Temperature Tolerance in Young Pacific Salmon, Genus Oncorhynchus[1]

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CONTENTS

ABSTRACT

Lethal limits of high and low temperatures were determined for the young of five species of Pacific salmon, the spring (*Oncorhynchus tshawytscha*), the pink (*O. gorbuscha*), the sockeye (*O. nerka*), the chum (*O. keta*) and the coho (*O. kisutch*).

For acclimation temperatures ranging from 5° to 24°C. significant differences between species in their resistance to high temperatures was obtained. The spring and coho were most resistant. The pink and chum salmon were least resistant, and the sockeye was distinguishable from the latter two by greater resistance for prolonged exposure to high temperatures. No species could tolerate temperatures exceeding 25.1°C. when exposed for one week.

A fanning-out of the opercula was shown to be directly correlated with the onset of death from a low temperature. By use of this criterion mixed lethal effects at low temperatures were demonstrated and found to be influenced by the size of the fish and by the salinity of the water. None of the species could withstand temperatures lower than 4°C. when acclimated to 20°C. and above. When taken from holding troughs as low as 5°C., coho and sockeye could not tolerate long exposure (four days) to 0°C.

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In a vertical gradient little difference in preferred temperature was observed, either betw species or for different acclimation temperatures. The 12° to 14°C. stratum was the region greatest concentration.

Specific differences in temperature responses are in keeping with taxonomic and ecologi distinctions.

INTRODUCTION

NO ACTIVITY of an animal escapes the effect of temperature. Distribution, develo ment, propagation and mere existence, each is influenced strongly in som manner by temperature. This influence must be met and surmounted eith through resistance or adaptation, external avoidance or internal control. It known that the upper and lower limits of temperature-tolerance in fish a extended through both adaptation and resistance (Fry, 1947a), and the varyin degrees of these two attributes separately and collectively distinguish the speci in this respect. By conducting experiments on tolerance to high and to lo temperatures among the young of the five North American species of Pacif salmon, the relative abilities of these species to cope with extremes of temperatur have been described in the following analysis.

Among earlier experiments, interest in the ability of fish to survive temper tures in the region of the freezing point of water and slightly below was express by Regnard (1895). In 1899, Maurel and Lagriffe while investigating both uppe and lower levels of temperature-tolerance, chiefly in fresh-water fish, conclude that these species were better adapted for resisting low than high temperatures Later investigators, dealing mainly with mortality from high temperatures, expressed the resistance in terms of the temperature reached before death when heated at a constant rate (Huntsman and Sparks, 1924), or averaged either the times to death (Loeb and Wasteneys, 1912) or the number of fish dead following a given exposure (Hathaway, 1927) at various constant temperatures. This quantitative expression of temperature-tolerance has been developed to provide a more inclusive treatment, borrowing from the methods of pharmacological procedure concerning dosage-mortality (Fry *et al.*, 1946).

The phenomenon of thermal adaptation in relation to previous temperature history provides the organism with greater scope for environmental experience. The term "acclimation" has been used to describe this effect, although "acclima tization" is apparently synonomous (Doudoroff, 1942; Heilbrunn, 1943; Brett, 1944). The importance of temperature-acclimation in nature and in experimental work has been stressed with significant emphasis (Doudoroff, 1945; Fry, 1947a). By working systematically with different temperature-acclimations the various levels of both upper and lower thermal tolerance can be determined within suffi cient statistical limits to permit accurate prediction. The development of precise methods of physiological measurement has set the stage for physiological description.

The close taxonomic relation of the Pacific salmons, genus *Oncorhynchus* (Milne, 1948) coupled with fairly distinct ecological habits, provides interest in the affinities which might be revealed by a rigorous analysis of their temperature tolerances. Similar work on other salmonoids is mounting (Fry *et al.*, 1946,

Fry, 1947b; Graham, 1949). With the gradual recording of such physiological attributes on a comparable basis within and between taxonomic groups of fishes, the role of temperature in the ecology, and possibly in the evolution, of these animals will become increasingly apparent.

ACKNOWLEDGMENTS

I am greatly indebted to Dr. F. E. J. Fry who has for a number of years both directed and stimulated research on temperature relations in fish. His wide variety of interests and knowledge in the problems of experimental biology has been the source of a wealth of suggestions and a constant inspiration.

The mathematical analysis of the data has been under the helpful guidance of Dr. D. B. DeLury of the Ontario Research Foundation.

The experiments were conducted in the Laboratory for Experimental Limnology which is operated jointly by the University of Toronto and the Department of Lands and Forests, located at the Southern Research Station of the Department of Lands and Forests, Maple, Ontario. The excellent facilities of the laboratory and the attention devoted to feeding and maintaining the stocks of fish by Messrs. D. Cucin, G. Stolfa and W. Sanderson contributed in a large measure to the successful execution of the research.

The salmon eggs were obtained through the courtesy of Mr. F. Neave, Pacific Biological Station, British Columbia, and of Mr. C. H. Ellis, Department of Fisheries, Washington. They have each responded to urgent pleas for additional shipments when transportation problems resulted in minor catastrophies.

The Fisheries Research Board of Canada has not only seen fit to grant extended leave of absence to the author, but has also given him financial assistance. It is a pleasure to acknowledge this support.

MATERIALS

Source

The five species of Pacific salmon common to the west coast of North America were obtained as eyed eggs from three hatcheries (Table I).

Each lot of approximately two thousand eggs was shipped in a fiberglass-insulated box, packed with ice and perforated to permit air exchange for the eggs. Successful shipment by air was possible if not more than thirty-six to forty-eight

TABLE I. The hatchery locations and dates of fertilizing, shipping and 50 per cent hatch for the five species of Pacific salmon.

Species	Hatchery	Fertilized	Shipped	50% Hatch
1. Spring	Dungeness, Wash.	30/8/49	20/10/49	9/11/49
2. Pink	" "	28/9/49	7/12/49	12/12/49
3. Sockeye	Issaquah, "	10/10/49	25/11/49	6/1/50
4. Chum	Nile Creek, B.C.	28/10/49	7/1/50	4/2/50
5. Coho	" " "	14/11/49	7/1/50	27/1/50

hours elapsed between packing and receiving. An initial mortality not exceeding 2 per cent inevitably followed the handling necessitated by shipment. With this exception, egg losses from other causes were exceptionally low in all cases where subsequent experiments were performed.

Feeding and Care of Young

The transition stage from alevin to free-swimming, feeding fry is a precarious one for young salmonids. The habit of feeding must be developed and encouraged, usually by frequent presentation of small particles of food. By directing a jet of water into a small aluminum screened basket containing finely ground beef or hog liver, adequate dispersal of the food over periods of 15 to 20 minutes, four times daily, was achieved. This routine was maintained for the first month of feeding, followed by reduction in feeding frequency and a change of diet, mainly in accordance with fish-cultural procedures for salmon currently practised in Washington State (Burrows, no date). The diet selected was a slight modification of one reported by Ellis (1948) which had been found to give best growth and least mortality for young spring salmon when tested on a variety of diets. A mixture of 50 per cent beef or hog liver together with 48 per cent ground "fish-pack" (haddock and cod fillet waste) and 2 per cent yeast was provided, up to the second month, followed by a reduction in liver to 30 per cent for the balance of the experimental period.

Mortality in the stock tanks with a constant temperature of 8.8° ± 0.2° was virtually negligible. No prophylactic treatments were introduced. At higher temperatures, 20°C. and above, up to 5 per cent mortality was observed in all species, and infrequent treatments (two to three times per month) with a 1 : 4,000 solution of Roccal were applied (Burrows). Two cases where disease became significant were encountered; one with five-month-old chum salmon, from a single tank, which necessitated discarding the remaining fish as well as one series of obviously discordant data; the other, with three-month-old sockeye, raised by stages to 24°C. and apparently incapable of deriving adequate nutrition from their diet at such an elevated temperature.

The pH of the well water supplying the laboratory was 7.3 with total solids amounting to 254.3 parts per million (Table II).

Retaining Troughs

The retaining troughs were each supplied with running water tapped from hot- and cold-water sources of relatively constant temperature and pressure. Adjustment of these with regular inspection permitted setting the temperature of a trough (above 9°C.) to within ± 0.1°C. of any desired temperature. Usually two, sometimes three, species were cultured in a divided trough.

Below 9°C. a refrigeration unit reduced the temperature in a single holding tank in which the five species were retained separately in cages of fine aluminum wire screening. Thus, different levels of temperature-acclimation were readily obtained with a high degree of accuracy and constancy.

TABLE II. Mineral constituents of the water supply used in these experiments, as reported by the Chief Provincial Analyst, Sept. 24, 1947.

	parts per million
Total solids	254.3
Alkalinity	202.0*
Total hardness	181.0*
Iron and Aluminum (oxides)	4.7
Iron	0.2
Calcium	89.5
Magnesium	14.1
Potassium and Sodium	36.8
Sulphates	45.1
Chlorides	13.0

*As carbonate of lime.

SIZE AND AGE OF EXPERIMENTAL FISH

Uniformity of conditions in every feature of the history of the young fish was maintained as far as possible. Keeping them in the same or similar troughs, at the same temperature, and presenting the same diet in sufficient quantity to ensure abundance, were the first precautions. The variations in response to subsequent high or low temperatures might then be considered as attributable to specific differences only.

The time for commencing experiments was set at three months after hatching (about two months after feeding commenced), and then continued for an additional two to three months. The fish from higher temperatures were used first. The knowledge that the young chum and pink salmon move to salt water early in their first year motivated making comparison of the species in the very young stages.

TABLE III. Mean fork-lengths, weights and ages of the salmon fry used in temperature-tolerance tests.

Species	Length (*cm.*)	Weight (*g.*)	Age (*months*)
	Upper temperature tolerance		
Spring	4.44 ± 0.40	1.03 ± 0.27	3.6
Pink	3.81 ± 0.29	0.30 ± 0.15	3.8
Sockeye	4.49 ± 0.84	0.87 ± 0.45	4.7
Chum	5.44 ± 0.89	1.62 ± 1.03	4.9
Coho	4.78 ± 0.60	1.37 ± 0.62	5.2
	Lower temperature tolerance		
Spring	4.72 ± 0.41		7.7
Sockeye	4.50 ± 0.53		5.8
Chum	5.09 ± 0.51		5.2
Coho	4.83 ± 0.45		5.4

In Table III mean fork-lengths and weights (with standard deviations) a the average age from hatching of all samples used in the temperature-toleran tests are compiled. No weights are included for the fish used in low-temperatu tests. These samples were not removed from the lethal tanks until some ti after death, and water absorption had affected their weight.

Lethal Baths

The lethal baths, six in number and measuring 22 inches square by 11 inch deep (Figure 1) were each constructed of galvanized iron coated inside wi aluminum paint and adapted for use in either upper or lower thermal-toleran tests. The addition of complete insulation with fiberglass of one-inch thickne was of value in reducing temperature variation to a minimum. Thermostatical controlled, 120-watt heater-coils in pyrex tubing counterbalanced a steady lo of heat, mainly from aeration and from a very slow exchange of water, equal the volume of the tank every twenty-four hours. In the low-temperature letha the heat loss was augmented by the addition of a layer of crushed ice, partition off on three sides of the tank by a removable galvanized iron sheet. A standardiz

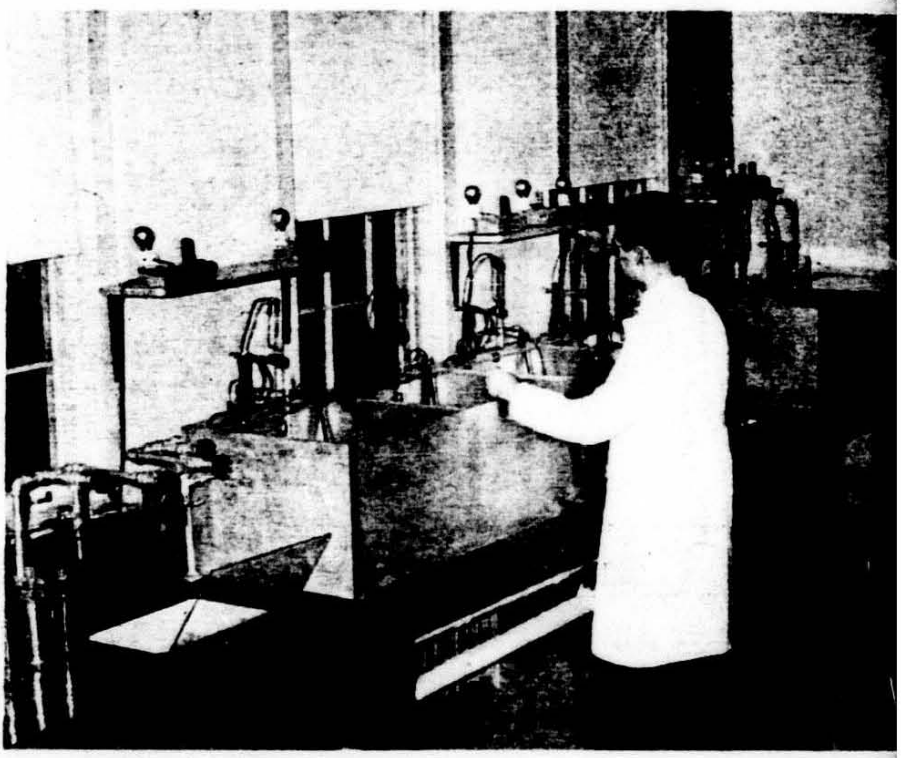

Figure 1. Apparatus for determining upper and lower lethal temperatures being assembl Two units, one with four, the other with two lethal baths are shown with connections for therm control, aeration and water exchange. (Photograph by Mr. W. P. Rice.)

thermometer calibrated in intervals of 0.1°C. was used throughout all experiments. Often no detectable change in the tank temperatures was apparent and, in general, variation did not exceed a range of more than 0.1°C. from any set level.

Under maximum loads of fish per tank (40) oxygen concentrations were never reduced below 93 per cent saturation (5.24 cc. O_2/1., 26.5°C.) in tests on high lethals, and not below 81 per cent (6.91 cc. O_2/1., 6.0°C.) in low-lethal experiments.

Preferred-Temperature Tanks

Two preferred-temperature tanks were used in a limited series of experiments concerning the region most frequented in a vertical temperature gradient by variously acclimated salmon fry. These tanks, illustrated in Figure 2, stand 36 inches high, with length and width measurements of 36 and 20 inches respectively. Plate glass facings held in angle iron edging and bolted to the main frame complete the outer structure. Water, usually of low temperature, is introduced at the bottom through a metal tube perforated uniformly over its length with small holes, the displaced water being drained off at the top. A coiled copper tube

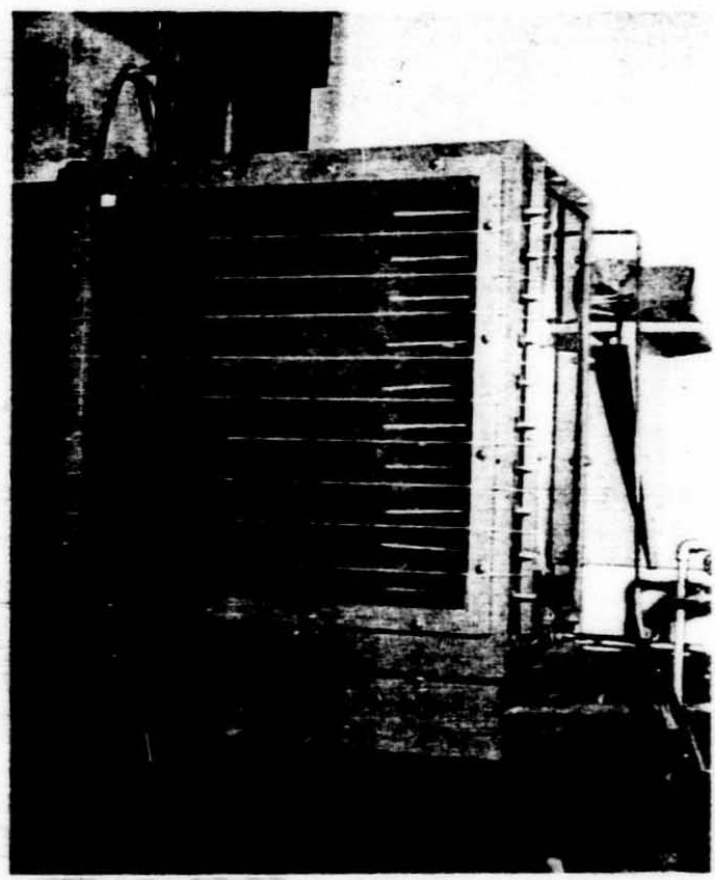

Figure 2. A preferendum tank divided into ten cells by white cord for recording of fish positions under low illumination. Thermometers are situated in the front right of each cell. A faint outline of the coiled copper tube for carrying hot water is visible within the tank.

forming a closed system conducts hot water in a downward spiral around the inside periphery of the tank in contradirection to the rising "cold front". By adjusting the temperature and rate of flow of the introduced cold water as well as that of the hot water within the copper coil, any desired temperature gradient can be obtained.

METHODS

The approach to the problem of describing the temperature-tolerance of young Pacific salmon has been to hold samples of each species at different non-lethal temperatures but otherwise similar conditions. These variously acclimated samples were later tested for their tolerance to high and to low temperatures, ranging from rapidly lethal to sublethal levels. The data were treated graphically or mathematically to distinguish such differences as might occur.

The application of these methods are considered below.

ACCLIMATION

As early as 1895 Davenport and Castle reported on the "acclimatization of organisms to high temperature", and threads of this principle have been variously woven around the theme of temperature relations in fish by Loeb and Wasteneys (1912), Hathaway (1927), Binet and Morin(1934), Sumner and Doudoroff (1938), and more generally by Weigmann (1929, 1930, 1936), Ogle and Mills (1933), Heilbrunn (1943), and others. Yet a great deal of experimental work has been done without adequate regard for the conditioning effects of temperature in the past-history of animals. A study of changes in heat-tolerance for the goldfish, *Carassius auratus*, from both low (4°C.) and moderately high (20°C.) temperature-acclimations (Brett, 1946) led to the general conclusion that rate of acclimation was related to metabolic rate. Thus, at low temperatures, acclimation proceeded at a slow rate but increased to a very rapid rate at high temperatures, probably in geometric progression. Conversely the loss of acclimation to any level of temperature was a comparatively slow process under most conditions.

The thermal history for the egg, alevin and early fry stage for each of the salmon species was 8.8°C. with very little variation. When the fry were about two months old the process of moving them through a series of temperature-acclimations was initiated. The *minimum* standards set for acclimation from the holding-trough temperature of 8.8°C. to any one of the following temperatures were:

To 5°C.	4 weeks at 5°C.
To 10°C.	3 weeks at 10°C.
To 15°C.	3 weeks at 15°C.
To 20°C.	1 week at 15°C., 2 weeks at 20°C.
To 23°C.(or 24°C.)	1 week at 15°C., 1 week at 20°C., 1 week at 23°C.

Repetition of some of the upper lethal-temperature experiments after one and even five months' lapse of time without further change in acclimation gave no significant change in heat-tolerance.

The first species to be investigated was the spring salmon. Preliminary tests indicated that a maximum acclimation temperature without significant loss in the holding troughs was close to 24°C. This was later substantiated by successfully culturing over 200 young spring salmon at 24°C., at which temperature they proved to be very active and to be good feeders, but had reduced growth rate when compared with groups from lower temperatures, particularly those from 15°C. A level of 24°C. was introduced, therefore, as the highest standard acclimation temperature for all species. By chance one of the most hardy of the five species had been used to set the standard for the others. The pink salmon, next in line by age for progressive acclimation to 24°C., fed poorly. Immediate lethal-temperature experiments following minimum acclimation standards for 24°C. showed a breaking away from the usual temperature-time mortality curve after prolonged exposure to a temperature of 24.5°C. Extrapolation of this divergent trend indicated that 24°C. bordered on 50 per cent lethality for prolonged exposure. Evidence of the unsatisfactory nature of such a high acclimation temperature was convincing in the sockeye fry. The latter species, while apparently more resistant than the pink salmon, showed a complete aversion to feeding in about 40 per cent of cases which later appeared as typical "pin heads", often obtained in hatcheries when young fish do not develop the feeding habit (although at an equable temperature). The growth of the remainder was curtailed almost completely and their activity was quite apparently reduced. Consequently, the acclimation temperature was lowered to 23°C. for the sockeye, the chum and the coho. Insufficient numbers of spring and pink salmon remained to provide comparison at the new acclimation level.

Upper Lethal Temperature

The method of lethal-temperature determination as conducted by Fry and associates has remained basically consistent from its inception (Brett, 1941), with the marked exception of the duration of exposure to given temperatures which produce some but not complete mortality. The analysis and interpretation of the data have changed and expanded considerably (see Fry *et al.* 1942; Brett, 1944; Doudoroff, 1945; Fry *et al.* 1946; Fry, 1947a; Hart, 1947, 1949). Without tracing the history of these changes, a discussion of the present treatment of temperature data and the current terminology used to describe the observations is necessary.

Each lethal bath is regulated to a constant temperature almost exactly 0.5°C. different from the temperature of an adjacent bath and appropriately chosen by preliminary tests to span the conditions from rapid to slow, partial, or non-lethal temperature effects. Records of the times to death for all fish are kept by close inspection. These latter have been called the *resistance times* which, if plotted graphically on normal axes in order of occurrence, take on the appearance of an asymmetrical S-curve. A series of such curves can be plotted, including the various temperatures investigated, as has been done for a sample of spring salmon fry from 10°C. acclimation in Figure 3*a*. It has been demonstrated (Bliss, 1935a, 1937) that many dosage-mortality curves can be resolved into straight-line

relations if the proper derivative of time is applied and the variation of response is normally distributed within the sample. By converting the axes into probability units as one variable (order of death) and logarithm of time as the other variable (time to death) a linear progression of points is frequently obtained (Figure 3*b*). The application of this principle to lethal-temperature experiments with fish was shown to be quite appropriate by Fry *et al.* (1946). The hundreds of observations which have since been made on many species of fish (Hart, 1947; 1949; present paper) and found to adhere closely to the above interpretation have added convincingly to the validity of the relation. The normality of the distribution permits application of standard statistical treatments. In addition the mean, median and mode, all coincide in a normal distribution, so the descriptive value of the single figure (50 per cent point) is evident.

From each lethal-temperature experiment a series of median resistance times may be plotted for the corresponding levels of temperature, in the manner of Figure 4*a*. It is apparent that for every state of acclimation the possibility of a series of such points exists. Thus, an overall picture of the effect of temperature can be constructed. Conveniently enough these curves, in the case of high-temperature tolerance, can be resolved into straight lines by using the logarithm of time against temperature (Figure 4*b*). A distinct break in the semilogarithmic plot, not otherwise evident, occurs at a progressively earlier point of time in the lines for lower levels of acclimation (usually below 20° to 15°C. for Pacific salmon). The discovery of this break (Fry *et al.*, 1946) and its variable occurrence with acclimation was most significant and has constituted the main difference in experimental procedure from that of earlier investigations. The definition of *lethal temperature* has been that temperature at which 50 per cent of the population is dead after indefinite exposure. The stumbling block in the past has been the duration of the experimental test. Doudoroff (1945) questioned the 14-hour period used by Fry *et al.* (1942) and shortened to 12 hours by Brett (1944). The answer, as indicated above, was provided when breaks in the resistance time-temperature relations showed that mortality from temperature as a primary cause had ceased. The duration, even as long as the seven-day period used for Pacific salmon should be governed by this factor since it varies for different species and different acclimations. As long as the resistance times continue to be finite the fish are considered to be in a *zone of resistance*. Beyond this lies the *zone of tolerance* (Fry, 1947a).

At one acclimation there are any number of resistance times but only one lethal temperature. To distinguish indices derived from high- and low-temperature experiments the terms *upper* and *lower* lethal temperatures are applied respectively.

Lower Lethal Temperature

Temperatures distributed from 0°C. (0.1° ± 0.1°C.) to 7°C. at one degree intervals were used in lower lethal-temperature determinations. One or two instances of experiments at fractional degrees are reported, but the use of 20 fish per tank from a limited total sample precluded carrying the investigation to a finer point.

There seems to be relatively little trouble in deciding when a fish has died from high temperature, except in a few instances (Brett, 1944; Hart, 1949). A cessation of respiratory movements and muscular contraction accompanied by complete loss of response to stimuli have been regarded as quite decisive criteria. Often no reference concerning such end points is included in reports on upper lethal-temperature experiments. A check on 180 Pacific salmon, including all species, by removing the fish to a lower temperature immediately after "death" was recorded, resulted in no recoveries. On the other hand the depressing effects of low temperatures, producing a type of "suspended animation", have been the source of considerable trouble in establishing satisfactory criteria of death from this cause. Usually groups of fish at a single low temperature have been removed from a large sample and tested for mortality by immersion in water of a more equable temperature, the recoveries being noted over the first 24 hours. This has been performed at intervals throughout the experiment, or, when only a small sample of fish was available, at the end of a given exposure time (Fry *et al.*, 1942; Brett, 1944). The lack of a more direct criterion of death has restricted operations in this field.

Close inspection of Pacific salmon revealed that with the approach of cold-death the characteristically immobile and closely compressed opercula of the chilled fish commence to fan out perceptibly. By systematically recording this symptom before removal of each sample to a testing tank (at 12°C.) for recoveries, a comparison of the "predicted" and "actual" mortalities was made. A highly significant correlation between the two was obtained (coefficient of correlation $r = +.90$, $P_{.01} = .37$), the cases of non-agreement being scattered almost entirely on the side of greater "actual" mortality. Unpredicted recovery was virtually non-existent. Consequently, the resistance times could be tabluated from direct observation of the fish in the lethal baths as in the upper lethal experiments, and the median resistance times plotted for different degrees of low temperature, a system hitherto not employed in low-temperature work.

In practice, lots of ten fish were placed in small plastic cylinders (6 inches long, 3 inches in diameter) capped at either end with plastic screening. Two such samples for each species from a given acclimation were inspected at each of the test temperatures. When the number of predicted dead in the first cylinder-regardless of the number dead in the second, had reached 50 per cent, the container was removed to running water at 12°C. The treatment of the second container varied in order to test the prediction value over a greater range of mortalities, but usually contained estimated deaths of between 50 and 100 per cent. The number of *actual* dead was recorded 24 hours later and excluded all fish showing perceptible activity—rarely a questionable category.

The median resistance times, determined by plotting the order of death (similar to upper temperature resistance), when transposed to a graph follow a pattern which has not been possible to convert into a more convenient form by any further resolution of the data. A solution for the problem of duration in the experiments on effects of high temperature was presented. Following the same line of reasoning, though not as apparent in this case, a flattening-out of the curves to become asymptopic with the time axis, is indicative of continued, pro-

longed survival of the sample under the corresponding temperature conditions. This latter inflection occurs by at least 5,000 minutes (three and a half days) in practically all cases, and considerably earlier for the *higher* acclimations. Consequently, a limit of 5,500 minutes was set for the duration of all low-temperature work.

A further use of the lethal temperature to delineate the biokinetic range of fishes has been illustrated by the construction of a trapezium relating upper and lower lethal temperatures to acclimation temperature (Fry *et al.*, 1942, and later references). For every stage of acclimation there is a corresponding lethal temperature. In the upper temperature region the lethal and acclimation temperatures approach each other, finally providing an *ultimate upper lethal temperature* (Fry *et al.*, 1946) beyond which no extension of temperature-tolerance is possible for the species as we know it. Such relations for the Pacific salmon have been illustrated in Figures 20 to 24.

Preferred Temperature

The specific aim of the investigation was to work out in some detail the limits of tolerance; it was also possible to carry out research on temperature selection but of a preliminary nature and consequently presented as such.

Measurements of the aggregation of fish in horizontal temperature gradients have been conducted effectively by Doudoroff (1938) and Sullivan (1949). Emphasis must be placed on the horizontal nature of these gradients since *vertical* gradients were employed for the Pacific salmon. One highly significant difference is apparent, namely, that a gravity gradient is inextricably involved in a vertical tank. It has been customary to reduce as far as possible all interfering factors when recording the responses of an organism to a gradient of a given identity. The methods employed in the present instance were to habituate ten previously acclimated fry to feeding freely in the preferendum tank for one week at the same acclimation temperature. No other control was instituted. Live *Daphnia* regularly introduced with finely ground food, and slowly swept around the tank by currents produced through aeration, served to scatter the fish in an irregular manner throughout the tank. Lighting during the habituation period was from overhead 150-watt bulbs and from sunlight through side windows (Figure 2).

On the day of an experiment, feeding was reduced (excessively fed fish tend to sink to the bottom when inactive) and only between 10 p.m. and midnight were observations on distribution in a temperature gradient recorded. At this time lighting, sufficient only to record positions accurately, was produced by two 3-candle-power sources, placed 35 to 40 inches on either side of a middle point of the tank. Any defence of territory which had been exhibited under full illumination was never displayed in the greatly reduced lighting of an experiment. The distribution in various thermal gradients was then noted by counting the number in each cell of known temperature.

Statistical Treatment

The various sources of variability in response to extremes of temperature and the statistical treatment of the data are presented in Appendix I. Most of the calculations were made by the method of analysis of variance.[1]

RESULTS

The actual levels of tolerance to extremes of temperature and the differences in response among the five species have constituted the main theme of study. These are presented by dealing with one aspect of temperature and considering the five species collectively under each heading.

Upper Limits of Temperature Tolerance

SPRING SALMON. Upper limits of temperature-tolerance include the resistance times and lethal temperatures for each acclimation. A typical series of mortality times at different test temperatures has been depicted in Figure 3*a* on normal axes, and on probit and logarithmic axes in Figure 3*b* for young spring salmon acclimated to 10°C. At 24.0°C. and below no deaths were recorded for that sample during the 10,000-minute (one week) duration of the experiment (Table IV). The lethal temperature therefore lies somewhere between 24.0° and 24.5°C. (Table XI). In the latter graph the mean of the logarithms of the times to death has been calculated. The very close approximation of the median and mean mortality times is apparent and in agreement with the findings of Fry *et al.*, 1946.

The median times to death have been plotted further in Figure 4*a*, illustrating the levelling-off of the median resistance times at lower temperatures to become parallel with the time axis. In Figure 4*b* the resolution of these data into a linear series of points has been achieved. The line *A*—*B* drawn almost at right angles to that for the median resistance times for 5°, 10° and 15°C. acclimations denotes the points of time at which continuation of the experiment provides no change in results (up to 10,000 minutes). Although the resistance times of the higher acclimated fish (20° and 24°C.) showed an increased tolerance at comparable times for periods of 1,000 minutes (about 17 hours) and less, continued exposure resulted in continued mortality to a level of death (line *B*—*C*) which finally equalled that of the lower acclimation of 15°C. This phenomenon has been observed repeatedly over the higher levels of acclimation for every species studied by Fry *et al.*, (1942, 1946) and Hart (1947, 1949). It is a characteristic, to a greater or lesser extent, of all the species considered in the following presentation.

PINK SALMON. The pink salmon were decidedly the most difficult of the five species to handle in fresh water after the first month of feeding, and for their length showed the least weight (Table III). The difficulties experienced in acclimating this species to 24°C. were noted earlier. Their intolerance to a temperature as high as 24.0°C. is also apparent in the distribution of their resistance

[1]Complete tables of data are included in a Ph.D. thesis, 1951, in the libraries of the Department of Zoology, University of Toronto, and the Pacific Biological Station, Nanaimo, B.C.

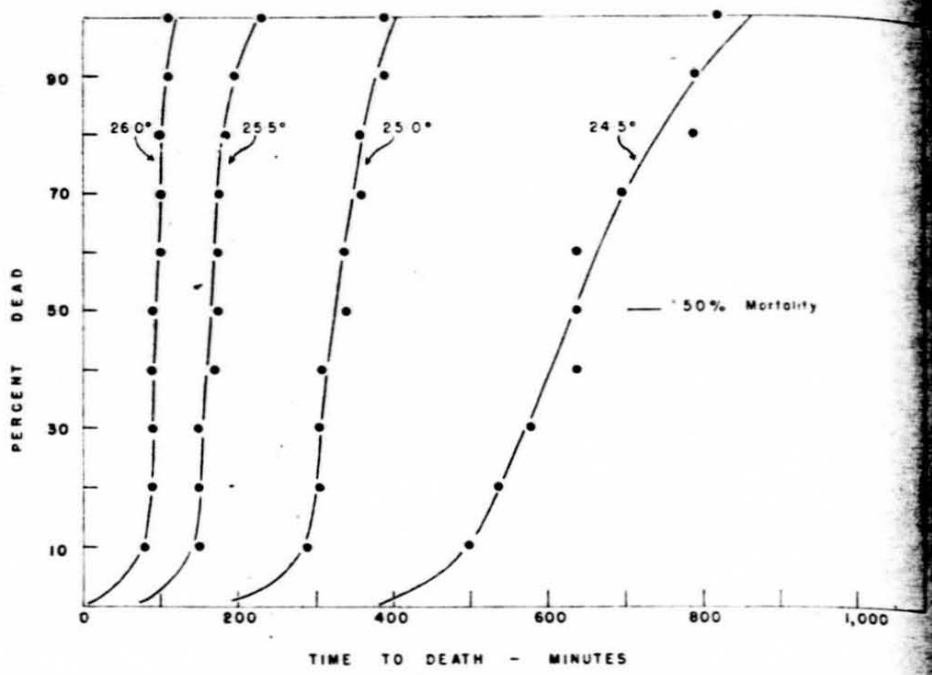

FIGURE 3a. Times to death at different lethaltest temperatures among young spring salmon acclimated to 10°C. Plotted on arithmetric axes.

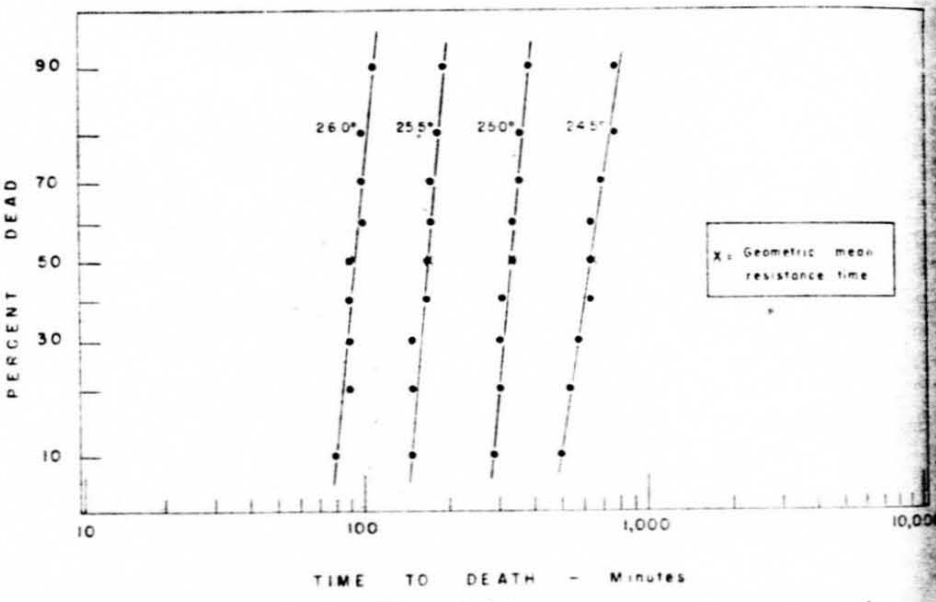

FIGURE 3b. Times to death at different lethaltest temperatures among young spring salmon acclimated to 10°C. Plotted on probit × logarithmic axes. Calculated geometric mean resistance times coincide with the median resistance times (at probit 5.0).

times following a minimum period of acclimation to this level of temperature (Table IV). After 2,000 minutes' exposure to 25.5°, 25.0° and 24.5°C., no increase in their tolerance over that for 20°C. acclimated samples was obtained.

The results for lower acclimations (Figure 5) were in accordance with general expectations except for the 5°C. group. Mortality was so rapid in lethal tanks with temperatures of 22.5° and 23.0°C. that it was decided to test the majority of the remainder of a limited sample at 21.0° and 19.0°C. Only four fish finally

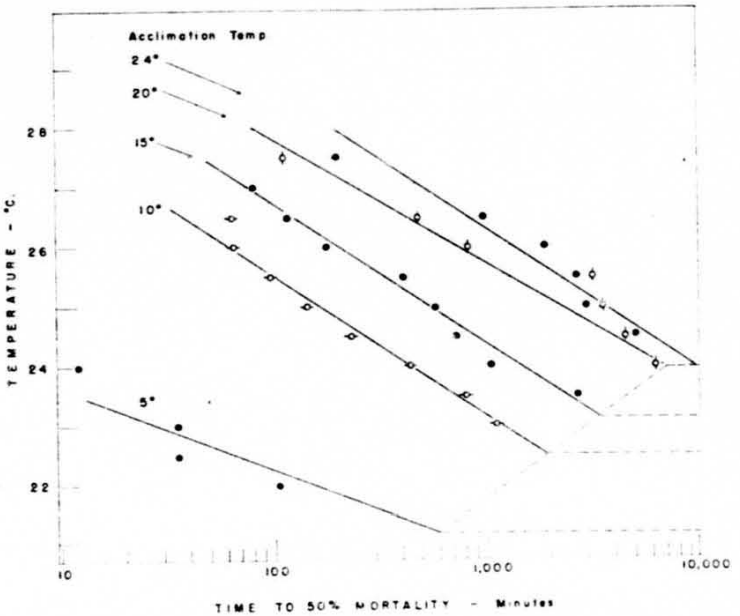

FIGURE 5. Median resistance times to high temperatures among young pink salmon acclimated to temperatures indicated. The encircled point for 5°C. acclimated pinks denotes the use of four fish instead of the normal ten in other points.

remained for an intermediate test at 22.0°C. The reliability of these data is therefore not as great as that for the more orderly points of higher acclimations.

SOCKEYE SALMON. Sockeye salmon, although held at a maximum acclimation of 23°C. (thus 1°C. lower than the highest for pink salmon), also showed an inherent intolerance for such levels of temperature as 24.0°C. and 24.5°C. after prolonged exposure (Table IV, Figure 6). However, of the five species, the sockeye are probably the best adapted to fresh water, often spending two years in lakes before migrating seaward. A completely fresh-water subspecies is also commonly reported (Dymond, 1936). The responses of the young sockeye to high temperatures were quite similar in pattern to those of a fresh-water species of Salmonidae, *Salvelinus fontinalis* (Fry *et al.*, 1946). The lines connecting median resistance times for acclimations of 20°C. and below are fairly regularly spaced and are not significantly different in slope to be distinguished as not parallel (Table X).

The apparent variation in slope for the 15°C. acclimated group is not beyond what might be expected from chance. This has been derived from a consideration of the total data mustered in the tables of analysis of variance (Tables VI to X). As a result, in this instance only, the line for the 15°C. acclimation has not been drawn by inspection as the best straight line for the plotted points, but as the most likely relation to be expected on the basis of the total data, that is, approximately parallel.

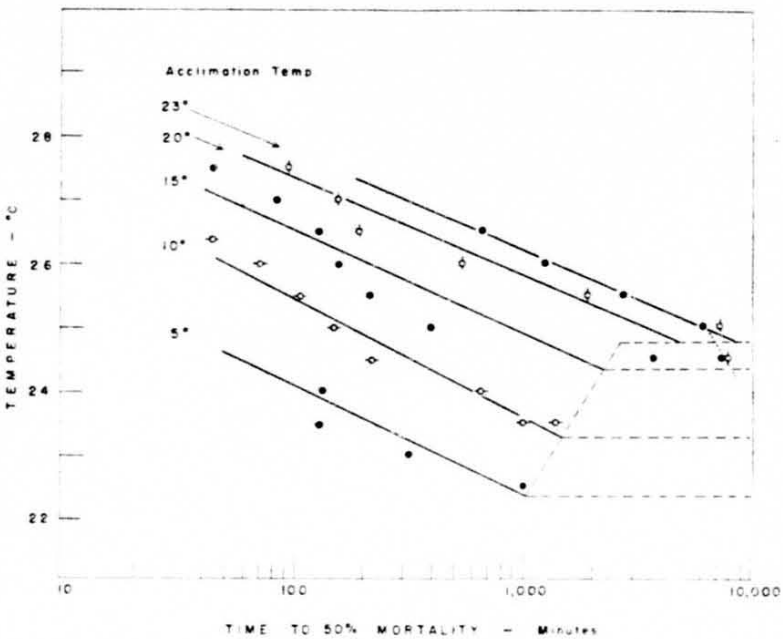

FIGURE 6. Median resistance times to high temperatures among young sockeye salmon acclimated to temperatures indicated. A somewhat lowered resistance among the 23° and 20°C. acclimated groups for prolonged exposure to 24.5°C., below the expected level, is indicated by the dotted line on the extreme right-hand side.

CHUM SALMON. The results of experiments with chum salmon are recorded in Table VII and Figure 9. This species showed the greatest amount of variability for experiments in which the mean resistance time approached or exceeded 1,000 minutes. Like the pink salmon, the chum normally move to sea quite early in life although some have been maintained in fresh water up to two years by Chin and Kuroda (1935). Acclimation to 23°C. was quite successful. However, after 9,000 minutes' (6 days') exposure to 24°C. in a temperature-tolerance test, half the sample had died, confirming the impossibility of acclimating this species to 24°C.

COHO SALMON. An accident in the early history of the coho salmon eliminated the stock of eggs held in one of two troughs. This loss, while unselective, necessitated some curtailment in the programme of study. At the time of experimentation

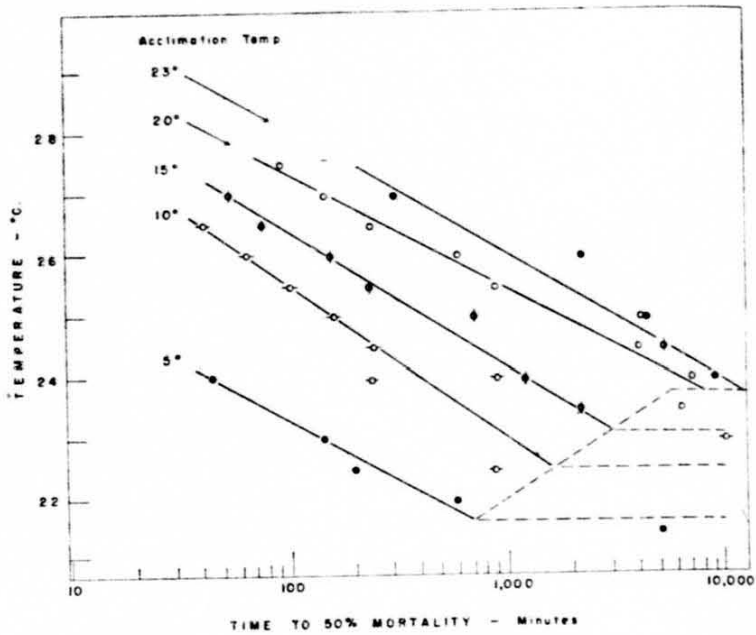

FIGURE 7. Median resistance times to high temperatures among young chum salmon acclimated to temperatures indicated.

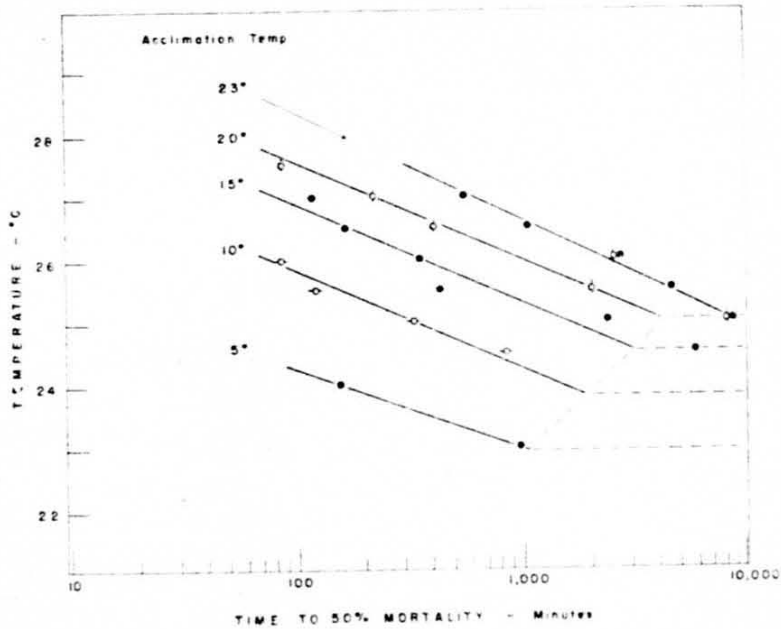

FIGURE 8. Median resistance times to high temperatures among young coho salmon acclimated to temperatures indicated.

with upper lethal temperatures, it was apparent that at higher acclimations the coho were conforming very closely to the reaction times already determined for the spring salmon. The remaining tests in this series were therefore bypassed to permit more intensive investigation in the relatively unexplored low-lethal range. In retrospect this was perhaps unfortunate as it now appears that a difference between coho and spring salmon, where such occurs, is present in their high-temperature tolerance from low acclimations.

The results for the coho appear in Table IV and Figure 8.

PROLONGED EXPOSURE. An experiment concerning long-term exposure to high temperatures was performed with sockeye and chum salmon acclimated to 5°C. These fish do not feed readily at critical high temperatures, so prolonged exposure would inevitably cause death from malnutrition, if from no other cause. In this respect the two species are alike. Since they differ in migratory habits, the sockeye remaining in fresh water usually for at least one year longer than chum salmon, it is quite possible that they differ in ability to tolerate fresh-water conditions (cf. Hoar and Bell, 1950). So far, few experiments with high temperatures have involved exposure times in excess of 10,000 minutes (approximately one week), particularly for fish from acclimation temperatures below 15°C. The results for 30,000 minutes' exposure for these two species are recorded in Figures 9 and 10. The sockeye typically showed no additional mortalities beyond 3,000 minutes. The pattern of mortality in the chum salmon was very similar to that for the sockeye, with no further deaths recorded between the end of the first day (1,440 minutes) and the end of a week (10,080 minutes). Beyond this period a rather unexpected but orderly progress of mortality appeared in the chum salmon samples. Speculation as to the cause of death might be made in the direction of the relative intolerance of this species to fresh water; further experimentation is desirable.

Lower Limits of Temperature Tolerance

SPRING SALMON. The method of determining the resistance times at low-lethal levels of temperature usually involved two samples of ten fish of the same species and acclimation for each test temperature. The removal of one sample with 50 per cent predicted dead changed each test before completion from a total of 20 to a total of 10 subjects, hence the plotting of the individual resistance times at 5 per cent intervals changed to 10 per cent intervals following the time of removing the first sample. The slight inconvenience involved was a result of checking "predicted" against "actual" dead. In this manner all mortalities from a single test temperature could be plotted in determining the time to 50 per cent mortality.

No size effect in Pacific salmon of the same age was demonstrated from data on heat-tolerance (Table XIII). Results of experiments on cold-tolerance, however, were strongly suggestive of a size influence. The method employed was not such that the individual fish, once dead, could be later identified when the whole sample was removed from a lethal bath. A group of fish, some living, some dead, was on hand. By considering only those cases in which at least three but not

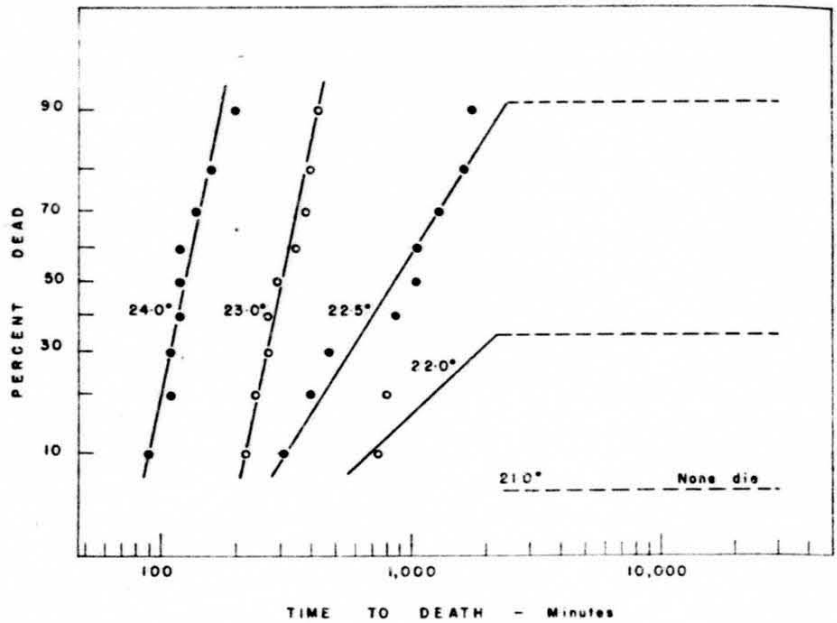

FIGURE 9. Times to death at various high temperatures among young sockeye salmon acclimated to 5°C. and tested for 30,000 minutes (approximately three weeks).

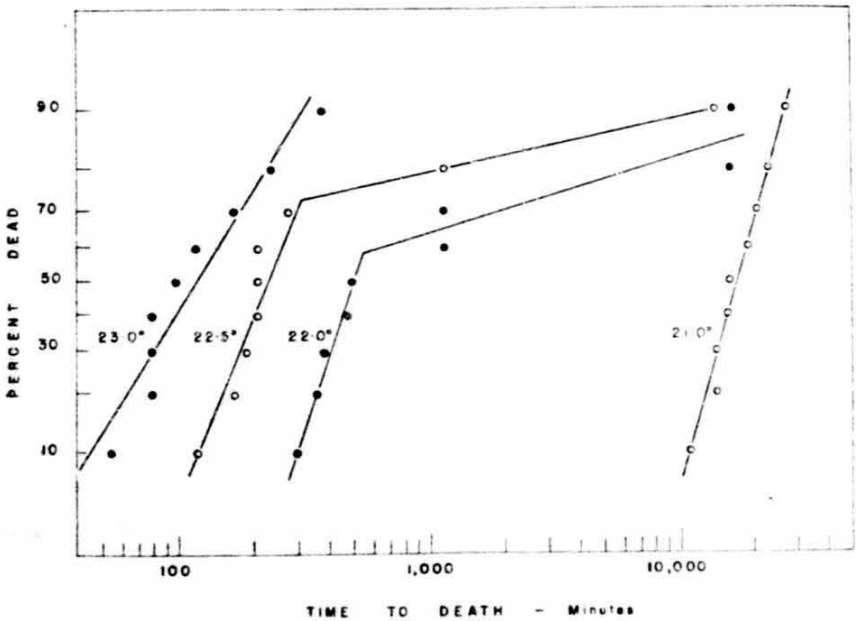

FIGURE 10. Times to death at various high temperatures among young chum salmon acclimated to 5°C. and tested for 30,000 minutes (approximately three weeks).

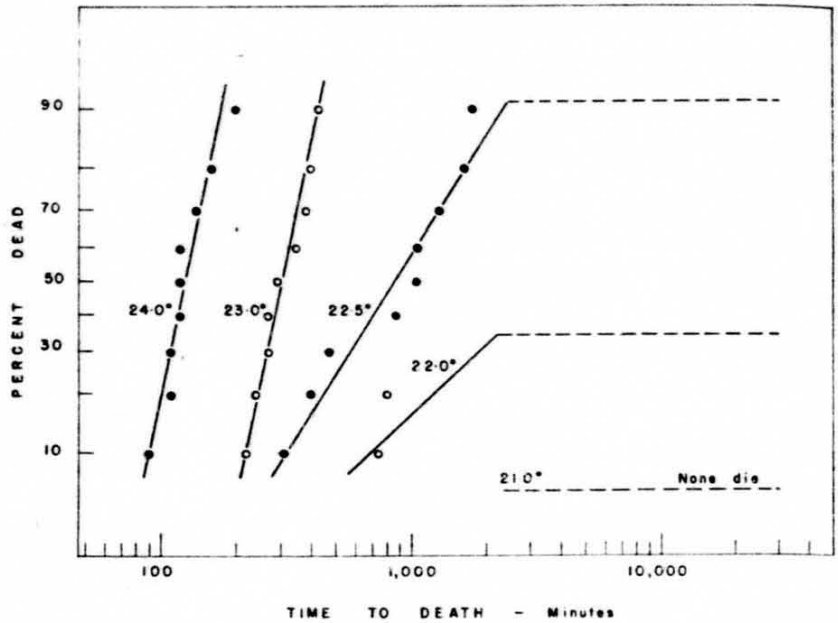

FIGURE 9. Times to death at various high temperatures among young sockeye salmon acclimated to 5°C. and tested for 30,000 minutes (approximately three weeks).

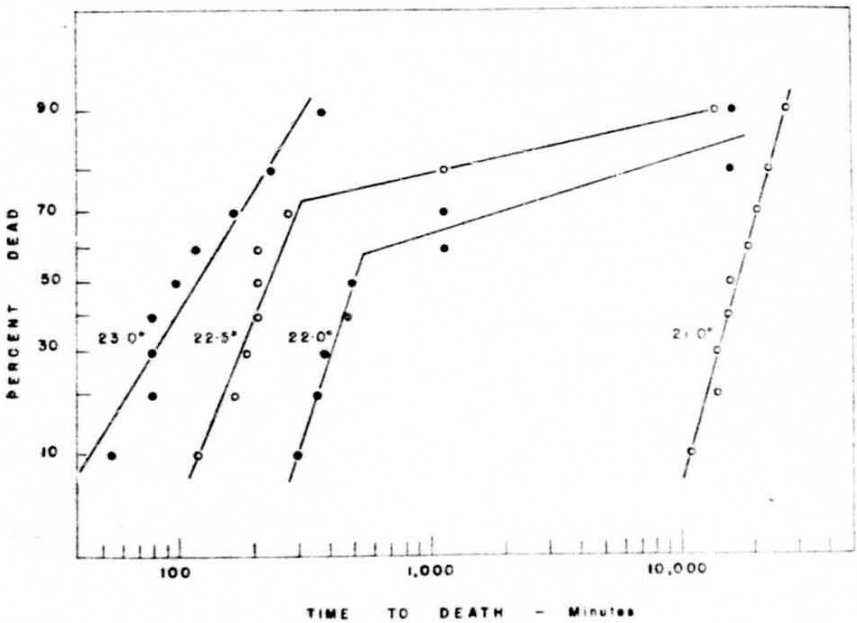

FIGURE 10. Times to death at various high temperatures among young chum salmon acclimated to 5°C. and tested for 30,000 minutes (approximately three weeks).

more than seven had succumbed, comparison of the sizes among all specimens showed nearly twice as many living fish above the mean length for the total sample as dead fish. Since the measurements were made some time after death, weights were not taken. The mean lengths and standard errors of the two groups were: living = 5.00 ± 0.046 cm., dead = 4.75 ± 0.043 cm. The data show a statistically significant difference (Table XIV).

Evidence was procured for two separate lethal effects of low temperature: the first, occurring only at the lowest temperatures, was very rapid and decisive, and was followed by a second series of deaths after considerable delay. Owing to the methods employed, the first mortalities could not be sorted out from the remainder. They constituted a relatively small fraction of the total dead. Unrecorded observation at the time of an experiment revealed an apparently distinct size factor in this primary phase of death, the smallest fish dying first. Confirmatory experiments will be required for adequate proof. The presence of these comparatively smaller fish in the samples of dead fish recorded above may constitute the main source of difference between the two major groups.

Preliminary tests with spring salmon, before the system of predicting death had been instituted, were performed at 0.5°C. intervals from 0° to 3°C. (Figure 11, 24° and 20°C. acclimations; Table V.) The reasoning, derived from experience with upper lethal experiments, was fallacious in part since the range of low temperatures causing death for 24°C. acclimated salmon was almost twice as great, within the same time limits (covering 6 rather than 3 degrees C.). The doubly "expensive" technique of closely spaced temperature tests coupled with sampling for dead throughout the experiment was replaced by the predicting system for tests at 1°C. interval in the remaining cases reported for the spring salmon.

The median resistance times follow a rather varied relation with increasing temperature (Figure 11), and are apparently complicated by more than one cause of death. The sigmoid shape of the curves is one characteristic which persists throughout the remaining species, particularly at higher acclimations.

PINK SALMON. Although a few preliminary tests were made with 10°C. acclimated pink salmon, 5½ months old, mortality in the fresh-water holding troughs appeared during the last two weeks in sufficient proportions to signify some intolerance to these conditions. Small samples were incapable of withstanding 5°C. Attempts to continue work with this species in fresh water were stopped.

SOCKEYE SALMON. The inability of Pacific salmon to tolerate sudden immersion in low temperatures was well exemplified in the work with young sockeye salmon. For survival in nature the necessity for this species to acclimate to low temperatures is emphasized by the results illustrated in Figure 12 (Table V). Even 10°C. acclimated samples succumbed to 2° and 3°C. within 4,000 minutes' (three days') exposure, and a temperature of 0°C. caused some mortality amongst those taken from holding tanks at 5°C.

The sigmoid pattern of the resistance time-temperature curves is most pronounced among the sockeye. An initial period of rapidly increasing resistance to low temperature (for 20° to 10°C. acclimation) is followed by relatively little

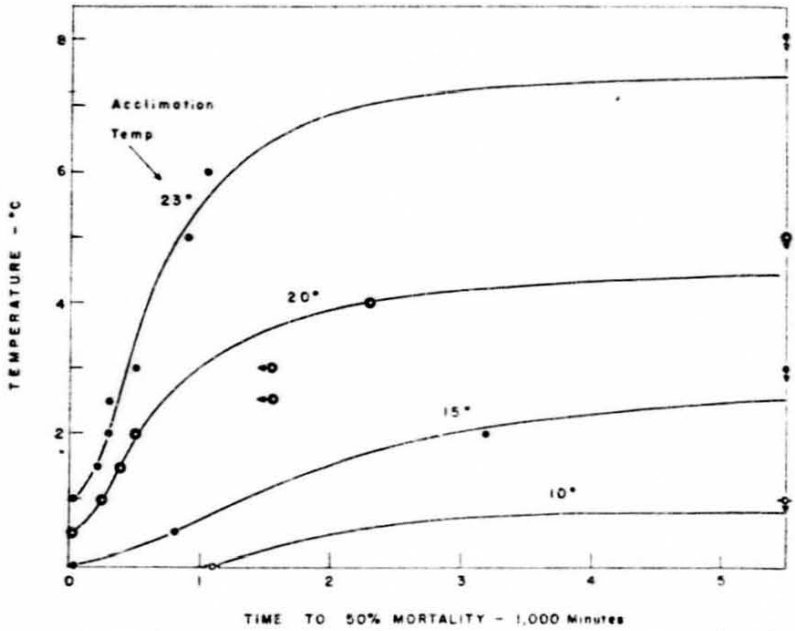

FIGURE 11. Median resistance times to low temperatures among young spring salmon acclimated to temperatures indicated. Arrows signify tests at temperatures which caused less than 50 per cent death for 5,500 minutes' exposure if placed above the line, or greater than 50 per cent by the time indicated if below the line.

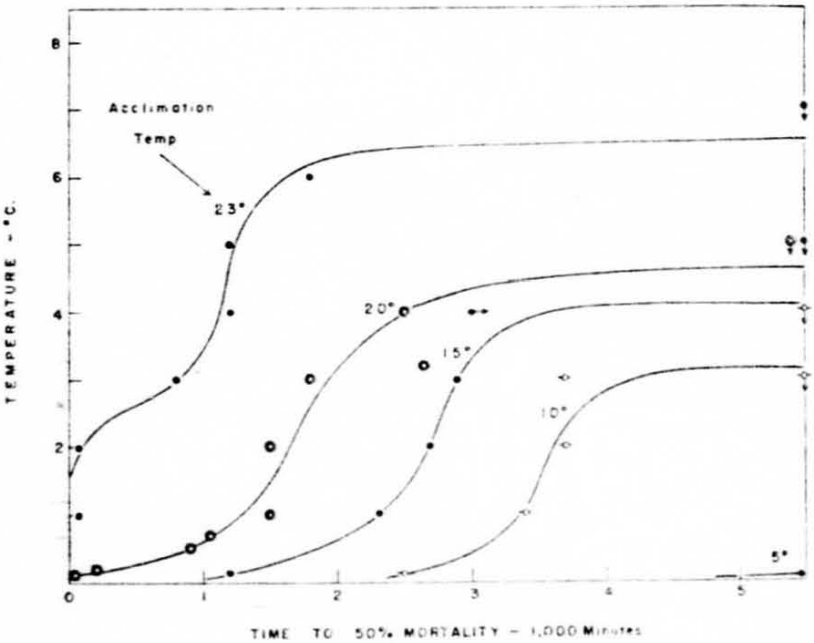

FIGURE 12. Median resistance times to low temperatures among young sockeye salmon acclimated to temperatures indicated. Arrows used as stated in Figure 11.

change in resistance for two and three degrees' increase in temperature. No mortality occurs within a degree or so above this latter zone even for a considerably increased exposure time. The presence of this "break" at an exposure time of at least 4,000 minutes for fish acclimated to 10°C. and higher (usually occurring at test temperatures of 2°C. and above) supports the overall experimental time of 5,500 minutes for lower lethal-temperature determinations.

CHUM SALMON. The greatest difference in response to low temperatures was exhibited by the chum salmon from different acclimations (Table V, Figure 13). When taken from 23°C. and put at 7°C., 50 per cent mortality occurred by 4,000 minutes. From 10°C., however, mortality at 0°C. was observed only toward the very end of the imposed test time. This was somewhat surprising in view of the scant but indicative findings on the intolerance discovered for the pink salmon, to which the chums appear to be most closely related (Milne, 1948).

COHO SALMON. The data and curves illustrating the median resistance times to low temperatures for coho salmon appear in Table V and Figure 14. The significant mortality among members of this species at 0°C. when acclimated to as low as 5°C. demonstrates how confined the coho are to temperatures above the freezing point of water. In general their reactions show the same trends as in the other Pacific salmon.

MIXED LETHAL EFFECTS. Brief mention was made of two distinct responses observed while studying the lethal effects of low temperatures. The presence of very rapid mortalities in contrast with delayed lethal effects, either between samples at slightly different temperatures, or within samples at given critical temperatures, was apparent from even superficial examination. Doudoroff (1942) observed somewhat similar phenomena among young greenfish, *Girella nigricans*, and distinguished between "primary chill-coma" and "secondary chill-coma". He records that "The initial shock was not manifest until several seconds after transfer to the low temperature, and apparently was not due to stimulation of the cutaneous sense organs, but was produced only when the low temperature had penetrated internally, probably to the central nervous system. Accordingly, it was more delayed in large specimens than in small ones". The discovery of a satisfactory criterion of cold-death among the Pacific salmon permitted a more critical study of the time-temperature-acclimation relations for death. Results for sockeye salmon will be presented, being more extensive but not unique. By plotting the data on probability × logarithmic paper it was possible to discriminate clearly between the two trends of death. From 23°C. acclimation (Figure 15*a*), 1°C. caused rapid death for all individuals (as presumably would lower temperatures); 2° to 3°C. split the samples into rapid and delayed deaths as shown; 4° to 6°C. resulted in only delayed deaths; above 6°C., less than 50 per cent mortality was obtained. From 20°C. acclimation (Figure 15*b*) 0°C. caused rapid mortality; 0.5°C. divided the sample into the two types of death; 1°C. and above caused delayed to no mortality. From 15°C. acclimation (Figure 15*c*) one case of mortality was observed for a lethal-test temperature of 0°C. followed by delayed to no mortality at higher temperatures. The time to 50 per cent. mortality is consequently affected by per cent occurrence of "primary" cold-deaths within the sample and by the size of the fish which chance to be present in that sample.

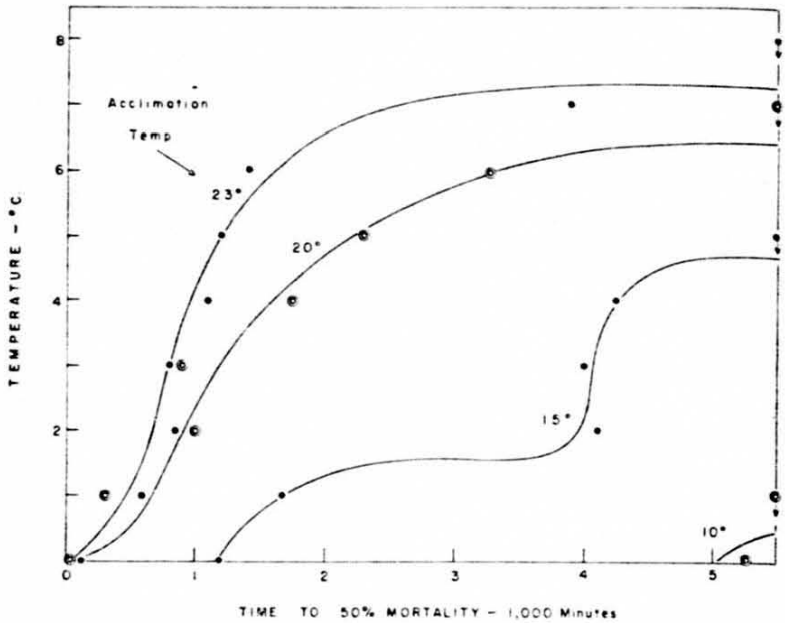

FIGURE 13. Median resistance times to low temperatures among young chum salmon acclimated to temperatures indicated. Arrows used as stated in Figure 11.

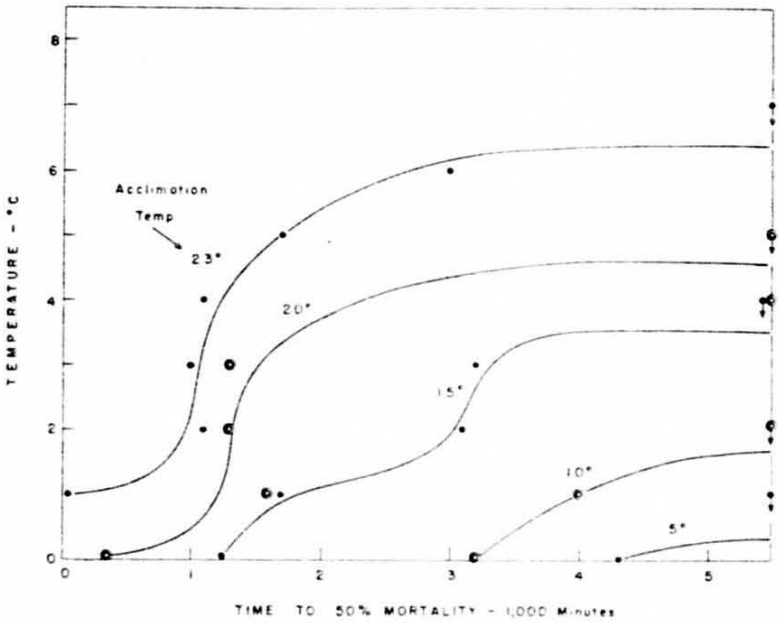

FIGURE 14. Median resistance times to low temperatures among young coho salmon acclimated to temperatures indicated. Arrows used as stated in Figure 11.

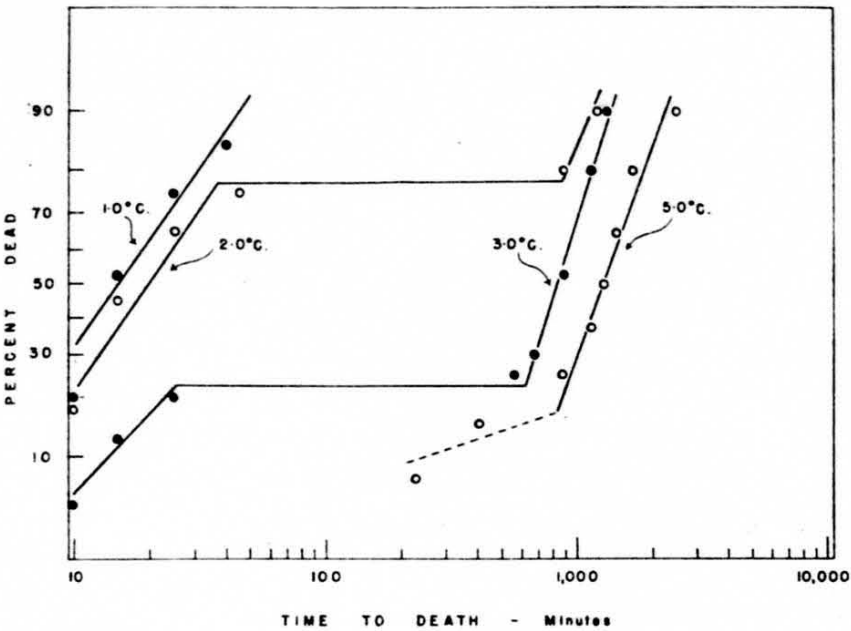

FIGURE 15*a*. Times to death at various low temperatures among young sockeye salmon acclimated to 23°C. Plotted on probit × logarithmic axes.

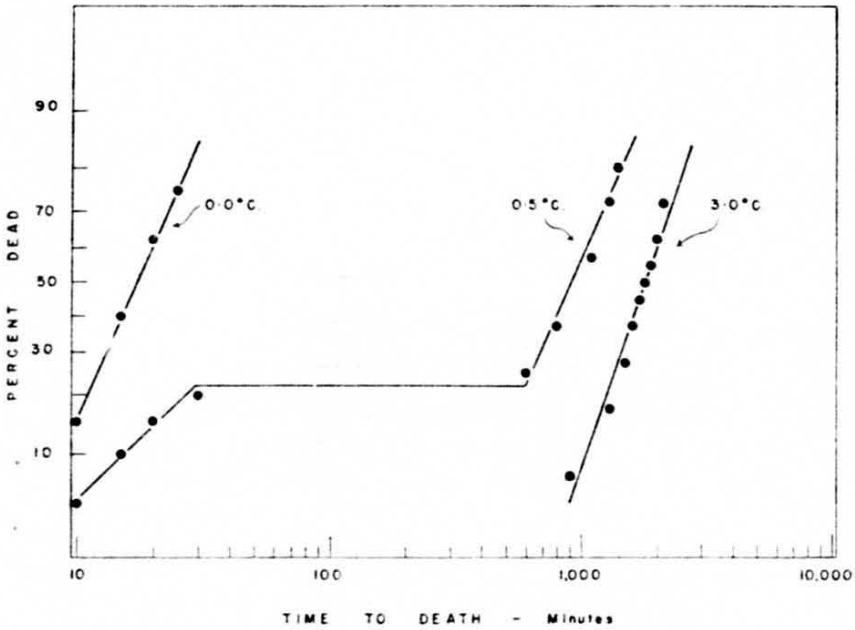

FIGURE 15*b*. Times to death at various low temperatures among young sockeye salmon acclimated to 20°C. Plotted on probit × logarithmic axes.

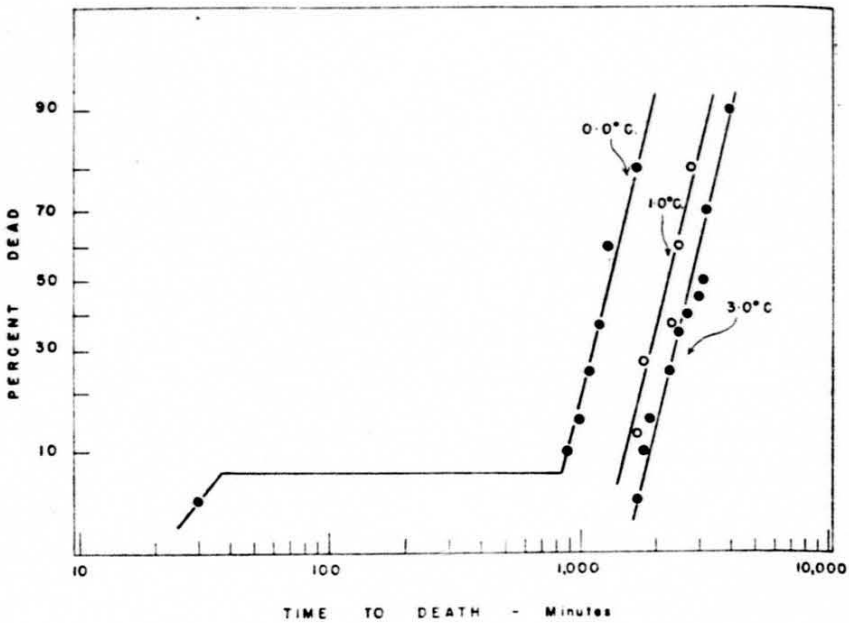

FIGURE 15c. Times to death at various low temperatures among young sockeye salmon acclimated to 15°C. Plotted on probit × logarithmic axes.

A reason for greater variability in cold-temperature resistance and the variety of inflections making up the median resistance time-temperature curves can be attributed partly to these causes.

The work of Doudoroff (1942, 1945) was suggestive of an osmoregulatory problem in the delayed cold-deaths, possibly acting as an accessory lethal factor. To test the hypothesis, a preliminary experiment using two groups of 20°C. acclimated sockeye, one in fresh water and the other in Atlantic sea water diluted to 9.9 parts per thousand, was performed at comparable low temperatures (Figure 16). Both rapid and delayed cold-deaths were observed at 0.2°C without significant difference in response within the two media. At 0.7°C. an indication of greater resistance among the last surviving members in the saline solution was present. At 3.2°C. a decided increase in the resistance was observed but not to the point of eliminating death from low temperature in part of the sample.

Two conclusions can be drawn from this experiment. At the lowest lethal levels of temperature a medium of salt water (slightly hypertonic) does not alter the course of death from that observed in fresh water. Such a medium does, however, reduce the lethal effects of low temperature for delayed cold-death when resistance times exceed 1,000 minutes (about 17 hours). From these it would seem that three causes for death are involved: one, a very rapid agent usually effective within 60 minutes of exposure, a second, not so rapid in action, and a third which is related to osmotic balance.

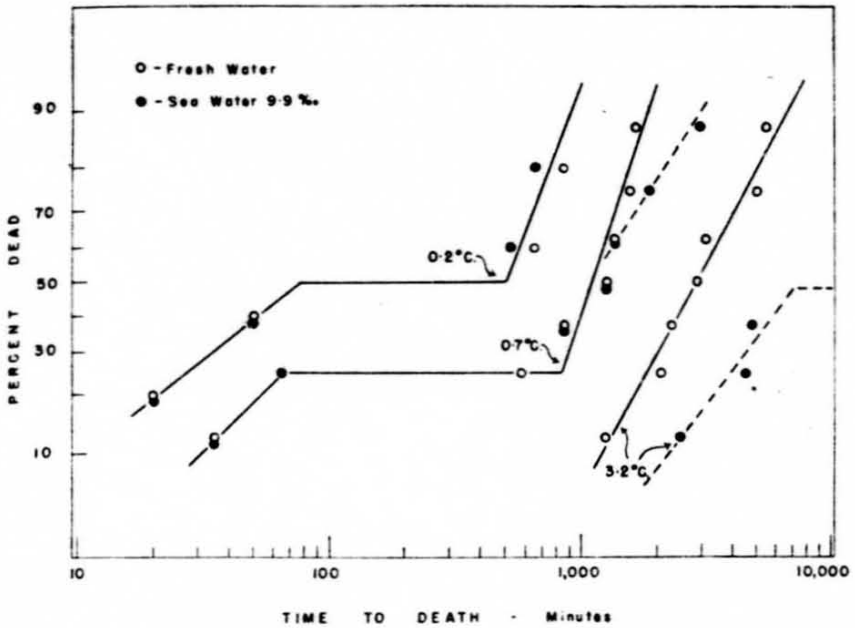

FIGURE 16. Times to death at various low temperatures for samples of young sockeye salmon acclimated to 20°C. and tested in fresh water and in sea water diluted to 9.9 parts per thousand (slightly hypertonic). Where a difference in response was apparent a broken line has been used for the salt-water data.

The observation that size appears to have some bearing on resistance to osmotic stress was reported by Wilder (1944) for sea-run speckled trout. Huntsman and Hoar (1940) using salinities of 20 and 28 parts per thousand concluded that Atlantic salmon parr "as they increase in size become more resistant to sea water". These findings in conjunction with the size effect among Pacific salmon at low lethal temperatures support the possibility of an osmotic factor acting as a lethal agent at low temperatures. Conversely the lack of any size relation in deaths from high temperatures might be taken to indicate other than osmotic factors involved in these mortalities.

PREFERRED TEMPERATURES

SPRING SALMON. Some of the inherent problems concerning the determination of preferred temperatures for fish in a vertical temperature gradient were outlined under "Methods". The presence of other gradients (gravity, distance from surfaces, etc.), if impossible to remove or control, must be accounted for by demonstrating the supression of these in relation to preference for some level of the identity under investigation. By changing the position of the temperature gradient within the tank, without altering other relations, it was possible to demonstrate the selective aggregation of spring salmon in the region of 12° to

13°C. (acclimation of 20°C.) despite a highly significant difference in their position within the tank. This distribution in space, varied yet remaining relatively constant with respect to temperature, has been depicted for this species in Figure 17. An attempt has been made to conform to the relative dimensions of the tank and distribution of the fish, such that the figure presents a "graphical picture" in every sense of the word.

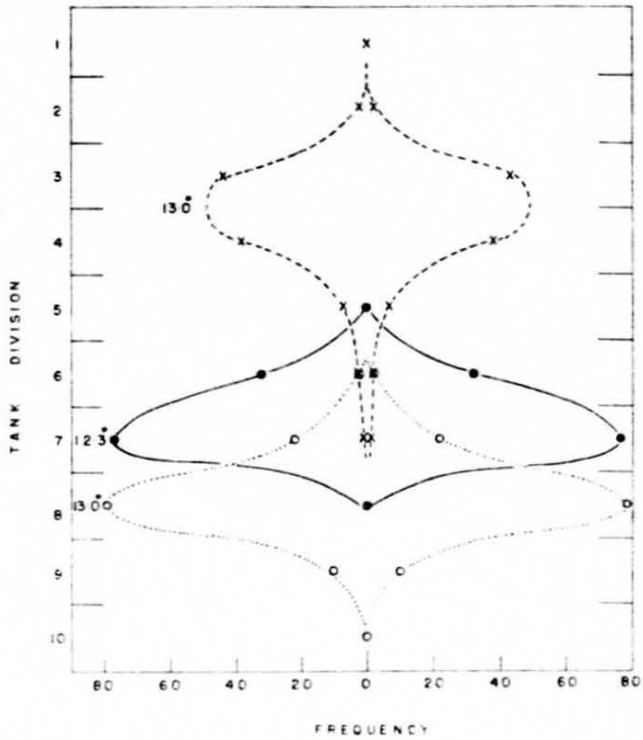

FIGURE 17. Frequency distribution of young spring salmon acclimated to 20°C. in three successive temperature gradients, using the same tank. The calculated preferred temperatures are noted for each distribution.

A few observations which apply to all the species are of significance. No matter what the gradient, even reaching the lethal level, the fish would make feeding excursions to the surface if the appropriate movements of the investigator, which always accompanied feeding, were made. On some, although infrequent, occasions, under the routine procedure of low illumination and night-time recording, no selected temperatures could be determined. Activity of the fish was too great, being more limited by the surfaces of the tank than by any other recognizable feature. No suggestion to account for the increased "excitability" on these particular occasions can be advanced. Adequate feeding and uniformly quiet conditions were always observed prior to introducing a temperature gradient. A natural "exploring" activity is a characteristic of the group.

PINK, SOCKEYE, CHUM AND COHO SALMON. The distribution in various temperature gradients as indicated by the preferred temperatures (mean and mode) for acclimations, mostly including 10°, 15° and 20°C. in the Pacific salmon species, are presented in the graphs used to display the zones of temperature tolerance (Figures 20 to 24). The variation in response between the different species was not sufficient to warrant dealing with them separately. Some general observations can be made.

Despite considerable difference in temperature-acclimation, amounting to 15°C., comparatively little difference in preferred temperature was observed experimentally. On the average no greater difference than 3°C. (11° to 14°C.) was displayed between means, and the region of greatest preference lay in the 12° to 14°C. stratum. The pink salmon from 20°C. acclimation constituted the only case in which a preferred temperature as high as 17.7°C. was observed. The general avoidance of temperatures above 15°C. for all species, in spite of acclimation to this level and to 20° and 24°C., was very marked. A tendency to show greater dispersal in the fish from higher acclimations is suggested by the somewhat larger standard deviations for these samples.

Only a record of the mode was available for 5°C. acclimated sockeye on final analysis. Unfortunately no more supporting data are on hand.

Comparison of Temperature Resistance

RESISTANCE TIMES TO HIGH TEMPERATURES. The extent to which acclimation affects the resistance times to high temperatures within each species and the levels of tolerance characteristic of each species have been presented graphically and in tabular form. If comparison is to be made a variety of questions may be posed:

(1) How do these species differ in their resistance times at different levels of acclimation?

(2) If a difference exists, is it a matter of a difference in slope (rate of change of resistance time with temperature), a difference in overall level of temperature resistance, or a difference in both slope and level?

(3) What measure of difference can be quoted and with what statistical significance?

(4) Can the postulated straight-line relation between the logarithm of the median resistance time and temperature be justified?

Each of these questions can be answered by the method of analysis of variance (Appendix I) which permits a sorting out of the sources of specific difference from those resulting from sample variability coupled with interaction of the factors of acclimation, lethal test temperature and resistance time. Three basic comparisons can be made involving two of the variates—species, lethals[1] or acclimations[2]—in as many cases as exist in terms of the third. These are now considered:

Species × *lethals*, for three levels of acclimation. The results from three analysis-of-variance tables (Table VI) demonstrate a highly significant difference

[1] Refers to lethal test temperatures. [2] Refers to acclimation temperatures.

between species ($P < .01$) which increases with lower levels of acclimation, that is, at a lower level of acclimation temperature for a comparable treatment of lethal temperature the difference is more distinct. This is the crucial test. Figures 18*a* and *b* illustrate this relation. A further analysis to determine which species are contributing to the difference is considered later. As might be expected, the

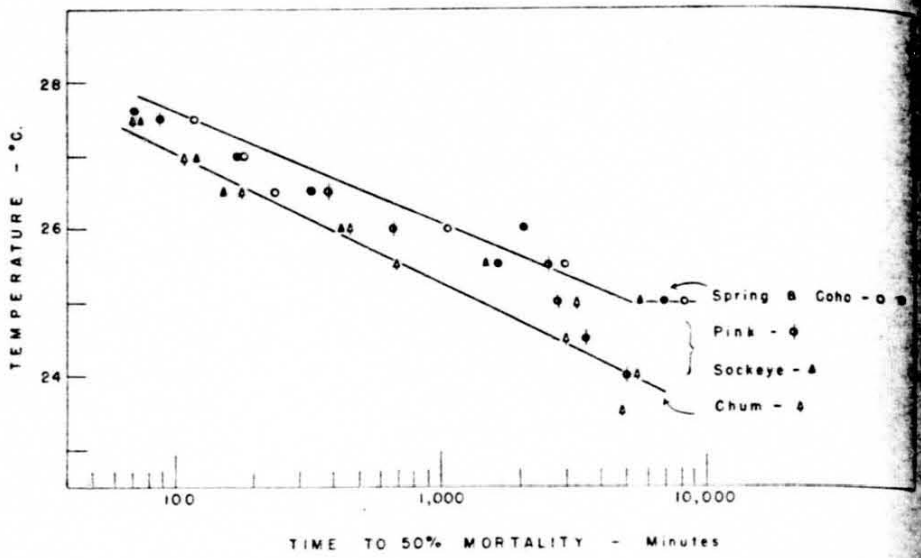

FIGURE 18*a*. Comparative median resistance times to high temperatures among young Pacific salmon acclimated to 20°C. Lines have been drawn for the most and least tolerant species.

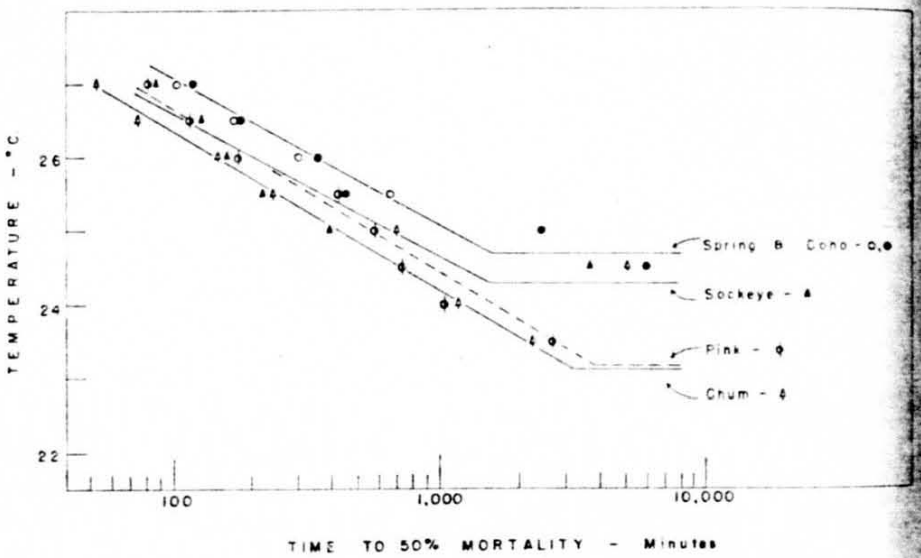

FIGURE 18*b*. Comparative median resistance times to high temperatures among young Pacific salmon acclimated to 15°C.

differences for all species in their response to different lethal test temperatures are also highly significant.

Species × *acclimations*, for four levels of lethal test temperature. This is a case in which the lumping of resistance times of three acclimations for single lethal levels is used to distinguish between species. It can be concluded from the results in Table VII that between species at each lethal level, and with some increase at lower levels, there is a consistent significant difference, yet not as great as that for a series of lethals at one acclimation. The increasing significance at lower levels of lethal temperature is inherent in the study of temperature effects. If a lethal temperature approaching that for boiling water were used, it would have little consequence what species or acclimation characterized the fish. In contrast, therefore, it is the temperatures which just cause distinct mortality that will best demonstrate specific and acclimation differences.

Lethals × *acclimations*, for different species. These five "4 × 3" analysis-of-variance tables (computed in Table VIII) complete the series of first-order comparisons. In essence they signify what has already been stated, namely, that temperatures of both acclimation and lethal level have highly significant effects in *each* of the species (former tables only applied to the *group* as a whole). One species, the sockeye ($P < .05$), does not show the same degree of significance as the others ($P < .01$). There is a greater variability in this species which is apparent from the diversity in the points plotted in Figure 6.

It is possible to draw up a table of second-order comparison, for example, *species* × *lethals* × *acclimations*. The very fact of a difference in temperature-response among the species which resulted in the use of somewhat different lethal levels of temperature, to bridge the cases from non-lethality to fairly rapid lethality, reduces this table for like comparisons below a feasible size. A survey of the three first-order tables, however, reveals that the error term, signified by the interaction component in each table, lies mainly between values of 1.0 and 3.0, and is sufficiently consistent to support the view that the interaction of *species* × *lethals* × *acclimations* would also be consistent throughout the relation.

A further step involves assigning the particular amount of variation which may be attributed to each independent comparison (degrees of freedom). Thus, in the first analysis concerning *species* × *lethals* at 20°C. acclimation (Table VI), the total sum of squares was 1249.02 with 29 degrees of freedom. It is possible to extract each independent comparison by the use of appropriate multipliers (Fisher and Yates, 1948; see Appendix). Since these are then the sums of squares for single degrees of freedom they also equal the mean square or variance (s^2) and can be compared for significance directly with the "error" by an F test. Such a searching analysis has been carried out in this particular case only. The findings from it concerning the *species* × *lethals*, and the statements already made by use of the more generalized analysis tables submitted, permit the application of the following conclusions to the whole problem (consult Table IX):

(1) No significant difference in response to upper lethal levels of temperature exists between spring and coho salmon (acclimated to 10°C. and above).

(2) Spring and coho salmon show a highly significant difference in response from that of either sockeye, pink or chum.

(3) Pink and chum salmon show a barely significant difference from each other, but not from sockeye in either case.

(4) There is a very highly significant "linear fitness" of the logarithmic relation for these data which accounts for 98 per cent of the variance; the balance can be attributed to curvature.

Of the total 29 degrees of freedom, 20 were assigned to the "error" component (Table VI). This component is constituted of the variability of the organisms resulting in slight deviations from the postulated relation and their interaction (*lethals* × *species*), plus the variations due to experimental procedure which can never be entirely eliminated. By the same method of using appropriate multipliers, 20 separate components each representing one degree of freedom were obtained. A study of these provided no value which in itself was significantly different from that to be expected from the variability of the material. Consequently it can be concluded that:

(5) The *slopes* of the lines relating resistance time to temperature for the five species are not significantly different (acclimations 10° to 20°C.) and

(6) The same relation can be applied to each species with equal confidence, differing only in the temperature *level* at which this relation exists.

RESISTANCE TIMES TO LOW TEMPERATURES. The constancy of relations in the upper temperature levels is in contrast to that for lower temperatures. No systematic testing of the latter data is possible from present knowledge of the subject. A graphical presentation of some of the responses among the young Pacific salmon taken from the same acclimation temperatures (Figure 19*a* and *b*) serves to illustrate this phenomenon. A fair similarity in pattern for 23°C. acclimated species becomes progressively more variable with decreasing acclimation. The chum salmon, which at first seemed to be among the most sensitive judging from high acclimations, were the least sensitive from an acclimation of 10°C.

The spring salmon were consistently the most sensitive to the lowest temperatures (0°C.), but showed a rapid increase in resistance at slightly higher temperatures.

A striking intolerance to low temperatures characterizes all five species. Further study in this field of temperature-responses will be necessary to clarify some of the complexity which has appeared in these findings. The possible influence of size has been pointed out. The mean fork-lengths of the fish used are reported in Table III.

ZONES OF THERMAL TOLERANCE

The concept of a zone of thermal tolerance bounded by upper and lower incipient lethal temperatures for the greatest possible range of temperature-acclimation, and terminated by ultimate lethal temperatures, was advanced by Fry *et al.* (1942) for the goldfish. The freezing point of water limited the minimum acclimation to 0°C. for fresh-water fish. By construction of a trapezium relating these confining temperatures, calculation of the area of the zone of tolerance in "degrees Centigrade squared" gave quantitative expression for an otherwise qualitative description. Various species of fish have since been described in this

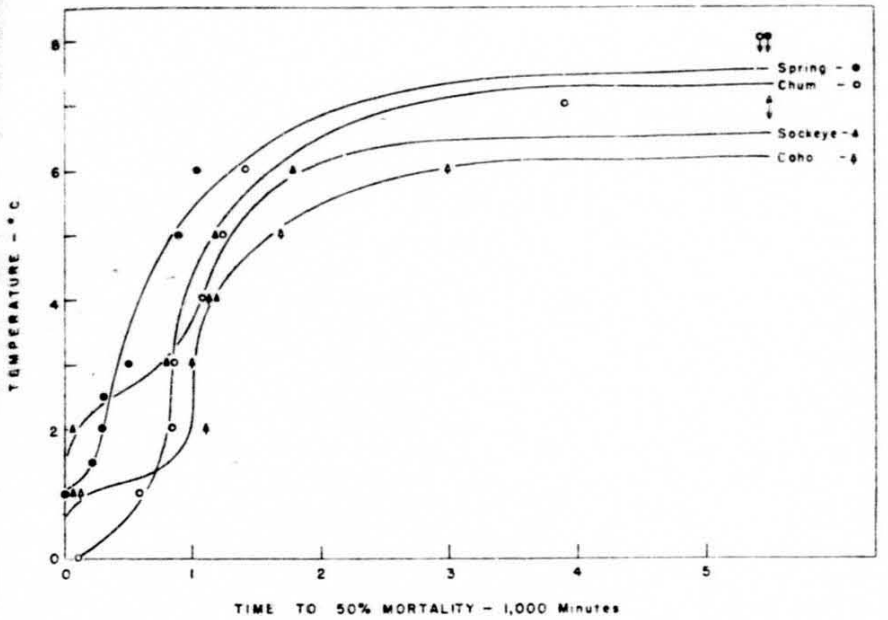

FIGURE 19*a*. Comparative median resistance times to low temperatures among young Pacific salmon acclimated to 23°C. Arrows used as stated in Figure 11.

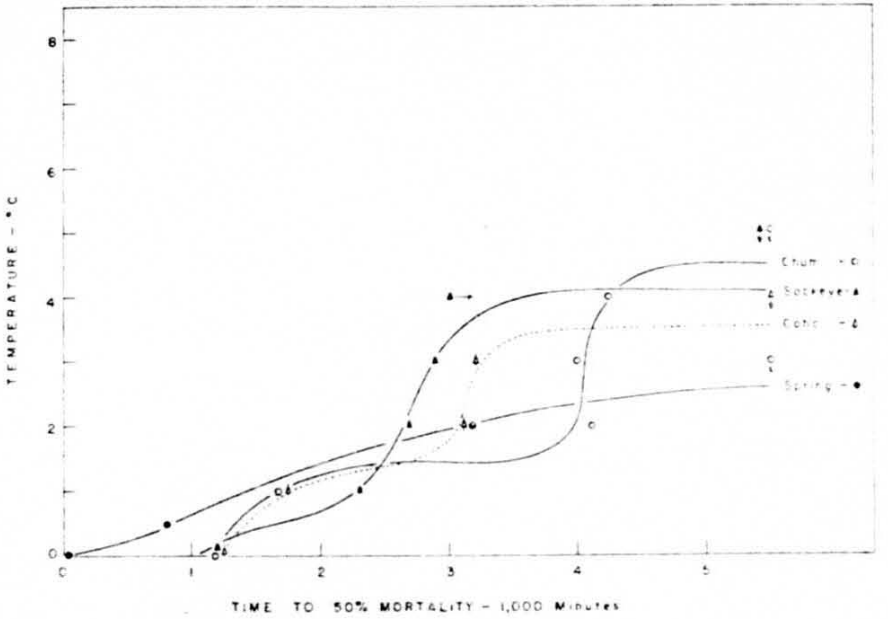

FIGURE 19*b*. Comparative median resistance times to low temperatures among young Pacific salmon acclimated to 15°C. Arrows used as stated in Figure 11.

fashion (Brett, 1944; Fry *et al.*, 1946; Hart, 1947, 1949), all conforming within slight variations to the original pattern for the goldfish. An increasing acclimation temperature has always resulted in an increasing lethal temperature in some fractional proportion. The construction of a diagonal line at an angle of 45° to the axes (Figure 20) has provided a ready means of determining the ultimate upper lethal temperature, since this line represents the locus of all points for which lethal temperature equals acclimation temperature. Characteristically, for upper incipient lethal temperatures, no species has been found to exploit the full possibility suggested by extrapolation of this linear relation (between upper lethal temperature and acclimation temperature), always dropping short as a result of uniform intolerance at the highest acclimations, providing a "plateau" in a graphical presentation. Although the resistance time to a high temperature may be lengthened through higher acclimation, it was pointed out that this resistance is finite and the lethal temperature remains unaltered for the relation designated by the "plateau". These relations are illustrated in Figures 20 to 24 for the Pacific salmon. Some new aspects, differing from the normal trapezium, are apparent. The lethal-temperature points are not always best represented by a straight line, particularly in the lower lethal bracket. A high degree of sensitivity to low temperatures among the Pacific salmon almost confines these species to acclimation temperatures above 0°C. Some death at this level was observed among 5°C. acclimated samples (Table XII). In a preliminary acclimation culture of spring salmon at 3°C., high mortality occurred in the presence of healthy sockeye, chum and coho salmon of the same temperature history. This intolerance of the springs might account for the rapid falling away of the upper lethal temperature for low acclimations, which is not apparent for the coho salmon, so similar in other respects.

The spring and coho salmon had the greatest tolerance and were practically identical in area (529 and 528 degrees C. squared respectively). The sockeye were intermediate (505 degrees C. squared) and the chum salmon least (468 degrees C. squared). The line relating upper lethal temperature to acclimation temperature for the pink salmon was very similar to that for the chum salmon. The apparent intolerance of the pinks to low temperatures would restrict their zone of tolerance even more than in the case of the chums, placing them lowest in order of eurythermal relations. With the possible exception of the pink salmon, and notwithstanding the variable nature of the lines relating lower lethal temperature to acclimation temperature, most of the difference in areas is a result of difference in upper lethal temperature.

DISCUSSION AND CONCLUSIONS

Time and Temperature

Division of response to extremes of temperature into zones of tolerance and resistance, previously set forth for other fishes, has been appropriate for similar distinctions among the Pacific salmon. Although the pattern of resistance to low temperatures was quite different from that for high temperatures, the same factors of tolerance and resistance were equally applicable.

Heilbrunn (1943) has compiled a considerable number of records concerning the temperature at which thermal death occurs in a wide variety of organisms. These have been taken largely from reviews by Kanitz (1915), Uvarov (1931), and Belehradek (1935). In criticism of these data Heilbrunn (p. 420) comments "there are very few useful records of heat (or cold) death temperatures. This is due to the fact that many authors have neglected the time factor". Death from

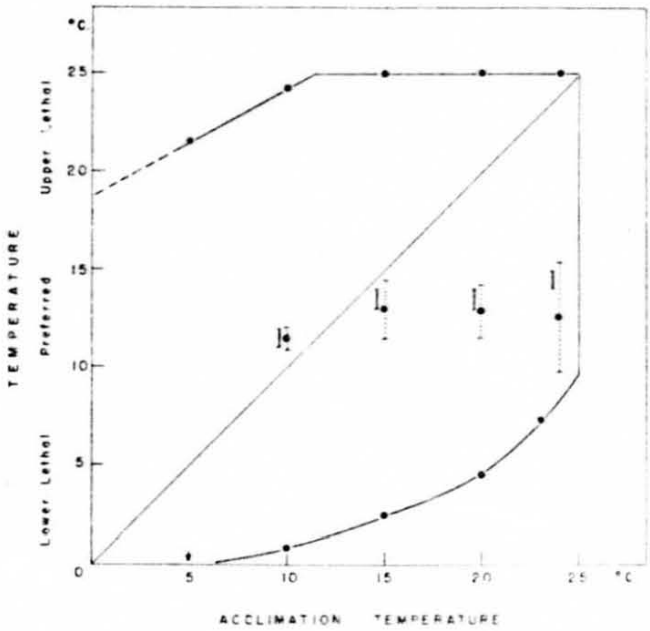

FIGURE 20. Thermal tolerance for young spring salmon in fresh water. The preferred temperatures are represented by a central point for the mean, with limits for one standard deviation dotted above and below. The degree Centigrade in which the mode occurred is represented by a solid vertical line. Where an experiment was performed but resulted in less than 50 per cent mortality a "V" has been inserted.

extremes of temperature is not just dependent on a threshold level below or above which an organism either lives or dies, but may be considered a resultant of both temperature and exposure time. At each lethal level of temperature there is a characteristic rate of dying (rate of mortification, Fry *et al.*, 1946) which may be influenced within limits by acclimation. A threshold level is approached, however, when the rate of dying approaches zero, as in the case calculated for half the sample at the incipient lethal temperature. Consideration of the time beyond which no more mortality may be expected from temperature as a primary cause has been extended in the present study for low lethal temperatures. Temperatures (high or low) which would not cause mortality, regardless of acclimation, can be determined from the zone of tolerance. Very similar limits can also be set

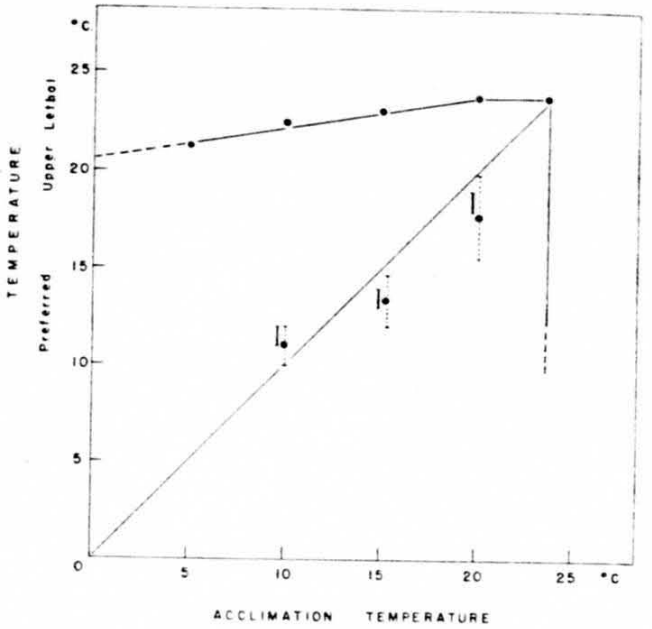

FIGURE 21. Thermal tolerance for young pink salmon. No lower lethal experiments were performed. Preferred temperatures plotted as in Figure 20.

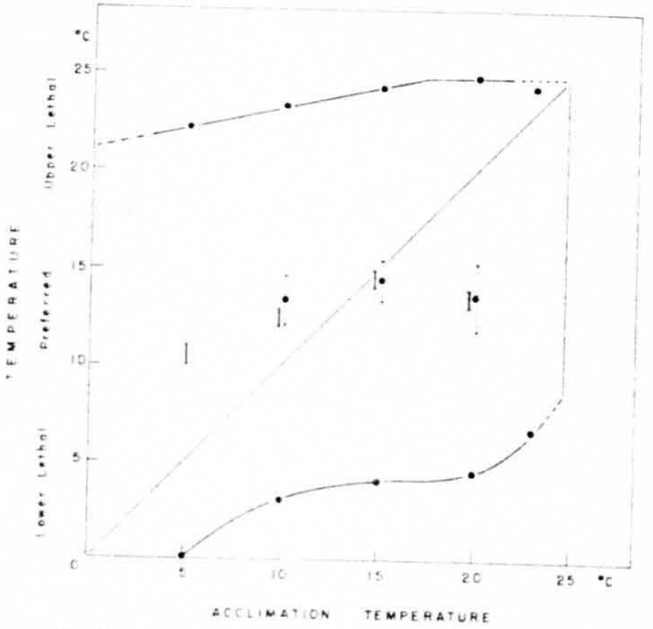

FIGURE 22. Thermal tolerance for young sockeye salmon. Preferred temperatures plotted as in Figure 20.

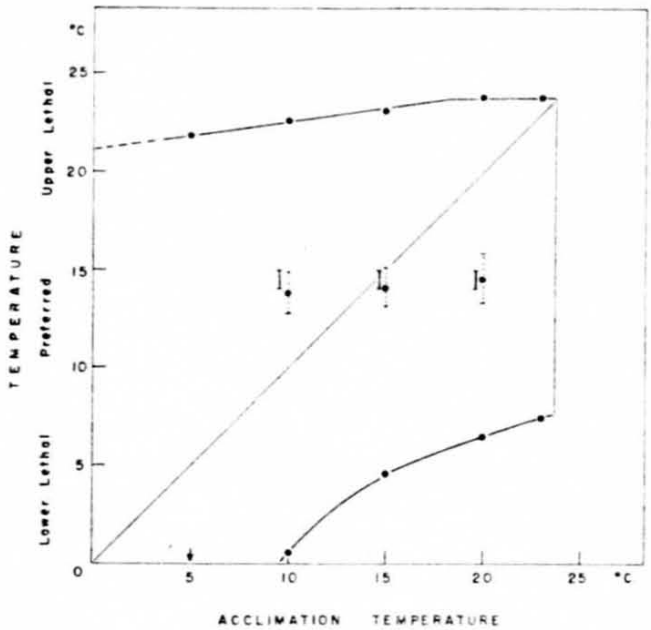

FIGURE 23. Thermal tolerance for young chum salmon. Preferred temperatures and "V" plotted as in Figure 20.

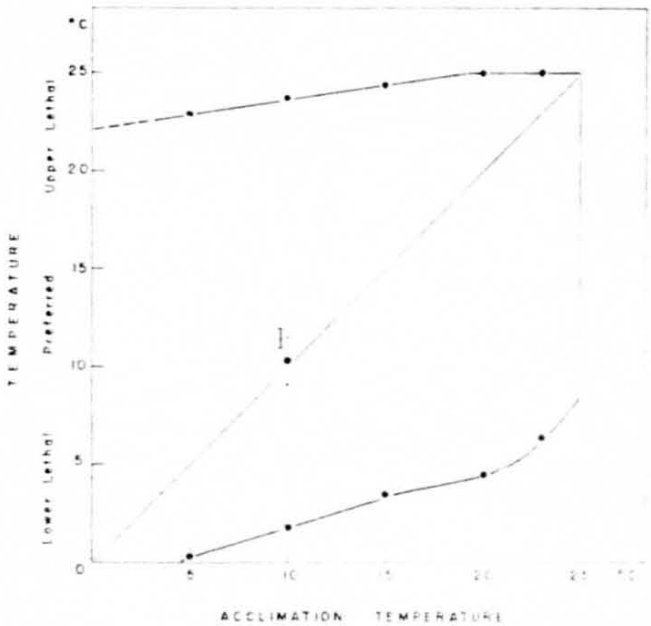

FIGURE 24. Thermal tolerance for young coho salmon. Preferred temperature plotted as in Figure 20.

by examining the resistance times for various acclimation temperatures as in Figure 25 for sockeye salmon. The points beyond which no more deaths are likely to occur have been indicated by a dotted line. Thus, temperatures between 8° and 21°C. are unlikely to cause death among sockeye no matter what the acclimation, nor what the exposure time. The higher or lower the temperature is above or below these limits, the longer the test time necessary to determine the lethal temperature. A somewhat different series of lethal temperatures would have been quoted if no experiments had been continued beyond 24 hours (1,440 min.) for both upper and lower lethal levels.

Future experiments on temperature-tolerance will continue to add to our knowledge of the factors affecting temperature relations in fish. In the investigation of Pacific salmon, emphasis has been placed on uniform treatment of all samples such that, although the methods and later analyses may change, the differences set forth should remain unaltered. One limitation should be pointed out. The results apply to the stocks of salmon from which the eggs were collected (Table I) and may therefore be traced back to comparatively few females. The possibility of variation both within and between local stocks cannot be disregarded.

Comparison with some Other Salmonoids

The resistance times for six species of salmonoids determined for samples acclimated to 20°C. have been plotted in Figure 26 (from Fry, 1947b; present paper). Data for only two of the species of *Oncorhynchus* are included. These two, the spring salmon (*O. tschawytscha*) and the chum salmon (*O. keta*) were respectively the most and least resistant to the same test temperatures; the remaining three species occur at intermediate positions (Figure 18). It will be seen that the two species of *Salmo* occupy positions distinct both from each other and from the remaining species. *Salmo salar*, the Atlantic salmon, is the most resistant of the salmonoid group. The members of the Pacific salmon species lie in a compact series intermediate between *Salmo* and *Cristivomer*, while *Salvelinus fontinalis* approaches the resistance of *Salmo trutta* at the highest test temperatures (28.0° and 28.5°C.) but drops below it for lower temperatures, falling within the range for *Oncorhynchus*. Except for the speckled trout (*Salvelinus fontinalis*), no further comparisons are warranted until more experimental data are obtained. A marked difference in the lower lethal relations between the speckled trout and the Pacific salmon exists, sufficient to suggest a qualitative difference rather than a purely quantitative one. The former was found to be resistant to low temperatures by Fry *et al.*, (1946) who report that the lower lethal temperature "was only just above 0°C. when the acclimation temperature was 24°C". This results in a comparatively large zone of tolerance (625 units) exceeding that for Pacific salmon which ranged from 468 to 529 units.

Some Ecological Relations

Limits of tolerance to extremes of temperature among young Pacific salmon have been determined. Significant differences exist. Whether these differences are sufficient to account for some of the distinctive habits which characterize the different species is a matter of conjecture.

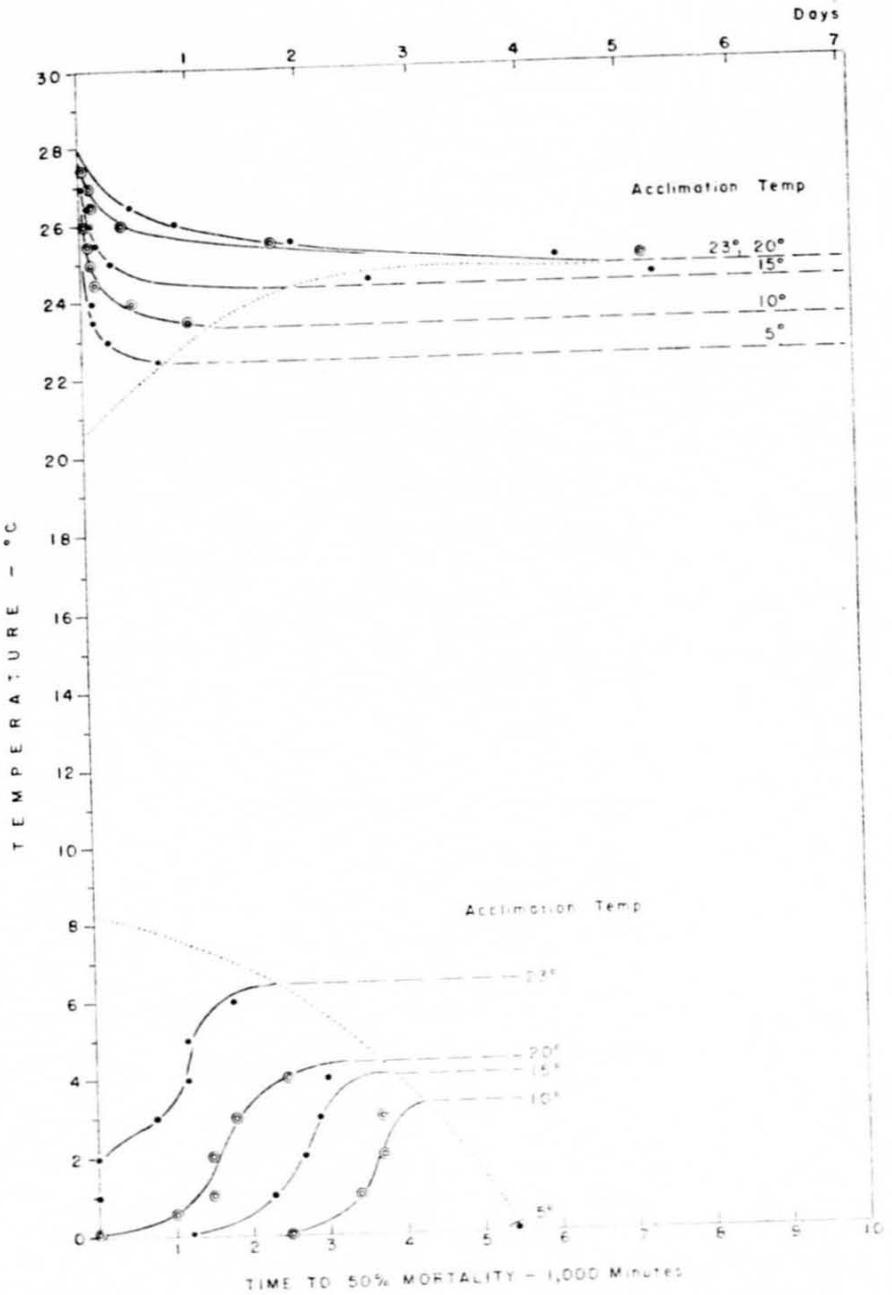

FIGURE 25. Median times to death at high and low temperatures for young sockeye. Broken lines indicate levels of temperature causing little or no mortality for continuous exposure. Dotted lines join the approximate points of inflection.

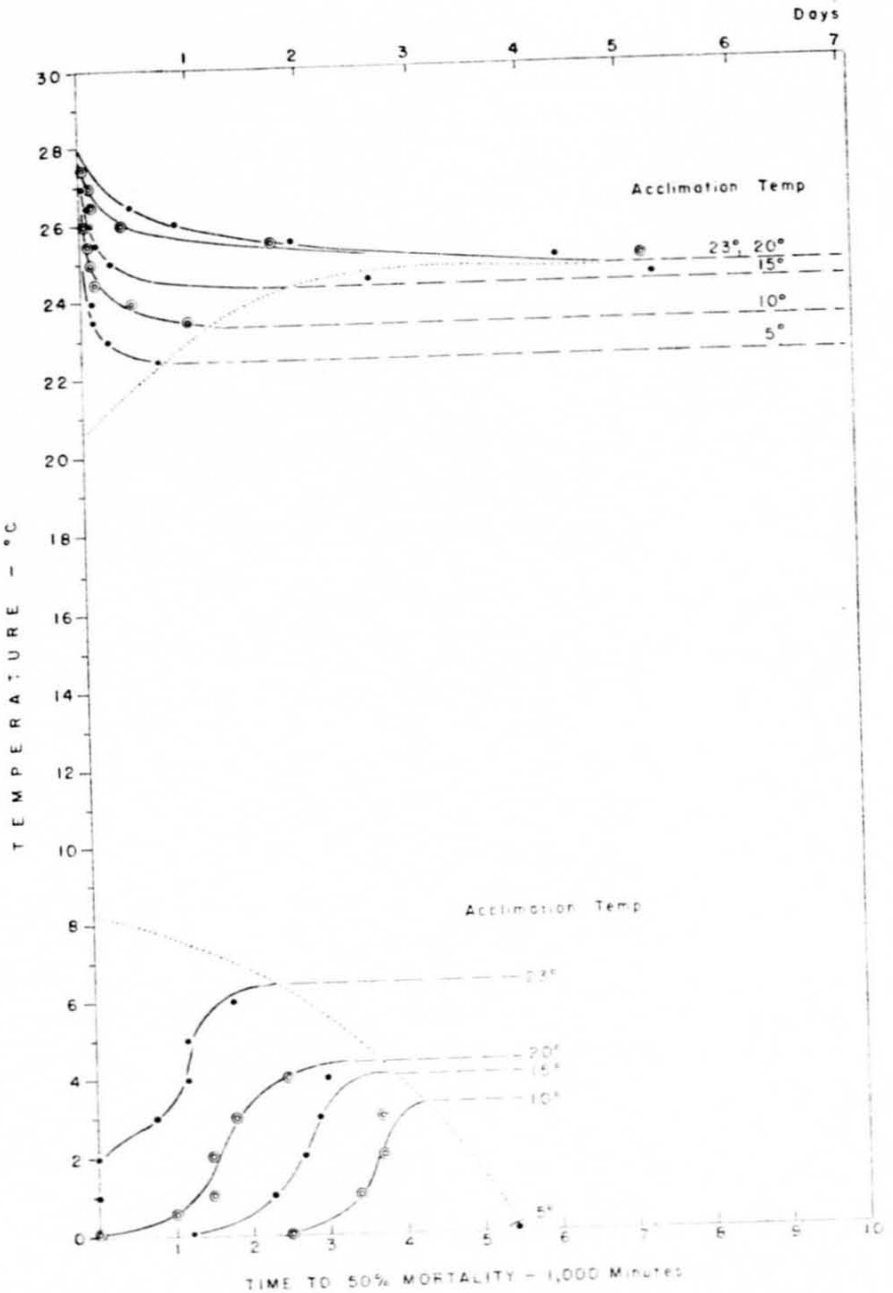

FIGURE 25. Median times to death at high and low temperatures for young sockeye. Broken lines indicate levels of temperature causing little or no mortality for continuous exposure. Dotted lines join the approximate points of inflection.

Young sockeye are usually lake-dwellers, frequenting the open water and subsurface regions, probably in the cooler temperatures in the vicinity of the thermocline (Ricker, 1937). During the summer they are rarely seen or caught in the shallow, littoral zones of the lake (Brett and McConnell, 1950) where young coho, shown to have a higher temperature-resistance, may be found on occasion in abundance. The sockeye migrate in the spring of the year shortly after the ice has left more northern lakes, or following rising spring temperatures

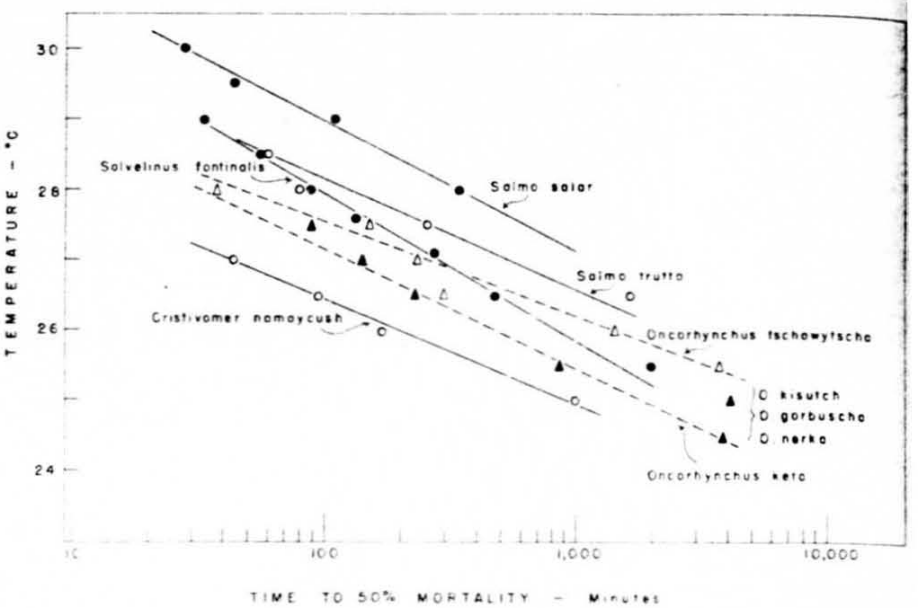

FIGURE 26. Median resistance times for different salmonoids acclimated to 20°C. Data for other than *Oncorhynchus* from Fry (1947).

in such a lake as Cultus which may remain "open" throughout the year (Foerster, 1937). Termination of the migration is closely correlated with ascending surface temperatures and the presence of a well established epilimnion which Foerster (1937) points out may form a temperature block. Coho continue to migrate downstream after sockeye have ceased to move.

Young pink and chum usually do not experience warm lake or river waters. Their migration to sea almost immediately after hatching in the spring of the year eliminates any such high-temperature experience at this stage of life. Their intolerance to fresh water would probably be more pronounced at higher temperatures. The limited success of Chin and Kuroda (1935) in holding chum salmon in fresh water beyond one year depended in part on low temperatures. The interaction of thermal and osmotic stresses requires investigation.

The spring salmon, although a first year migrant to sea, may be found during the summer in streams and rivers, and is more like the coho in this res-

pect. These two species were the most tolerant to high temperatures. The problem of their relative abilities to remain active at high and low temperatures must remain unanswered for the present. Observations, both field and laboratory, indicate a greater ability to feed and sport (jumping, darting, etc.) in warm waters among the spring and coho than among the remaining three species.

On the basis of morphological studies, rates of growth and life history considerations, Milne (1948) concluded that the pink and chum salmon appeared to be the most specialized, and the spring and coho probably the most primitive. This grouping of the species within the genus is in accord with distinctions made on the basis of temperature-tolerance.

SUMMARY

Five species of Pacific salmon are found in North American waters, the spring (*Oncorhynchus tschawytscha*), the pink (*O. gorbuscha*), the sockeye (*O. nerka*), the chum (*O. keta*) and the coho (*O. kisutch*). Young of these species, averaging 4 to 5 cm. in length and 1 gm. in weight, were used in a series of experiments concerning tolerance to high and to low temperatures.

Two months after hatching, each stock of fish was divided into five groups for acclimation to 5°, 10°, 15°, 20° and 23°C. (24°C. in spring and pink); acclimation and lethal-temperature experiments continued throughout the following four months. Resistance times were determined at intervals of 0.5°C. for high temperatures and 1.0°C. for low temperatures (0.0°C. and above). Upper lethal temperatures were calculated for exposures of 10,000 minutes (one week) and lower lethals for exposures of 5,500 minutes (approximately four days).

RESISTANCE TO HIGH TEMPERATURES. A statistical analysis, using the methods of analysis of variance for test temperatures ranging from 24.5° to 27.5°C. and acclimations of 10° to 20°C., for all species, led to the following conclusions:

(1) No significant difference in response to upper lethal levels of temperature exists between spring and coho salmon.

(2) Spring and coho salmon show a highly significant difference in response from that of either sockeye, pink or chum ($P < .01$).

(3) Pink and chum salmon show a barely significant difference from each other, but not from sockeye in either case ($P = .05$).

(4) There is a very highly significant ($P < .01$) linear fit of the logarithmic relation for these data (logarithm of the median time to death in relation to the temperature causing that death).

(5) The slopes of the lines relating log resistance time to temperature for the five species are not significantly different.

(6) The same relation can be applied to each species with equal confidence, differing only in the temperature level at which this relation exists.

The ultimate upper lethal temperatures for each species were: spring—25.1°C., coho—25.0°C., sockeye—24.4°C., pink—23.9°C., chum—23.8°C.

CRITERION OF DEATH AT LOW TEMPERATURES. It was discovered that with the approach of death from a low temperature the opercula commence to fan out perceptibly. This criterion was shown to be significant when compared with data

from recovery tests at warmer temperatures. Thus, the individual times to death at given low temperatures could be recorded and median resistance times determined for each sample.

RESISTANCE TO LOW TEMPERATURES. The relation between resistance time to low temperature and the level of that temperature has not been resolved into a simple equation as has been the case for heat-tolerance relations. A variable but usually sigmoid to double sigmoid pattern characterized the curves when plotted on normal axes. An initial period of rapidly increasing resistance to low temperature was followed by relatively little change in resistance for two and three degrees increase in temperature (15° to 23°C. acclimation). No mortality usually occurred within a degree or so above this latter zone for a considerably increased exposure time up to 5,500 minutes.

The young salmon were very sensitive to low temperatures. Among the four species tested, the coho and sockeye salmon could not tolerate long exposure (four days) to 0°C. even when taken from holding temperatures as low as 5°C.

The lower lethal temperatures for the highest acclimation, 23°C., were: spring—7.4°C., coho—6.4°C., sockeye—6.7°C., chum—7.3°C.

MIXED LETHAL EFFECT OF LOW TEMPERATURES. From acclimation temperatures of 20°C. and above, mixed responses were noted in the lethal baths. A rapid death of all fish occurred at the lowest temperatures. Temperatures slightly above this level caused rapid death in part of the sample followed by a long delay and then death of the remainder. Temperatures somewhat higher, yet still low enough to cause death, did so only after prolonged exposure. By plotting the data on probability × logarithmic paper it was possible to discriminate clearly between the two trends of death. Exposure to the same low temperatures in lethal baths containing 9.9% sea water instead of fresh water (slightly hypertonic) resulted in partially increased tolerance among sockeye salmon.

It therefore appears that three causes for death may be involved: one, a very rapid agent usually effective within 60 minutes of exposure, a second not so rapid in action, and a third which is related to osmotic balance.

SIZE EFFECT. No significant difference in the size of the first and last fish to die from high temperatures was present. For death from low temperatures, however, the size distribution of the dead fish from samples in which 30 to 50 per cent of the fish died showed a significantly lower mean length than in the balance of living fish. The earlier death among smaller fish appeared to be partly the result of greater susceptibility to "rapid" cold-death.

ZONES OF THERMAL TOLERANCE. The zones of thermal tolerance were constructed graphically and the areas calculated in units of degrees Centigrade squared. The high degree of sensitivity to low temperatures, almost confining these species to acclimation temperatures above 0°C., results in a comparatively low thermal tolerance rating. The spring and coho were almost identical, with 529 and 528 units respectively; the sockeye was next with 505 units; followed by the chum with 468 units. Lacking low lethal-temperature data, the pink salmon cannot be included, but one preliminary experiment suggested a lower resistance than in the chum salmon, and consequently a smaller zone of tolerance. Even

the most tolerant of the Pacific salmon was considerably below the speckled trout *Salvelinus fontinalis*, with a calculated area of 625 units.

PREFERRED TEMPERATURES. Comparatively little difference in preferred temperature was recorded experimentally, either between species or for differences in acclimation amounting to 15°C. in some instances. On the average, no greater difference than 3°C. (11° to 14°C.) was displayed between means from different acclimations. The region of greatest preference lay in the 12° to 14°C. stratum.

COMPARISON OF TEMPERATURE TOLERANCE. In making comparisons, most emphasis was placed on the resistance times to high temperatures. Experiments by previous investigators with other salmonoids demonstrate a greater heat resistance in two species of *Salmo* and lesser resistance in *Cristivomer namaycush* than in the *Oncorhynchus* group (all acclimated to 20°C.). *Salvelinus fontinalis* is more resistant to temperatures of 27°C. and above than any of the Pacific salmon; however, below this level, the generic distinction does not apply.

GENERAL CONCLUSIONS. The species of Pacific salmon are comparatively stenothermal. An intolerance to low temperatures particularly restricts their biokinetic range. For prolonged exposure (up to one week) to high temperatures the spring and coho salmon were most resistant, the sockeye intermediate and the pink and chum salmon least resistant. These differences are in keeping with taxonomic conclusions and certain ecological distinctions.

REFERENCES

BELEHRADEK, J. Temperature and living matter. *Protoplasma Monographien*, **8**: 1-229, Berlin, 1935.

BINET, L., AND G. MORIN. Action de la chaleur sur les poissons. *Jour. de Physiol. et de Pathol. Gén.*, **32**: 272-379, 1934.

BLISS, C. I. The calculation of the dosage-mortality curve. *Ann. Appl. Biol.*, **22**: 134-167, 1935a.

The comparison of dosage-mortality data. *Ann. Appl. Biol.*, **22**: 307-333, 1935b.

Calculation of the time-mortality curve. *Ann. Appl. Biol.*, **24**: 815-852, 1937.

BOLTT, J. R. Tempering versus acclimation in the planting of speckled trout. *Trans. Am. Fish. Soc.*, **70**: 397-403, 1941.

Some lethal temperature relations of Algonquin Park fishes. *Univ. Toronto Stud., Biol. Ser.*, No. 53 (*Pub. Ont. Fish. Res. Lab.*, No. 63), 49 pp., 1944.

Rate of gain of heat-tolerance in goldfish (*Carassius auratus*). *Univ. Toronto Stud., Biol. Ser.*, No. 52 (*Pub. Ont. Fish. Res. Lab.*, No. 64), 9-28, 1946.

BURROWS, R. E. Fish-cultural procedures for use at the Grand Coulee stations. *Western Fish-Cultural Investigations*, etc., 46 pp., Portland, Oregon (no date, mimeographed).

CHIN, Z., AND K. KUROTA. Sur l'élevage de saumon (*Oncorhynchus keta*). Walbaum) pendant les longues durées dans l'eau douce. *Keijo Jono. Med.*, **6**: 30-40, Keijo, Japan, 1935.

DAVENPORT, C. B., AND W. E. CASTLE. Studies on morphogenesis. III. On the acclimatization of organisms to high temperature. *Arch. Entwicklungsmech.*, **2**: 227-249, 1895.

DAY, F. Effects of elevated temperature on fishes. *U.S. Fish. Comm. Bull.*, **5**: 142-144, 1885.

DEHORNOFF, P. Reactions of marine fishes to temperature gradients. *Biol. Bull.*, **75**: 494-509, 1938.

The resistance and acclimation of marine fishes to temperature changes. I. Experiments with *Gillichthys mirabilis* (Ayres). *Biol. Bull.*, **83**: 219-244, 1942.

The resistance and acclimatization of marine fishes to temperature changes. II. Experiments with *Fundulus* and *Atherinops*. *Biol. Bull.*, **88**: 2-, 194-206, 1945.

DUMONT, J. R. Some fresh-water fishes of British Columbia. *Rep. B.C. Comm. Fish.*, **1935**: 66-73, 1936.

ELLIS, C. H. Salmon hatcheries. *Ann. Rep. Washington, Dept. Fish.*, **1948**, 59-82, 1948.

FISHER, R. A. The design of experiments. 4th ed., Edinburgh: Oliver and Boyd, 1945.

FOERSTER, R. E. The relation of temperature to the seaward migration of young sockeye salmon (*Oncorhynchus nerka*). *J. Biol. Bd. Can.*, **3**(5), 421-438, 1937.

FRY, F. E. J. Effects of the environment on animal activity. *Univ. Toronto Stud., Biol. Ser.*, No. 55 (*Pub. Ont. Fish. Res. Lab.*, No. 68), 62 pp., 1947a.

———. Temperature relations of salmonoids. *Proc. Nat. Com. Fish. Cult., 10th meet.*, **1947**, Appendix "D", 1947b.

FRY, F. E. J., J. R. BRETT AND G. H. CLAWSON. Lethal limits of temperature for young goldfish. *Rev. Can. de Biol.*, **1**, 50-56, 1942.

FRY, F. E. J., J. S. HART AND K. F. WALKER. Lethal temperature relations for a sample of young speckled trout, *Salvelinus fontinalis*. *Univ. Toronto Stud., Biol. Ser.*, No. 54 (*Pub. Ont. Fish. Res. Lab.*, No. 66), 9-35, 1946.

GRAHAM, J. M. Some effects of temperature and oxygen pressure on the metabolism and activity of the speckled trout, *Salvelinus fontinalis*. *Can. Jour. Res.*, D, **27**, 270-288, 1949.

HART, J. S. Lethal temperature relations of certain fish of the Toronto region. *Trans. Roy. Soc. Can.*, **41**(3), 57-71, 1947.

———. Geographic variations of some physiological and morphological characteristics in fish. *Ph.D. thesis, Dept. of Zool., Univ. of Toronto*, 175 pp., 1949.

HATHAWAY, E. S. Quantitative study of the changes produced by acclimation on the tolerance of high temperatures by fishes and amphibians. *Bull. U.S. Bur. Fish.*, **43**(2), 169-192, 1927.

HEILBRUNN, L. V. An outline of general physiology. 2nd ed., Philadelphia: W. B. Saunders, 748 pp., 1943.

HOAR, W. S., AND G. M. BELL. The thyroid gland in relation to seaward migration of Pacific salmon. *Can. Jour. Res.*, D, **28**, 126-136, 1950.

HUNTSMAN, A. G. Death of salmon and trout with high temperature. *J. Fish. Res. Bd. Can.*, **5**, 485-501, 1942.

HUNTSMAN, A. G., AND W. S. HOAR. Resistance of Atlantic salmon to sea water. *Jour. Fish. Res. Bd. Can.*, **4**, 409-411, 1940.

HUNTSMAN, A. G., AND M. I. SPARKS. Limiting factors for marine animals. III. Relative resistance to high temperatures. *Contr. Can. Biol.*, **2**, 95-114, 1924.

KANITZ, A. Temperatur und Lebensvorgange. Berlin, 1915.

LITCHFIELD, J. T., JR., AND F. WILCOXON. A simplified method of evaluating dose-effect experiments. *Jour. Pharm. and Exp. Therapeut.*, **95**(2), 99-113, 1949.

LOEB, J., AND H. WASTENEYS. On the adaptation of fish (*Fundulus*) to higher temperatures. *Jour. Exp. Zool.*, **12**, 543-557, 1912.

MAUREL, E., AND LAGRIFFE. Action comparée de la chaleur et du froid sur certains poissons. *C. R. Soc. Biol.*, **51**, 915-918, 1899.

MILNE, D. J. The growth, morphology and relationship of the species of Pacific salmon and the steelhead trout. *Ph.D. Thesis, Dept. Zool., McGill Univ.*, 101 pp., 1948.

OGLE, C., AND C. A. MILLS. Animal adaptation to environmental temperature conditions. *Amer. Jour. Physiol.*, **103**, 606-612, 1933.

REGNARD, P. Action des très basses températures sur les animaux aquatiques. *C. R. Soc. Biol.*, 2, 652-653, 1895.

RICKER, W. E. Physical and chemical characteristics of Cultus Lake, British Columbia. *J. Biol. Bd. Can.*, **3**, 363-402, 1937.

SNEDECOR, G. W. Statistical methods. 4th ed., 1-485, Iowa State College Press, 1946.

SUMNER, F. B., AND P. DOUDOROFF. Some experiments upon temperature acclimatization and respiratory metabolism in fishes. *Biol. Bull.*, **74**, 403-429, 1938.

SULLIVAN, C. M. Aspects of the physiology of temperature selection in speckled trout (*Salvelinus fontinalis*). *Ph.D. thesis, Dept. of Zool., Univ. of Toronto*, 1-177, 1949.

UVAROV, B. P. Insects and climate. *Trans. Entomol. Soc. London*, **79**, Part 1, 1931.

WEIGMANN, R. Die Wirkung starker Abkühlung auf Amphibien und Reptilien. *Zeitschr. f. wissenschaft. zool.*, **134**, 641-692, 1929.

Weitere Untersuchungen über die Kältebeständigkeit poikilothermer Wirbeltiere. *Zeitschr. f. wissenschaft. Zool.*, **136**, 195-209, 1930.

Zur Kältebeständigkeit poikilothermer Tiere, Untersuchung an Schnecken und Fischen. *Biol. Zentralblatt*, **56**, 301-322, 1936.

WILDER, D. G. A comparative study of anadromous and fresh water populations of the eastern speckled trout (*Salvelinus fontinalis*). *Ph.D. thesis, Univ. of Toronto*, 1944.

YATES, F. The design and analysis of factorial experiments. Harpenden: Imperial Bureau of Soil Science, 1937.

APPENDIX I. STATISTICAL PROCEDURE

The methods of measuring the various indices, resistance time, lethal temperature, etc., have been outlined. The variability which was observed within the samples and its application to the variability which might be expected for the species, together with an analysis of the significance of the specific differences, must be considered on statistical grounds. For convenience these may be dealt with under separate headings.

Sources of Variability

The sources of variability fall roughly into two categories, controlled and uncontrolled. Among the controlled may be listed *acclimation temperature, lethal bath temperature* and *diet*. These are strictly problems in experimental procedure, and their constancy is a matter of precision of technique.

Among the uncontrolled, the variables of *sex, size* and *age* form a group of somewhat different character from the remainder. It was impossible to determine the sex readily at such an immature stage as the fry, and consequently its effect, if present, falls into the uncontrolled individual variability. It seems unlikely that any concern should be expressed over this lack of separation since sex differences in thermal tolerance have rarely been demonstrated even in mature fish (Hart, 1949), but recent work on the guppie does indicate a possible sex factor linked with an inherent size difference between sexes (M. B. Gibson, personal communication).

The age within species was identical for any one experiment and only slightly different between species (Table II). Control of this variable was observed, therefore, to the greatest degree experimentally possible. Size differences have usually been associated with age differences and their separate influences not analysed. In general, for upper lethal experiments, where size has been found to produce an effect, the larger fish apparently have been more susceptible (Day, 1885; Huntsman and Sparks, 1924; Huntsman, 1942); however, no size effect has been reported under imposed experimental conditions providing uniform acclimations for gobies (Sumner and Doudoroff, 1938), speckled trout (Fry *et al.*, 1946) and various fresh-water fish (Hart, 1947). Analysis of the size differences for upper temperature-tolerance in Pacific salmon also has shown no effect (Table XIII). Nevertheless tolerance to low temperature appears to be complicated by size.

The remaining uncontrolled factors are those which may be attributed to individual variation despite similar acclimation, lethal-bath temperature and diet, and the interaction of chance variates. All of these are lumped as "error" components. The smaller the "error", the greater the chances become for detecting differences which might otherwise be masked through contaminating effects. It is towards the elimination of these latter confounding elements that the design of experiments and the methods of analysis of variance have contributed so profoundly (consult: Yates, 1937; Fisher 1945, 1948; Snedecor, 1946, and others).

Resistance Times

The design of the experiments outlined has been to treat five species (lots) of Pacific salmon with five levels of temperature-acclimation (treatments) and to test samples of these species for comparison of resistance to temperature. Since the same acclimations and lethal-test temperatures were used for each species, it was possible to consider the data for the following relations and the differences exhibited thereby, for example,

1. Species × lethals, for each acclimation,
2. Species × acclimations, for each lethal level,
3. Lethals × acclimations, for each species.

From a measure of the unresolved variability (error) the significance of the resolved components may be tested (F test) using the following method of compilation:

1. Comparison of species by times to death at high temperatures.

Analysis of variance.

Lethals = lethal test temperatures.

Acclimations = acclimation temperatures.

x = log median resistance time. Median resistance time determined by plotting the individual times to death on probability × logarithmic paper. This is equivalent to the geometric mean time to death (Fry *et al.*, 1946); by use of the median time, however, cases in which less than 100 per cent (but more than 50 per cent) mortality occurred can be used for comparison.

Tx = total median resistance times.

S = sum of all items.

G = grand total.

d.f. = degrees of freedom = $(n - 1)$, $(k - 1)$.

c = correction factor = G^2/nk.

Symbolic Table:

		Species					
		1	2	3	...	n	
Lethals or Acclimations	1	$Tx1$				Txn	S_1
	2	⋮					S_2
	3	⋮					
	k	Txk					S_k
		S_1	S_2	...		S_n	G

Sum of Squares:

$$(1)\ \text{Between species} = \underset{1}{\overset{n}{S}}\left[\underset{1}{\overset{k}{S}}(Tx)^2/k\right] - c$$

$$(2)\ \text{Between lethals} = \underset{1}{\overset{k}{S}}\left[\underset{1}{\overset{n}{S}}(Tx)^2/n\right] - c$$

$$(3)\ \text{Error} = (4) - [(1) + (2)]$$

$$(4)\ \text{Total} = \underset{1}{\overset{nk}{S}}(Tx)^2 - c$$

2. Table of multipliers used for orthogonal comparison between each species using total log median resistance times for all lethals and all acclimations per species.

Species	1	2	3	4	5
Multipliers	1	1	0	− 1	− 1
	1	1	− 4	1	1
	1	− 1	0	1	− 1
	1	− 1	0	− 1	1

3. Table of multipliers used for orthogonal comparison between lethals when testing for linear fitness (Fisher and Yates, 1948).

Lethals	1	2	3	4	5	6
Multipliers	5	3	1	− 1	− 3	− 5
	5	− 1	− 4	− 4	− 1	5
	5	− 7	− 4	4	7	− 5
	1	− 3	2	2	− 3	1
	1	− 5	10	− 10	5	− 1

Lethal Temperatures

The lethal temperature is the temperature for one particular resistance time, theoretically *infinite time*, for half the population. If sufficiently large samples could be used in a series of lethal baths of very finely divided temperature difference, and the experiment continued until mortality from an "over-dose" of temperature had ceased, the temperature of the tank containing just 50 per cent of the original sample would be the lethal temperature. Furthermore, it might

well be expected that the susceptibility to the different levels of temperature (dosage), as measured by the number of survivors, would be normally distributed, if considered in relation to the logarithm of the corresponding temperature (following Bliss, 1935a, b). Just as the individual susceptibility to temperature is normally distributed[1] when measured by time to die at a given temperature, so the mortalities in the samples are also normally distributed[1] with temperature. This relation has provided a means of testing the results from small samples for agreement with the postulated normal distribution by a chi-square test (Bliss, 1935b) and of estimating the lethal temperature with limits of error (standard error in this case) for the population from which the sample has been taken. The rather lengthy mathematics involved have been simplified by graphical means (Litchfield and Wilcoxon, 1949).

Application of the above methods is limited for the present lethal-temperature determinations. Three or more cases in which between 0 and 100 per cent survival persists at the end of an experiment are required to provide sufficient independent comparisons for the particular table of chi-square to be used. To achieve these comparisons an increment of temperature difference smaller than the currently used 0.5°C. interval would be required, probably of the order of 0.2° or 0.1°C. The cases in which appropriate testing of the data has been applied are indicated in the appropriate tables. Otherwise the best straight line has been fitted by eye and the limits of variability corresponding to ± (5.0 − 4.0) *on Probit scale square root of the number in the sample* quoted, that is, a tentative standard error.

Preferred Temperatures

Temperature selection in a gradient has frequently been expressed by illustrating graphically the number of times one or more fish have been observed in given sections or compartments of a test tank (Doudoroff, 1938; Sullivan, 1949). The preferred or selected temperature has been either the temperature interval in which the modal group occurred or the temperature corresponding to the mean position occupied by the fish. If the mean and the mode correspond, distribution of the fish above and below the preferred temperature tends to be more symmetrical than might be expected if they are separate. In the figures on preferred temperatures the mean ± the standard deviation, and the mode have been presented. The method for calculating the mean was simply to multiply the number of fish recorded for each cell by the temperature at the midpoint of that cell and divide by the total number of observations, extending the calculations to determine the standard deviation in the usual fashion.

[1]Since the relation is best described by a straight line when expressed in terms of probability units.

TABLE IV. Median resistances time to high temperatures among young Pacific salmon acclimated to various temperatures.

	Acclim. Temp. (°C.)	Lethal test temperatures in °C. and median resistance times in minutes											
		27.5	27.0	26.5	26.0	25.5	25.0	24.5	24.0	23.5	23.0	22.5	22.0
	21	330	655	1120	2440	5100	12300						
	20	150	235	305	1460	3750	10700						
Spring	15		100	170	300	665	2500						
	10			52	95	175	335	660					
	5						38	48	75	90	175	205	
	21	200		985	1900	2660	2920	5000					
	20	110	170	190	820	3260	3500	4500	6200				
Pink	15		80	115	180	420	580	725	1040	2700			
	10			65	65	95	145	235	440	800	1100		
	5								12		35	35	105
	23			675	1215	2760	6190	7410					
	20	95	150	185	550	1920	7300	7900					
Sockeye	15	45	85	130	160	210	400	3740					
	10			12	72	105	150	175	660	1200			
	5								135	130	340	1000	
	23		305		2200		4260		9000				
	20	90	112	235	590	880	4200	3900	7000	6200			
Chum	15		52	75	150	210	700	5100	1200	2300	8000		
	10			40	63	100	160	245	900		9800	880	
	5								45		145	200	650
	23	360	590	1100	2810	4760	9000						
	20	90	230	420	2630	2100	8800						
Coho	15		122	170	360	115	2430	6000					
	10				88	125	340	880					
	5								160		1000		

TABLE V. Median resistance times to low temperatures among young Pacific salmon acclimated to various temperatures. (" > " signifies the median resistance time was less than the time entered: " < " more than time entered.)

	Acclim. Temp. (°C.)	Lethal test temperatures in °C. and median resistance times in minutes										
		0.0	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.0	6.0	7.0
Spring	23			15	200	300	300	500		900	1100	
	20		15	250	400	500	< 1500	< 1500	2300			
	15		800			3200						
	10	1200										
Sockeye	23			10		15		790	1200	1200	1800	
	20	15	900	1500		1500		1800	2500			
	15	1200		2300		2700		2900	3000 >			
	10	2500		3400		3700		3700				
	5	5500										
Chum	23	10		600		850		800	1100	1200	1400	3900
	20	15		300		1000		900	1750	2300	3280	
	15	1350		1850		4200		4000	4500			
	10	5500										
Coho	23			45		1100		1000	1100	1700	3000	
	20	350		1600		1300		1300	5500			
	15	1250		1700		3100		3200				
	10	3200		4000								
	5	4300										

TABLE VI. Results from analysis of variance of resistance times (logarithms) to various lethal-test temperatures among species of Pacific salmon acclimated to different temperatures.

Acclim. Temp.	Comparisons	Sum of squares	Degrees of freedom	Variance	Value of F ratio	Value for F = .05	Value for F = .01
20°C.	Between species (spring, coho, sockeye, pink, chum)	51.332	4	12.833	5.15**	2.87	4.43
	Between lethals (25.0, 25.5, 26.0, 26.5, 27.0, 27.5°C.)	1147.743	5	229.549	91.93**	2.71	4.10
	Species × lethals (error)	49.944	20	2.497			
	Total	1249.019	29				
15°C.	Between species (spring, coho, sockeye, pink, chum)	77.646	4	19.412	9.43**	3.01	4.77
	Between lethals (25.0, 25.5, 26.0, 26.5, 27.0°C.)	320.367	4	80.092	38.82**	3.01	4.77
	Species × lethals (error)	33.009	16	2.063			
	Total	431.021	24				
10°C.	Between species (spring, sockeye, pink, chum)	28.424	3	9.475	14.05**	3.86	6.99
	Between lethals (24.5, 25.0, 25.5, 26.0°C.)	78.379	3	26.116	38.75**	3.86	6.99
	Species × lethals (error)	6.062	9	0.674			
	Total	112.864	15				

** Highly significant.

TABLE VII. Results from analysis of variance-of-resistance times (logarithms) for variously acclimated Pacific salmon when tested at given lethal temperatures.

Lethal Temp.	Comparisons	Sum of squares	Degrees of freedom	Variance	Value of F ratio	Value for F = .05	Value for F = .01
26.5°C.	Between species (spring, coho, sockeye, pink, chum)	17.149	4	4.287	4.02*	3.84	7.01
	Between acclimations (20, 15, 10°C.)	167.633	2	83.817	78.55**	4.46	8.65
	Species × acclimations (error)	8.533	8	1.067			
	Total	193.316	14				
26.0°C.	Between species (spring, coho, sockeye, pink, chum)	37.100	4	9.275	6.98**	3.84	7.01
	Between acclimations (20, 15, 10°C.)	318.434	2	159.217	119.80**	4.46	8.65
	Species × acclimations (error)	10.629	8	1.329			
	Total	366.162	14				
25.5°C.	Between species (spring, coho, sockeye, pink, chum)	35.104	4	8.776	4.48*	3.84	7.01
	Between acclimations (20, 15, 10°C.)	388.772	2	194.385	99.13**	4.46	8.65
	Species × acclimations (error)	15.692	8	1.961			
	Total	439.567	14				
25.0°C.	Between species (spring, coho, sockeye, pink, chum)	89.173	4	22.293	6.21*	3.84	7.01
	Between acclimations (20, 15, 10°C.)	549.067	2	274.534	76.51**	4.46	8.65
	Species × acclimations (error)	28.702	8	3.588			
	Total	666.942	14				

* Significant.

** Highly significant.

TABLE VIII. Results from analysis of variance-of-resistance times (logarithms) to various lethal-test temperatures at different acclimations for five species of Pacific salmon.

Species	Comparisons	Sum of squares	Degrees of Freedom	Variance	Value of F ratio	Value for F = .05	Value for F = .01
Spring	Between lethals (26.5, 26.0, 25.5, 25.0°C.)	226.246	3	75.416	25.89**	4.76	9.78
	Between acclimations (20, 15, 10°C.)	288.676	2	144.338	49.55**	5.14	10.92
	Lethals × acclimations (error)	17.476	6	2.913			
	Total	532.398	11				
Coho	Between lethals (26.5, 26.0, 25.5, 25.0°C.)	184.087	3	61.362	29.26**	4.76	9.78
	Between acclimations (20, 15, 10°C.)	320.222	2	160.111	76.33**	5.14	10.92
	Lethals × acclimations (error)	12.580	6	2.097			
	Total	516.889	11				
Sockeye	Between lethals (26.5, 26.0, 25.5, 25.0°C.)	134.045	3	44.682	6.03*	4.76	9.78
	Between acclimations (20, 15, 10°C.)	262.406	2	131.203	17.76**	5.14	10.92
	Lethals × acclimations (error)	44.483	6	7.414			
	Total	440.933	11				
Pink	Between lethals (26.5, 26.0, 25.5, 25.0°C.)	81.676	3	27.225	12.48**	4.76	9.78
	Between acclimations (20, 15, 10°C.)	300.421	2	150.210	69.71	5.14	10.92
	Lethals × acclimations (error)	13.091	6	2.182			
	Total	395.188	11				
Chum	Between lethals (26.5, 26.0, 25.5, 25.0°C.)	120.262	3	40.087	15.79**	4.76	9.78
	Between acclimations (20, 15, 10°C.)	209.318	2	104.659	41.22	5.14	10.92
	Lethals × acclimations (error)	15.234	6	2.539			
	Total	344.814	11				

* Significant. ** Highly significant.

TABLE IX. Comparison between each species of Pacific salmon acclimated to 20°C. and subjected to various lethal-test temperatures from 25.0° to 27.5°C. This constitutes a breakdown of the significant difference between species to single degrees of freedom and a comparison for significance with the error term, 2.497, with 20 degrees of freedom, recorded in table VI.

Comparisons (orthogonal)	Variance	F ratio	F(P = .05)	F(P = .01)
1. (Spring—coho)—(pink—chum)	33.051	13.22**	4.35	8.10
2. (Spring—coho—pink—chum)—(4 sockeye)	6.360	2.54	"	"
3. (Spring—pink)—(coho—chum)	7.508	3.02	"	"
4. (Spring—chum)—(coho—pink)	4.410	1.76	"	"
Comparisons (non-orthogonal)				
1. (Spring)—(coho)	0.204	0.08	4.35	8.10
2. (Spring—coho)—(2 sockeye)	21.6	8.65**	"	"
3. (Pink—chum)—(2 sockeye)	0.002	0.001	"	"
4. (Pink)—(chum)	11.8	4.72*	"	"

* Significant. ** Highly significant.

TABLE X. Comparison between linear and quadratic components for relation of logarithm of resistance time to lethal-test temperature. This constitutes a breakdown of the significant difference between lethals recorded in table VI.

Components	Variance	F ratio	F(P = .05)	F(P = .01)
1. Linear components	1125.133	450.**	4.35	8.10
2. Quadratic component (1st)	15.429	6.180*	"	"
3. Quadratic component (2nd)	0.656	0.262	"	"
4. Quadratic component (3rd)	0.114	0.045	"	"
5. Quadratic component (4th)	6.022	2.410	"	"
Curvature (av. of 2 to 5)		2.22	2.87	4.43

* Significant. ** Highly significant.

Table XIII. Mean lengths and order of death of 820 young Pacific salmon used in upper lethal experiments.

Species	Order of death and mean lengths (cm.)					Significance of difference between means 1 and 5		
	1	2	3	4	5	t.	d.f.	P.
Spring	4.43	4.40	4.50	4.40	4.46	0.027	74	0.9
Pink	3.78	3.81	3.78	3.83	3.84	0.065	62	0.9
Sockeye	4.37	4.61	4.53	4.52	4.41	0.032	70	0.9
Chum	5.64	5.56	5.43	5.43	5.15	0.148	66	0.9
Coho	4.72	4.95	4.84	4.84	4.57	0.108	46	0.9

Table XIV. Comparison of mean lengths of live and dead Pacific Salmon from experiments on low-temperature tolerance.

	Mean length	Standard error	Number
	cm.	*cm.*	
Live	5.003	0.046	170
Dead	4.748	0.043	171
Mean (diff.)	0.255		
S.E. (diff.)		0.063	
Normal deviate	4.05		
Probability	.0001		