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TEMPERATURE PREFERENCES BY TWO SPECIES OF FISH AND THE INFLUENCE OF TEMPERATURE ON FISH DISTRIBUTION [1]

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ABSTRACT

Experiments were conducted to determine the preferred temperatures and the final preferenda of *Pimephales promelas* and *Lepomis cyanellus*. Specimens of *P. promelas* and *L. cyanellus* were acclimated to five and four different temperatures for 30 days. A gradient tank was built permitting a temperature gradient of 2° C. per chamber. Openings in the partitions between the chambers let the fish move freely throughout the tank. One fish was used to a test and ten tests for each acclimation level. Recordings of the position of each fish were made every 15 seconds for 40 minutes. The temperatures corresponding to the modes were averaged to obtain the preferred temperature for the acclimation level. *Pimephales promelas* and *L. cyanellus* acclimated rapidly to high temperatures but required about two weeks

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to acclimate to low temperatures. Preferred temperatures increased as the acclimation temperatures increased for both species until the final preferendum was reached. The final preferendum was significantly higher for *L. cyanellus* than for *P. promelas.* The final preferenda of fishes may have important effects upon distribution in environments. The importance of temperature in field work is emphasized and problems for future investigations are discussed.

The study reported herein was to determine the preferred temperatures and the final preferenda of two Oklahoma fishes, *Pimephales promelas* (Rafinesque), the fathead minnow, and *Lepomis cyanellus* Rafinesque, the green sunfish. The study was made possible through Research Grant No. WP-67 (C2) from the National Institutes of Health. Interest was centered primarily with behavioral reactions of fish to temperature changes rather than physiological effects of temperature. Fry (1952) and Doudoroff (1957) have made available excellent articles about the physiological effects of temperature on fish.

Chidester (1924) reviewed the literature on fish migration and concluded that temperature was the primary physical factor in the migration of fish. Sprugel (1951), while investigating the effect of temperature on the distribution of fish, found that bluegills would leave the higher oxygenated levels in lakes and penetrate lower strata where the dissolved oxygen was much reduced. The length of time spent at the lower levels was limited. Dendy (1946) found that dissolved oxygen levels of 1.5 ppm did not necessarily constitute a barrier to fish. He also found that pressure did not influence distribution as strongly as did temperature.

The temperature range within which organisms are able to perform normal metabolic activities is known as the zone of tolerance. The ranges above and below the zone of tolerance are known as the upper and lower incipient lethal levels. The zone of tolerance can be extended to higher or lower temperature ranges by acclimation. Many workers (Fry, Hart, and Walker, 1946; Fry, 1947; Brett, 1952; Fry and Gibson, 1953; Ferguson, 1958) believed that as the acclimation temperature increased, the upper incipient lethal level also rose. However, a point is reached where a further rise in acclimation temperature does not increase the upper lethal level. The final lethal level is known as the ultimate upper incipient lethal level. The lower lethal level also can be raised or lowered by acclimation. Organisms can survive the upper incipient lethal level for a short time, but will be killed. The time of survival is called the resistance time.

Somewhere between the upper and lower incipient lethal levels there is a preferred temperature for any particular organism. Fry (1947) has explained the preferred temperature as the region, in an infinite range of temperature, at which a given population will congregate with more or less precision. The preferred temperature is dependent upon the thermal history of the organism.

MATERIALS AND METHODS

The species of fish used in the experiments were chosen because they were locally common, fairly hardy, and easily kept in the laboratory. Both were collected from farm ponds near Stillwater, Oklahoma. All fish were kept in tap water that had been previously aerated at least 24 hours.

After an adjustment and observation period of one week, the specimens were transferred to aquaria, 16 x 9 x 10 inches in size, for acclimation to the test temperatures. Fifteen fish whose average total length was 55 mm were placed in each aquarium. The largest fish tested was 74 mm in total length. Heated aquaria were kept at constant temperatures by using heaters with thermostats. Cooled aquaria were placed in household refrigerators. To attain near freezing temperatures, a refrigerator with a large compressor was used. This refrigerator was also used to store the cooled water for the actual experiments. Adjustment of the apparatus permitted a selection of desired temperatures with a variance of 0.5° C.

The fathead minnows were fed a diet of commercial poultry food and powdered egg, finely ground and mixed. Live Daphnia and a supplement of the poultry mixture were fed to the green sunfish.

Tests were begun after an acclimation period of 30 days and continued until some specimens were in the acclimation aquaria for eighty days The preferred temperature experiments were run in a gradient tank similar to one used by Doudoroff (1938). The gradient tank was divided into eight chambers, the outside dimensions being 81.5 x 9.5 x 9.75 inches. The material used was high-grade redwood which was economical, watertight, and easily shaped. The chambers were separated by aluminum sheets but openings (2 x 3 inches) permitted the specimens to move freely from one chamber to another. Each chamber was equipped with two inlet tubes located near the bottom to permit entrance of heated and cooled water. A wire screen was used as a false floor to bar the fish from the inlet tubes and from the mixing water below the screen. The tank was designed to reduce corners and thus deny "hiding place" for the fish (Fig. 1). An outlet tube was placed at the water line of each chamber to prevent horizontal currents. The rate of flow to each chamber was controlled by 0.25-inch brass valves. The tank was illuminated by two, four-foot, 30 watt fluorescent lamps placed end to end.

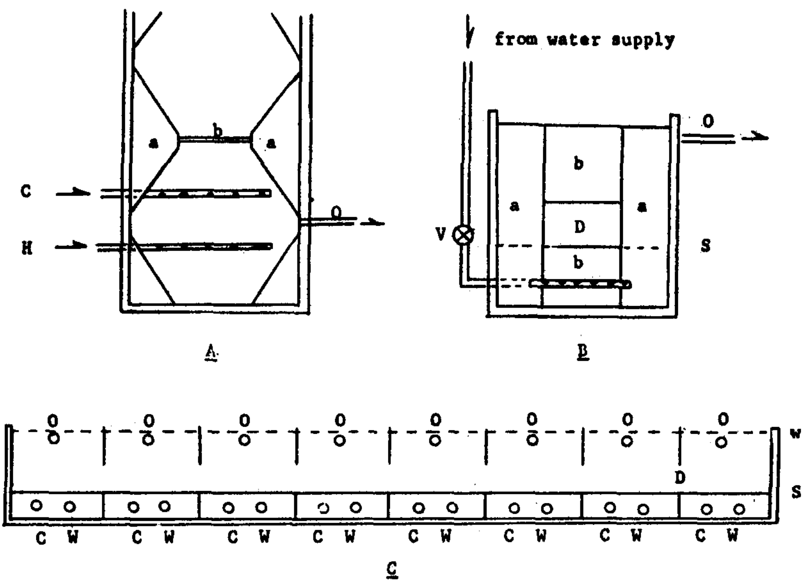

Figure 1: Diagram of gradient tank. A, top view; B, end view; C, side view. a and b - partitions, D - opening between chambers, S - screen, V - valve controlling water supply, and w - water level. W, C, and O - warm water supply, cooled water supply, and outlet tubes, respectively.

Cooled water was supplied to the gradient tank from a large refrigerator. Heated water was supplied from a large refrigerator liner containing a 1320 watt immersion type water heater. Both water supplies were aerated constantly to insure ample dissolved oxygen. Warmed and cooled water was delivered to the gradient tank by gravity flow. A gradient of 2° C. between each chamber was possible.

The tests were conducted in an air conditioned room whose temperature varied from 21.7 to 25.0° C. The temperature gradient was established and included the temperature at which the fish had been acclimated. A fish was introduced into the chamber containing the water possessing the appropriate acclimation temperature. Fish acclimated to 4° C. were introduced into the gradient tank at 6° C., the coolest temperature attain-able with the apparatus. One fish per test was used to eliminate any bias due to schooling. The specimen was allowed to move freely through the tank for a period of thirty minutes, permitting orientation to the tank and to the gradient. At the end of the thirty minute period, records were made for forty minutes by recording the chamber number containing the fish at each fifteen second period. The temperatures in the chambers were checked at ten minute intervals and the gradient was re-established if necessary. The gradient was shifted, in some tests, to make certain the fish was responding to the temperature and not to a particular chamber. Length in millimeters and weight in grams was recorded of each fish after a test was completed. Dissolved oxygen and hydrogen-ion concentration measurements were recorded for both end chambers to assure no significant gradient of the two factors existed.

Specimens of *P. promelas* were acclimated at 4° C., 10° C., 15° C., 22° C., and 30° C. and those of *L. cyanellus* at 4° C., 10° C., 22° C., and 30° C. Ten tests per species were run at each acclimation temperature. The numbers of observations of specimens of each species in each chamber were totaled and the mean computed. The mean was converted into a percentage of total observations and the percentage values graphed. The preferred temperature for each acclimation level was determined by averaging the modes for each of the ten tests. A resulting mean is shown on each percentage graph.

PREFERRED TEMPERATURE AND THE FINAL PREFERENDUM

Both species adjusted readily to temperature change Dobie *et al.* (1956) stated that the fathead minnow can tolerate sudden temperature changes as great as 11° C. We found that both species were able to tolerate temperature changes from room temperature (21° C.) to 30° C. and to 10° C., the fish required a holding period of two weeks near 10° C.

Particular caution was taken with the gradient tank to avoid gradients other than temperature. Dissolved oxygen determinations were made for each test. All determinations showed that the oxygen level was near or above the staturation level. Whitmore *et al.* (1960) found no avoidance to oxygen levels above 6 ppm. Moss and Scott (1961) found the highest critical level of dissolved oxygen was 1.4 ppm at 35° C. for the largemouth bass. No oxygen concentration in the preferred temperature studies were below 7.5 ppm. Therefore, it was assumed that there was no effect of oxygen variation in the gradient tank.

The pH varied between 7.8 and 8.5 but was the same in the end chambers in each test. No evidence was found of a gradient in the hydrogen-ion concentration.

Tests were conducted in the tank without a temperature gradient to learn if one or more chambers were preferred by the fish. No evidence of a preference was found. The high points at the end of the graph (Fig 2) are believed to be caused by the greater distance a fish had to travel to enter and leave the end chambers. The chambers may have afforded a false sense of security for the test fish.

Specimens of the fathead minnow were acclimated to 4° C., 10° C., 15° C., 22° C., and 30° C. The preferred temperatures found for specimens acclimated to the above temperatures were 8.8° C., 15.2° C, 23.3° C., 20.7° C., and 22.6° C., respectively (Figs. 3-7). The preferred temperatures do not coincide with the modes because they were averaged to obtain the preferred temperature. The movements of the specimens were deliberate and slow to moderate. Back-and-forth movements in the gradient tank were common throughout the experiments with more time being spent in the chambers of preferred temperature.

Specimens of the green sunfish were acclimated to 4° C., 10° C., 22° C., and 30° C. The preferred temperatures found for the specimens acclimated to the above temperatures were 10.6° C., and 27.0° C., and 26.8° C. respectively (Figs. 8-11).

All fish moved erratically with short jerky movements in the gradient tank. The degree of excitement of the fish could be estimated by the rate of movement of the pectoral fins. During periods of rapid movement, the pectorals moved rapidly, whereas, if the specimens were resting, there

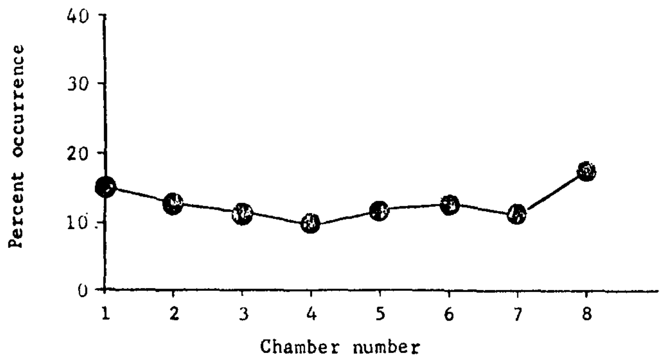

Figure 2: Average frequency of occurrence in chambers for five fish tested without a gradient.

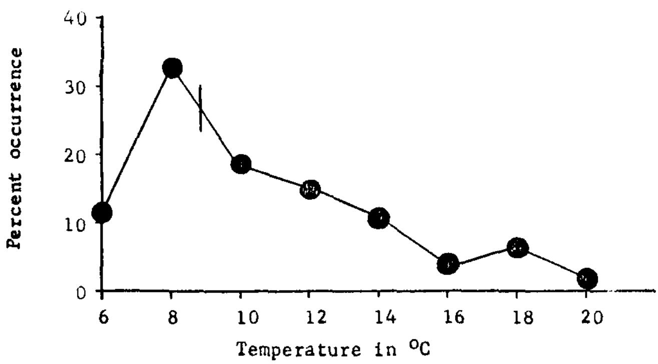

Figure 3 Average frequency of occurrence in chambers at different temperature levels for P. promelas acclimated to 4°C. Vertical slash indicates computed preferred temperature.

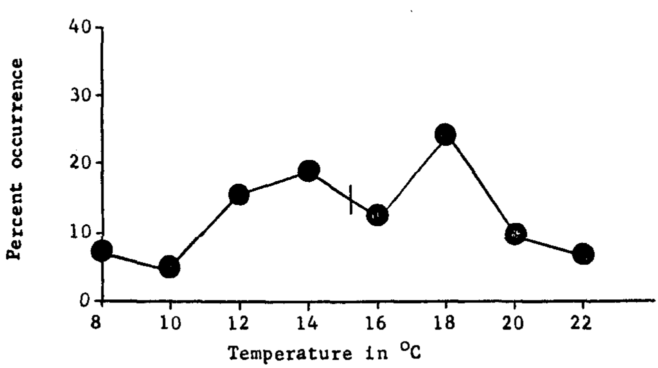

Figure 4: Average frequency of occurrence in chambers at different temperature levels for P. promelas acclimated to 10°C. Vertical slash indicates computed preferred temperature.

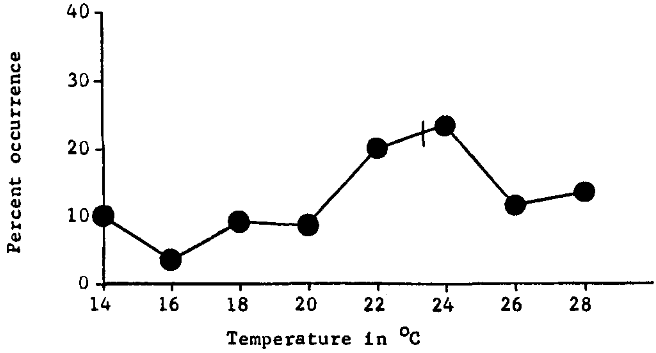

Figure 5: Average frequency of occurrence in chambers at different temperature levels for P. promelas acclimated to 15°C. Vertical slash indicates computed preferred temperature.

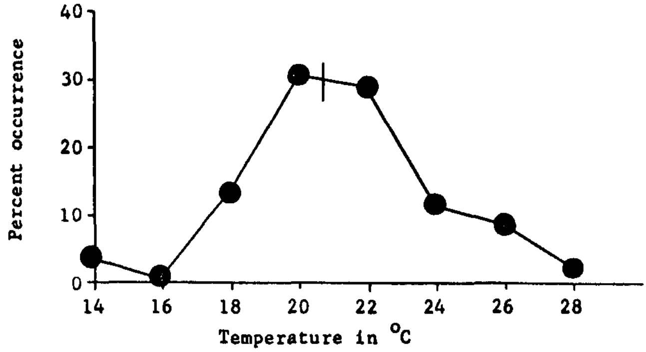

Figure 6: Average frequency of occurrence in chambers at different temperature levels for P. promelas acclimated to 22°C. Vertical slash indicates computed preferred temperature.

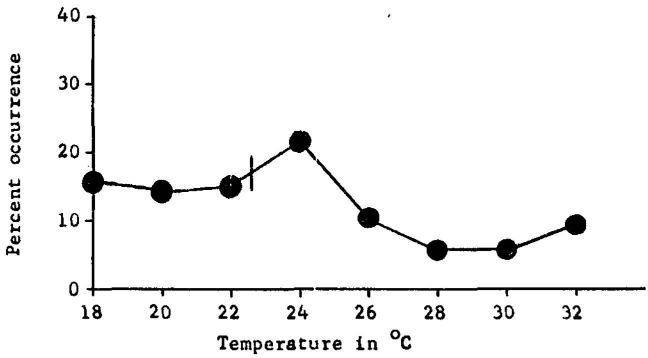

Figure 7: Average frequency of occurrence in chambers at different temperature levels for P. promelas acclimated to 30°C. Vertical slash indicates computed preferred temperature.

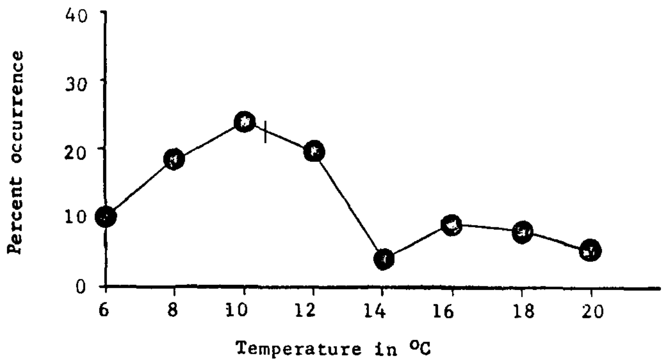

Figure 8: Average frequency of occurrence in chambers at different temperature levels for L. cyanellus acclimated to 4°C. Vertical slash indicates computed preferred temperature.

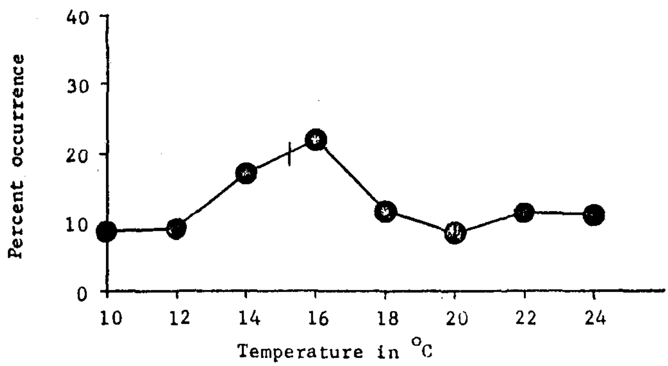

Figure 9: Average frequency of occurrence in chambers at different temperature levels for L. cyanellus acclimated to 10 °C. Vertical slash indicates computed preferred temperature.

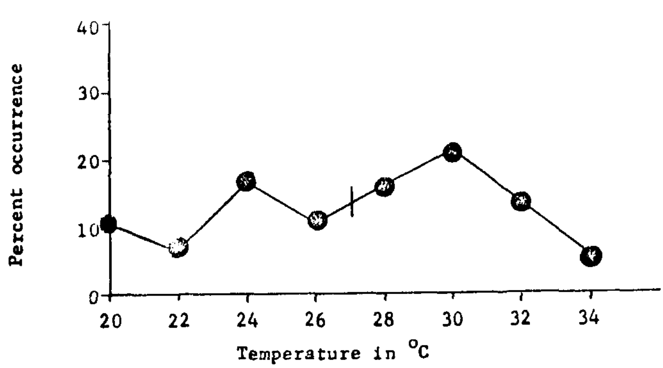

Figure 10: Average frequency of occurrence in chambers at different temperature levels for L. cyanellus acclimated to 22°C. Vertical slash indicates computed preferred temperature.

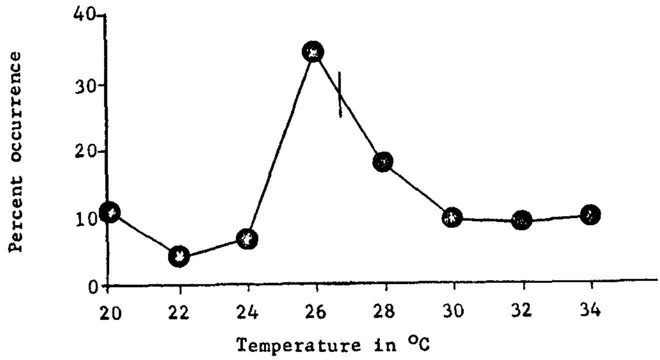

Figure 11: Average frequency of occurrence in chambers at different temperature levels for L. cyanellus acclimated to 30°C. Vertical slash indicates computed preferred temperature.

was little movement of the pectorals. The green sunfishes appeared to be curious concerning their surroundings. Specimens were observed many times trying either to enter or to bite the small piece of exposed outlet tube. At times a fish would actually put its snout into the tube of 0.25-inch diameter. Although the action did not alter the test, it was an interesting incident observed with the green sunfish.

DETERMINATION OF THE FINAL PREFERENDUM

The theory that fish acclimated to a low temperature selected a preferred temperature somewhat higher than their acclimation temperature and that fish acclimated to a high temperature select a preferred temperature somewhat lower than their acclimation temperature is supported. Somewhere between the high and low temperatures lies what is known as the final preferendum. Fry (1947) refers to the final preferendum as that temperature around which all individuals will ultimately congregate, regardless of their experience.

The diagonal line (Figs. 12-13) is determined by points of concidence of acclimation and preferred temperatures. The intersection of the line with the preferred temperature cuve is the final preferendum.

The curves in figures thirteen and fourteen representing the green sunfish and the fathead minnow are similar to those of other workers (Doudoroff, 1938; Fry, 1947; Brett, 1952; Pitt et al., 1956), but the final preferenda varied greatly. The final preferenda for the green sunfish and the fathead minnow were found to be 27.3° C., and 23.4° C., respectively.

DISCUSSION

A question concerning the length and rate of acclimation among fishes arose early in the study. Brett (1956) said the rate of change in the ability to tolerate higher temperatures was relatively rapid. Less than 24 hours were required for acclimation at temperatures above 20° C., while 20 days were necessary for some of the lower temperatures. Brett (1956) concluded the ability of a fish to acclimate was governed by the rate of metabolism. When the rate of metabolism is decreased by low temperatures, the ability to acclimate to low temperatures also decreased (Brett, 1956). Doudoroff (1942, 1945) came to the same conclusions while studying marine species. Our results support the conclusions.

The ability to tolerate temperature extremes in Oklahoma is emphasized throughout the life history of the green sunfish and the fathead minnow. Wallen (1955), in temperature study on Oklahoma farm ponds, reported maximum and minimum surface temperatures from 35.8° C., to 0.4° C. and bottom temperatures, at five foot depths, from 27.22° C. to 4.9° C. Any species of fish living and reproducing in a pond whose life history covers

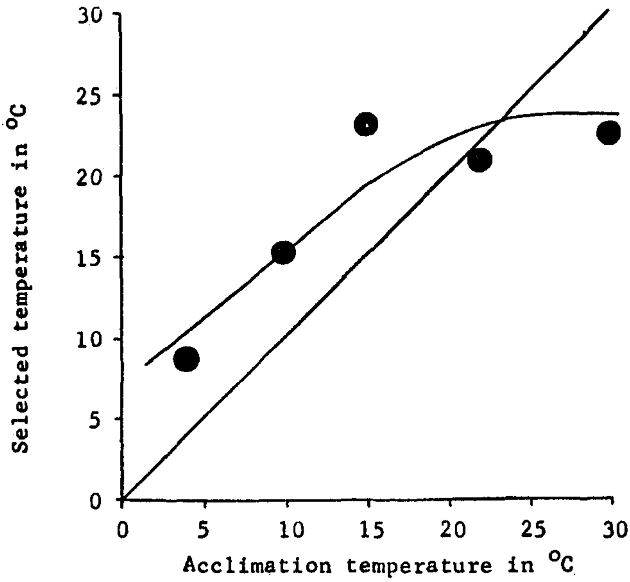

Figure 12: Preferred temperature in relation to acclimation temperature for P. promelas. The final preferendum is located at the intersection of the preferred temperature curve and the 45° line.

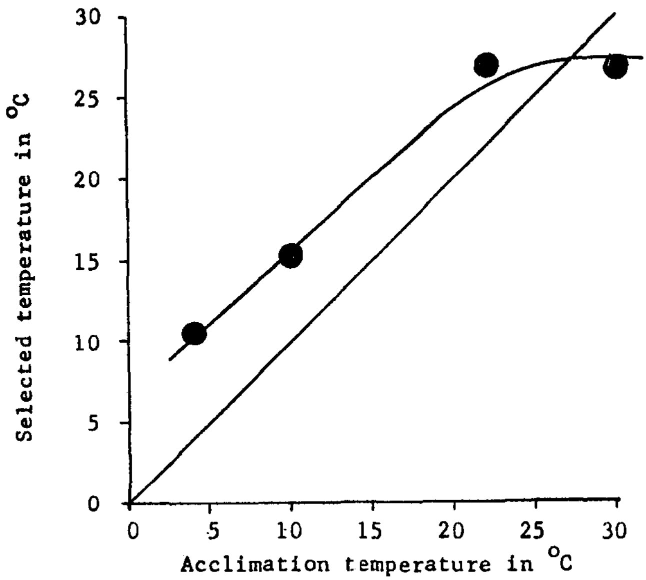

Figure 13: Preferred temperature in relation to acclimation temperature for L. cyanellus. The final preferendum is located at the intersection of the preferred temperature curve and the 45° line.

a span of one or more years must be capable of adjusting over a period of time, to wide temperature ranges.

Thermal acclimation seems to play a major role in the determination of preferred temperatures. Doudoroff (1938), considered the effect of acclimation to be temporary and stated that "a specimen could become 'adapted' to, and establish an apparent 'preference' for any temperature within a fairly wide range."

The tests conducted here were not sufficiently long to validate Doudoroff's statement, although it seems likely that an organism could adjust to any temperature within its zone of tolerance. The preferred temperature of an organism is of interest in laboratory experiments while its function in field conditions seems less understood. The preferred temperature provides a means of determining the final preferendum which is of major importance.

VALUE OF THE FINAL PREFERENDUM

By usage, a preferred temperature follows a period of acclimation, therefore a preferred temperature must apply to laboratory practice, because an acclimation design would be rare for open water. One preferred temperature contributes little information toward the thermal requirements of an organism. A series of preferred temperatures for a particular species offers the approach to the determination of the final preferendum. Since the preferred temperature is dependent upon an organism's thermal history and the final preferendum is not, final preferendum could play an important role in the vertical distribution of fish.

Fry and Hart (1948) found that the highest activity of goldfish occurred near its preferred temperature and concluded there was usually a close correlation between the optimum for activity and the final preferendum.

Ferguson (1958) found a correlation between the final preferenda of laboratory studies and the temperature selections in the field by the same species of fish, particularly in the lower temperature range. The laboratory temperatures were consistantly higher than the field temperatures. The variations were attributed in part to the use of fry and fingerlings in laboratory experiments. (Ferguson, 1958.)

The results reveal the importance of the final preferendum in relation to field work. It was found that the final preferendum for the fathead minnow was significantly lower than the final preferendum for the green sunfish. It is probable they are separated in bodies of water.

FUTURE PROBLEMS FOR INVESTIGATION

The restricted scope of the study left questions unanswered. The validity of the preferred temperature should be determined. The determination would require a laboratory with facilities for long-term studies. Aditional or modified tests involving municipal and industrial pollution mght reveal important effects about the temperature changes in streams. More information is needed concerning vertical temperature selection by fish in lakes, the effects of wastes in temperature selection, the influence of temperature in habitat selection, and the effects of severe temperature changes upon the physiological functions of fish.

Information regarding temperature requirements could be useful to the sport fisherman, the commercial fisheries, the aquatic biologist, and in the establishment of water standards.

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THE SUITABILITIES AND RELATIVE RESISTANCES OF TWELVE SPECIES OF FISH AS BIOASSAY ANIMALS FOR OIL-REFINERY EFFLUENTS [1]

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A grant from the National Institutes of Health was awarded to W. H. Irwin and Troy C. Dorris of Oklahoma State University for research concerning the resistances of fishes to oil-refinery effluents. The study began in February, 1960, and a supplemental grant which became effective September, 1961, was awarded to permit an increase in the number of species to be included. The study reported was done under the supplemental grant and concerns 12 species of fish as toxicity bioassay animals. Observations were made concerning collection, transportation, maintenance, and test reactions of each species. The literature was searched to provide information relating to the suitability of each species investigated. The median tolerance limit of each species to oil-refinery wastes was calculated and submitted to statistical analysis.

Some species of fish are more suitable as bioassay animals than others. A major requirement is that a species be available in adequate supply at all times

[1] Contribution No. 353 from the Department of Zoology, Oklahoma State University.