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## HEAT RESISTANCE EXPERIMENTS WITH THE LONGEAR SUNFISH, *LEPOMIS MEGALOTIS* (RAFINESQUE)

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### INTRODUCTION

A fish withstands the lethal effects of an intolerably high temperature for a certain period of time; this resistance time (Brett, 1952) depends on the severity of the temperature and the previous thermal history of the fish. The pattern formed by a plot of median-log-resistance times and the higher test temperatures has generally been found to be described by a single straight line (Fry, Hart, and Walker, 1946; Brett, 1952; Hart, 1952). At lower test temperatures an abrupt change in slope of the response pattern occurs giving a new line which is more nearly parallel to the time axis. The intercept of this line and the other, more steeply sloped line marks the transition from resistance to tolerance (Brett, 1956). Gibson (1954), in experiments with the guppy, *Poecilia reticulata* Peters, remarked that in the lower range of lethal temperatures near this transition (incipient-upper-lethal temperature), the mortality pattern was not complete even after 10,000 minutes exposure. Brett (1952) obtained comparable results for the chum salmon, *Oncorhynchus keta* (Walbaum).

The second author and his associates, in past experiments involving several orders of fishes, became aware of additional complexities in the upper-lethal-temperature response. These observations were instrumental in the conception and design of the present experiment whose object was to determine more precisely the nature of this response and to investigate the upper-lethal-temperature relationships of *Lepomis megalotis* (Rafinesque), a centrarchid which has been previously neglected as a subject for lethal-temperature experimentation. Fish acclimated to three different temperatures were tested because it was suspected that the characteristics of the three resistance patterns might differ.

### METHODS AND MATERIALS

The experimental fish were reared from eggs collected from nests in the shallow, upper reaches of the Middle Fork of the White River, two and one half miles south of Sulphur City, Washington Co., Arkansas (T15N-R29W-Sec34), during May and June, 1964. The eggs, upon arrival at the laboratory, were placed in constant-temperature tanks filled with aged and aerated tap water. The fry were fed (twice daily), first, brine shrimp and later, as they grew larger, various combinations of brine shrimp, mosquito larvae, and a cooked mixture consisting of high-protein baby cereal and blended calf liver (Gordon, 1950). Both

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eggs and young fish were subjected to constant, "cool-white" fluorescent light. The hatching and rearing temperatures, although varied in some cases, never exceeded the final acclimation temperatures.

When the young fish had reached a convenient size (standard length of 12 mm or more), they were assigned to either an acclimation temperature of 25.0, 30.0, or 35.0°C. Duration of acclimation periods prior to testing was at least one week. Acclimation temperatures, checked by thermometer twice or more daily, never deviated more than  $\pm 0.1^\circ\text{C}$ , and deviation to even this extent was rare. Fish to be used in proximal experiments were separated from the others by placing them in a net suspended in the acclimation tank, and they were not fed for 24 hours or more before being subjected to lethal temperatures.

A modification of the method described by Brett (1952) was used to determine resistance times. Lethal baths — 2-gallon goldfish drums filled with heated water from Great House Springs (4 miles north of Fayetteville) — were arranged in a graded series, the water of each differing  $0.1^\circ\text{C}$  from that in the bowl on either side. Lethal test temperatures ranged from 35.0 to 36.9°C for fish acclimated to 25.0°C, from 36.0 to 39.0°C for fish acclimated to 30.0°C, and from 37.3 to 41.5°C for 35.0°C-acclimated fish.

Constant water temperature of each lethal bath — as in the acclimation tanks — was maintained by strong aeration and an aquarium heater controlled by a contact thermometer through a relay. A standardized thermometer calibrated in intervals of  $0.1^\circ\text{C}$  was used to check bath temperatures. Variation of bath temperature did not exceed  $\pm 0.05^\circ\text{C}$  from the set value. Any deviation was corrected as soon as it was detected.

Samples of 10 (20 in tests of fish acclimated to 30.0°C) individuals were taken randomly from the holding nets and placed directly into the lethal baths. A continuous watch was kept the first few days of each experiment, and the resistance time of each fish was recorded to the nearest 5 seconds. [See Brett (1952) for description and criteria of heat-death.] After the first few days of the experiments, the fish were checked at intervals not exceeding 12 hours. The dead fish were removed from the goldfish drum, and standard length was measured and recorded. The separating of these young fish by sex was considered impractical.

Inspection of the plotted resistance times on a per fish basis (semilogarithmic axes) for each acclimation group indicated that a statistical scrutiny of sample variances was appropriate.

It was assumed that the resistance time,  $t$ , had the log-normal density function,

$$f(t) = (t\beta \sqrt{2\pi})^{-1} \exp \left\{ - (\ln t - \ln \alpha)^2 / 2\beta^2 \right\}$$

$$\text{where } \alpha, \beta > 0; t > 0$$

i.e., the logarithm of the resistance time was normally distributed. This

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resulted in mean resistance time and variance of the resistance time being, respectively,

$$E\{t\} = \alpha \exp(\beta^2/2) \quad \text{and}$$

$$\text{var}\{t\} = \alpha^2 \exp\{2\beta^2\} - \alpha^2 \exp\{\beta^2\}.$$

However, letting  $z = \ln_e t$ , then  $E(z) = \ln \alpha$ , and

$$\text{var}(z) = \beta^2, \text{ or, if } y = \log_{10} t = \log_{10} e \cdot \ln_e t,$$

$$E(y) = \log_{10} e \cdot \ln \alpha \text{ and } \text{var}(y) = (\log_{10} e)^2 \beta^2.$$

If the assumptions were correct, then either  $\log_{10} t$  or  $\ln t$  had a normal distribution, the only difference being that the parameters differed by a scale factor,  $\log_{10} e$ ; i.e.,

$$\ln t \sim N\{\ln \alpha, \beta^2\}$$

$$\log t \sim N\{\log_{10} e \cdot \ln \alpha, (\log_{10} e)^2 \cdot \beta^2\}.$$

In order to test for heterogeneity of variance, sample variances were calculated in the usual manner from log resistance times for samples of size  $n$  drawn at random from  $K$  normal populations. The common degrees of freedom associated with each  $s^2$  were then  $= n - 1$ . The test statistic used for testing  $H_0: \sigma_1^2 = \sigma_2^2 = \dots = \sigma_K^2$  was the maximum F-ratio,

$$F_{\max} = s_{\max}^2 / s_{\min}^2.$$

For  $K \leq 12$ , the 0.05 critical values used for this test statistic were those given by David (1952). For  $K > 12$ , a normal approximation suggested by Hartly (1950) was applied. This approximation suggested that

$$\ln s^2 \sim N\{\ln \sigma^2, 2/(\nu - 1)\}, \text{ approximately, or}$$

$$(\ln s^2 - \ln \sigma^2) \sqrt{2/(\nu - 1)} \sim N(0,1). \text{ Hence,}$$

$$\ln F_{\max} / \sqrt{2/(\nu - 1)} = (\ln s_{\max}^2 - \ln s_{\min}^2) / \sqrt{2/(\nu - 1)} =$$

$$(\ln s_{\max}^2 - \ln \sigma^2) / \sqrt{2/(\nu - 1)} - (\ln s_{\min}^2 - \ln \sigma^2) / \sqrt{2/(\nu - 1)} :$$

$$z_{\max} - z_{\min} = W_{(K)}, \text{ i.e., when } H_0: \sigma_1^2 = \sigma_2^2 = \dots = \sigma_K^2 =$$

$\sigma^2$  is true, then  $\ln F_{\max} / \sqrt{2/(\nu - 1)}$  is distributed as the range of

$K$   $N(0, 1)$  variates. Hence, if  $W_{(K)} 1 - \alpha$  is taken to be the  $1 - \alpha$

percentage point for the distribution of range, then

$$Pr \left\{ F_{\max} = s_{\max}^2 / s_{\min}^2 \leq e^{W_{(K)} 1 - \alpha \sqrt{2/(\nu - 1)}} \right\} = 1 - \alpha$$

so that  $\exp \left\{ W_{(K)} 1 - \alpha \sqrt{2/(\nu - 1)} \right\} = \Lambda_{(K)} 1 - \alpha$  provides an ap-

proximate  $1 - \alpha$  percentage point for using  $\frac{s^2_{\max}}{s^2_{\min}}$  as a test statistic.

In actual practice  $\Lambda_{(K) 1 - \alpha}$  calculated by this approximation

was too small so that the probability of rejecting  $H_0$  in error was somewhat larger than  $\alpha$  when these approximate critical values were used. The most accurate values of  $W_{(K) 1 - \alpha}$  appeared to be those

by Harter, Clemm, and Guthrie (1959). Percentage points of the studentized range were given but for  $d.f. = +\infty$ ; the percentage points were actually those of the range  $W$ .

With a consideration of these factors in mind, multiple range tests for equality of variances were performed. We reverted to the Newman-Keuls procedure of holding  $1 - \alpha$  constant regardless of  $K$

rather than following Duncan's approach of using  $1 - (1 - \alpha)^{K - 1}$  as the significance level for a test involving  $K$  variances (cf. Duncan, 1955).

In order to determine whether fish size was associated with resistance to upper-lethal temperatures, Kendall's rank-correlation test (Siegel, 1956) was applied for each test sample.

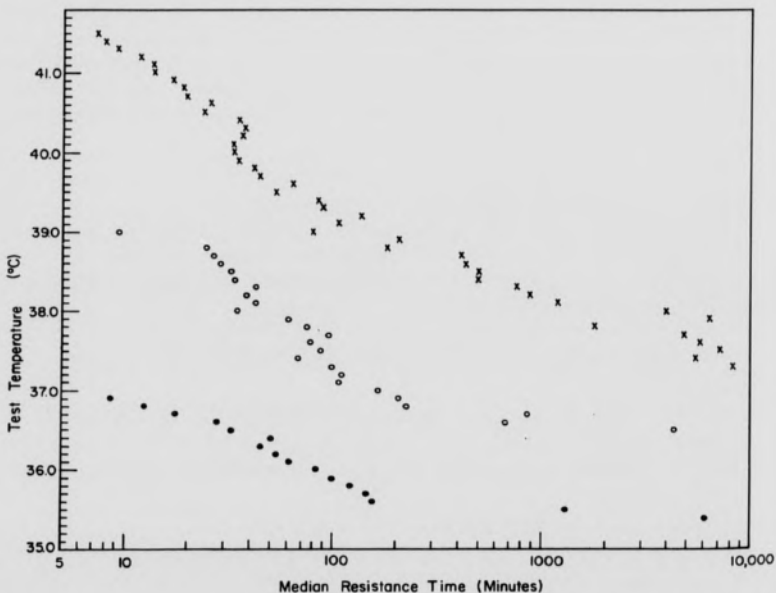


Figure 1. Median-resistance times for young longear sunfish acclimated to 25.0°C (o), 30.0°C (o), and 35.0°C (x).

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## RESULTS

A semilogarithmic plot of sample-median-resistance times versus test temperatures indicated the general nature of the response of the three acclimation groups to upper-lethal temperatures (Figure 1). The scatter of the plotted medians reflected the variability of individual resistance times.

Medians could be determined only for those samples in which at least  $n/2 + 1$  fish had died before that experiment was terminated. All fish acclimated to 35.0°C and subjected to resistance tests eventually died; at 37.3°C, the lowest test temperature, the last fish died after 24,831 minutes, the longest resistance time for any 35.0°C-acclimated fish. Some 25.0- and 30.0°C-acclimated fish survived more than 11 and more than 8 days, respectively, at the lower test temperatures (Table 1).

25.0°C Acclimation				30.0°C Acclimation			
Test Temp. (°C)	No. Tested	No. Surviving	Test Duration (Min.)	Test Temp. (°C)	No. Tested	No. Surviving	Test Duration (Min.)
35.8	10	1	15,877	36.6	20	1	12,129
35.7	10	1	15,871	36.5	20	8	11,775
35.6	10	2	17,536	36.4	20	17	11,784
35.5	10	3	17,537	36.3	20	16	11,798
35.4	10	4	17,556	36.2	20	19	11,814
35.3	10	6	17,562	36.1	20	15	11,814
35.2	10	7	17,569	36.0	20	17	11,882
35.1	10	7	17,574				
35.0	10	10	17,590				

TABLE 1

Numbers of fish acclimated to 25.0 and 30.0°C surviving tests at less severe test temperatures.

Using the 14-hour-test duration criterion of Fry, Brett, and Clawson (1942), the incipient-upper-lethal temperatures (Fry *et al.*, 1946) were estimated to be 35.5, 36.6, and 38.2°C for fish acclimated to 25.0, 30.0, and 35.0°C, respectively. For this range of acclimation temperatures, the incipient-upper-lethal temperature of young longear sunfish increased about 1.3°C for a 5.0°C increase in acclimation temperature.

In general, sample variances of individual logarithmic resistance times were lowest for fish acclimated to 35.0°C and were highest for fish acclimated to 25.0°C (Figures 2 and 3). Excluded from these figures were samples in which one or more fish were still alive at the end of the experiment. Also, the 39.0°C-test sample from the 30.0°C-acclimation group was not included here (nor in the statistical analysis)

since it consisted of 10, rather than 20 fish as in all other tests for this acclimation.

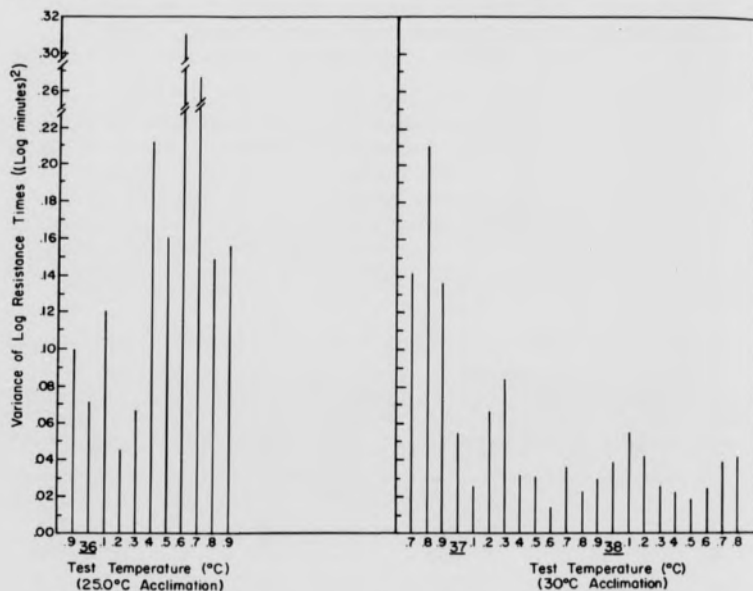


Figure 2. Variances of individual logarithmic-resistance times of young longear sunfish acclimated to 25.0°C (left) and 30.0°C (right).

The sample variances of the 25.0°C-acclimation group were accepted as homogeneous at the 5% level of significance. The 30.0°C-acclimation group had heterogeneous variances ( $p < 0.05$ ) which were found to represent 5 populations of variances at the 5% level of significance. The 35.0°C-acclimated fish displayed an even greater differentiation of sample variances than did the 30.0°C group; these heterogeneous ( $p < 0.05$ ) variances were found to comprise 6 variance populations ( $p < 0.05$ ).

In general, larger fish were more resistant to the upper-lethal-test temperatures. Although smaller size was correlated to greater resistance in a few samples from each acclimation group, no negative correlation was significant at the 5% level of confidence. For the 25.0°C-acclimation group, significant ( $p < 0.05$ ), positive correlation was exhibited in samples tested at 36.2 and 36.8°C. Larger size was significantly ( $p < 0.05$ ) correlated with greater resistance in the 30.0°C-acclimated fish tested at 36.7, 36.9, 37.1, 37.2, 37.3, 37.4, 37.5, 37.7, 37.8, 38.1, and 38.8°C; and, in the 35.0°C-acclimated fish tested at 38.4, 38.9, 39.0, 39.2, 39.3, 39.6, 39.8, 39.9, and 41.0°C.

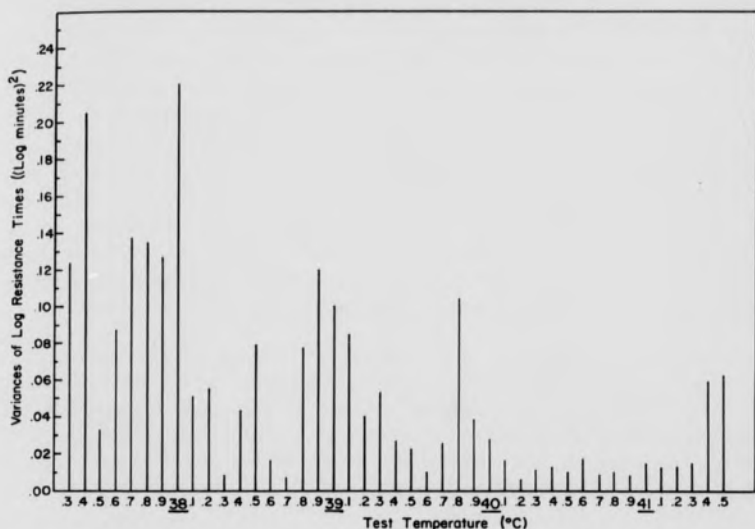
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Figure 3. Variances of individual logarithmic-resistance times of young longear sunfish acclimated to 35.0°C.

## DISCUSSION AND CONCLUSIONS

Hart (1952) has estimated incipient-upper-lethal temperatures of two centrarchids—the bluegill, *L. macrochirus* Rafinesque, and the largemouth bass, *Micropterus salmoides* (Lacépède). For bluegills from Welaka, Florida, 33.8°C was the tentatively estimated upper lethal of fish acclimated to 30.0°C. The upper-lethal temperatures of largemouth bass from Put-in-Bay, Ohio, were reported to be 34.5 and 36.4°C for acclimation temperatures of 25.0 and 30.0°C, respectively. (No information was reported for 25.0°C-acclimated bluegills nor for either species acclimated to 35.0°C.) Young longear sunfish were more heat tolerant than either of these fishes; the longear is a species typical of shallow, sunny, headwater habitats in which summer temperatures are higher than in usual habitats of bluegill and largemouth bass.

For young longear sunfish the increase in incipient-upper-lethal temperature for a 5°C increase in acclimation temperature was about 1.3°C. Using Hart's (1952) estimates of upper-lethal temperatures for acclimations of 25.0 and 30.0°C, this value was 1.9°C for largemouth bass.

The upper-lethal response of young longear sunfish acclimated to higher temperatures (30.0 and 35.0°C) was characterized by heterogeneous variances of log resistance times. Variances of different magnitudes were associated with different regions in the test-temperature range, forming a variance pattern. This phenomenon was more ap-



parent in the 35.0°C-acclimation group than in the 30.0°C group (Figures 2 and 3). The variance pattern of the former became more evident when it was noted that the high variances at 38.4, 38.5, and 39.8°C were caused by the abnormally low resistance time of one fish in each of these samples. The variance pattern of the fish acclimated to 35.0°C, then, consisted of at least 5 regions: (1) 41.5-41.4°C, (2) 41.3-40.1°C, (3) 40.0-38.8°C, (4) 38.7-38.3°C, and (5) 38.2-37.3°C. Region (3) may have constituted two separate variance regions. Region (4) marked the most evident "break" in the variance pattern (and, therefore, in the resistance pattern) and might be termed an "isthmus" of resistance. This region, or at least the lower part of this region, seemed to be synonymous with the transition point discussed by Brett (1956).

A similar pattern of variability was reflected in unpublished data collected by the second author and his associates on the heat resistance of the Ozark minnow, *Dionda nubila* (Forbes); the black bullhead, *Ictalurus melas* (Rafinesque); the channel catfish, *I. punctatus* (Rafinesque); the mosquito fish, *Gambusia affinis* (Baird and Girard); and the green sunfish, *Lepomis cyanellus* Rafinesque — fishes representing three orders.

One hypothesis that can be formed to explain the phenomenon of temperature-variance association to form a pattern is that the observations leading to a particular sample variance all arose from a single normal population, but that the distribution of individual resistance times simply "spread out" or "clumped" in response to the particular test temperature for some unaccountable reason.

A second and perhaps more appropriate hypothesis takes the following form: Suppose  $E\{s_{\min}^2\} = \sigma^2$ . Suppose then that the observations leading to  $s_{\max}^2$  did not all have a common mean, but that

they represented a sample from a mixture of  $p$  normal populations, each with variance  $\sigma^2$  but with means  $\mu_1, \mu_2, \dots, \mu_p$ , with  $n_1$  observations having arisen from population with mean  $\mu_1$ ,  $n_2$  from population with mean  $\mu_2$ , etc. It was required that

It was required that  $\sum_{i=1}^p n_i = n$ , the total sample size. If this sampling hypothesis is true, then

$$E\{s_{\max}^2\} = \sigma^2 (v + 2\lambda)/v$$

where

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$$\lambda = \sum_{i=1}^p n_i (\mu_i - \gamma)^2 / 2 \sigma^2$$

where

$$\gamma = \sum_{i=1}^p n_i \mu_i / n \quad (\text{the average mean})$$

Hence,

$$\mathcal{E}\{s_{\max}^2\} = \sigma^2 + \sum_{i=1}^p n_i (\mu_i - \gamma)^2 / v$$

It is noteworthy that the second term is non-negative and has a value of zero only if  $\mu_1 = \mu_2 = \dots = \mu_k$  in which case  $\mathcal{E}\{s_{\max}^2\} = \sigma^2$ .

The more the  $\mu_i$  are dispersed, the greater this second term and hence

$s_{\max}^2$ . But if  $\mathcal{E}\{s_{\max}^2\}$  is large, then the probability is small that

$s_{\max}^2$  is small or that  $s_{\max}^2 / s_{\min}^2$  is small enough not to exceed the

critical value so that  $H_0$  is accepted.

If this second hypothesis is true, we can only assume that test temperatures in the low variance regions created or, more precisely, discovered, one or a few physiological populations. On the other hand, test temperatures in the high variance regions allowed the expression of a greater number of populations.

If this, in fact, represents the actual situation, best-fit lines do not fully describe the upper-lethal-temperature response. Perhaps a series of overlapping regression lines would lend more meaningful expression to patterns of upper-lethal-temperature resistance.

In general, young longear sunfish of larger size were more resistant than smaller individuals to upper lethal temperatures. This indicated that thermal resistance may have changed during development. (Hart (1952) found that larger bluegills (*L. macrochirus* Rafinesque) were more resistant to upper lethal temperatures than smaller individuals.

The size-resistance correlation was strongest in the test-temperature region (region 3) associated with high variances just above the isthmus region (region 4).

SUMMARY

Upper-lethal-temperature experiments with young longear sunfish, *Lepomis megalotis* (Rafinesque), reared from eggs in the laboratory were performed to determine more precisely the nature of the upper-lethal-temperature response in fishes and to determine the upper-lethal-temperature relationships of this centrarchid.

The young fish were reared in constant-temperature baths and, prior to lethal tests, were acclimated a minimum of 1 week to one of three temperatures — 25.0, 30.0, and 35.0°C. Upper-lethal tests were performed at 0.1°C intervals in a manner similar to that employed by Brett (1952). Constant light was maintained during growth, acclimation, and testing.

The incipient-upper-lethal temperatures of young longear sunfish were estimated to be 35.5, 36.6, and 38.2°C for fish acclimated to 25.0, 30.0, and 35.0°C, respectively.

A maximum F-ratio test indicated that sample variances of log-resistance times were heterogeneous for fish acclimated to either 30.0 or 35.0°C, but not for fish acclimated to 25.0°C. The sample variances of 30.0- and 35.0°C-acclimated fish represented several variance populations.

Size of variance was associated with test temperature in the 35.0°C-acclimated fish (to a lesser extent, in the 30.0°C-acclimated fish), forming a variance pattern. It is suggested that different test temperatures allowed the expression of different numbers of physiological populations.

In general, larger young longear sunfish were more resistant to upper lethal temperatures than their smaller siblings of similar age.

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