QUANTITATIVE STUDY OF THE CHANGES PRODUCED BY ACCLIMATIZATION IN THE TOLERANCE OF HIGH TEMPERATURES BY FISHES AND AMPHIBIANS

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INTRODUCTION

A great many observations have been made by numerous experimenters on the maximum temperatures tolerated by organisms. In 1895 Davenport and Castle listed thermal death points that had been determined for 69 species of plants and animals; and the number of species studied, especially of microorganisms, has been increased greatly since that time. Quantitative studies on acclimatization as a factor in the tolerance of high temperatures began with the observations of Flourens (1846) and Ehrenberg (1859), who reported organisms living in hot springs at temperatures of 85° and 98° C., when closely allied species in the waters of the same region ordinarily were exposed to temperatures no higher than 40° C. Schwartz (1884) and Aderhold (1888) found that Euglena collected in summer ceased activity when the temperature fell to 5° or 6° C., while those collected in winter remained active at 0° C.

The most notable work on experimental acclimation of microorganisms is that of Dallinger (1880) on flagellates. By gradually raising the temperature of his cultures, he was able, within a period of several years, to change their limits of tolerance from 23° to 70° C. Jollos (1921) has shown that protozoans acclimated to high temperatures and to certain chemicals may retain their increased tolerance for many generations in the absence of the acclimating agent.

Pioneer work on acclimatization of vertebrates was that of Davenport and Castle (1895). They tested the tolerance of toad tadpoles by raising the temperature of the surrounding water at the rate of about 25° C. in 10 minutes, and noted the

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temperature at which the tadpoles went into heat rigor. They found that individuals reared at 15° C. went into rigor at 40.3°, while for those that had been kept at 25° C. for 28 days the limit of tolerance was 2.3° higher. The increased resistance was only partly lost in 17 days at 15° C. Loeb and Wasteneys (1912) tested the duration of survival at high temperatures of Fundulus that had been kept at 10° and 27° C. Individuals that had been kept at 10° C. died in four hours at 25° C., while 30 hours' exposure to 27° C. enabled the fishes to survive indefinitely at 35° C. By short exposures on successive days to temperatures that normally would have killed the fishes the tolerance limit was raised to 39° C., and the added resistance was not wholly lost in several weeks at a temperature near the freezing point.

A few suggestions have been made regarding the nature of the changes involved in acclimatization. Davenport, in 1897, suggested that increase in tolerance might be caused by a lowering of the water content of protoplasm, with consequent raising of the temperature necessary to cause coagulation. Loeb and Wasteneys (1912) compare acclimatization with the annealing of glass, while Miss Behre (1918) has presented evidence indicating that an adjustment in metabolic rate is an important factor in acclimatization. She shows that raising or lowering the temperature of Planaria alters the rate of metabolism, but that continued exposure to high or low temperature is accompanied by a gradual return to the original rate.

In spite of the large amount of work done on temperature tolerance, the methods employed have been so varied that there seems to be no satisfactory way of comparing, quantitatively, the results obtained by the various workers, and hence there is no basis for an accurate comparison of different animals. The purposes of the present study are as follows:

1. To find a method of studying, quantitatively, the changes in tolerance produced by acclimatization, which will be applicable to a wide variety of kinds of animals.

2. By the use of this method to determine, for each of several different animals, the relative amounts by which the tolerance of high temperatures can be modified, and to see whether the power of adjustment bears any definite relation to the ecology of the animals and to their taxonomic position.

3. To afford, by the work on tolerance, a point of departure for further experiments on the physiological nature of acclimatization.

This work was undertaken at the suggestion of Prof. A. S. Pearse, to whom the writer is under great obligation for advice and assistance. The writer is indebted to Dr. J. R. Roebuck and to Dr. F. G. Hall for suggestions regarding construction of apparatus, and to Dr. Willis H. Rich for reading and criticizing the manuscript. The work was supported, in part, by funds provided by the Bureau of Fisheries.

GENERAL PLAN OF THE EXPERIMENTS

MATERIAL

Five species of animals were used in these experiments—the yellow perch, *Perca flavescens* (Mitchill); the large-mouthed black bass, *Micropterus salmoides* (Lacépedè); the bluegill, *Lepomis incisor* (Mitchill); the sunfish (pumpkinseed), *Eupomotus gibbosus* (Linnaeus); and the tadpoles of the toad, *Bufo americanus* Le Conte.

Several considerations led to the choice of these animals. In the first place, it was decided that this introductory work should be done on the members of a well-defined group, within which the relation of ecology to modifiability of tolerance could be studied effectively. An attempt to compare animals belonging to different phyla involves such profound differences in structure and physiology that several members of each great group must be studied in detail in order to obtain results that are of ecological significance, so it was thought that each of the larger groups should be considered in a separate series of experiments. The present study, therefore, is limited to gill-breathing vertebrates.

The fishes named were chosen for the reasons that they are abundant and easily obtained in Lake Mendota, and that they represent a fairly wide range of habitat preferences (Pearse, 1918 and 1921; Pearse and Achtenberg, 1920), *Micropterus salmoides*, *Eupomotus gibbosus*, and *Lepomis incisor* being essentially shallow-water fishes, while *Perca flavescens* is, by preference, an inhabitant of lower levels. Toad tadpoles were studied as another type of gill-breathing vertebrate because, commonly living in small bodies of water, they would be expected to withstand rather wide and rapid fluctuations in temperature.

In most of the experiments on fishes, individuals between 1 and 2 years of age were used, those less than 1 year old being very sensitive and hard to handle, while older specimens could be used only in very small numbers because of limited space in the constant-temperature baths. The young fishes possessed the further advantage that they were not passing through the physiological changes that accompany the different phases of the annual reproductive cycle. All of the tadpoles used had easily visible hind legs. They developed rapidly during the experiments, but, unless otherwise stated, none of them had visible front legs at the times of the tests.

All of the fishes used were seined from Lake Mendota. The tadpoles were obtained in some large, shallow pools in South Madison, several collections being made in the course of the work. All specimens were brought to the laboratory as soon as possible after being collected and were kept in large aquaria and battery jars with a constant flow or frequent changes of water. No animals were used in the tests until they had been in the laboratory at least 48 hours, as it was found that in the first day or two after being brought in their resistance to heat was extremely variable.

METHOD OF MEASURING TOLERANCE

In this quantitative study it was necessary (1) to decide what should be considered as constituting tolerance and (2) to find a fairly accurate, numerical means of expressing the degrees of tolerance possessed by different individuals. After a number of preliminary experiments it was decided to express tolerance in terms of the maximum temperatures that could be survived for periods of 1 minute, 4 minutes, 15 minutes, 1 hour, 4 hours, and 24 hours. The 24-hour tests were considered the most significant and the others served as checks upon them. The maximum temperatures that could be endured for the various lengths of time are referred to as the "24-hour tolerance limit," the "4-hour tolerance limit," etc. The general method of procedure was as follows:

1. Tests were made (usually simultaneously) at two or three constant temperatures, 2° C. apart, the lowest of which was believed to be near the upper limit of tolerance of the individuals tested.

2. Observations were made 1 minute, 4 minutes, 15 minutes, 1 hour, 4 hours, and 24 hours, respectively, after the beginning of each test.

3. At every observation the condition of each individual was recorded, and the time of the last reading at which an individual was alive was recorded as its survival time.

Two reasons led to the choice of the method just described—(1) it is relatively simple and is applicable to tests on a considerable number of individuals; (2) the time periods into which the tests were divided (1 minute, 4 minutes, etc.), constitute, roughly, a geometric progression. Therefore, the degrees of tolerance represented by survival for the various lengths of time belong to different orders of magnitude, and the method affords a quantitative basis for the comparison of the tolerance of various individuals and species.

Fishes were considered dead when respiratory movements had ceased, as no case of recovery following cessation of respiration was ever observed. In tadpoles no great effort was made to distinguish between heat rigor and death. The distinction proved to be unimportant, for many observations showed that recovery from rigor never occurred except during the first hour of the test. Failure to move in response to repeated mechanical stimulation with a blunt glass rod was taken as evidence that a specimen was dead or in rigor.

In the early stages of the work it became apparent that, under the conditions employed, the tolerance limits for the members of a given species that had received a certain treatment lay within rather definite limits. For example, all normal bluegills and sunfishes survived at 34° and none at 36°. In other cases the thermal death points were less definite, but, on the average, an increase of 2° in the trial temperatures was sufficient to bring about a change from 90 per cent survival to 90 per cent death. However, the tolerance limit of the majority of the individuals was found not to be a satisfactory basis for comparisons, as the tolerance of the average individual may fall above or below that of the mode. For this reason the average tolerance limit was computed for each series, the following equation being used:

$$\frac{tP_1 + (t-2)P_2 + (t-4)P_3}{100} = T$$

In this equation, t is the highest temperature tolerated for the given period by any of the individuals tested; $t-2$ and $t-4$ are temperatures, respectively, 2° and 4° below t; P_1, P_2, and P_3 are the percentages of individuals for which t, $(t-2)$, and $(t-4)$ are, respectively, the maximum temperatures tolerated; and T is the average of the tolerance limits of the individuals in the series.

The manner of applying the foregoing formula may be illustrated by the case of the normal bass tested at 30°, 32°, 34°, and 36°. The percentages of individuals surviving at these temperatures were as follows:

Degrees	Per cent
30	100
32	86
34	26
36	0

Hence, $t=34°$. From these results it seems fair to assume that the 14 per cent that died at 32° would have survived at 30°; hence, $P_3 = 14$. Furthermore, the

26 per cent of survivals at 34° indicates that out of the 86 per cent that survived at 32°, 26 per cent would have survived at 34°. Hence, $P_1 = 26$ and $P_2 = 60$, and T equals approximately 32.3°. The advantage of this average tolerance limit over the simpler majority limit, as a basis for measuring acclimatization, is illustrated in Table 1, which shows that the majority tolerance lies distinctly below that of the average in the cases of bass and toad tadpoles, and decidedly above it in the case of the perch.

TABLE 1.—*Maximum temperatures tolerated for 24 hours by a majority and an average of normal individuals*

	Majority	Average
	° C.	° C.
Perch	30	29.6
Bass	32	32.2
Bluegill	34	34.0
Sunfish	34	34.0
Toad tadpoles	36	36.3

METHOD OF ACCLIMATIZATION

In attempting to test the relative ability of various animals to become acclimated, it was decided to make the following determinations for each species:

1. The tolerance limits of individuals that had been living at room temperature. These individuals are referred to as normals.

2. The extent to which tolerance limits were raised by exposure for varying lengths of time to a temperature of 30° C.

3. The extent to which tolerance limits were lowered by exposure for varying lengths of time to a temperature of 10° C.

The temperature in the aquaria in which the normal animals lived averaged between 22° and 23° C. The members of all the different species showed decided stimulation when transferred to 30° C., and a temperature of 10° C. was low enough to decrease activity and feeding very markedly.

The acclimatization periods used for fishes were 1 day, 4 days, and, when practicable, 16 days. In the work on toad tadpoles, periods of 1 hour, 4 hours, 1 day, 4 days, and 8 days were employed, development occurring too rapidly to permit the use of 16-day periods with the individuals available.

DETAILS OF METHODS

APPARATUS

The principal pieces of apparatus used consisted of (*a*) a cold box, which was kept at about 10° C.; (*b*) a series of seven constant-temperature baths, adjustable for any desired heat above room temperature; and (*c*) an aeration system, by which air was bubbled through the jars of water containing the fishes.

The cold box consisted of outer and inner wooden cases, inclosing an outer and an inner sheet-iron tank. The jars containing the specimens stood in water in the inner sheet-iron tank, which, in turn, was surrounded by the water filling the outer tank. Insulation was furnished by the inner and outer wooden cases, the double glass doors of which permitted good illumination. The baths for use at higher

temperatures were wooden tubs, each large enough to hold four or five of the battery jars containing the specimens.   An electric heating unit was placed in the bottom, so that stratification of the water was prevented almost entirely by the convection currents; and the temperature was controlled by a mercury thermostat, which operated a relay in the heating circuit.   The aeration system was not needed in the work on tadpoles but was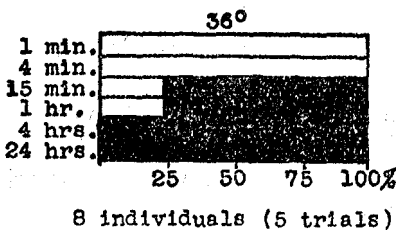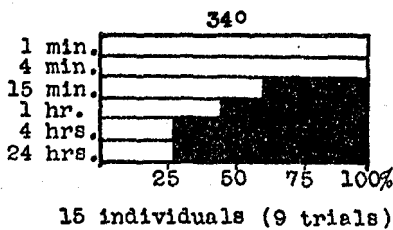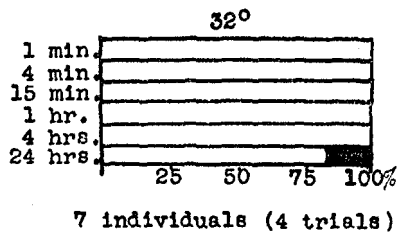 essential for the fishes, because the limited capacity of the constant-temperature baths and the need of testing separately a large number of individuals necessitated the use of rather small jars for the specimens.   Air at low pressure was supplied by a specially designed suction pump, from which a rubber tube led to each specimen jar, where a fine glass tip delivered the bubbles in a stream that was strong enough to stir the water well.   At or below room temperature this system normally furnished ample aeration, the respiration of the fishes apparently being normal.

Fig. 1.—Effects of high temperatures on "normal" large-mouthed black bass. The specimens were transferred directly from room temperature to the test temperatures (32°, 34°, and 36°, respectively). The six lines in each rectangle represent successive observations on the same individuals. The lengths of the sectors in each line show the percentages of individuals that were living or dead at the time of each observation. ☐ living;   ■ dead

ACCLIMATIZATION CONDITIONS

Acclimatization was carried on in battery jars, different sizes being used, according to the size and number of the specimens. Tadpoles were run in lots of 10 to 20 without artificial aeration; fishes, in lots of 1 to 4 with aeration. In all cases the jars used were large enough, so that the fishes could turn around without touching the sides of the vessel. All specimens were fed during the acclimatization periods.

TEST CONDITIONS

Lake Mendota water, drawn from the university taps, was used in all the tests, being brought to the desired temperature and then stirred vigorously to remove excess air. Ordinarily, it was probably just about saturated with oxygen at the beginning of each experiment.

The tests on the fishes were made in battery jars, about 1,800 cubic centimeters of water being used for yearling fishes and about 5 liters for the larger individuals. Tadpoles usually were tested in groups of three in specimen jars containing 250 cubic centimeters of water.   Transfers were made directly from the acclimatization temperatures to the test temperatures, and there was no feeding during the 24-hour test periods.

DEGREE OF ACCURACY MAINTAINED

Acclimatization periods listed as 24 hours include a few as low as 22 and a few as high as 27 hours.   The ordinary variation in the so-called 4-day periods was between 92 and 100 hours, while periods of from 15 to 17 days were included in the 16-day

group. During the test periods, especially during the early hours, observations usually were made exactly on schedule times, or within a very few minutes of them.

In acclimatization at 30° specimens were discarded if the temperature was found to have risen above 31°. The work of Loeb and Wasteneys (1912) had shown that exposure for a short time to a high temperature produced lasting results, and, as will be seen later, experiments in this series bore out their conculsion; so it was felt that an exposure to a temperature of 31° for even a short time was enough
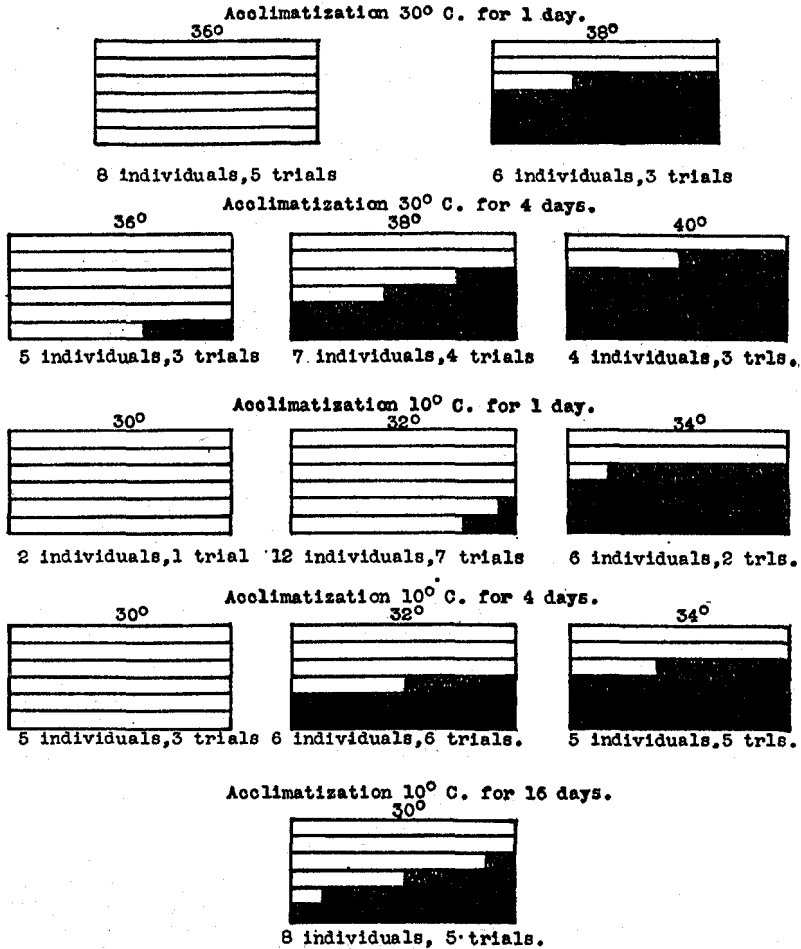
Fig. 2.—Effects of high temperatures on large-mouthed black bass that had been acclimatized to 30° C. or 10° C. For explanation of symbols see Figure 1

to invalidate the experiment. During the test periods any fluctuations of temperature, either upward or downward, were important. The normal variations were 0.2° to 0.3° C. They seldom were more than this, except when the apparatus was seriously out of order, and if at any time in a test the temperature was found to be as much as 1° above or below that specified the results were discarded. This rule was very important, because it was found that a drop of 1° or 2° for a short time during the early hours of a test apparently enabled a fish to become adjusted and to

survive when all other individuals tested under the same conditions had died; and, conversely, a rise of 1° for only a short time sometimes was followed by the death of fishes that normally would have been expected to survive.

So far as possible, uniform aeration was maintained, the streams of bubbles being controlled by the use of clamps on the rubber air tubes. A number of experiments in which it appeared that the deaths might have been due to weakness or stoppage of the aeration were discarded.
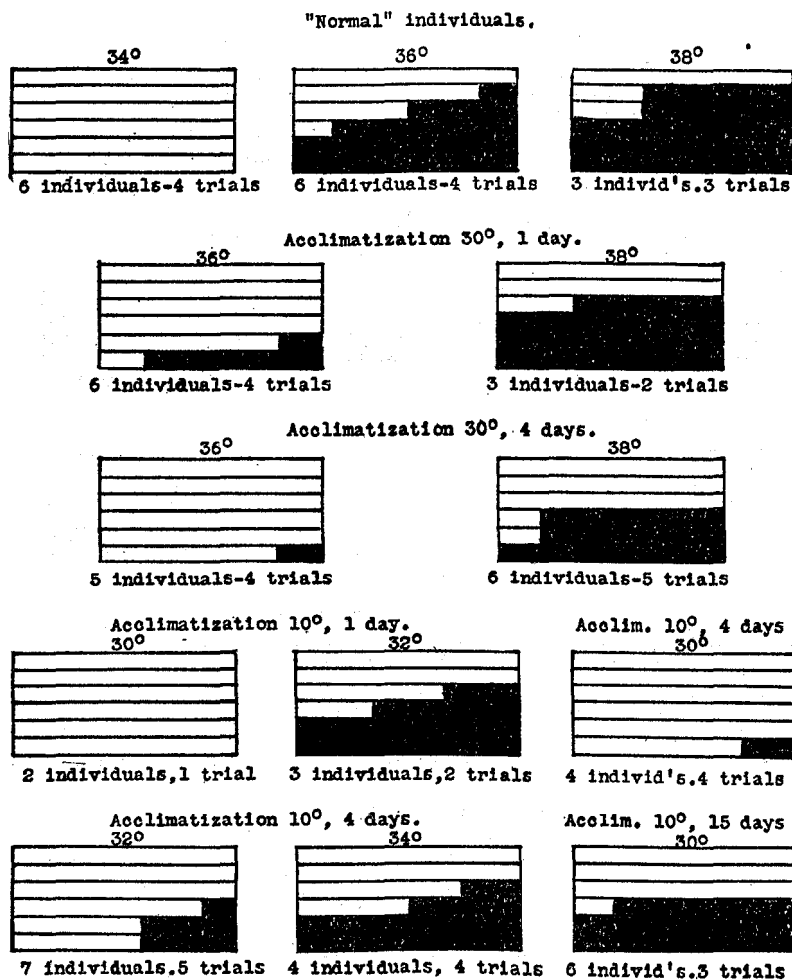
FIG. 3.—Effects of high temperatures on young bluegills. For explanation of symbols see Figure 1

NUMBER OF TRIALS

The term "trial" as used in this paper refers to a 24-hour test at a given temperature on the specimens in one container. The term "series" is used to designate the group of trials performed at a given temperature on similar individuals of the same species which had received the same preliminary treatment. The various trials in one series usually were made on different days, although in some instances

two of them were run simultaneously. The numbers of trials and individuals in the various series were very unequal, being small when all the individuals died quickly or survived easily and much larger at some crucial points in the experiments in which the results were seriously in doubt.

RECORDS

The record of each trial included (1) remarks on any peculiarities of the specimens at the beginning of the test (for example, size and condition), (2) temperature
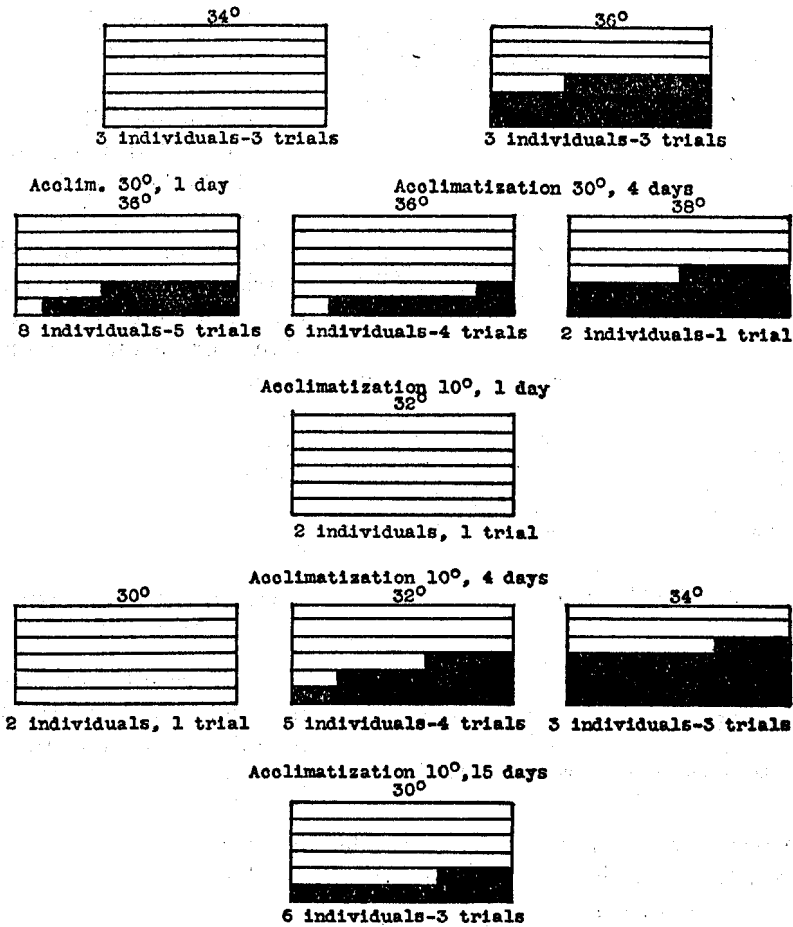
FIG. 4.—Effects of high temperatures on young sunfishes (pumpkinseed). For explanation of symbols see Figure 1

and exact duration of acclimatization, (3) notes on the behavior of the specimens immediately following transfer to the trial temperature, and (4) notations made at the time of each observation. The latter included a statement of the exact time at which the observation was made and a brief description of the condition of each of the specimens as indicated by its appearance and behavior, together with notations on any of the experimental conditions that seemed important.

RESULTS

The details of the results obtained in the work on yearling fishes and on toad tadpoles are shown diagrammatically in Figures 1 to 7. These diagrams present the results of 180 trials on a total of 247 fishes and 62 trials on a total of 181 tadpoles. These trials comprise 46 series on fishes and 24 series on tadpoles. In addition to the experiments represented in the diagrams, a relatively small number of trials was performed on mature sunfishes and perch and on perch, bluegills, and sunfishes less than 1 year old.

The figures given above include only experiments successfully completed under the conditions that have been described and at temperatures that seem to be significant in indicating the limits of tolerance of the animals. They are exclusive of a considerable number of trials conducted at temperatures that were found to be distinctly above or below the limits of tolerance of the species. Such trials were of some interest, however, in indicating the consistency with which, under the experimental conditions, a graded series of temperatures affected the animals.

"Normal" individuals.

32° — 6 individuals-6 trials

34° — 2 individuals-2 trials

Acclimatization 30°, 1 day

32° — 5 individuals-5 trials

34° — 3 individuals-3 trials

Acclimatization 10°, 1 day

30° — 4 individuals-4 trials

32° — 6 individuals-6 trials

Acclimatization 10°, 4 days

30° — 10 individuals-10 trials

32° — 6 individuals-6 trials
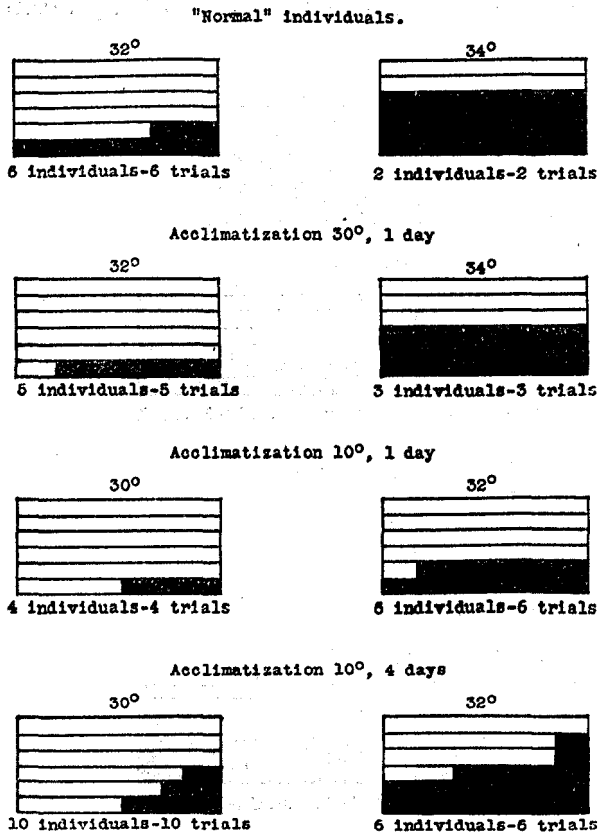
FIG. 5.—Effects of high temperatures on yellow perch. For explanation of symbols see Figure 1

LIMITS OF TOLERANCE IN NORMAL INDIVIDUALS

The results obtained with normal individuals of the five species of animals, which are summarized in Table 2 and in Figure 8, may be stated briefly as follows:

1. The perch, which is typically a dweller in fairly deep water, has decidedly the lowest resistance to high temperatures of any of the animals studied.

2. The large-mouthed black bass, bluegill, and sunfish, which are, in the main, shallow-water fishes, have resistances that are notably higher than that of the perch.

3. Toad tadpoles, which normally live in shallow pools that are subject to wide fluctuations in temperature, have a higher resistance than any of the fishes.

TABLE 2.—*Maximum temperatures, in degrees centigrade, tolerated for various lengths of time by normal individuals of different species*

Animal	24-hour tolerance	4-hour tolerance	1-hour tolerance	15-minute tolerance	4-minute tolerance
Perch	29.6	31.5	32.0	32.0	34.0
Bass	32.2	32.4	33.4	33.7	36.0 +?
Bluegill	34.0	34.0	34.3	35.7	36.4
Sunfish	34.0	34.0	35.3	36.0	36.0 +?
Toad tadpole	36.3	37.3	37.4	38.6	39.2

The following features of the data contained in Table 2 and Figure 8 seem worthy of mention:

1. In each species there was a perfectly orderly progression, from the relatively low temperatures that were tolerated for 24 hours to the higher temperatures, which could be survived for only four minutes.

Individuals in 2-legged stage

| 36° | 38° | 40° |

12 individuals
4 trials

18 individuals
6 trials

15 individuals
5 trials

Individuals in early 4-legged stage

| 36° | 38° | 40° |

9 individuals
3 trials

9 individuals
3 trials

9 individuals
3 trials

Individuals in which tail was being resorbed

| 36° | 38° | 40° |

3 individuals
1 trial

3 individuals
1 trial

3 individuals
1 trial
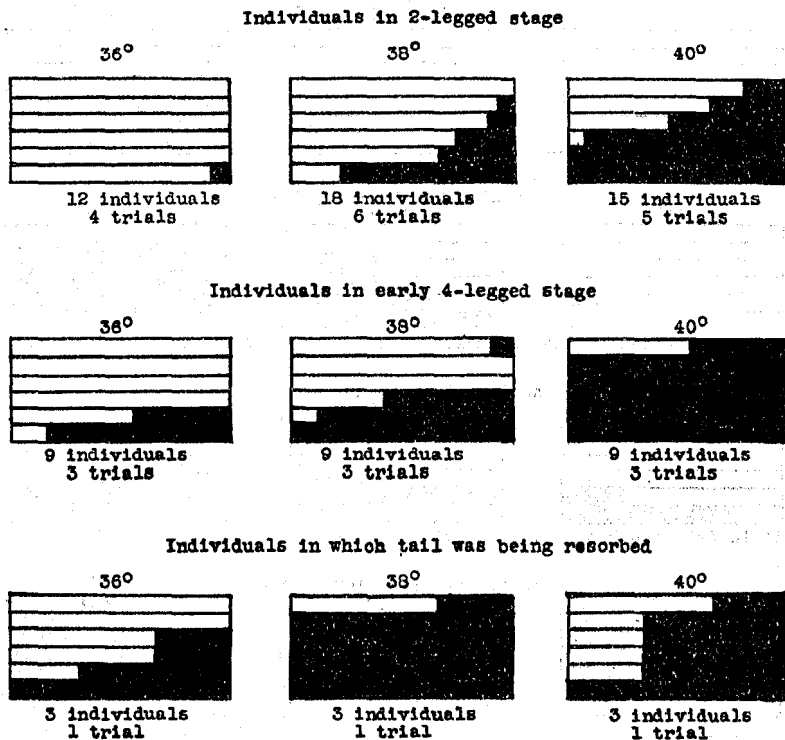
FIG. 6.—Effects of high temperatures on "normal" toad tadpoles in various stages of development. The symbols are the same as in Figure 1, except that the black sectors include individuals in heat rigor as well as those actually dead

2. For every time period, with one minor exception, the relative degrees of heat tolerated by the five species of animals fell in the same sequence—that is, the ascending order of tolerance was (a) perch, (b) bass, (c) bluegill, (d) sunfish, (e) toad tadpoles.

A few tests were made in the endeavor to determine the relation of age to tolerance of high temperatures. Perch 6 months old showed about the same resistance as those 18 months old, usually tolerating 30° C. (86° F.) but not 32° C. (89.6° F.).

In the work on fishes 2 or more years old the results were complicated by the fact that the volume of water in relation to the size of the fish was found to be an important factor in determining the temperatures survived. Two perch were tested at 30° C. in jars containing 2½ liters of water and two others in jars containing about 6 liters of water. Each of the fishes in the small jars died in a little more than one hour, while both of those in the large jars survived the 24-hour test in good condition. Similar results were obtained with mature sunfishes, death at 30° C. occur-
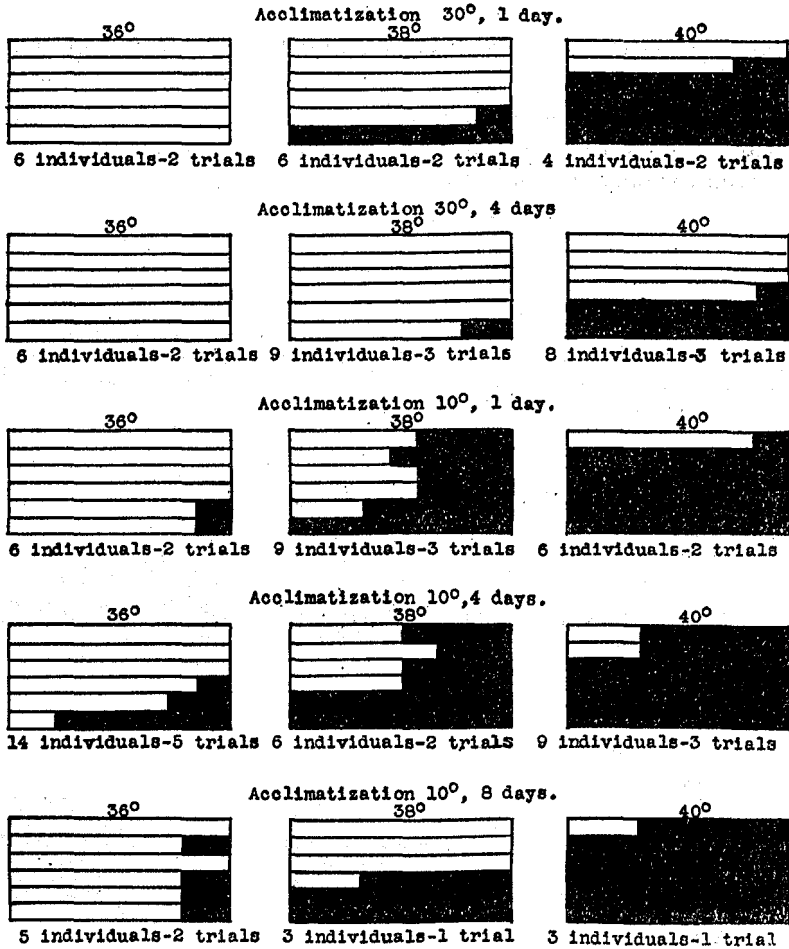
FIG. 7.—Effects of high temperatures on toad tadpoles that had been acclimatized to 30° C. or to 10° C. For explanation of symbols see Figures 1 and 6

ring in from 16 to 22 hours in the small jars, while the 24-hour test period was easily survived in the larger volume of water. It was found, however, that mature sunfishes could live for weeks, with infrequent change of water, in small jars at 30° C., after spending the first 24 hours at that tempertature in the larger container. These facts suggest that the dissolved content of the water may be of great importance as a cause of death of normal individuals if the volume of water is relatively small.

In tadpoles the results seem to indicate a definite relation between phases of the life cycle and temperature tolerance. For purposes of comparison, the tadpoles

used were divided into three classes—(1) those having hind legs in various stages of development but no visible front legs, (2) those having four legs visible but not yet beginning to resorb the tail, and (3) those in which the tail was being lost. Many times, in the work on the two-legged stage, young or retarded individuals were included in the same trials with those that were farther advanced in development, and there seemed to be no appreciable difference in heat tolerance between them; but about the time of the appearance of the front legs there was a sharp fall in the resistance, and this became still more marked as the tail was being resorbed. Evidence on this point was obtained by special trials conducted on individuals in the later stages of metamorphosis, the data being presented in Table 3 and Figure 6.

TABLE 3.—*Maximum temperatures, in degrees centigrade, tolerated for various lengths of time by normal toad tadpoles in various stages of development*

Stage of development	24-hour tolerance	4-hour tolerance	1-hour tolerance	15-minute tolerance	4-minute tolerance
Two legs	36.2	37.3	37.4	38.6	39.3
Four legs	34.2	35.1	36.9	38.0	38.0
Losing tail	34.0	34.7	35.3	35.3	36.0

To rule out asphyxiation as a possible cause of death of the most advanced individuals, solid objects were supplied on which they could crawl out and get air. The data indicate that for each of the five time periods the tolerance limits of the three stages of tadpoles fell in the same order—namely, two-legged individuals highest, early four-legged stage intermediate, and those losing their tails lowest.

MODIFICATION OF TOLERANCE BY ACCLIMATIZATION

In making determinations of tolerance on individuals that had been exposed to temperatures of 30° C. (86° F.) and 10° C. (50° F.) the same methods of procedure and computation were used as in the case of normal individuals. The details of the results are shown in Figures 2, 3, 4, 5, and 7 and in Tables 4 and 5, while Figure 9 compares the results secured with the various animals.
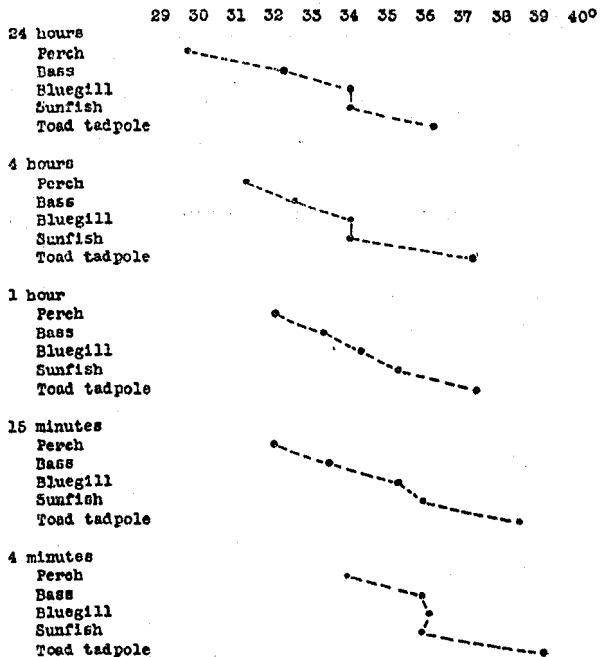

FIG. 8.—Averages of maximum temperatures tolerated for various lengths of time by normal individuals of different species. Complete data on tolerance of bass and sunfish for four minutes are not available. In both of these cases the tolerance may be a little greater than that shown

TABLE 4.—*Maximum temperatures, in degrees centigrade, tolerated for various lengths of time by normal individuals and by those that had been acclimatized for different lengths of time at 10° and 30° C. The signs +? or —? indicate that the limit of tolerance was shown to be as high (or low) as the figure given and was possibly higher (or lower)*

Temperature and duration of acclimatization	24-hour tolerance	4-hour tolerance	1-hour tolerance	15-minute tolerance	4-minute tolerance
Perch:					
30°, 1 day	30.3	32.0	32.0	34.0	34.0+?
Normals	29.6	31.5	32.0	32.0	34.0
10°, 1 day	29.0	30.3	32.0		
10°, 4 days	29.0	29.4	31.3	31.7	31.7+?
Bass:					
30°, 4 days	35.2	36.0	36.9	37.4	39.0
30°, 1 day	36.0	36.0	36.0	36.7	38.0
Normals	32.2	32.4	33.4	33.7	36.0+?
10°, 1 day	31.5	31.8	32.0	32.3	34.0
10°, 4 days	30.0	30.0	31.0	32.8	34.0
10°, 16 days	28.0—?	28.3	29.0	29.8	30.0
Bluegills:					
30°, 4 days	35.6	36.3	36.3	38.0	38.0+?
30°, 1 day	34.3	35.7	36.0	36.7	38.0+?
Normals	34.0	34.0	34.3	35.7	36.4
10°, 1 day	30.0	30.0	30.7	32.6	34.0+?
10°, 4 days	30.6	31.1	32.7	33.5	34.0+?
10°, 16 days	28.0—?	28.0—?	28.3	30.0+?	30.0+?
Sunfish:					
30°, 4 days	34.3	35.7	38.0		
30°, 1 day	34.2	34.8	36.0+?	38.0	38.0
Normals	34.0	34.0	35.3	36.0	36.0+?
10°, 1 day	32.0+?				
10°, 4 days	30.0	30.4	31.2	33.4	34.0+?
10°, 16 days	28.0—?	29.4	30.0+?		
Toad tadpoles:					
30°, 4 days	37.6	38.0	39.8	40.0+?	40.0+?
30°, 1 day	36.0	37.7	38.0	38.0	39.5
Normals	36.3	37.3	37.4	38.6	39.2
10°, 1 day	35.7	36.3	37.1	37.1	37.1
10°, 4 days	34.4	35.6	36.7	37.0	38.0

TABLE 5.—*Numbers of degrees centigrade by which tolerances of different species were modified by acclimatization at 30° and at 10° C. The data in this table represent the differences between the temperatures tolerated by normal and acclimatized individuals, as shown in Table 4*

Temperature and duration of acclimatization	24-hour tolerance	4-hour tolerance	1-hour tolerance	15-minute tolerance	4-minute tolerance
Perch:					
30°, 1 day	0.7	0.5	0.0	2.0	0.0
10°, 1 day	—.6	—1.2	.0		
10°, 4 days	—.6	—.9	—.7	—.3	—2.3
Bass:					
30°, 4 days	3.0	3.6	3.5	3.7	3.0
30°, 1 day	3.8	3.6	2.6	3.0	2.0
10°, 1 day	—.7	—.6	—1.4	—1.4	—2.0
10°, 4 days	—2.2	—2.4	—2.4	—.9	—2.0
10°, 16 days	—4.2	—4.1	—4.4	—3.9	—6.0
Bluegills:					
30°, 4 days	1.6	2.3	2.0	2.3	1.6
30°, 1 day	.3	1.7	1.7	1.0	1.6
10°, 1 day	—4.0	—4.0	—3.6	—3.1	—2.4
10°, 4 days	—3.4	—2.9	—1.6	—2.2	—2.4
10°, 16 days	—6.0	—6.0	—6.0	—5.7	—6.4
Sunfish:					
30°, 4 days	.3	1.7	2.7		
30°, 1 day	.2	.8	.7		
10°, 1 day	(?)				
10°, 4 days	—4.0	—3.6	—4.1		
10°, 16 days	—6.0	—4.6	—5.3		
Toad tadpoles:					
30°, 4 days	1.3	.7	2.4	1.4	.8
30°, 1 day	—.3	.4	.6	—.6	.3
10°, 1 day	—.6	—1.0	—.3	—1.5	—2.1
10°, 4 days	—1.9	—1.7	—.7	—1.6	—1.2

RESULTS OF EXPERIMENTS ON FISHES

The facts regarding fishes shown in Figure 9 may be summarized as follows: (1) The loss in tolerance by acclimatization at 10° C. was most rapid in sunfishes and bluegills, the species that have the highest normal resistance; it was less marked in the bass, and was decidedly the least in the perch, which has the lowest normal tolerance. (2) The gain in tolerance at 30° C. was rapid in the bass and relatively slow in the sunfishes, bluegills, and perch.

Four facts regarding the data on which these conclusions are based may be noted: (1) With a few exceptions (probably due to experimental error), the results indicate a continuous increase in tolerance by acclimatization at 30° C. and a continuous decrease at 10° C. (2) The tolerance limits determined for the different periods of time (24 hours, 4 hours, 1 hour, 15 minutes, and 4 minutes), involving, as they do, more than one set of individuals and more than one set of experimental conditions, serve as a check on each other and for the most part corroborate each other. (3) The percentage of results that are far off from the mode in any series is small. (4) Observations on behavior (which will be presented later), although hard to express in numerical terms, support the conclusions.

Inasmuch as the initial tolerance of bluegills and sunfishes was relatively high (34° C.; 93.2° F.)

Fig. 9.—Extent to which acclimatization alters the tolerance of each species. The center line in each figure indicates the average tolerance limit of "normal" individuals. The lengths of the bars extending to the right show the average amounts of increase in tolerance produced by acclimatization; those to the left show decreases

and their gain in tolerance by acclimatization at 30° C. quite slow, it seemed possible that their gain in tolerance was slow because the acclimatization temperature of 30° was not high enough to evoke a vigorous reaction. This appeared the more probable because Loeb and Wasteneys (1912) secured their greatest changes in tolerance by exposing fishes, for short periods of time, to very high temperatures; so it was thought desirable to determine the effect of acclimatization at the tolerance limit of each species.
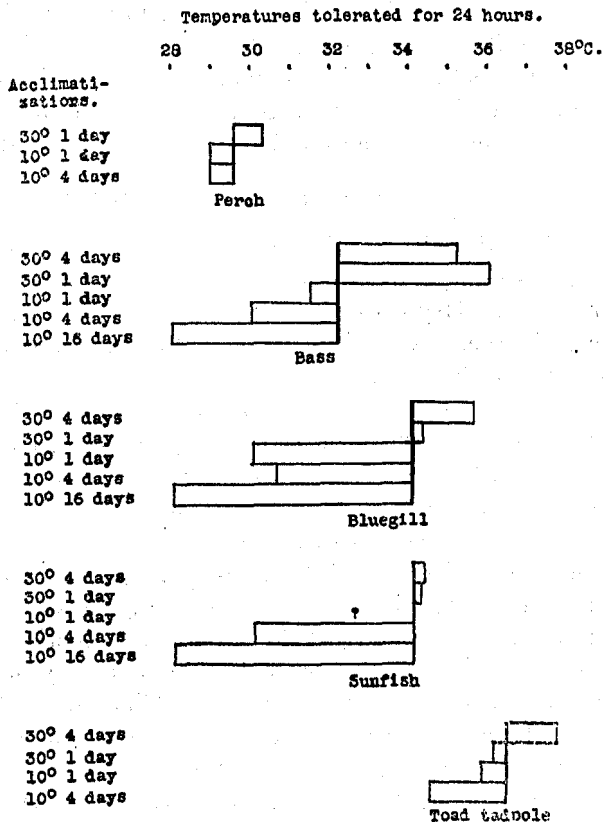
Accordingly, bass, bluegills, and sunfishes that had been at 30° C. for one day were exposed to 36° C. (96.8° F.), their tolerance limit, and then tested at higher temperatures. It was found that one day at 36° enabled the bass to tolerate 38° C. (100.4° F.) for 24 hours or more; while four days' acclimatization was required to produce the same results in bluegills and sunfishes. These results indicate that the tolerance of bluegills and sunfishes can be raised substantially if a high enough temperature is used in acclimatization, but they also furnish confirmatory evidence of of the still greater modifiability of the bass. When, in this connection, it is recalled that the acclimatization temperature of 30° C. (86° F.) (which was the only one used for the perch) was the normal tolerance limit of the species, the conclusion that the perch is the least modifiable of the animals studied seems to be confirmed.

MODIFICATIONS OF TOLERANCE IN TOAD TADPOLES

The detailed results obtained with tadpoles are shown in Tables 4 and 5 and in Figures 7 and 9, in which it appears that the changes in tolerance were clearly marked but slow. Exposure to 30° C. (86° F.) for 24 hours produced no significant change in resistance; while in 4 days at 30° C. the gain in tolerance was less than half that shown by the bass.

As in the case of bluegills and sunfishes, the slowness of the gain in tolerance appears to be due, at least in part, to the fact that the acclimatization temperature of 30° C. was too far below the normal tolerance limit to be very effective. This view was supported by several tests. Fifteen individuals in five different lots were exposed for one day to a temperature of 36° C. (96.8° F.) their tolerance limit. They were then tried at 38° C. (100.4° F.), and all but one survived for 24 hours. On being transferred to 40° C. (104° F.), however, all died. These results seem to indicate that one day at 36° C. was as effective in raising the resistance of tadpoles as four days at 30° C., and that the capacity of tadpoles for increase of tolerance is not very different from that of bluegills and sunfishes.

The most distinctive feature of the results obtained with tadpoles is the slowness with which resistance was lost through exposure to low temperature. It will be recalled that in those species of fishes having the highest normal resistance there was a rapid lowering of the tolerance limit by exposure to 10° C. (50° F.), while in the species having low normal resistance the decrease in tolerance was slow. Tadpoles, on the other hand, with a normal resistance 2.3° higher than that of any of the fishes, lost tolerance more slowly than any of the fishes except the perch. Table 6 brings out this contrast.

TABLE 6.—*Relation between normal tolerance limits of different species and the number of degrees centigrade by which the tolerance limit was lowered by exposure to 10° C. for four days*

Species	Normal 24-hour tolerance limit	Amount of lowering of tolerance limit
Perch	29.6	0.6
Bass	32.2	2.2
Bluegill	34.0	3.4
Sunfish	34.0	4.0
Toad tadpole	36.3	1.9

BEHAVIOR AT HIGH TEMPERATURES

Observations on the behavior and apparent condition of the experimental animals, while difficult to reduce to quantitative terms, are of some interest because they support, in some important respects, the conclusions drawn from the data on survival, and they give some clues as to the nature of the acclimatization process. Three effects commonly followed the transfer of normal fishes to high temperatures:

1. Increase in general activity.

2. Disturbances of equilibrium such as (a) elevation of the tail, so that the body stood at an angle of from 30 to 90° to the horizontal; (b) a persistent tendency of the fish to rise to the surface, tail first, constant use of the fins being required to keep the fish at the bottom; (c) a rolling from side to side, or rotation on the longitudinal axis; (d) floating at the surface.

3. Increase of respiratory movements. Sometimes there was merely an increase in respiratory rate, but in other cases there was also an increase in amplitude of respiratory movements, so that breathing was labored, the fishes often coming to the surface to gulp air.

The disturbance of equilibrium usually occurred within 1 to 4 minutes after the fishes were transferred to the higher temperature, although sometimes it was delayed longer, while the increase in respiratory movements usually became pronounced within the first 15 minutes of the test. In cases where the fishes survived the 24-hour test, normal equilibrium usually was regained in from 1 to 4 hours, while the labored breathing commonly disappeared in from 4 to 20 hours. The original rate of respiratory movements seldom, if ever, was regained during the 24-hour test period.

The extent to which the effects described were manifested by normal fishes varied greatly, according to the test temperatures, and the description that follows represents the usual results in all the species except the perch.

1. At the highest temperature that most of the normal individuals could tolerate for 24 hours there was some disturbance of equilibrium and respiration, followed by complete recovery.

2. At a temperature that a minority of the individuals could survive there was marked initial disturbance of equilibrium and respiration. This was followed by complete recovery in some cases; in other cases equilibrium was regained temporarily, but death occurred several hours later; in still others there was no recovery.

3. At a temperature that none of the individuals could survive there was great disturbance of both equilibrium and respiration, with no signs of recovery except, perhaps, temporary improvement in equilibrium.

The behavior of perch resembled, in a general way, that of the three other species, but their recovery of normal respiration was slower. A bass, when placed at 32° C. (89.6° F.), or a bluegill or sunfish at 34° C. (93.2° F.), their respective limits of tolerance, usually resumed light, easy breathing within 10 to 12 hours; while a perch placed at 30° C. (86° F.), its limit of tolerance, often would continue noticeably labored respiratory movements for two or three days. These facts apparently support the conclusion, previously reached, that the perch has the least power of adjustment to increases in temperature of any of the fishes studied.

The disturbances in equilibrium and respiration occurring within the first 15 minutes of a test are referred to as the "initial shock." Table 7 summarizes the

observations on initial shock and the subsequent effects in normal and acclimatized bass tested at their tolerance limits and at temperatures above these limits. The following facts, which appear in Table 7, are fairly typical of what was observed in all the species of animals:

1. Acclimatization at 10° C. (50° F.), and 30° C. (86° F.) not only changed the limits of tolerance of fishes but also changed their behavior at high temperatures.

2. Acclimatization at 30° C. greatly reduced the initial shock, even at temperatures at which many individuals eventually died. Individuals that did show shock effects rarely survived.

3. Acclimatization at 10° C. greatly increased initial shock, and large numbers of individuals, after showing great disturbance of equilibrium and respiration, were able to make at least a temporary recovery.

TABLE 7.—*Incidence of and recovery from initial shock in large-mouthed black bass; the figures in the table refer to percentages of individuals*

Acclimatization	Test temperatures, ° C.	Showed no shock effects		Showed shock effects					
				Recovered normal equilibrium and respiration		Recovered normal equilibrium but not respiration		No apparent recovery	
		Lived	Died	Lived	Died	Lived	Died	Lived	Died
Normals	32	0	0	100	0	0	0	0	0
	34	0	0	26	0	0	20	0	54
	36	0	0	0	12	0	0	0	88
30°, 1 day	36	88	0	12	0	0	0	0	0
	38	0	17	0	17	0	0	0	66
30°, 4 days	36	0	0	60	40	0	0	0	0
	38	0	57	0	0	0	0	0	43
10°, 1 day	30	100	0	0	0	0	0	0	0
	32	0	0	75	0	0	25	0	0
	34	0	0	0	0	0	0	0	100
10°, 4 days	30	0	0	100	0	0	0	0	0
	32	0	0	0	0	0	83	0	17
	34	0	0	0	0	0	40	0	60
10°, 16 days	30	0	0	0	0	0	40	0	60

The results obtained with tadpoles paralleled fairly closely those obtained with fishes. While there was not the tendency to rise to the surface, which was noticed in fishes, the loss of equilibrium often was very pronounced. The increase in temporary initial shock by acclimatization at 10° C. was clearly noted, several individuals (fig. 7) being observed to go into heat rigor within the first few minutes of a test and resume normal movement before the end of the first hour.

The significance of the shock effects is not clear. The loss in equilibrium and tendency to rise to the surface, which was noted in fishes, resembled the behavior noted by Hall (1924) in fishes that were subjected to high concentration of CO_2 and low concentrations of oxygen. Whether the effect was due to the secretion of gas into the swim bladder, or simply to the expansion by heat of the gas already there, seems uncertain. In any case, the loss of equilibrium does not appear necessarily to be closely connected with the immediate cause of death of fishes or tadpoles, as death often occurred several hours after complete recovery of equilibrium.

DISCUSSION

Before taking up a discussion of the results obtained, some possible complicating factors in the experiments will be considered. In the first place, it may be asked whether the methods used constitute an adequate test of the temperature tolerance of the animals. Ideal procedure might, like the methods of Dallinger (1880) and Jollos (1921), working with microorganisms, test the ability of an animal to reproduce at a given temperature, but obviously such a method was impracticable in the present instance. Thermal death points have been employed in various ways by different experimenters. The method of Davenport and Castle (1895) involves the cumulative effects of a series of increasing temperatures; and as some animals succumb very quickly when an injurious temperature is reached, while others linger for some time in a dying condition, the use of steadily increasing temperatures does not seem to afford a very satisfactory means of comparing the thermal death points of different animals. Loeb and Wasteneys (1912) measured tolerance in terms of the number of minutes that their fishes survived at a given temperature; and while this method seems quite satisfactory in theory it is difficult to apply in a long series of experiments, as its accurate use would necessitate observations at frequent intervals throughout both day and night. In the present experiments the use of fixed time periods and several constant temperatures made it possible to determine the maximum temperature tolerated for each of the time periods, these maximum temperatures serving as checks on each other.

The desirablility of continuing the tests for more than 24 hours was considered, because it was realized that survival for one day at a given temperature does not necessarily imply the ability to tolerate it indefinitely. There was strong objection, however, to lengthening the experiments, as Saprolegnia often develops on the fishes very rapidly at or above 30° C, and a slight growth of this fungus seems to reduce materially their resistance to high temperatures. Then, too, at the end of a 24-hour test most of the individuals were either dead or apparently in good condition, so it was thought that the one-day period afforded a reasonably satisfactory basis for comparison with minimum likelihood of the introduction of complicating factors.

The question also may be asked whether any of the results noted can have been due to selection. There were only two points in the handling of the fishes before the test periods at which there were significant numbers of deaths. The first was the time when the fishes were brought into the laboratory, when a considerable mortality occurred among yearling fishes. The deaths at this time apparently were due to the shock of handling, and not to temperature, as almost all the perch (which are quite sensitive to heat, poor aeration, and foulness of the water) survived the transfer, while the young bluegills, which are much more resistant to heat and foulness of water than the perch, suffered a mortality of more than 50 per cent. The sunfishes stood the change considerably better than the bluegills, while the loss among bass was probably less than 5 per cent. The deaths of older fishes, following transfer, were negligible. The other case where deaths occurred to a notable extent was during the acclimatization of perch at 30° C. As this selection during acclimatization might have been expected to raise the average resistance of the survivors, it merely serves to emphasize the fact that the perch has relatively little capacity for increase in tolerance.

The precise cause of death at high temperatures is an important question, to which only a partial answer can be made at present. Mayer (1917) considers that it is due mainly to accumulation of acid in the tissues. The work of a number of investigators has shown that several factors, some external and some internal, may be involved. Loeb and Wasteneys (1912) showed that slight changes in the salinity of the surrounding water caused marked changes in the temperature tolerance of Fundulus. The importance of dissolved oxygen and carbon dioxide as limiting factors in the life and distribution of fishes has been stressed by several writers (Juday and Wagner, 1908; Birge and Juday, 1911; Wells, 1913 and 1915; and Pearse, 1918). That hydrogen-ion concentration is an important factor in the tolerance of low oxygen tensions by fishes is maintained by Wells (1913 and 1915). The influence of pH on the distribution of fishes has been pointed out by Shelford (1923) and Coker (1925); while the work of Shelford and Allee (1913), Shelford and Powers (1915), and Powers (1921) indicates that many (but not all) fishes can discriminate between different degrees of acidity and show definite positive and negative reactions to pH gradients. It appears that there is a good deal of difference between the pH preferences of various species, and the observations of Miss Jewell (1922) indicate that the reactions of individual fishes to pH may be modified by acclimatization.

There is a considerable amount of evidence to the effect that changes in the composition of blood of fishes may play a large part in survival under unfavorable conditions. Packard (1905, 1907, and 1908) materially lengthened or shortened the survival time of Fundulus in oxygen-free water by injections of sodium carbonate, acetic acid, or other substances. Birge and Juday (1911) suggested that if a fish were able to change the alkali reserve of its blood it would better be able to tolerate oxygen deficiency; and this theory is supported by Powers (1922 and 1922a), who has secured evidence indicating that the ability of fishes to absorb dissolved oxygen varies with the pH of the water, but that the effect is relatively slight in cosmopolitan fishes. Krogh and Leitch (1919) showed that the blood of carp, pike, and eels can unload oxygen at lower oxygen tensions than can the blood of trout and some marine fishes.

In the present experiments the changes from low to high temperatures necessarily involved changes in concentration of dissolved oxygen; and from the literature cited it appears that in many cases the death of fishes at high temperatures must be regarded as the result of the combined action of a number of factors, some internal and some external. However, the strong aeration in these experiments was designed to reduce to a minimum the fluctuations in oxygen and carbon dioxide tension during the tests, and there are two reasons for beleiving that temperature was the limiting environmental factor in the tolerance of the animals tested:

1. While the death symptoms commonly seemed to indicate asphyxiation, this appeared to be due to internal rather than external causes. Of two fishes in the same jar, frequently one would be dying (apparently from asphyxiation), while a similar individual, which had received the same preliminary treatment, would be breathing easily and would survive in good condition.

2. The results obtained in the 4-minute and 15-minute test periods, in which there was very little opportunity for change in the dissolved content of the water, ran almost perfectly parallel with the results obtained in the longer test periods.

Forbes and Richardson (1908), Everman and Clark (1920), Pearse and Achtenberg (1920), and Pearse (1921) agree that perch are to be regarded as typically lake fishes, with a decided preference for deep, cool water. When obliged to desert the deepest parts of a lake on account of summer stagnation, perch remain, for the most part, at the bottom in the region of the thermocline (Pearse and Achtenberg, 1920). It was not surprising, therefore, to find that normal perch had a low limit of temperture tolerance and relatively little ability to become adjusted to higher temperatures. Neither did it seem strange that such resistance as the perch did possess was very slightly reduced by exposure to a temperature of 10° C. The large-mouthed black bass, sunfish, and bluegill, on the contrary, are to be regarded as typically shallow-water fishes (Pearse, 1921). They were collected in large numbers from waters 3 to 5 feet deep, in places where abundance of aquatic vegetation impeded circulation of water, so that the temperature often was considerably higher than that of most of the surface of the lake. Sunfishes, indeed, seldom are found in waters without vegetation (Pearse, 1921). The results in the present experiments seem to indicate a correlation between the high temperatures occurring in the shallow portions of a lake and a high degree of tolerance and modifiability possessed by the fishes that usually inhabit these waters.

It is doubtful, however, whether differences in tolerance and in capacity for acclimatization are explainable wholly on ecological grounds. It is of interest to note that bluegills and sunfishes (which are closely related in taxonomic position and have similar but not identical habitat preferences) showed the same normal limit of tolerance and practically the same degree of modifiability. Bass, on the other hand, with habitat preferences quite similar to those of the other centrarchids, appeared to have a limit of tolerance distinctly lower than that of the bluegills and sunfishes, but a greater capacity for rapid increase in resistance by exposure to high temperatures. Although all three species of shallow-water fishes suffered rapid reduction in tolerance by exposure to low temperature in the laboratory, this change might be expected to occur only very slowly in a state of nature because of the relative slowness of fluctuation in the temperature of a lake.

The conditions of life of toad tadpoles are somewhat different from those of fishes. The shallow pools that they commonly inhabit present the possibilities of wide diurnal fluctuations in temperature and of protracted chilling during periods of cool, cloudy weather. As previously noted, the present experiments seem to indicate that normal tadpoles have a very high tolerance limit, with moderate capacity for further increase in resistance, while their loss of tolerance at 10° C. is relatively slow. When it is recalled that, among fishes, high tolerance limits were subject to rapid lowering by exposure to cold, the possession by tadpoles of a very high and relatively stable degree of tolerance appears to be a distinct adjustment to the variable temperature conditions under which they live.

Regarding the physiological nature of the changes involved in acclimatization, little that is new can be said at present. Evidence secured by Davenport and Castle (1895) and by Loeb and Wasteneys (1912) indicates that resistance to high temperatures gained by acclimatization is lost very slowly even at very low temperatures, and several observations to the same effect were made on both fishes and tadpoles in the course of the present work. The heavy breathing of fishes transferred to high

temperatures, indicative of increased respiratory metabolism (Gardner and Leetham, 1914 and 1914a; Rubner, 1924), was followed by a return to nearly normal breathing as the fishes became adjusted to their new environment. This suggests that acclimatization involves either an increase in the ability of fishes to absorb dissolved oxygen, or a decrease in metabolic rate, such as was observed by Miss Behre (1918) in Planaria. Experiments to test the latter possibility are now in progress.

SUMMARY

1. The maximum temperatures tolerated for 24 hours by normal animals belonging to five different species were found to be as follows: Perch, 29.6° C. (85.3° F.); large-mouthed black bass, 32.2° C. (90° F.); bluegill, 34° C. (93.2° F.); sunfish, 34° C. (93.2° F.); toad tadpole, 36.3° C. (97.3° F.). For the most part the tolerance limits of the different species are correlated with the temperatures to which they are exposed in their normal habitats.

2. Within each species individuals of different ages appeared to have about the same limits of tolerance, except in the case of tadpoles, which underwent a marked loss of resistance during the later stages of metamorphosis.

3. Continued exposure to high or low temperatures progressively raised or lowered the limit of tolerance of each species.

4. The fishes that inhabit shallow water (bass, bluegill, and sunfish) underwent a change of tolerance by acclimatization much more readily than the perch, which is typically an inhabitant of deep, cool water.

5. Some of the minor differences in tolerance and in modifiability of resistance among the various species of fishes seem to be specific, showing no apparent correlation with ecological factors.

6. Toad tadpoles differed from the fishes studied, in that, while having a very high limit of tolerance, their resistance to heat was reduced very slowly by exposure to low temperature.

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