HEAT TOLERANCE OF CHANNEL CATFISH
Ictalurus punctatus [1]

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ABSTRACT

Three lots of 6-day-old to 11½-month-old channel catfish from a common initial source but reared under different thermal conditions were utilized to investigate several heat-tolerance relationships. During the experimental period the fish were held in constant-temperature tanks and samples of fish were subjected to a range of sub-lethal and lethal temperatures in test baths.

The results were studied by plotting time to death of individual fish and means of test samples on semi-log paper, probability paper in some instances, and subjecting pertinent data to statistical analysis.

Fish acclimated to 26.0, 30.0, and 34.0C had upper lethal temperatures of approximately 36.6, 37.3, and 37.8C respectively. Differences in resistance times were related to age but not to size.

The results of prolonged exposure to "semi-lethal" test temperatures indicated that channel catfish may die from the direct results of heat for at least 13¾ days after initial exposure and at slightly lower test temperatures die from the indirect results of heat, due to an increased rate of metabolism resulting in starvation.

The data indicated the presence of approximately six "lethal effects" that separated the fish into physiological populations on the basis of their response to lethal temperatures.

INTRODUCTION

Researchers have long been interested in the effects of temperature on ecology and distribution of fishes. Temperature is important in many natural processes such as reproduction, assimilation of food, respiration, and activity. Many studies have been concerned with determining the upper and lower thermal limits. It has been found that these limits vary from species to species and within species these limits are affected by a number of factors (Fry, 1964).

In considering the temperature relationships of fish, certain terms should be understood. The "zone of tolerance" (Fry, Brett, and Clawson, 1942) is the range of temperatures at which fish can live indefinitely and is bounded by the upper and lower lethal limits. Fry, Hart, and Walker (1946) identified a "zone of resistance" in which fish die as a result of lethal temperatures. "Resistance time" of fish is the time prior to death at a lethal temperature. The temperature at which 50% of the test fish of a given acclimation die from the effects of heat has been designated the "incipient-upper-lethal temperature" and separates the zone of tolerance from the zone of resistance. The "ultimate-incipient-upper-lethal temperature" is the highest temperature to which a species can be acclimated without thermal mortality. For the purposes of this paper, "semi-lethal" temperatures are defined as those temperatures, immediately above and below the incipient-upper-lethal temperature, that will kill only part of a population of fish.

Heat-resistance times have been plotted on semi-log paper, and a connection of either means or medians of samples tested at differing lethal temperatures formed "resistance lines" that were plotted as

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straight lines, indicating one log-normal distribution of death times (Fry et. al. 1946; Hart, 1952). Other investigators have noted that fish tested at certain temperatures often appeared to die from more than one mechanism of death or "lethal effect" (Brett, 1952; Gibson, 1954; Hagen, 1964; Neill, Strawn, and Dunn, 1966; Tyler, 1966).

"Temperature acclimation" involves physiological change, within genetically controlled limits, and occurs over a period of a few days. Levels of temperature acclimation are easily changed in the laboratory and have a profound effect on resistance times and on the upper-lethal temperature.

The present study was designed to add to the knowledge of the lethal temperature relationships of channel catfish. The data provided should enable fishery biologists to predict the survival of channel catfish unable to escape water heated to a high temperature by industry and when stocked in the hot, shallow water in farm ponds and on fish farms.

MATERIALS AND METHODS

Source of Fish

Three lots of channel catfish, all spawned and hatched on the state hatchery at Centerton, Arkansas, were used in the experiments. The first lot consisted of 27 fish, 70-119 mm. long (all fish measurements in this paper refer to standard length), left over from a 1965 experiment by Mr. Boyce W. West (see West, 1966). He obtained these fish from the Centerton State Fish Hatchery as larvae on June 22, 1965, and they were reared in the laboratory at controlled temperatures ranging from 25.0 to 30.0C. On May 1, 1966, these fish were placed in 30.0C water for the present study.

The second lot, obtained May 11, 1966, consisted of 685 fish grown on the Joe Hogan State Fish Hatchery at Lonoke, Arkansas. These fish were 73-120 millimeters long and were approximately the same age as the preceding lot. However, they had a varied thermal history at the hatchery prior to being placed in the laboratory. Upon arrival at the laboratory, they were given a prophylactic treatment consisting of a 1:2000 formalin dip followed by a 3.0% sodium chloride dip, and were then placed in 30.0C water.

The third lot consisted of approximately 4,000 larvae obtained from the Centerton hatchery on June 29, 1966. These fish had hatched approximately two days before and were being held in 28.4C water. They were transported in water of approximately 29.0C and upon arrival at the laboratory were given a mild sodium chloride dip and placed in 30.0C water. The three lots will be referred to as the West, Lonoke, and Centerton fish, respectively. All of the experiments were conducted May 17 to October 12, 1966.

Holding Tanks

In the laboratory the fish were maintained in constant-temperature tanks. Each tank contained approximately 60 gallons of Fayetteville, Arkansas city water and two large undersand aquarium filters covered with 2 inches of sand. The water in each tank was kept constant at the acclimation temperature by a thermoregulator, sensitive to temperature changes of 0.01C, which was connected to a transistorized relay unit that controlled one 100- and one 150-watt submersible heaters. Compressed air dispersed through four air stones helped to maintain a uniform temperature throughout the tank by circulating the water. The water temperature was checked either once or twice a day with a standardized themometer with 0.1C divisions, and the water never varied over ±0.1C. Two 20-watt Sylvania "gro-lux" fluorescent lights suspended 8 inches from the water surface supplied constant illumination.

Diet and Feeding

All lots of fish were fed a formula consisting of high protein baby cereal and blended calf liver (Gordon, 1950). The frequency of feeding varied from several times a day with the younger fish to either once or twice daily with older fish. The feeding was timed to precede the test time by 18 to 24 hours in order to minimize any effects that time of feeding may have on heat resistance.

Lethal Baths

The test apparatus consisted of fifteen 2-gallon goldfish bowls filled with aged tap water (Figure 1). The water was preheated to the selected

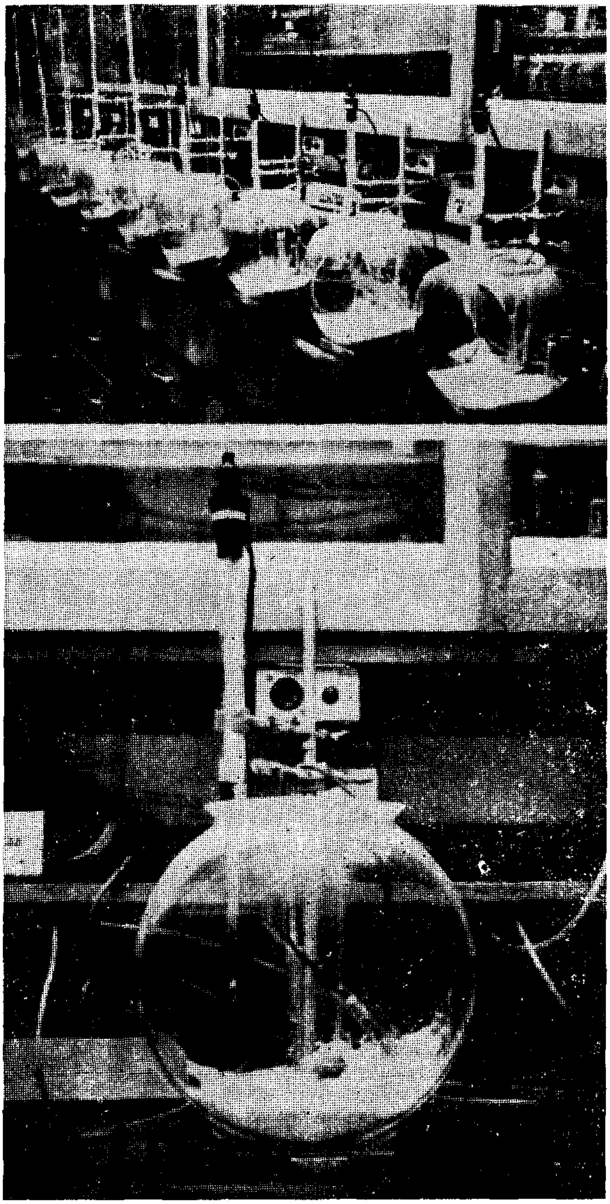

Figure 1. Photograph of part of the battery of lethal baths (above) and close-up of one of the baths (below). The heater, air stone, sand, thermoregulator and relay can be seen.

test temperature and maintained at that temperature by a thermoregulator connected to a 50-watt heater by a relay. The same standardized thermometer used to check the temperature of the holding tanks was used to check the bath temperatures. Deviations never exceeded ±0.05C from the set value and rarely exceeded ±0.02C.

Each bath contained an under-sand filter covered with 1 inch of sand. Compressed air dispersed through an air stone kept the water circulating to provide a uniform temperature throughout the bath. Overhead General Electric cool-white fluorescent lights supplied constant illumination during the tests. An effort was made to randomize the baths used as much as possible for the various tests.

Determination of Heat Resistance and Tolerance

Heat resistance and tolerance was determined by placing samples of 10 Centerton fish or 15 Lonoke fish in the lethal baths. All entries into the lethal baths were made between the hours of 10 a.m. and 4 p.m. The time to death of each fish was recorded to the nearest tenth of a minute. All values between 1,000 and 5,000 minutes were later rounded to the nearest minute. The time for each test sample was kept individually on an elapsed-time meter. A constant watch was kept on the test fish for the first 5,000 minutes. Those fish surviving longer than 5,000 minutes were checked at least every 8 hours, and the time of death of any dead fish was estimated. Upon removal from the baths the dead fish were measured to the nearest millimeter. Records were also maintained of the test temperature, age of the fish, acclimation time, date and time of testing, time of feeding, and test bath used. The fish could not be sexed due to the lack of gonadal development. Notes were made of the relative physical conditions of the fish. In the long-term resistance experiments the fish were fed in the lethal baths to maintain their physical condition. The water was also partially changed daily to eliminate excess metabolic products.

On June 20th the resistance time of 11½-month-old Lonoke fish acclimated to 25.0, 30.0, and 35.0C for 11, 23, and 7 days, respectively were tested at a series of four lethal temperatures for each acclimation. The original plans were to acclimate Centerton fish also to 25.0, 30.0, and 35.0C. However, in addition to these experiments, these fish were to be used for some acclimation-rate experiments and because it was not certain that channel catfish could withstand a quick change of 25.0 to 35.0C, acclimation temperatures of 26.0, 30.0, and 34.0C were selected for all the studies with the Centerton fish.

Experiments with the Centerton fish were started July 3rd when samples of 6-day-old fry, acclimated to 30.0C for 4½ days, were subjected to a range of test temperatures to determine their approximate upper-lethal temperature. On August 10-23, Centerton fish (now 44-57 days old), acclimated to 26.0, 30.0, and 34.0C, for 27, 42, and 47 days, respectively, were subjected to test temperatures at 0.2C increments ranging from non-lethal temperatures to temperatures that would be lethal in less than two minutes. Controls were also maintained in the test baths at the acclimation temperatures.

Method Used to Determine Heat Death

Unlike the "chill coma" of cold death, the time of heat death is rather apparent (Wells, 1914; Doudoroff, 1942; Brett, 1952). A complete loss of response to stimuli coupled with a cessation of muscular contraction and respiratory movements indicate death (Brett, 1952). In the present experiments the fish were considered dead when no movement of any type could be detected after a few seconds of examination.

Sometimes, at "rapidly lethal" test temperatures, channel catfish and bullheads exhibited movement after removal from the lethal baths (Hart, 1952). In the present study this was a frequent occurrence at the highest test temperatures. However, if the fish were returned to the lethal bath, no further movement occurred. If they were placed in water at acclimation temperature, a few shortly recovered and displayed nearly normal swimming movements but died approximately one hour later, indicating that irreversible damage had occurred as a result of the heat.

Regardless of the later reaction of the fish, in all instances the time of death was recorded as the time at which visible movement had ceased while in the lethal bath. The resulting error between the recorded death time and actual death time is not believed to have been more than a few seconds.

RESULTS

Resistance and Tolerance

The resistance times of the Lonoke fish (Figure 2) were tested for normality of distribution, utilizing transformed scores, $Y = \log_{10}$ resistance time. Out of the 15 sets of data only these for an acclimation temperature of 35.0C and a test temperature of 39.0C showed slightly significant deviations from normality. Therefore, it appears that the data are consistent with the long-standing hypothesis that resistance times are log-normal in distribution.

The resistance times of the 11½-month-old Lonoke fish (Figure 2) and the 44-57-day-old Centerton fish (Figure 3) were plotted on a per fish basis, and then the means of the resistance times of both lots of fish were compared (Figure 4). The two lots of fish were directly comparable only at 30.0C acclimation, but the trends at the other acclimations support the relationships indicated.

The Lonoke fish had resistance lines with steeper slopes and they appeared to have greater resistance to the higher experimental temperatures than the Centerton fish. However, at lower lethal test temperatures the Centerton fish appeared to have greater resistance.

The means of two-10-fish samples of eleven-month-old West fish tested June 1st (Figure 4) were similar in individual death times and means to Lonoke fish at the two common test temperatures of 38.0 and 40.0C. Chi-square tests (1% level) indicated no significant difference in the resistance time of the two lots (38.0 C-$X^2 = \overset{0.125}{23}$ d.f.; 40.0 C-$X^2 = \overset{0.343}{23}$ d.f.).

A comparison of survival at semi-lethal temperatures of Centerton fry, acclimated to 30.0C (Table 1) and 44-57-day-old Centerton fingerlings, acclimated to 30.0C (Table 2) indicates the fingerlings were slightly more resistant at these temperatures than the fry.

The fry were more variable than the older fish in the length of time to heat death and many of them developed deformed vertebral columns at temperatures as low as 35.5C. Only a few of the 44-57-day-old fish developed this condition, and it did not occur at temperatures below 36.0C.

Although there were age and/or size differences between lots, there was no indication of any correlation of size and resistance time between fish of the same age in any of the test groups.

Brett (1952) and Gibson (1954) noted that the mortality pattern was not complete at 10,000 minutes in their test fish. To determine effects of prolonged exposure to semi-lethal temperatures, Centerton fish acclimated at 26.0C were kept in the lethal baths for 37,650 minutes (26 days). Out of the 75 fish surviving at 10,000 minutes 15 died by 20,000 minutes, three between 20,000 and 30,000 minutes, and three between 30,000 and 37,650 minutes (Table 2). Most of the mortality therefore was complete by 20,000 minutes (13¾ days). However, even though the fish were fed once daily in the lethal baths, the maintenance requirements at temperatures above approximately 36.0 C were greater than the food intake, and they were so emaciated by 37,650 minutes that mortality would have occurred from starvation.

The incipient-upper-lethal temperatures for the 44-57 day-old Centerton fish, based on a test duration of 7,260 minutes, were estimated to be 36.6 for 26.0 C acclimation, 37.3 for 30.0 C acclimation, and 37.8 for 34.0 C acclimation.

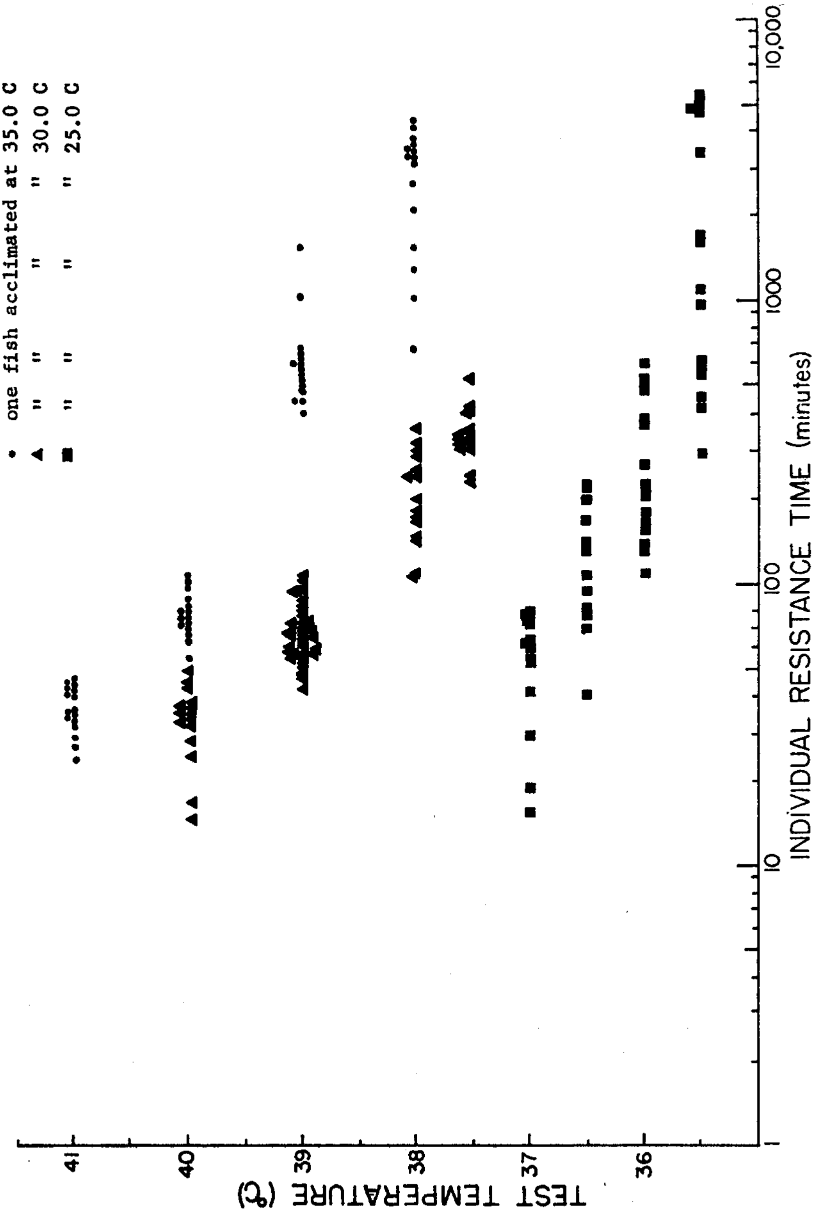

Figure 2. Individual Resistance Times of Lonoke Fish Acclimated at 25.0, 30.0, and 35.0C. Fifteen fish were used per sample except acclimation 30.0C-test temperature 39.0C which included 4 samples of 15 fish each. Standard length was 73-120 mm.

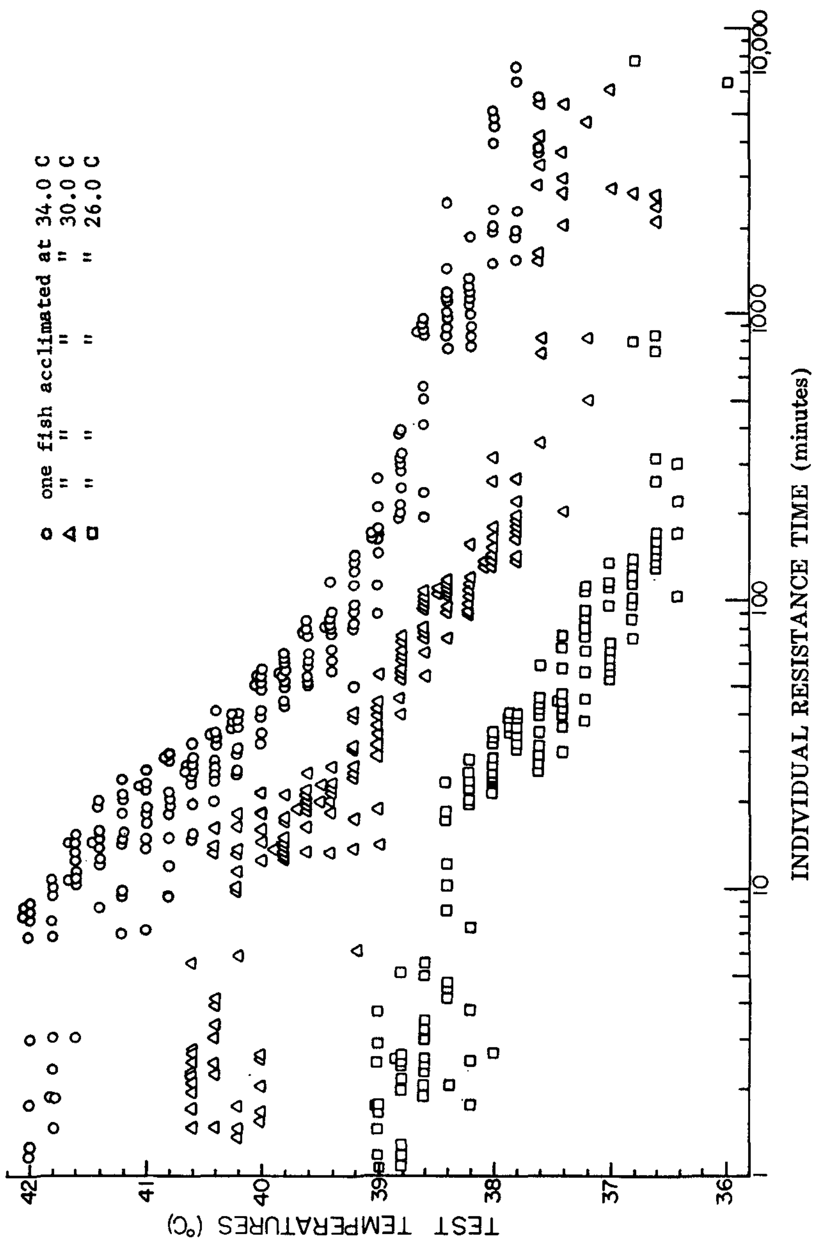

Figure 3. Individual Resistance Times of Centerton Fish (44-57 days old) at 26.0, 30.0, and 34.0C Acclimations. Samples of ten fish were tested at 0.2C increments. (Standard length was 13-39 mm.)

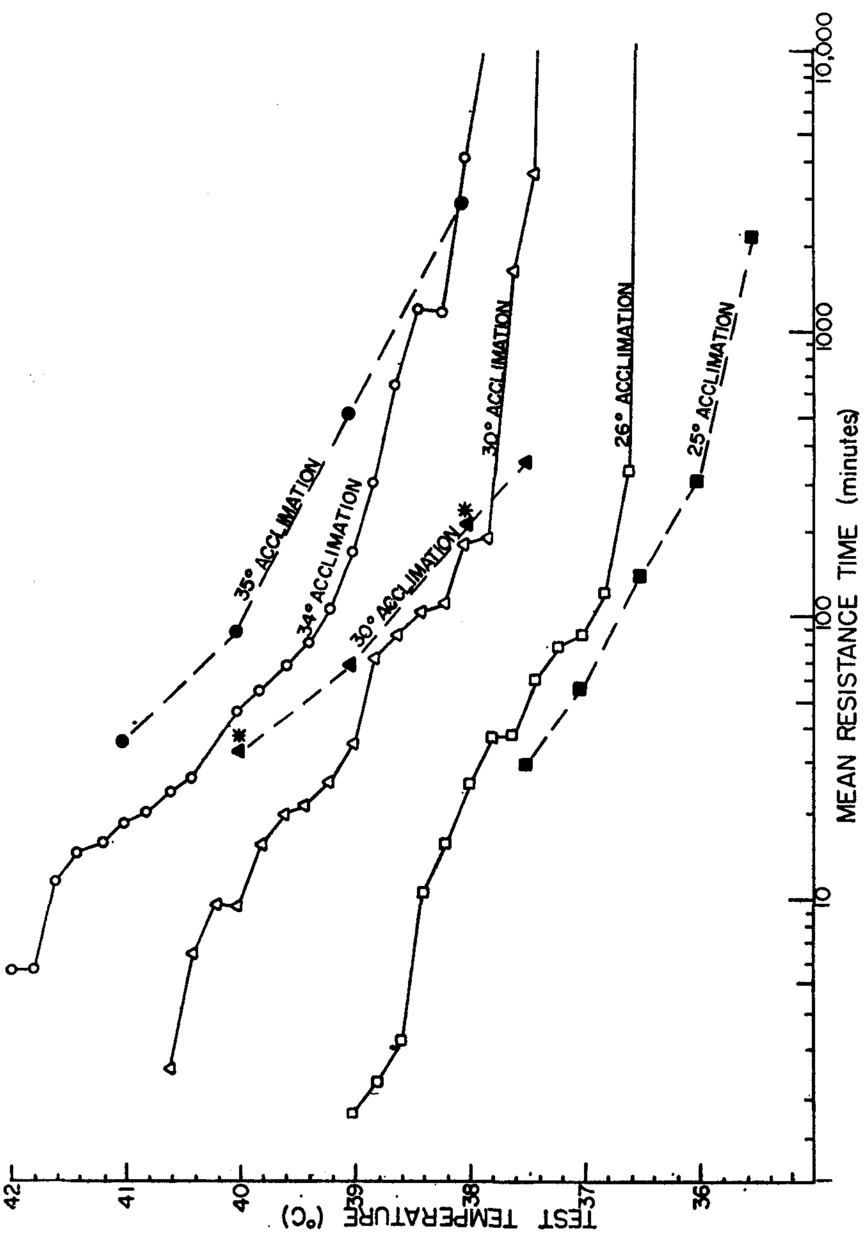

Figure 4. A Comparison of the Mean Resistance Times of the Lonoke (dashed lines) and Centerton Fish (solid lines). Means of two samples of the West fish are represented by *. (The West fish were acclimated to 30.0C for 31 days, the standard length was 79-119 mm.)

TABLE 1 — SURVIVAL OF 6-DAY-OLD CENTERTON FRY, ACCLIMATED TO 30.0 C AND TESTED AT SEMI-LETHAL TEST TEMPERATURES. (STANDARD LENGTH WAS 12-16 MM.)

Test Temp.	Resistance Time in Minutes	Abnormal Conditions of Surviving Fish
30.0° (Control)	all alive at 10,000	
35.5°	1 dead at 7,560 9 alive at 10,000	1 fish with a deformed vertebral column
36.0°	all alive at 10,000	
36.1°	3 dead 4,968-5,753 7 alive at 10,000	2 fish with deformed vertebral columns
36.3°	1 dead 5,347 9 alive at 10,000	2 fish with deformed vertebral columns
36.5°	4 dead 300-2,980 6 alive at 10,000	
37.0°	4 dead 300-8,000 6 alive at 10,000	6 fish with deformed vertebral columns

TABLE 2 — SURVIVAL OF 44-57-DAY-OLD CENTERTON FISH ACCLIMATED TO 26.0, 30.0 AND 34.0 C AND TESTED AT SEMI-LETHAL AND SUB-LETHAL TEMPERATURES (10 FISH PER SAMPLE AT 0 MINUTES AND STANDARD LENGTH WAS 13-39MM.)

	26.0 C Acclimation Number of Fish Surviving			
Test Temp. (°C)	To 10,000 Minutes	To 20,000 Minutes	To 30,000 Minutes	To 37,650 Minutes
Controls				
26.0	10	10	10	9*
Experimentals				
35.0	10	10	10	10
35.2	10	10	10	9
35.4	10	10	10	10
35.6	10	6	5	5
35.8	10	8	8	8
36.0	9	5	5	4**
36.2	10	6	4	4***
36.4	6	5	5	4****
	75	60	57	54

* Dead fish was in poor flesh.

** At 37,650 minutes one fish was in poor flesh and three fish had deformed vertebral columns.

*** One (1) fish dead at 11,973 minutes had a deformed vertebral column.

**** The four surviving fish were in poor condition and had deformed vertebral columns.

30.0 C Acclimation		34.0 C Acclimation	
Test Temp. (°C)	No. Surviving To 7,260 Min.*	Test Temp. (°C)	No. Surviving To 10,000 Min.*
Controls		Controls	
30.0	10	34.0	10
Experimentals		Experimentals	
36.0	10	37.0	10
36.2	10	37.2	10
36.4	10	37.4	10
36.6	7	37.6	7
36.8	9	37.8	4
37.0	8	38.0	2
37.2	7		
37.4	4		
37.6	1		
37.8	0		

* No abnormal fish noted at termination of test.

Lethal Effects

An examination of the resistance times of the Centerton fish at all three acclimations (Figure 3) revealed that at least three lethal effects were present. From this figure and plots on probability paper (not shown) the following observations were made. At the test temperatures used, mortality from the first lethal effect, which will be referred to as lethal effect I, occurred from 1.2 to 3.1 minutes for 34.0 C-acclimated fish, 1.4 to 4.3 minutes for 30.0 C-acclimated fish, and 1.1 to 4.8 minutes for 26.0 C-acclimated fish. Tyler (1966) attributed these early deaths to "shock." Lethal effect II started at 6.9 minutes for 34.0 C-acclimated fish, 10.2 minutes for 30.0 C-acclimation and approximately 10.2 for 26.0 C-acclimation. At each acclimation some fish would die from lethal effect I, some from II, and, in a few instances one or more fish died within a transitional zone apparently as a result of both lethal effects. A further indication of this dual effect was the splitting or broadening of the lethal effect II line as it approached the transitional zone with lethal effect I.

Lethal effect II formed nearly straight lines and accounted for over half of the fish dying from the lethal temperatures. The mortality from lethal effect II stopped by about 200 minutes.

The remainder of deaths were more confused and appeared to result from several lethal effects. There was a clumping of deaths at approximately 200-500 minutes, one between about 720 and 1,450 minutes, another in the area of 2,000-3,000 minutes and one in the area of 5,500 to 7,300 minutes. An examination of the data plotted on probability paper also indicated an uneven response but this method failed to delineate specific lethal effects.

Multiple lethal effects were not easy to discern in the Lonoke fish (Figure 2) because of the narrow range of test temperatures and limited number of fish tested.

DISCUSSION

Heat Resistance and Tolerance

Acclimation temperature had a profound effect upon the tolerance of channel catfish to high temperatures, as has been found with other species. The incipient-upper-lethal temperature for 44-57-day-old catfish was estimated to be 36.6, 37.3, and 37.8, respectively, for 26.0, 30.0, and 34.0 C acclimations. This is considerably higher than the 33.5 C ultimate-incipient-upper-lethal temperature given by Hart (1952) for channel catfish tested at Welaka, Florida, and Put-in-Bay, Ohio.

The thermal history, prior to acclimation, of the test fish used in our experiments did not appear to influence the resistance time since the Lonoke and West fish, with similar sizes, ages, and genetic backgrounds but contrasting thermal histories, had similar resistance times.

There are many references concerning the effect of size and age on thermal resistance. In most of the records the size and age varied together and the separate effects could not be determined. Hart (1952), in summarizing the literature on this subject noted a wide variety of results, and it appears that the effects of size and age differ from species to species. He found no relationship between size and order of death in the channel catfish he tested.

In this study there were no detectable differences within the test lots related to size of fish. Therefore, the differences between lots are believed to be due to age differences. These differences are complex and many questions are left unanswered by this study. The incomplete evidence gathered did indicate that the different lethal effects may change independently with age. The 44-57-day-old Centerton fingerlings had the greatest resistance at slowly-lethal temperatures followed by six-day-old Centerton fry, and 11½-month-old Lonoke fish had the least. On the other hand, the Lonoke fish had greater resistance at higher temperatures than the 44-57-day-old Centerton fish. Bishai (1963) found the resistance time of *Tilapia nilotica* fry was greater than that of either the larvae or adults at test temperatures of 38.0 and 39.0 C.

There was no change in the average resistance times of the Lonoke fish over the period tested as was noted in the Centerton fish. Heat resistance levels of the Centerton fish changed rapidly at first but leveled off by about 10 days of age.

It appears that after a certain period of growth has passed heat resistance in the channel catfish does not change. However, due to the relatively short period of testing, confirmatory evidence is necessary before weight can be put on this finding. A study that followed a group of fish from an early stage to maturity could make a valuable contribution to this area of knowledge.

During tests in the lethal baths, a relatively large percentage of the six-day-old Centerton fry developed deformed vertebral columns. A lesser percentage of Centerton fish at 44-57 days old and none of the still older Lonoke fish developed this condition. West (1966) also noted this condition in channel catfish raised from fry to fingerlings at his highest growth temperature of 36.0 C. This may be a further manifestation of the differences in response to heat, due to age.

Lethal Effects

Though most samples of Lonoke fish (Figure 2) were log-normal in distribution of resistance time, the more thorough study of the Centerton fish (Figure 3) revealed that resistance time is more accurately described as consisting of several cojoining log-normal populations. When the fish were dying from one lethal effect, variance was low. When two or more lethal effects were causing deaths, the variance was much greater, and time lapses appeared between those fish dying from one effect and those dying from another (Figure 3). Inspection of the data obtained at high lethal temperatures, plotted on semi-log paper and on probability paper, revealed two distinct physiological populations on the basis of their response to lethal temperatures. At lower lethal temperatures there appeared to be at least four more groupings of death times that could be due to lethal effects. Neill, Strawn, and Dunn (1965) described five variance populations in 30.0 C-acclimated longear sunfish (*Lepomis Megalotis* Rafinesque) that were ascribed to differing lethal effects. They believed areas of high variance were due to the expression of more than one lethal effect.

The basic physiological mechanisms of heat and cold death long have been investigated, but to date there is no entirely satisfactory explanation of them. There is some evidence that the essential tissue most sensitive to heat shock is that of the nervous system (Brett, 1956). The behavior of those fish dying from lethal effect I in the present study very strongly suggested a nervous-system failure. These fish exhibited rapid movements and died in various rigid positions. Those fish dying from later lethal effects died slowly and quietly and were always limp.

SUMMARY AND CONCLUSIONS

Three lots of channel catfish, comprising two size and age groups and having separate thermal histories, were tested for several factors affecting heat tolerance.

Heat tolerance was apparently determined by genetic background, acclimation level, and to a lesser extent by age. Thermal history prior to acclimation and size did not appear to influence heat tolerance.

The fingerling fish had the highest upper-lethal temperatures. However, at "quickly lethal" temperatures older fish had greater resistance.

The upper lethal temperatures for 44-57-day-old fish were approximately 36.6, 37.3, and 37.8 for 26.0, 30.0, and 34.0 C acclimations.

There appeared to be at least six lethal effects that tended to separate the fish into physiological populations on the basis of their heat resistance.

Heat death may occur up to 13¾ days after initial exposure. High temperatures may indirectly cause mortalities over a still longer period of time by increasing the rate of metabolism beyond the fishes ability to consume food.

ACKNOWLEDGMENTS

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TEMPERATURE SELECTION AND HEAT RESISTANCE OF THE MOSQUITO FISH, *Gambusia affinis*[1]

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ABSTRACT

The average selected temperature of a sample of *Gambusia affinis* previously acclimated to 20°C decreased from 28-29°C to about 27°C after the fish had lived in a temperature-gradient tank for one month. The final thermal distribution of the fish varied with sex and developmental stage. Males selected lower temperatures than did adult females. The young fish, although more scattered than the adults, occurred mostly at temperatures higher than those selected by the adults. These results may indicate temperature separation of young and adult fish in nature which would reduce intraspecific predation and competition. Heat resistance of adult females taken from the 26.2°C-compartment of the temperature-gradient tank was higher than the resistance of 28.0°C acclimated fish. The temperature-gradient females either had attained a high acclimation level by temporarily venturing above 28.0°C or had accumulated high resistance by living at varying temperatures below 28.0°C.

INTRODUCTION

The mosquito fish, *Gambusia affinis* (Baird and Girard), is native of many lowland ponds, lakes, and streams in southeastern areas of the United States. It has been widely introduced in other areas of the world as a mosquito control agent. Hart (1952) reported that this species has high heat tolerance in conformity with the high summer temperatures of its habitat — the surface regions of shallow, marshy waters. Adult females are more heat resistant than adult males; fry are more resistant than the adults of either sex (Hagen, 1964). He suggested that the observed differences may have adaptive value. The high heat tolerance of females allows them to withstand high temperatures when gravid, thereby ensuring survival of the population during hot summers. High heat tolerance of the fry permits them to live in very shallow, warm water in which predation and intraspecific competition would be reduced. Both Hart (1952) and Hagen (1964) worked with fish acclimated to constant temperatures; however, the temperature-gradient is comparable to the natural environment of the species.

Objectives of the present study were to investigate temperature selection in *G. affinis* and to evaluate heat tolerance of the fish living in a thermal gradient.

MATERIALS AND METHODS

Gambusia affinis were collected late in the winter of 1965-1966 from Little Wildcat Creek, a spring-fed tributary of the Illinois River about five miles northwest of Fayetteville, Arkansas. The fish were held in

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