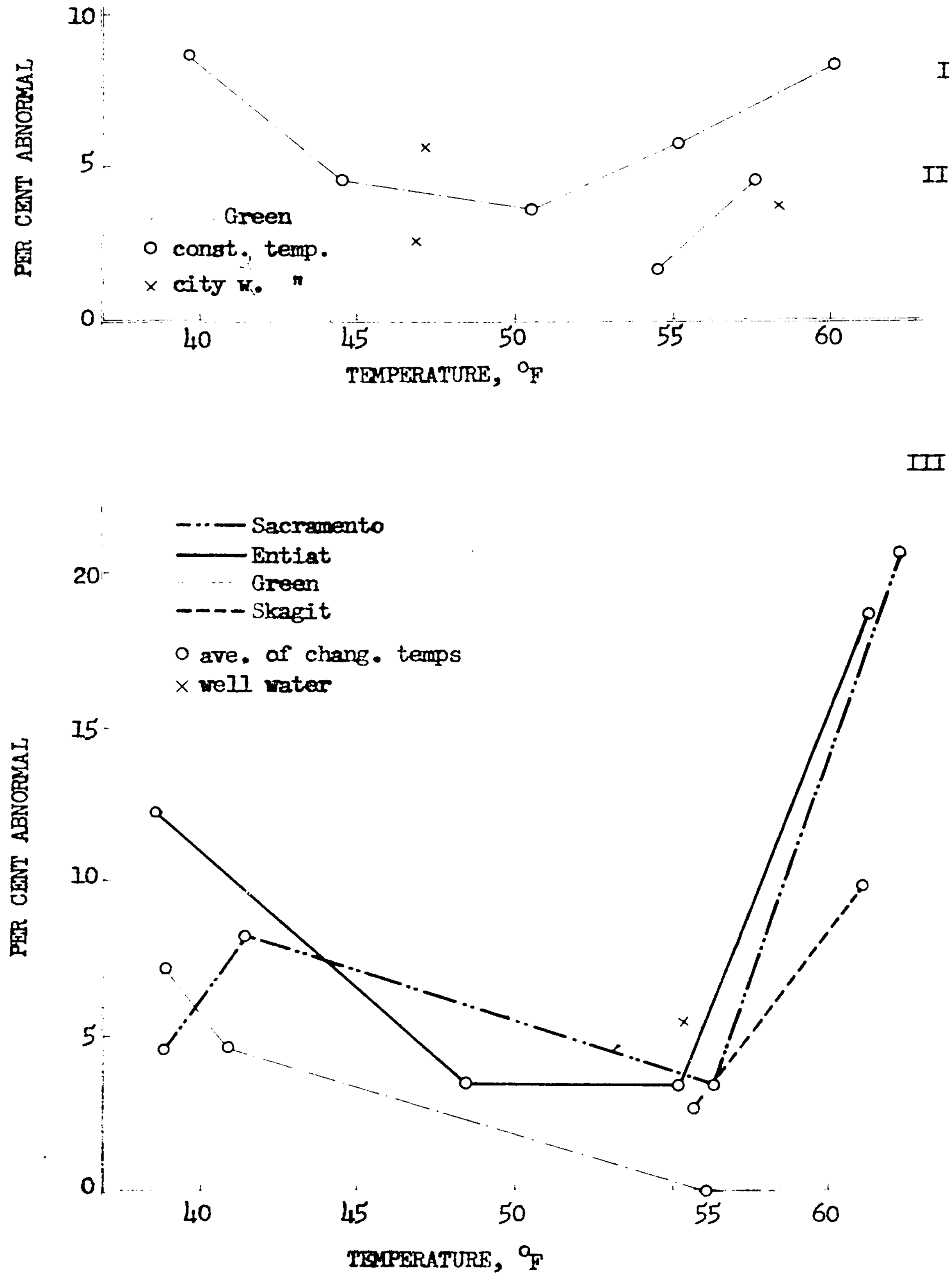


Figure 25. Temperature and Per Cent of Chinook Salmon with Abnormal Vertebrae for Lots of Experiment I, II and III

about one vertebra greater in the offspring than in the parents. If this difference is due to phenotypical variation caused by some factor in the environment, it is assumed that the effect is equal for all lots.

In conclusion, the lowest number of vertebrae are found at the intermediate temperatures in the range from 45° to 55°F. The average number of vertebrae is about 66 for Sacramento, 68 for Skagit, 69 for Green and 72 for Entiat. Above 60° and below 40°F the number of individuals with abnormal vertebrae increases. Low oxygen content of water during incubation increases the number of vertebrae.

Dorsal rays. In counting the rays of the dorsal fin all elements were included. Usually, in systematics, the small rays at the front of the fin that are less than one-half the length of the longest rays are not counted. When estimating temperature effects, there is no reason for not counting all elements. The base of all rays showed clearly in both the radiographs and the stained specimens, but sometimes the longest rays could not be measured.

The number of dorsal rays reported for these experiments is greater than the number reported in the literature. Jordan and Evermann (1896) list 11 dorsal rays for the species; Foerster and Pritchard (1935), 11 to 14; Clemens and Wilby (1946), 10 to 14. The observed values for Experiments I, II and III ranged from 13 to 18. For all three experiments the dorsal ray counts are recorded in Table 21 and are shown graphically in Figure 26. The curves are consistent for both the constant temperature and changing temperature experiments with the maximum number of rays in the 45° to 55°F temperature range. This is opposite to the effect of temperature upon the number of vertebrae. Tåning (1952) reported

TABLE 21

Numbers of Dorsal Rays for Chinook Salmon in Experiments I, II and III

Exp.	Lot	Temp., °F	13	14	15	16	17	18	$\bar{x}$	n	$s_{\bar{x}}$
I	G 2	39.8		10	91	32			15.17	133	.047
	3	44.7			34	147	10		15.87	191	.034
	5	50.6			27	191	33		16.02	251	.031
	6	55.1			9	41			15.82	50	.055
	7	60.2		3	10				14.77	13	.122
	4	47.4*			18	169	17		16.00	204	.029
	8	47.0			32	188	14		15.92	234	.029
II	G 3	54.6	2	11	65	14			14.99	92	.063
	5	57.8	5	56	16	1			14.17	78	.062
	4	58.5*	2	42	37				14.43	81	.061
III	Sa 1	39.0	1	17	17	4			14.62	39	.114
	2	41.6		1	5				14.83	6	.166
	4	56.5		12	140	57	2		15.23	211	.038
	5	62.2	2	8	11				14.43	21	.148
	6	55.5**		25	143	88	2		15.26	258	.040
	G 1	39.0		2	3	4			15.22	9	.278
	4	56.3			14	12	2		15.57	28	.120
	6#	55.4**		1	28	36	2		15.58	67	.071
	Sk 4	55.9			14	26	1	1	15.74	42	.097
	5	61.3			11	6	1		15.44	18	.145
	6#	54.7**			8	32	3		15.88	43	.076
	E 1	38.8			14	11	5		15.70	30	.137
	3	48.4			3	16	2		15.95	21	.109
	4	55.2		1	36	133	16		15.88	186	.039
	5	61.3		1	68	86	5		15.59	160	.044
	6#	55.2**			19	159	46	1	16.13	225	.036

* At city water temperature

**At well water temperature

\# Water of low oxygen content during incubation

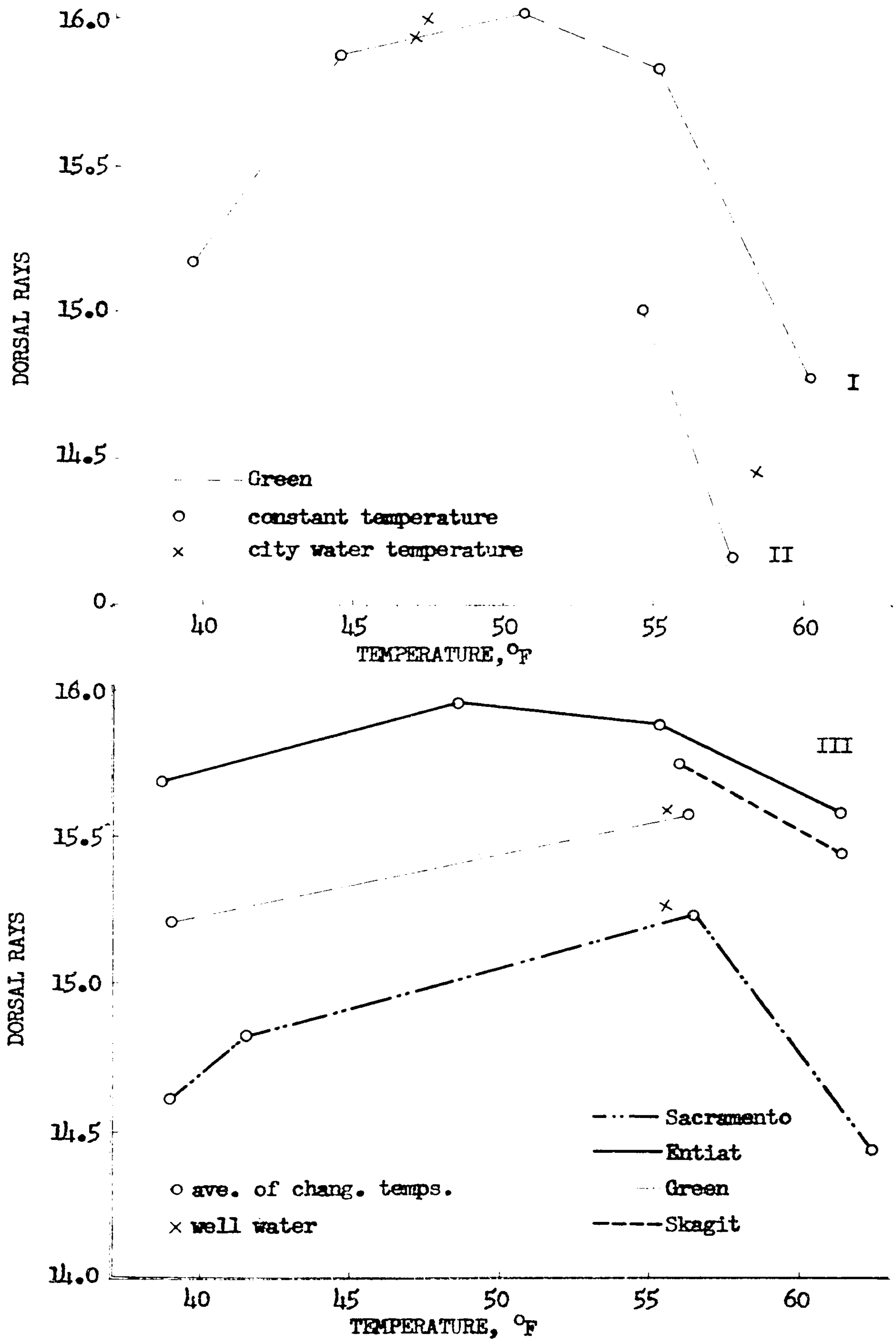


Figure 26. **Average Number of Dorsal Rays and Temperature for Lots of Experiments I, II and III**

a similar situation for the sea trout.

In conclusion, the maximum number of dorsal rays occurred in the temperature range of 45° to 55°F; this is opposite to the effect of temperature upon vertebrae.

Anal rays. In counting the rays of the anal fin all elements were included. The same argument for using all the elements of the dorsal fin also prevails for the anal fin and, likewise, the number of anal rays reported for these experiments is greater than reported in the literature.

Jordan and Evermann (1896) list 16 anal rays for the species. Other authors give the following number: Schultz (1931), 15 to 16; Foerster and Pritchard (1935), 16 to 18; Clemens and Wilby (1946), 15 to 19. The observed values for Experiments I, II and III ranged from 16 to 21 (for lots other than E 6). The counts of the anal rays are tabulated in Table 22 and shown graphically in Figure 27. Maximum values are in the range of 45° to 55°F with lower values on either side of this range. For the sea trout Tåning (1952) also found that the number of anal rays was greatest at intermediate temperatures.

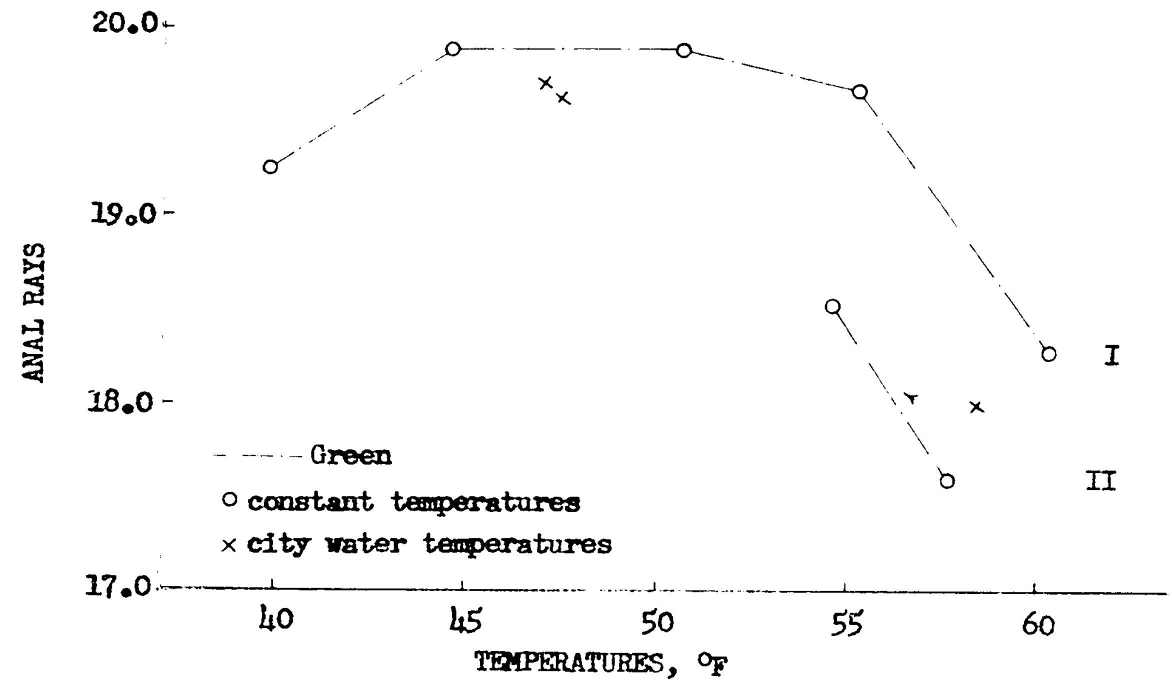
In conclusion, as with the dorsal rays the maximum number of anal rays occurred in the temperature range of 45° to 55°F.

TABLE 22

Numbers of Anal Rays for Chinook Salmon of Experiments I, II and III

Exp.	Lot	Temp. °F	16	17	18	19	20	21	22	23	$\bar{x}$	n	$s_{\bar{x}}$
I	G 2	39.8			15	62	40	2			19.24	119	.063
	3	44.7				45	124	20			19.87	189	.042
	5	50.6			1	51	164	21			19.86	237	.036
	6	55.1				16	27	1			19.66	44	.079
	7	60.2		1	6	4					18.27	11	.195
	4	47.4*			2	85	118	6			19.61	211	.039
	8	47.0			1	63	117	8			19.70	189	.040
II	G 3	54.6		2	18	24					18.50	44	.089
	5	57.8	1	38	43	5					17.60	87	.066
	4	58.5*		5	33	4					17.98	42	.072
III	Sa 1	39.0	1	11	15	6					17.79	33	.136
	4	56.5		9	123	93	11	1			18.46	237	.043
	5	62.2		17	11	1					17.45	29	.106
	6	55.5**		21	167	101	3				18.29	292	.036
	G 1	39.0			7	6					18.46	13	.144
	4	56.3			5	15	9				19.14	29	.129
	6	55.4**		1	23	49	10				18.82	83	.071
	Sk 4	55.9			11	46	8				18.95	65	.067
	5	61.3			8	12					18.60	20	.112
	6#	54.7**			9	54	11	1			19.05	75	.066
	E 1	38.8		1	15	20	2				18.61	38	.102
	3	48.4			3	13	7	1			19.25	24	.150
	4	55.2		1	20	149	82				19.24	252	.038
	5	61.3		15	89	86	25	6			18.63	221	.059
	6#	55.2**			9	19	78	185	69	5	20.82	365	.048

* At city water temperature
**At well water temperature
\# Water of low oxygen content during incubation



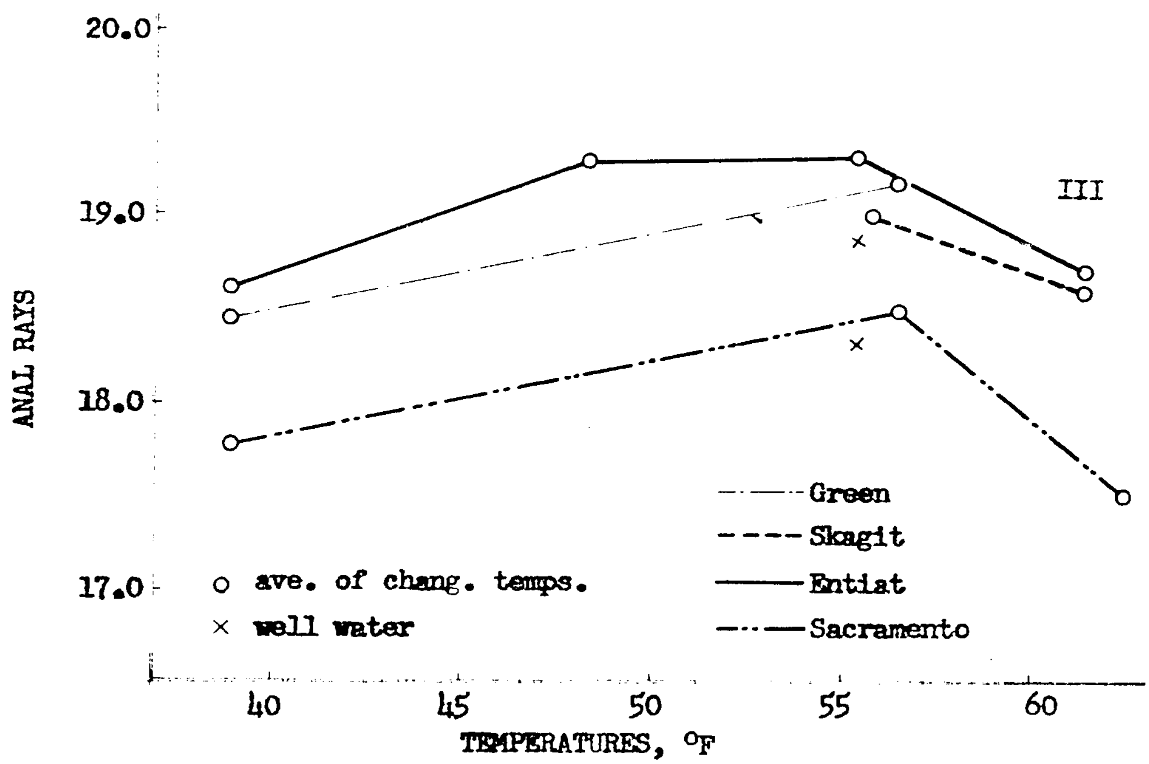


Figure 27. Average Number of Anal Rays and Temperatures for Lots of Experiments I, II and III

VII. SUMMARY

The observations from three experiments upon the effects of temperature on young chinook salmon are as follows:

Rate of development

(1) The temperature coefficients u, Q_{10} and x are not constant for the relationship of temperature to the number of days to hatching. The values for the coefficients are considerably greater at low than at high temperatures with a critical temperature about 47°F.

(2) For lots reared at constant temperatures in the range from 39.8° to 57.8°F, the temperature summation rule, the Belehradek equation and the logistic curve fit equally well to the relationship of temperature to the number of days to hatching.

For the temperature summation rule, $y(x-a) = k$, where $\underline{y}$ = the number of days to the time when 50 per cent of the eggs are hatched at an incubation temperature of $\underline{x}$, $\underline{a}$ is the threshold temperature and $\underline{k}$ is the temperature summation constant, new methods for estimating $\underline{a}$ and $\underline{k}$ and a confidence interval for $\underline{a}$ are given. The value for $\underline{k}$ is shown to be equal to the reciprocal of the regression of the speed of development on temperature; one estimate of $\underline{a}$ is $\frac{x_1y_1 - x_2y_2}{y_1 - y_2}$; a second estimate of $\underline{a}$ is $\bar{x} - k(\frac{1}{y})$; the confidence interval for the second estimate of $\underline{a}$ is also given. For the four races of chinook salmon the values for $\underline{a}$ range from 31.1° to 32.7°F; for $\underline{k}$, from 815 to 1020.

The $\underline{b}$ value of Bělehrádek's equation is 0.97 for the data corrected for a threshold temperature of 33.8°F and 1.12 for the uncorrected data, that is the threshold temperature is assumed to be 32°F or 0°C.

Following is the equation of the logistic curve that best fits the temperature-development relationship for lots reared at constant temperatures:

$$y = \frac{1 + e^{2.46-0.242x}}{.0396}$$

when the incubation temperature, x, is in degrees centigrade.

(3) The chinook salmon eggs from the Sacramento River develop 8 per cent faster than those from the Entiat River. The rate of development of the eggs from the Skagit and the Green Rivers is intermediate.

(4) Using data from all lots regardless of race, mortality rate, temperature pattern, or year the equation that best fits the relationship of the number of days to hatching, y, and the incubation temperature, x, in degrees centigrade is the logistic curve of the form

$$y = \frac{1 + e^{2.306-.2022x}}{.04404}$$

for which the standard error of estimate is 3.14.

(5) Water of low oxygen content during incubation increases the number of days to hatching about 18 per cent at average water temperatures of 55°F.

(6) The shortest hatching period occurs in lots reared in the temperature range 40° to 58°F for which the 5-95 percentile range is less than five days.

(7) Short hatching periods are associated with high survivals.

Mortality

(1) For the lots reared at 34°F or 65°F and higher none of the eggs survived to the hatching stage.

(2) One hundred per cent mortality occurs during the yolk-sac stage in lots reared at 60° and 62½°F.

(3) At constant temperatures of 55° and 57½°F the lots hatch successfully but during the yolk-sac stage, mortality increases to 50 per cent or greater.

(4) The mortality rate is low at all stages of development for lots reared at temperatures between 40° and 55°F.

Abnormal fry

In the temperature range 40° to 55°F the number of abnormal fry averages 4.6 per cent per lot and at 60°F and higher there is a nine-fold or greater increase.

Growth

(1) At hatching the fry reared at 40°F are larger than those reared at higher temperatures.

(2) The growth rate for lots reared at constant temperature is greatest at 55°F and decreases in relation to the distance from the optimum for lots at other temperatures.

(3) For lots reared at city water temperatures, the fish are smaller at the 20th week of the experiment than the fish reared at a constant temperature of 55°F, but are of the same size by the 46th week. Most rapid growth occurs when the temperature is near 60°F.

Meristic characters

(1) For lots reared at constant temperatures the average number of vertebrae is fewer in the temperature range from 45° to 55°F than at either higher or lower temperatures.

(2) For the Sacramento, Skagit, Green and Entiat races the number of vertebrae average 66, 68, 69 and 72 and range from 63 to 78.

(3) For lots reared at temperatures above 60°F and below 40°F the number of individuals with abnormal vertebrae increase.

(4) Water of low oxygen content during the incubation period increases the average number of vertebrae per lot as much as 2.4.

(5) The average number of both dorsal and anal rays is greater for the lots reared in the temperature range 45° to 55°F than for lots reared at either higher or lower temperatures. This is the opposite of the effect of temperature upon the number of vertebrae.

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