UNIVERSITY OF TORONTO STUDIES
BIOLOGICAL SERIES, No. 54

LETHAL TEMPERATURE RELATIONS FOR A SAMPLE OF YOUNG SPECKLED TROUT, *SALVELINUS FONTINALIS*

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PUBLICATIONS OF THE
ONTARIO FISHERIES RESEARCH LABORATORY, No. 66

TORONTO
THE UNIVERSITY OF TORONTO PRESS
1946

CONTENTS

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Introduction

Lethal temperatures have been given quantitative assessment in the laboratory in various ways. Such measurements have been the temperature reached before death when heated at a certain constant rate (e.g. Huntsman and Sparks, 1924), the average survival time at a given constant temperature (Loeb and Wasteneys, 1912), and the temperature at which a given percentage survive a given length of time (Hathaway, 1927).

While all these indices are valid for physiological comparison insofar as it may be presumed that identical material treated identically in any one of these ways should always give an identical result, nevertheless no one of them can give a complete answer as to the nature of the difference between groups which when treated identically, behave in a different manner. These indices taken singly are still less satisfactory as laboratory measures designed to predict the limitations natural conditions may impose on the organism.

The range of any environmental factor in which life of an organism is possible at all may be divided into two zones. Within certain limits the animal can live indefinitely. We propose to call this zone the *zone of tolerance*, and the levels demarcating this zone the *upper* and *lower incipient lethal levels* respectively. At levels beyond the zone of tolerance the organism will be able to exist for a period of time that will depend on the level of the lethal factor. The length of time that an organism can resist the effects of a level of an environmental factor which is beyond its zone of tolerance for that factor we propose to call the *resistance time*. Applying these general definitions to temperature in particular we propose to speak of a zone of *thermal tolerance* bounded by *upper* and *lower* incipient lethal temperatures[1] and a zone of *thermal resistance* beyond these temperatures.

[1]These are equivalent to the ultimate median tolerance limits of Doudoroff, 1945. We prefer not to use the word 'ultimate' since it has already been adopted in another sense in the 'ultimate' lethal temperature (Fry, Brett and Clawson, 1942).

These definitions are of course but a restatement of facts which have been mentioned previously by many workers. However, there does not appear to have been presented heretofore any case in which these lethal effects of temperature have been given so complete and quantitative expression for a single species as we present here for yearling speckled trout.

ACKNOWLEDGEMENTS

The speckled trout used in these experiments were provided by the Ontario Department of Game and Fisheries. We wish to acknowledge the kind co-operation of Mr. H. H. MacKay, Biologist and Director of Fish Culture, who made the arrangements for the supply of trout and arranged for the storage of some of them in Toronto. We also wish to acknowledge the valuable guidance given us by Professor G. F. M. Smith during the course of the statistical treatment of our results.

MATERIAL AND METHODS

The experiments were carried out in May and June on yearling fish obtained from the Codrington, Ontario, hatchery. The methods employed were essentially those used previously by Brett (1941, 1944) and Fry, Brett, and Clawson (1942) which in turn were adapted from those used by Loeb and Wasteneys (1912) and Hathaway (1927).

Previous to determining the lethal effects of temperature the fish were acclimated to various constant temperatures. At least one day per degree temperature difference between the holding temperature and the acclimation temperature was allowed for thermal adaptation. During the holding and the acclimation period the fish were fed fresh liver.

To determine the effects of a given temperature, a sample of from five to ten fish was placed directly from the acclimation bath into another thermostat maintained at the desired temperature. The water in these thermostats was aerated by compressed air and changed either by siphoning and replacing, or by introducing a continuous flow. The baths used were two feet square and seven inches deep, made of galvanized iron and coated inside with aluminum paint. Glass-covered electric heaters were used to maintain

the temperature within approximately ± 0.1°C. Experiments in which the temperature was constantly changing were carried out in the same tanks but hot or cold tap water was run in as desired to bring about the temperature changes.

The time at which each fish died was noted. Contrary to the previous practice in our laboratory the experiments were not terminated at an arbitrary period since it was found that at some temperatures all the fish would die although many hours might elapse before any mortality occurred at all.[2] In general the experiments were continued until the subjects were all dead or sufficient time had elapsed for death to have occurred as determined by extrapolation from results at higher temperatures.

Various instances have been reported in the literature in which a relation has been shown between the size of fish and their sensitivity to extremes of temperature (e.g. Huntsman and Sparks, 1924). In these cases so far as the authors are aware resistance to heat diminishes as size increases. Because of the existence of this relationship, records were kept in our experiments of the weight of each fish in relation to the order of death. These data are marshalled in table 1.

TABLE 1.—Size of the trout used arranged in order of death. Values given are number of fish in each size class.

Order of death	Weight class grams												Mean weight grams
	2.0 – 3.9	4.0 – 5.9	6.0 – 7.9	8.0 – 9.9	10.0 – 11.9	12.0 – 13.9	14.0 – 15.9	16.0 – 17.9	18.0 – 19.9	20.0 – 21.9	22.0 – 23.9	24.0 – 25.9	
1	11	16	7	4	2	5	1	0	0	0	1	1	7.12
2	7	12	10	5	7	4	3	0	0	0	0	0	7.74
3	5	16	9	5	3	6	2	2	0	0	0	0	8.27
4	4	17	9	8	3	2	3	2	0	0	0	0	7.66
5	4	9	9	9	7	3	5	0	1	0	0	1	8.60

Significance of difference of frequency distribution in rows 1 and 5

$\chi^2 = 48 \times 48 \times 0.0047842 = 11.023 \quad n^1 = 12 \quad P = 0.5.$

Significance of difference of means in rows 1 and 5

$S = 4.472 \quad N = 48 \quad S\bar{x} = 0.9123 \quad \bar{x}_5 - \bar{x}_1 = 1.673$

$t = \dfrac{1.673}{0.912} = 1.83 \quad n^1 = 94 \quad P = 0.08.$

[2] Doudoroff, 1945, has recently pointed this out for Marine species.

TABLE 2.—Comparison of the median survival time with the arithmetic a geometric mean survival times. The values given are the average of experiments.

	Median	A.M.	G.M.
Av. time min..............	423.9	448.0	423.0
Cases greater than Median ..		23	10
Cases equal to Median......		2	7
Cases shorter than Median ..		2	10

Neither the chi square test for distribution nor the "t" test the difference between the means show any significant differen between the size of the first and the size of the last fish to die in o experiments. This apparent conflict with the results in the lite ature is possibly due to the fact that all the fish in our sampl were of the same age, but the matter might profitably be inves gated further.

As has been pointed out (Bliss, 1937), the temporal order which deaths occur in a population on which some constant leth factor is exerting its effect is a statistical problem and if the prop derivative of time can be found the order will fit the normal cur of probability. As will be recalled, a property of the normal cur is that the median and the mean coincide. Using this property a criterion it was found that the derivative of time which gav normal distribution of the deaths was the logarithm. A demo stration of this fact is given in table 2 where the geometric a arithmetic mean times of death are compared with median tim obtained by interpolation. It will be noted in table 2 that th arithmetic mean time was found to be much more frequently long than the median time than shorter, while the geometric mean ti was shorter than the median time as frequently as it was long Figure 1 also demonstrates the goodness of fit of the logarithm the time of death to the normal probability curve. On the basis these comparisons the geometric mean mortality time has be considered equivalent to the median mortality time and has be calculated to represent it. All calculations of experimental e have also been centred about the geometric mean.

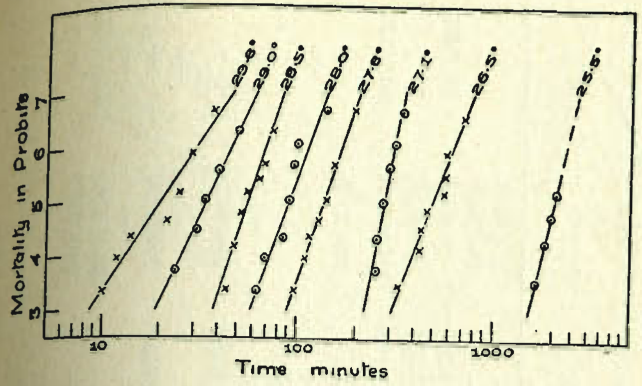

FIGURE 1.—Time-mortality curves for 20°C. acclimated fish exposed to various lethal levels of temperature, plotted as probits against log time. Probits taken from Fisher and Yates (1943).

Where the time of death of one or more individuals was lost and it was thus impossible to calculate the geometric mean, the known points were plotted as probits against the logarithm of time and the median obtained by inspection. The same procedure was also followed when more than 50% but not 100% of the individuals died before the experiment was terminated.

RESISTANCE TIMES IN RELATION TO LETHAL LEVELS AND THERMAL HISTORY

In order to determine the length of time that 50% of the population could withstand various lethal levels of temperature, the series of experiments were carried out that are summarized in table 3. To illustrate the relations between these values, figures 2 and 4 have been prepared.

TABLE 3.—Summary of experimental data relating to resistance time. Values are geometric mean survival time except where indicated. The figures in brackets are the variance of the logarithm of the geometric mean time.

Test temp. °C.	Acclimation temperature 3°C.	11°C.	15°C.	20°C.	22°C.	24°C.	25°C.
30.0	—	—	—	—	—	15(.0329)	—
29.5	—	—	—	—	—	36(.0056)	—
29.0	—	—	—	34(.0133)	47(.0174)	49(.0215)	51(.0201)
28.5	—	—	28(.0077)	57(.0066)	77(.0311)	86(.0106)	—
28.0	—	24(.0500)	{40(.0096) 51(.0220)	89(.0782)	130(.0194)	197(.0068)	194(.0120)
27.7	—	—	—	—	—	252(.0718)	—
27.6	—	—	—	136(.0073)	—	—	—
27.5	—	—	96(.0019)	—	269(.0119)	—	—
27.1	—	78(.0120)	—	277(.0017)	—	—	—
27.0	—	—	{108(.0047) 151(.0105)	—	519(.0032)	745(.0039)	—
26.6	—	96(.0067)	—	—	—	—	—
26.5	—	—	196(.0053)	481(.0104)	1010(.0223)	750(.1390)	—
26.3	—	136(.0175)	—	—	—	—	—
26.0	39(.1651)	217(.0038)	437(.0057)	—	—	2340(.0309)	2256(.0501)
25.5	—	—	859*	2000*	—	5040(.0306)	—
25.0	—	620*	—	—	—	—	—
24.0	303(.1697)	—	—	—	—	—	—
23.5	550*	—	—	—	—	—	—

* Median mortality time estimated by plotting probits.

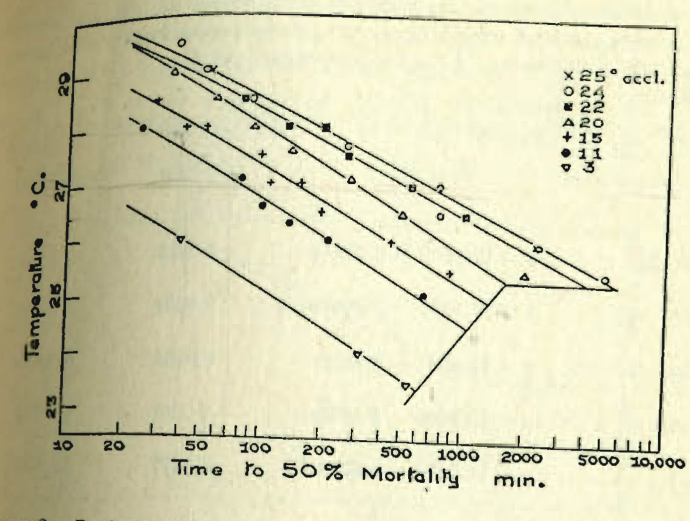

FIGURE 2.—Resistance times for various temperatures in the lethal range plotted for various levels of thermal acclimation. The lines drawn represent the regression equations given in table 4 except that for the 3°C. acclimation level which was drawn by eye.

In figure 2 the median survival time is plotted against the lethal temperature on semilogarithmic paper, a different symbol being assigned to each level of acclimation. No points are plotted where 50% mortality occurred in an interval less than 20 minutes. These were discarded in order to avoid to some extent relying on data derived from experiments in which a significant proportion of the time may have passed before the subjects had been heated to the temperature of the bath.

As displayed in figure 2 each array of points can be fitted quite well by a straight line, the two lines for the 24° and 25° acclimation levels coinciding. The series of straight lines for the four lower acclimation levels are parallel within the accepted limits of probability. The lines for the three upper levels of acclimation have a slope which differs significantly from that displayed by the four lower ones. The formulae for the regression lines plotted in figure 2 are given in table 4.

TABLE 4.—Formulae for the regression lines describing the thermal resistance of yearling speckled trout acclimated to various temperatures and measured in Toronto tap water. (x is the temperature in deg. C., y is log time in minutes).

Acclimation temperature °C.	Formula	σslope	σestimate
24, 25.......	$y = 17.8467 - 0.5567x$	0.0362	0.1631
22..........	$y = 17.1967 - 0.5367x$	0.0238	0.0500
20..........	$y = 15.0331 - 0.4661x$	0.0269	0.0500
15..........	$y = 15.1846 - 0.4833x$	0.0269	0.0755
11..........	$y = 14.6256 - 0.4728x$	0.0277	0.0610

It will be noted that the lower ends of the regression lines in figure 2 are terminated abruptly by two boundary lines, one roughly at right angles to the four lower regression lines and the other horizontal. The regression lines have no meaning beyond those boundaries because in the region beyond them 50% mortality does not occur no matter how prolonged the experience may be. The boundary lines thus separate the zone of resistance from the zone of tolerance and delineate the upper incipient lethal temperature (page 9) for the various levels of thermal adaptation. The rising section of the boundary represents the region where the upper lethal temperature increases in response to an increase in acclimation temperature, the horizontal portion, the ***ultimate upper incipient lethal temperature***, the temperature beyond which no increase in lethal temperature results from further increase in acclimation temperature (Fry *et al.*, 1942).

Three features in figure 2, namely the parallel slopes of the resistance lines over the region where the upper lethal temperature increases in response to an increase in acclimation temperature, the change of slope at higher acclimation temperatures, and the entry into the zone of tolerance at a lower exposure time at lower acclimation temperatures, also appear to be present in the data for Girella

nigricans and *Fundulus parvipinnis* (Doudoroff, 1942, 1945). Doudoroff's data have been replotted in figure 3.[3] It seems reasonable to believe that the similarity displayed by this careful work on marine fishes lends support to the interpretation placed on the findings here for the speckled trout. The response of the goldfish also appears to be the same (page 33).

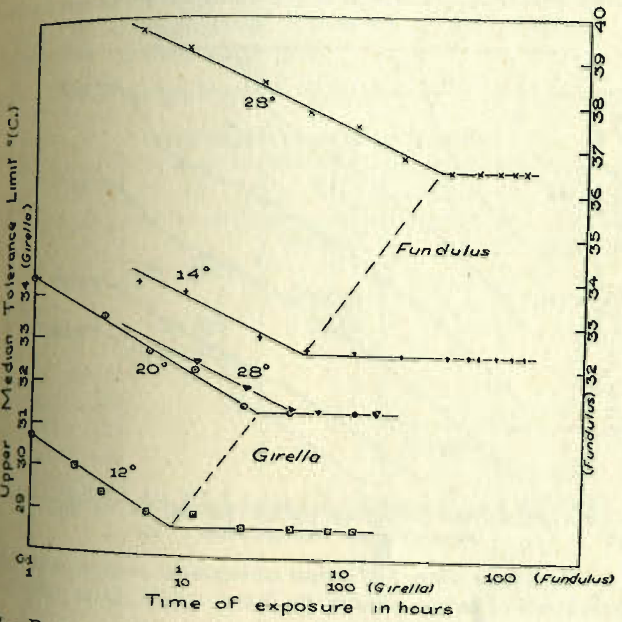

FIGURE 3.—Resistance time for *Fundulus parvipinnis* and *Girella nigricans* plotted from data given by Doudoroff (1942, 1945). Values for *Fundulus* acclimated to 20°C. have not been plotted since they appear to be atypical.

The extent to which the resistance time at a given lethal level of temperature is dependent on the thermal history of the fish is also illustrated in figure 4 which is derived from values read off the

[3]It should be noted that the axes of the graph in which Doudoroff's data are shown are labelled somewhat differently from those of figure 3. Consultation with his papers will show that his method of computation differs slightly from ours. However, the same data treated in the two ways should give the same regression line for resistance. The horizontal portion of Doudoroff's curves does not of course appear without method of computation.

regression lines in figure 2. In figure 4 resistance times are plot against acclimation temperatures for various lethal levels of te perature. The proportional rate of increase in resistance ti attendant upon an increase in acclimation temperature wo appear to subside gradually until the acclimation level has reach a stage where the upper lethal temperature approximates the te perature in question. There is then a sudden increase in rate a

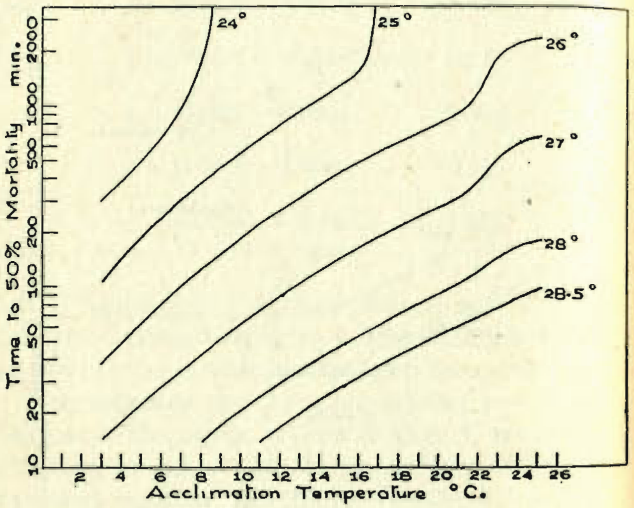

FIGURE 4.—Resistance times for various acclimation temperatures plotted various levels of temperature in the lethal range. Data taken from regression lines in figure 2.

the resistance time quickly becomes infinite. At temperature lev just above the ultimate upper incipient lethal temperature (page this increase in rate begins but is suppressed, the rate of incre subsiding to zero. At considerably higher temperatures the pha of sharp increase at the higher levels appears to be entirely s pressed.

Upper Lethal Temperatures

The percentage of fish which survive for a presumably indefi period at various temperatures and for various levels of acclima are given in table 5. Each experiment involving less than 5

mortality was continued well beyond the time indicated for that statistic when the regression lines in figure 2 were extrapolated. The precise time that each sample was exposed is given in table 5. Further discussion of the upper lethal temperatures is continued on page 20.

TABLE 5.—Summary of experimental data relating to upper and lower median incipient lethal temperatures. Values are per cent mortality. Figures in brackets are test times in minutes.

Upper test temp. °C.	Acclimation temperature degrees C.					Lower test temp. °C.
	3	11	20	22	24, 25	
25.5	–	–	67(2070)	–	100(5040)	
25.1	–	100(620)	–	–	–	
25.0	–	–	0(2300)	–	0(5000)	
24.7	–	17(1500)	0(2200)	–	–	
24.3	–	30(1200)	–	–	–	
24.0	100(300)	–	–	–	–	
23.5	80(1500)	–	–	–	–	
23.3	20(1900)	–	–			
23.0	20(2000)	–	–	0(1400)	–	0.0
22.5	0(2000)	–	–	–	100(4300)	0.2
				–	83(2900)	0.6
Est. upper incipient lethal temp. °C.	23.4	24.5	25.3	–	25.3	

Lower Lethal Temperatures

No extensive series of lower lethal temperature determinations were carried out since preliminary work showed that for this species it was below 0°C. throughout nearly the whole of the biokinetic range. It was only just above 0°C. when the acclimation temperature was 24°C. Since the ultimate upper lethal temperature is only 25.3°C. there is evidently little room for further loss of cold tolerance. Such data as were obtained are recorded in table 5.

THERMAL TOLERANCE

The thermal tolerance of this population of yearling speckl[...] trout is shown in figure 5 in the manner originally adopted for th[...] goldfish (Fry *et al.*, 1942). The area contained by the trapezoid [...] 625 units, just about half the tolerance displayed by goldfish [...] similar age.

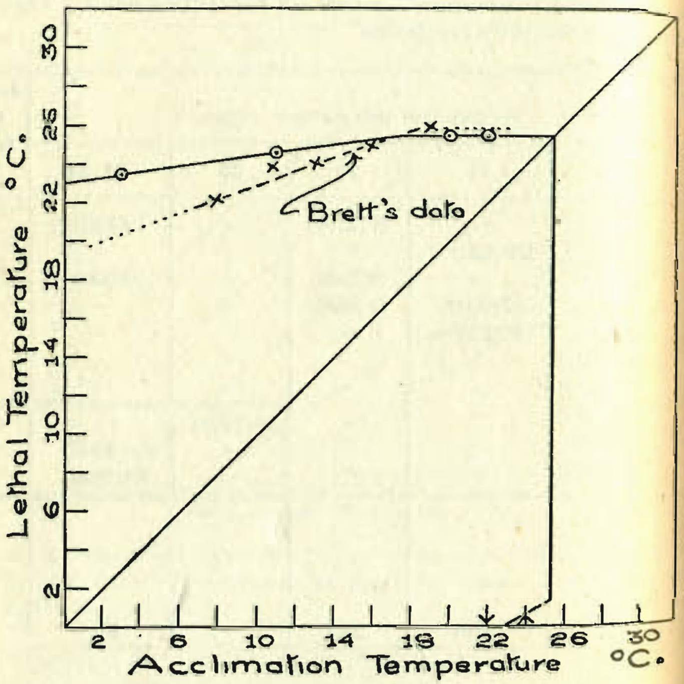

FIGURE 5.—Thermal tolerance of a sample of yearling speckled trout meas[...] in May and June in Toronto tap water. The dotted line indicates the tr[...] of Brett's (1941) data for another population of slightly older yearlin[...] measured in Opeongo lake water, and for further data (Brett ms.) he s[...] sequently gathered on yearlings from the same source as our own, but [...] necessarily of the same stock.

In these trout the upper lethal temperature increases only o[...] degree for seven degrees change in acclimation temperature in co[...] trast with the slope of one in three found for the goldfish and for th[...]

bullhead (Brett, 1944), and indicated by the data for *Fundulus* and *Girella* (Doudoroff, 1942, 1945). A similar lack of marked change of the upper lethal temperature in response to changes in acclimation temperature is displayed by the minnow, *Pfrille neogaea* (Brett, 1944). Brett (1941, 1944 ms.) gives data for other populations of speckled trout which indicate that in those populations the change in lethal temperature with change in acclimation temperature was more nearly of the order of one degree in lethal for three degrees in acclimation temperature. The nature and extent of this difference require a thorough exploration.[4]

Comparison of our data with Brett's shows that the ultimate upper incipient lethal temperatures are of the same order, while at a lower level of acclimation temperature the lethal temperature for Brett's samples is considerably lower than that found for our population at a similar level of thermal adaptation. Thus the flatter slope of the relation between lethal temperature and acclimation temperature in the present case indicates a greater and not a lesser thermal tolerance. Perhaps the ratio of change in lethal temperature to change in acclimation temperature reflects inversely the rate at which thermal adaptation can take place.

The ultimate upper lethal temperature of 25.3°C. (78°F.) is in close agreement with the maximum for this species commonly accepted by fish culturists and supports the conclusions of Embody (1921) and Ricker (1934).

TIME TO DEATH IN CHANGING LETHAL LEVELS OF TEMPERATURE

While as a matter of analytical convenience it may be desirable to determine the thermal resistance of an organism by measuring the length of time a sample can withstand a particular constant temperature, such data have little or no direct application in the field. Any thermal death in nature is almost certain to be brought about under changing temperature conditions. Fortunately for all practical purposes at least, the relation between the time to death

[4] The difference between our results and those of Brett does not appear to be due to the age of the fish since we have now determined the thermal resistance at the 5° acclimation level for a sample of our stock held for a further period of nine months and have found values concordant with our other observations. These fish were older than the oldest on which Brett made his determinations.

at a constant lethal level of temperature to that at changing le levels is not a complex one. It appears to be the sum of the therm experience at each lethal level. This was first pointed out by Jaco (1919).

In order to determine whether this relationship also held for t speckled trout, a series of experiments was performed in which t same sample was exposed in succession for various lengths of ti to two or more levels of temperature, each of which would be ult mately lethal, and the time to death noted. The observed times death were then compared with theoretical times calculated in th following manner.

It was assumed that the length of time a fish could exist at given lethal level of temperature depended on the rate at whi mortification[5] proceeded at that temperature, and that this rate mortification was constant throughout the time necessary to brin about the death of the animal. Thus if at a given temperature fish could exist for 100 minutes, the rate of mortification would b assumed to be 1% per minute, and it would be taken that 50 of the condition leading ultimately to the death of the organis would be brought about by the unfavourable temperature in t first 50 minutes, and the remaining 50% in the last 50 minutes. O the basis of this assumption then, what may be termed the *min rate of mortification* at any lethal level of temperature is the re procal of the resistance time in minutes at that temperature, and t degree of mortification resulting from a given exposure to th temperature would be the minute rate of mortification multipl by the exposure time in minutes. If the animal were exposed known lengths of time to a series of temperatures for which t minute rates of mortification were also known, then the degree mortification resulting from each exposure could be calculated a these fractions summed. When the total of the fractions was un the animal would have received a lethal dosage. In using this the we have multiplied the reciprocals by 100 in order to use percenta rather than fractions.

The experiments carried out to test this theory were of t

[5]The term mortification is used here to mean the degree to which an orga has proceeded towards death. The median mortality point is the point at w half the fish have achieved 100% mortification.

types. In some the fish were dropped into water at a given lethal level of temperature and exposed continuously to that temperature for a given length of time. Subsequently the temperature was changed suddenly to another lethal level and maintained constantly at that level until death occurred or until another shift in temperature was made. These steplike changes in temperature were used in the first experiments. In the later experiments the temperature was changed continuously, a time-temperature record being kept until the death of the animals.

TABLE 6.—Comparison of the length of time required to bring about death in yearling speckled trout exposed successively to two or more lethal levels of temperature for various periods of time with the theoretical resistance time.

Acclimation temperature °C.	Thermal experience °C.	Obs. time to death min.	Theoretical time to death min.
11	27.1,33 min to 26.3 till death	102 ± 18	107
11	26.3,60 min to 27.1 till death	112 ± 10	100
11	25.8,90 min to 26.1,40 min. to 26.5 till death	190 ± 22	177
20	26.5,230 min to 28.0 till death	291 ± 16	281
20	27.0,125 min. to 28.0 till death	183 ± 20	179
20	27.5,75 min. to 28.0 till death	128 ± 10	122

Error given is 2σ.

The results of the step experiments are summarized in table 6. The theoretical times to death under the experimental conditions were each calculated as in the following example which is the first case given in table 6. The median mortality time for each temperature experience was calculated from the appropriate regression line for the state of thermal acclimation of the subjects (table 4). Thus in the example below, the fish were acclimated to 11°C. and the formula for the regression line is log time = 14.626 − 0.4728 temp. and the median mortality times for the temperatures used are 27.1°C. 65 minutes, and 26.3°C. 155 minutes. The fish were first exposed to 27.1°C. for 33 minutes which represents a theoretical mortification of 51%. It should then have taken an additional 79 minutes' exposure at 26.3°C. to bring the sample to the median mortality point, making a total time of 112 minutes for the whole experiment. The observed time to the median mortality point was 102 minutes. The observed time lies within twice the standard deviation of the theoretical time and thus within the accepted limits of experimental error.

Table 7 summarizes the results of experiments in which the trout were in temperatures that changed continuously and steadily rather than by steps. Under these conditions the theoretical lethal experience cannot be calculated quite as simply as in the step experiments although it is still an integration of the area under the time-mortification rate curve. Instead of calculating the theoretical time to death and comparing it with the observed time as was done in table 6, the theoretical progress of mortification that would be accounted for in the observed time could have been calculated and compared with the observed mortification of 100%. To return to the example these calculations could have been made as follows: The median mortality times at the temperatures used were 65 minutes at 27.1°C. and 155 minutes at 26.3°C. The actual times spent at each temperature were 33 minutes at 27.1°C. and 69 minutes at 26.3°C. These represent 51% and 45% mortification respectively and together total 96%. The observed mortification was of course 100% and the discrepancy between the observed and the expected result is 4%. The experimental error is 6%.

The calculation for the step experiment above amounts to the addition of two rectangles whose bases are the exposure times at each temperature and whose heights are 100 times the minute rate of mortification at each temperature. With a continuously changing temperature following no formulated course, the mortification time curve is irregular and the area under it is not amenable to calculation by formula and in these cases was therefore found by counting squares. Figure 6 illustrates the method of calculating the degree of mortification for the comparisons given in table 7. The 'y'-axis of a piece of arithmetic paper is marked off in equal parts from zero to 100 to represent the percentage minute rate of mortification. The 'x'-axis is marked off in minutes. Points representing the

TABLE 7.—Observed time to death in changing temperatures compared with theoretical times.

Acclimation temperature °C.	Thermal experience	Obs. time to 50% mortality min.	Calculated per cent mortification	Calc. time to 50% mortality min.
A. Temperature rise beginning within lethal level				
11	25.0-30.0 in 25 min.	22.5 ± .9	98^{+22}_{-17}	22.5
11	25.0-29.2 in 32 min.	29.9 ± .5	91 ± 6	30.7
11	25.0-28.0 in 80 min.	79.6 ± .5	99 ± 3	79.6
11	25.0-28.0-26.0 in 77 min.	*74 ± 3	101 ± 2	72.0
B. Temperature rise beginning at acclimation level				
11	11.0-28.8 in 376 min.	365 ± 3	220 ± 30	347
24	24.0-30.0-29.0 in 130 min.	127 ± .5	154 ± 7	118

*By inspection from probits.

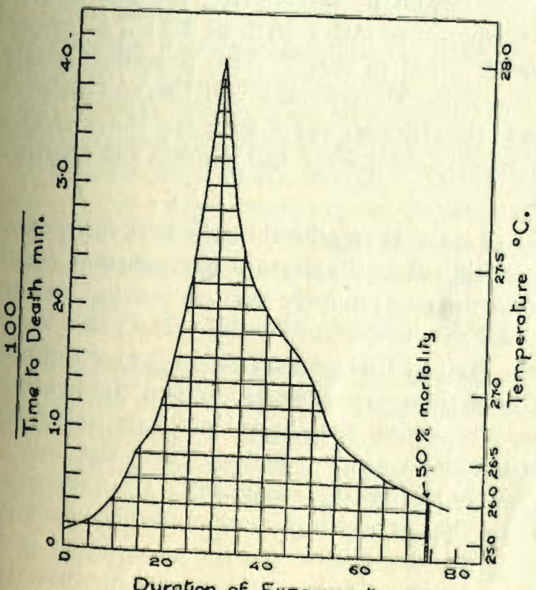

FIGURE 6.—Illustration of the method used to plot the course of mortification when the lethal level of temperature was continuously changing. Each square indicated under the curve indicates 1% of the theoretical mortification. This graph represents the experiment summarized in line 4, table 7.

mortification rate for lethal levels of temperature at degree and half-degree intervals are marked off and assigned to the appropriate lethal levels. In figure 6 this scale is drawn to the right-hand side. This scale of course changes for each acclimation level.

For a given experiment, a time-temperature curve is drawn and the time read off as each of the significant degree and half-degree intervals is passed. These temperatures and times are then plotted as in figure 6. A perpendicular is erected at the geometric mean survival time and the area under the curve at that point found by counting squares, the unit being one minute by one per cent mortification. Areas and times were also found for 2σ above and below the geometric mean time to give a measure of the experimental error.

Only the first four experiments listed in table 7 are comparable to the step experiments listed in table 6. As before, each of these experiments was begun by introducing the fish direct from their acclimation temperature into a bath at 25°C., a temperature that would be slowly lethal to them. This procedure was adopted to remove the possibility of undetermined thermal adaptation. The results of these experiments agree with the theoretical well within the limits of experimental error and confirm the results of the step experiments.

It may be of interest to note that the first three experiments in table 7 were designed to illustrate a phenomenon which has been remarked on in the past, namely that on heating an animal slowly the temperature attained before death takes place varies with the heating rate. Half of this generalization is given quantitative expression in these three experiments. When the heating is started at a temperature above the upper incipient lethal temperature undetermined acclimation is ruled out and the more rapid the rate the higher the temperature attained. The precise temperature attained will be that at which the area under the time-temperature curve assigned to the proper mortification rates reaches 100% mortification. These three examples give a quantitative basis for only half the generalization because if the heating was begun at a level below the upper lethal temperature acclimation occurring during the thermal experience before the lethal temperature was

reached would not be accounted for by any data presented in this report.

In the last two experiments listed in table 7 heating was begun at the acclimation temperature and allowed for the possibility of further thermal adaptation as mentioned above before a lethal level was reached. In the first of these, heating was begun from the base temperature of 11°C. and about four hours were passed before the zone of temperature experience lethal to trout, acclimated to 11°C., was reached. As will be seen, these fish did not die at the time when the death of trout acclimated to 11°C. would be expected, the area under the curve amounting to 220 instead of the expected 100. This discrepancy can be explained by assuming that the thermal experience between 11°C. and 24.5°C. resulted in the acclimation of the fish to a higher temperature.

While nothing is proved by the process, it can be calculated that an acclimation level equivalent to that attained at a steady temperature of just under 16°C. would provide the correct scale of reciprocals to translate the experimental time-temperature curve into 100% mortification. This acclimation temperature can be derived from figure 4 as follows. The calculated mortification on the basis of 11°C. acclimation was 220, the theoretical value on the basis of the correct acclimation level should be 100. The time to death at a given lethal level of temperature, say 27°C. when the trout are adapted to 11°C. is 74 minutes. The actual time they would have lived at that temperature with their new level of acclimation would have been $\frac{220}{100} \times 74 = 163$ minutes. The acclimation temperature which would allow a resistance time of 163 minutes at 27°C. is 16°C. By virtue of the fact that within the acclimation and the lethal range under consideration, the log time-temperature curves for mortality (figure 2) can be described by parallel lines, the resistance times at acclimation levels of 16°C. and 11°C. maintain the same ratio at all the lethal levels involved. Therefore nothing is necessary but to reassign the temperature points to reciprocals appropriate for 16°C. acclimation rather than 11°C.

The final changing temperature experiment gave results which are discordant with all other data gathered during the investigation. Unfortunately this discrepancy was not discovered in time to repeat the experiment. The experiment performed was to warm tr acclimated to 24°C. from that temperature at a rate of one deg per twenty minutes until a temperature of 30°C. was reached. Th were then cooled at approximately the same rate until death o curred. The area under the curve when plotted as in figure amounted to 154 instead of the expected 100.

While this experiment is similar to the previous one in that t rise in temperature was started from the acclimation temperatu there is no reason to suppose from the data presented in figures and 4 that any increase in resistance would take place during th temperature experience before entering the lethal range.

A possible explanation might be the tempering effect, the lessen ing of shock by introducing the animal gradually to the lethal rang However, Brett (1941) went to pains to demonstrate the absence any such effect in the very species used in these experiments.

There is some justification for considering this discordant findi to be an unfortunate accident and it will be considered so pendi further work. The temperature attained was one at which tro can at best live only a short time and this circumstance could le to considerable error. Furthermore it will be noted in figure 2 th one sample of 24°C. acclimated fish lived at 29.5° more than times as long as would be expected on the basis of the gene regression line and one might expect another sample of similar s to do likewise.

THE ACCUMULATION OF LETHAL EXPERIENCE

Speckled trout exposed to lethal levels of temperature in natu are likely to be submitted to them repeatedly each day during t diurnal heating of the streams or perhaps in still waters dur nocturnal migrations to the shallows. These possibilities point the need for determining whether sub-lethal exposure to lethal lev of temperature on one day would be added to a second exposure the succeeding day. Only one experiment was performed to exp this possibility but the results from it were quite conclusive.

A sample of five trout acclimated to 20°C. was exposed to 27 for three hours (60% mortification) and then returned to 20°C 22 hours. They were then subjected to 27°C. for five hours

expected median mortality time, with no deaths resulting. The experiment was then discontinued for it was evident that any residual effect of the exposure on the previous day was not such as to reduce the survival time. Indeed since none of the fish had died within the expected median mortality time there is some hint of a gain in acclimation as a result of the previous sublethal exposure.

HEAT DEATH OF SPECKLED TROUT IN NATURE

We are indebted to Professor A. G. Huntsman for a record of the heat death of speckled trout along with other species in the Pollet and Petitcodiac rivers over the period September 12-16, 1942 (Huntsman, 1946). A copy of the stream temperature record taken at this point covering the period September 11-13 is reproduced in figure 7.

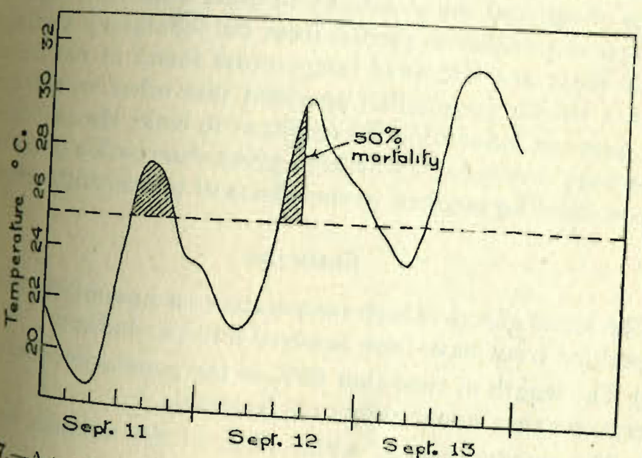

FIGURE 7.—A transcription of part of the temperature record for the Petitcodiac river given by Huntsman (1946). The horizontal dashed line indicates the ultimate incipient upper lethal temperature of speckled trout. The shaded portions above this line indicate the thermal experience that trout similar to our sample would have resisted before undergoing a mortality of 50% assuming an acclimation temperature of 24°C.

Assuming the trout acclimated to 24°C. before entering lethal levels of temperature, speckled trout similar in behaviour to our sample would have survived through September 11 and would have commenced to die about 3.00 p.m. on September 12. In this one case at least our laboratory findings are in quite close agreement with conditions in nature.

CONCLUSIONS

These experiments, if the results are confirmed by further work on other species, point the way to the reconciliation of the three methods which have been used for describing the lethal effects of high temperatures on fish. The work recorded here is only preliminary and not only incomplete in that further data on some of the points discussed would have been desirable but also because one pertinent section, rate of thermal acclimation, has been entirely omitted.

Further, if these findings are extended to a determination of the influence of age and the possibility of other influences explored, it would appear possible to predict from the laboratory findings the probable effect any course of temperature found in nature would have on a similar population, provided that other environmental factors were not influencing the result; or to make the statement in another way, to determine whether a given observation of mortality in nature could be ascribed to the effects of temperature alone.

SUMMARY

1. The lethal effects of high temperature on a population of yearling speckled trout have been resolved into two indices.

(*a*) The length of time that 50% of the population could resist temperatures that would ultimately be lethal.

(*b*) The temperature at which 50% of the animals could be presumed to exist indefinitely.

2. Each index was measured on a series of samples acclimated at temperatures of 3, 11, 15, 20, 22, 24 and 25 degrees Centigrade.

3. Lethal effects of low temperatures did not rise above 0°C. until the acclimation temperature had reached approximately 24°C.

4. The rate of dying in changing temperatures above the upper lethal temperature can be ascribed to the additive effect of the thermal experience undergone at each temperature.

5. A sample of this population acclimated to 20°C. exposed to lethal experience amounting to 60% mortification had recovered completely from this experience within 22 hours after being returned to its acclimation temperature.

6. These laboratory findings agree closely with a record of heat death of speckled trout in nature given by Huntsman (1946).

7. No correlation was found between size and order of death at a given lethal level of temperature in this group of fish which were all of the same age.

APPENDIX

THERMAL RESISTANCE OF YOUNG GOLDFISH (*CARASSIUS AURATUS* L.) AS INDICATED BY THE DATA OF FRY, BRETT AND CLAWSON (1942)

Doudoroff (1945) has recently raised the question as to whether a 14-hour period is of sufficient length to give a measure of the thermal tolerance of fish. He demonstrated that this was certainly not so for *Fundulus parvipinnis* and *Girella nigricans*. Unfortunately he was not able to form any opinion regarding the goldfish, since Fry, Brett and Clawson (1942) did not publish their data in a form that would allow him to determine at what experimental time the upper lethal temperature became stable.

In order to remedy this deficiency, we have taken the liberty in the absence of Brett and Clawson of retabulating the original data on the goldfish in the form used by Doudoroff (table 8). These data are also illustrated in figure 8.

TABLE 8.—Per cent survival of young goldfish at various high temperatures in relation to acclimation temperature and duration of exposure.

Accl. temp. °C.	Number of fish	Test temp. °C.	Duration of exposure hours 0.5	1	3	6	12	24	Median survival time min.
41	5	42.0	100	100	100	20	0	0	240
40	8	41.5	100	100	100	63	0	0	500
38	4	43.0	25	0	0	0	0	0	25
	8	41.5	100	75	50	0	0	0	130
	8	40.0	100	100	100	100	100	100	
Median survival temp..			42.4	42.0	41.5	41.0	41.0	41.0 °C.	
32	5	41.0	40	0	0	0	0	0	30
	5	40.0	100	80	0	0	0	0	85
	8	39.0	100	100	88	63	63	..	
	8	38.0	100	100	100	100	100	100	
Median survival temp..			40.9	40.0	40.2	39.3	39.2	39.2 °C.	
24	10	37.0	10	10	10	10	10	..	
	5	36.0	100	100	80	80	80	..	
	10	35.0	100	100	100	90	90	..	
Median survival temp..			36.6	36.6	36.2	36.2	36.2 °C.		
17	8	34.0	12	12	12	12	12	12	
	8	33.0	100	100	88	88	88	88	
	8	32.0	100	100	100	100	100	100	
Median survival temp..			33.8	33.8	33.7	33.7	33.7	33.7 °C.	
10	8	31.5	25	25	25	25	25	25	
	7	30.0	86	86	86	86	86	86	
Median survival temp..			30.8	30.8	30.8	30.8	30.8	30.8 °C.	
2	5	29.0	0	0	0	0	0	0	
	5	28.0	40	40	40	40	40	40	
	7	27.0	1 died but time not recorded.						

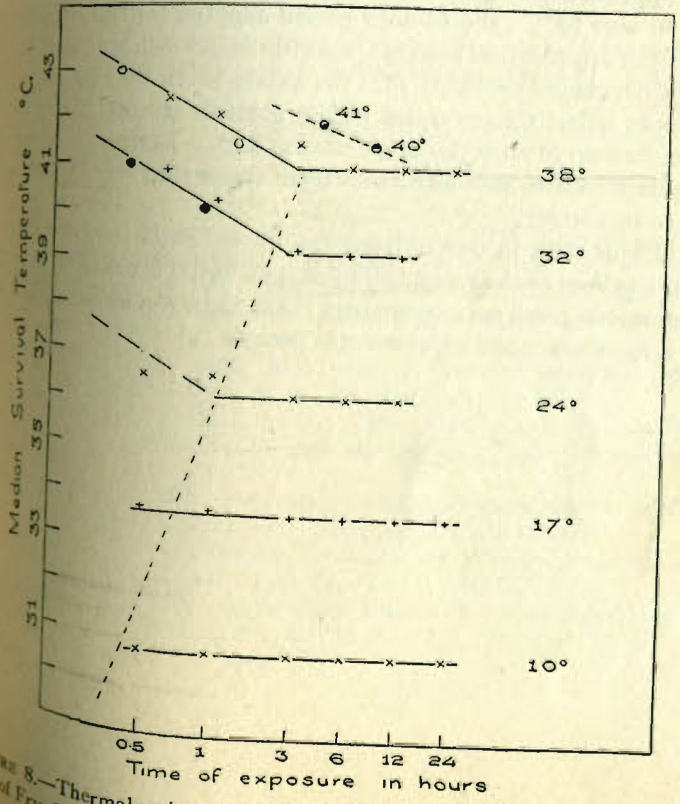

FIGURE 8.—Thermal resistance of the goldfish as illustrated by the original data of Fry, Brett and Clawson (1942). Plotted from the data in table 8.

The data in the zone of resistance are somewhat scanty but as they are they point to the same type of behaviour as has been demonstrated for *Girella nigricans*, *Fundulus parvipinnis* and *Salvelinus fontinalis*. It is particularly interesting to note that as in *Girella* and *Salvelinus*, the goldfish continue to show an increased resistance after the plateau of tolerance has been reached. The striking feature about the goldfish data is that the zone of resistance is tremendously curtailed in this species in comparison with the others cited. Even at acclimation levels of 40°C. and 41°C., the zone of tolerance appears to be entered within 24 hours and at an acclimation level of 10°C. within something of the order of half an hour.

It appears from figure 8 that, with the exception of acclimation levels of over 38°C., the 14-hour period adopted for the determination of the upper lethal temperatures of the goldfish was ample. It may be concluded therefore that the values given by Fry, *et al.*, for the upper lethal temperatures of the goldfish represent the limits of tolerance up to an acclimation level of 38°C. Their interpretation of the limit of tolerance at acclimations above that level would [illegible] seem to be correct.

Unfortunately in determining the lower lethal temperature [illegible] record was kept of the mortality by stages, and it is possible that the temperatures given may be slightly lower than would have resulted from a more extended experimental period.

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