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Some Relationships of Upper Thermal Tolerances to Preference and Avoidance Responses of the Bluegill

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ABSTRACT

Temperature tolerance, preference, and avoidance studies were conducted on the bluegill (*Lepomis macrochirus*) in Conowingo Pond, lower Susquehanna River, Pa. Upper incipient lethal temperatures of 23.3, 29.3, and 35.8°C were determined for bluegill (mean total length 90 mm) acclimated at 1, 13, and 27°C, respectively. The results agree with published information on bluegill. Median resistance temperatures were plotted against linear regressions of preference and avoidance temperatures. Preferred temperatures were 4 to 5°C less than median resistance temperatures. Bluegill acclimated at temperatures ⩾13°C avoided short-term (96 hr) lethal temperatures. Median resistance time for bluegill acclimated at 13 and 27°C leveled off and exceeded 96 hr near the avoidance temperature. Avoidance temperatures exceeded lethal temperatures at acclimation temperatures <13°C. No mortality occurred with bluegill acclimated at 1°C and subjected to a temperature differential ⩽21°C. Low thermal responsiveness was observed most frequently in avoidance studies conducted at acclimation temperatures <10°C and in preference studies conducted at acclimation temperatures <20°C.

The ecology of Conowingo Pond, on the lower Susquehanna River in Pennsylvania, and connecting waters has been under study since June 1966. The research, conducted by Ichthyological Associates, Inc., was designed to determine the impact, if any, of the operation of the Peach Bottom Atomic Power Station Units 2 and 3 on aquatic life in the pond. Experimental studies initiated in November 1972 were designed to provide predictive data on (1) the behavior of fishes in or near the thermal discharge and (2) the effect of changes in temperature in or near the plume which may occur as a result of station operations.

Considerable information has accumulated on the effects of temperature on fishes in bibliographies by Beltz et al. (1974), Coutant, Huber, and Pfuderer (1972), Coutant, Pfuderer, and Collier (1974), Coutant and Pfuderer (1973), Raney and Menzel (1969), and Raney, Menzel, and Weller (1974). Specific studies on preference and avoidance behavior of bluegill in response to a thermal gradient are available in Peterson, Schutsky, and Allison (1974a; 1974b) and Peterson and Schutsky (1975). Specific studies on temperature tolerance of bluegill are available in Banner and Van Arman (1973), Christianson and Tichenor (1968), Hart (1952), Hathaway (1927), Hickman and Dewey (1973), and Holland et al. (1974).

An important question concerning the operation of the plant was the relationship between thermal resistance and certain behavioral responses to temperature in the bluegill and whether these responses defined ranges of thermal tolerance. The objectives of the study were to determine whether thermal preference and avoidance responses were closely related to upper levels of thermal tolerance and whether mortality could be predicted using behavioral responses to a thermal gradient.

MATERIALS AND METHODS

Test specimens ranging from 52 to 159 mm in total length (mean 90 mm) were collected by seine in Conowingo Pond and transported to the laboratory in 16-liter insulated and aerated bait carriers. Water temperatures varied less than ±0.5°C during transport, and fish reached the laboratory within 3 hr.

Fish were held in the laboratory for 24 hr at their field acclimation temperature (the temperature of Conowingo Pond at the time of their capture) before they were tested. The holding tank was equipped with a 25-kg m^{-1} sec^{-1} compressor and a constant-temperature circulator adjusted to maintain the acclimation temperature. The water temperature in the tank was monitored with a 7-day recording thermometer. Temperatures fluctuated less than 0.5°C

Peterson and Schutsky: Ichthyological Associates, Inc., Drumore, Pennsylvania.

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during the test period, dissolved oxygen was maintained close to saturation, and pH was 7.4 to 7.9. Water used in the laboratory was from Muddy Run Pumped Storage Pond, a receiving body of Conowingo Pond. This water was filtered, passed through an ultraviolet sterilizer and temperature-control units, and recycled.

Preliminary studies (Peterson, Schutsky, and Allison, 1974a) indicated a high incidence of myxobacteria, *Chondrococcus columnaris* and *Aeromonas* sp., in tests conducted at temperatures $\geqslant$15.6°C. Using the ultraviolet irradiating units effectively controlled the spread of the pathogens in subsequent tests; i.e., no mortality was observed in control specimens.

Partitioned transparent plexiglass aquariums with a filtered-water flow system were used to test the effects of rapid temperature change. The flow rate was approximately 5 liters/min. These aquariums were of two sizes, a large unit divided into four 30-cm^3 compartments and a smaller unit divided by removable framed nylon screens into eight 15-cm^3 compartments. Each compartment was aerated. An automatic timer was adjusted weekly to simulate the natural photoperiod, with 0.5-hr periods of dusk and dawn. A light level of 215 lux at the surface of the water was maintained by lamps provided with Vita-Lite fluorescent bulbs (Duro-Test Corp., North Bergen, N. J.), the spectral energy distribution of which was similar to natural daylight.

All tests were conducted using equal numbers (usually 10) of control and experimental specimens. Jensen (1972a) indicated that increasing sample size significantly reduced the standard error of an estimated LT$_{50}$ (temperature lethal to one-half the specimens) until the sample size reached about 30 fish. However, the studies by Peterson, Schutsky, and Allison (1974a) suggest that crowding may adversely affect tests at acclimation temperatures $\geqslant$15.6°C.

The procedure used to determine lethal temperatures was similar to that used by Brett (1952) and later by Edsall and Colby (1970) and Edsall, Rottiers, and Brown (1970). Fish were transferred directly from the holding tank to plexiglass aquariums at the test temperature. Control fish were transferred in a similar manner to plexiglass aquariums at their acclimation temperature. The temperature of the test water was measured at the beginning of the test and at 1, 5, 10, 15, 30, and 45 min and 1, 2, 3, 4, 24, 48, 72, and 96 hr. Responses to rapid temperature change (loss of equilibrium and mortality) were recorded when water temperatures were taken. Additional temperatures were often recorded between 4 and 96 hr. Tests were terminated when all specimens died or at the end of 96 hr. Cessation of opercular movement was used as the criterion for death.

Jensen (1972a; 1972b) indicated that, to increase precision in estimating an LT$_{50}$ for a small sample, test temperatures should be as close to the projected LT$_{50}$ value as possible. We hypothesized that avoidance temperatures would approximate LT$_{50}$. Thus experimental temperatures at or near avoidance temperatures were selected on the basis of preliminary tests. Generally, test temperatures at 0.5°C intervals higher and lower than the avoidance temperature were used. Responses to sublethal (highest test temperature at which no death occurred), upper incipient lethal (test temperature at which 50% of the fish died), and ultimate upper lethal temperature (lowest test temperature at which 100% of the fish died) were determined for each acclimation temperature (Hickman and Dewey, 1973).

Mortality curves were determined by the method of Fry, Hart, and Walker (1946). Resistance times of specimens were plotted as accumulated percentage mortality on semilog-probability paper. Median resistance times were determined graphically for three acclimation temperatures (1, 13, and 27°C).

The preference unit used was a modification of that developed by Meldrim and Gift (1971). The unit consisted of a trough 3.7 m long, 22.9 cm wide, and 22.9 cm deep, with a bottom of 24-gauge, type 304 stainless steel. Water was introduced at one end of the trough from a temperature-controlled circulating bath. As the water flowed the length of the trough, it was heated by three banks of infrared bulbs located beneath the stainless-steel bottom to form a horizontal thermal gradient. Each bank consisted of four 250-watt bulbs connected to a dimmer switch and a temperature regulator. The water drained from the opposite end of the trough and was filtered, aerated, and returned to the temperature-controlled bath. Thus, by regulating the intensity of each of the light banks and the temperature of the water bath, we maintained the desired thermal gradient.

Lighting was provided by three Vita-Lites extending the length of the trough. Tests were conducted at 430 lux, measured at the surface of the water. The pH was determined at the conclusion of each test.

In a test, the trough was filled with water of the acclimation temperature to a depth of 2.5 to 5.0 cm to permit horizontal but restrict vertical movement of test fishes. Acclimation temperature is that at which a fish was collected and held in the laboratory before testing. Fish were placed in the center of the trough and allowed free movement for 10 to 30 min before a thermal gradient of as much as 22°C was established. Observations were made at 10-min intervals, and the temperature at the position of each fish was recorded by the use of 23 thermistors spaced equidistant throughout the length of the trough and connected to a central readout. Median body position (Fahmy, 1972) was used to determine the temperature at the position of each fish. Temperature at the upper and lower end of the gradient was recorded at this time. The test was usually terminated when more than one-half the specimens selected the same temperature continuously for 30 min. If a preferred temperature was not selected within 3 hr, the test was terminated because the test fishes may have reacclimated to a temperature other than the original acclimation temperature.

The avoidance design used was a modification of that employed by Meldrim and Gift (1971). Two temperature-controlled circulating baths were used as storage tanks. Water from each bath flowed by gravity into opposite ends of the troughs, drained at the centers, and was filtered and

recirculated to the temperature baths. The 5-μ filter consisted of a polypropylene bag filled with activated charcoal. Dye tests with malachite oxylate showed a sharp boundary at the center drain. The apparatus was thus divided into thermally distinct quadrants.

The methods used follow those of Meldrim and Gift (1971). Equal numbers of fish were placed into each quadrant. Two diagonally opposite quadrants contained water of the acclimation temperature (T) while the remaining quadrants contained water of increased temperature (T+). After a 5- to 30-min orientation period, the amount of time spent by each fish in each quadrant was measured for a trial period of 10 min. The number of times a fish entered each quadrant was multiplied by the amount of time it spent there to give a frequency distribution for each quadrant. A two-tailed t-test was used to determine if a significant difference ($P \leqslant 0.05$) existed between the two frequency distributions. If both the (T+) quadrants were significantly avoided, the test was terminated. If the avoidance of one or both of the T+ quadrants was nonsignificant, the T and T+ temperatures were increased 2 to 3°C, and the same fish were then tested at the new temperatures.

Oxygen and pH were measured at the beginning and end of each test. The thermal conditions were monitored by a 24-channel temperature recorder connected to thermocouples at 20-cm intervals along the inner sides of both troughs.

A closed-circuit television system was used for observations. This allowed the unit to be completely enclosed for light control and permitted movement around the trough. The tests were recorded on videotape for subsequent analysis with the temperature-recorder printout. Tests were conducted at a light level of 430 lux at the water surface.

RESULTS AND DISCUSSION

A typical mortality curve for bluegill is presented in Fig. 1. Complexity of mortality curves increased with resistance time. Complex curves were best represented by several straight lines (Fig. 1). A similar relationship was found by Edsall and Colby (1970) in temperature tolerance studies with the cisco, and by Tyler (1966) in a review of temperature tolerance studies on fishes and invertebrates.

The relationship between median resistance time, lethal temperature, and acclimation temperature is shown in Fig. 2. Median resistance temperature, considered to be the upper incipient lethal temperature for a 96-hr exposure, is represented by the point on each curve where resistance time exceeds 96 hr.

Sublethal, upper incipient lethal, and ultimate upper lethal temperatures were directly proportional to acclimation temperature (Table 1) and increased 0.5°C for each 1°C increase in acclimation temperature. The results of our study were compared with those of other investigators by interpolation of data presented in Table 1. Upper incipient lethal temperatures for bluegill acclimated to 20 and 22°C agree (±1°C) with those reported by Hart (1952) (31.5°C) and Hathaway (1927) (31°C). The results also agree (±1°C) with upper incipient lethal temperatures (27.5, 33, and 36°C) reported by Banner and Van Arman (1973) for bluegill acclimated to 12.1, 19, and 26°C, respectively. Ultimate upper lethal temperatures (33 and 33.9°C) for bluegill acclimated to 20 and 22°C were similar to those reported by Hickman and Dewey (1973) (33.5°C) and Hathaway (1927) (34°C). Christianson and Tichenor (1968) also reported similar 24-hr upper incipient lethal temperatures (31 and 34°C) for bluegill acclimated to 15 and 30°C.

Preference and avoidance data were analyzed separately by a stepwise multiple regression to determine relationships with acclimation temperature (X) and mean total length of fish (X_1). The acclimation temperature accounted for most of the variation in the dependent variable in preference and avoidance studies. Although the variance contributed by the length of the fish was significant ($P \leqslant 0.05$), inclusion of the variable in the multiple regression equation resulted in only a slight reduction in the standard error of the estimate. Consequently the 95% confidence intervals on the population mean of the preferred and avoided temperatures were computed excluding length from the estimates. Confidence intervals were plotted with temperature-tolerance data (Fig. 3).

The equation that best describes the preference temperature of the bluegill is

$$\begin{aligned} Y_P &= 18.918 + 0.437X \\ R^2 &= 0.785 \\ s_{y \cdot x} &= 1.762 \end{aligned} \tag{1}$$

The avoidance temperature is best described by the following equation:

$$\begin{aligned} Y_A &= 27.392 + 0.226X \\ R^2 &= 0.443 \\ s_{y \cdot x} &= 2.043 \end{aligned} \tag{2}$$

Preference temperatures were directly proportional to acclimation temperatures and consistently lower (2.8 to 8.2°C) than avoidance temperatures (Table 1). We should note, however, that preferred temperatures corresponding to acclimation temperatures <8°C could not be determined because of the high incidence of low thermal responsiveness at lower acclimation temperatures. Low thermal responsiveness (LTR), which was defined by Meldrim and Gift (1971) as the inability of a fish to avoid areas in a thermal gradient which produce stressful conditions, has been observed with many species of fish at various acclimation temperatures.

At acclimation temperatures <20°C, fish often showed no distinct preference but continued to select the highest temperature available. This normally led to LTR if the temperature differential in the gradient approached or exceeded the predicted LTR temperatures (Fig. 4). We found a similar response in four other centrarchids tested from Conowingo Pond: green sunfish (*Lepomis cyanellus*),

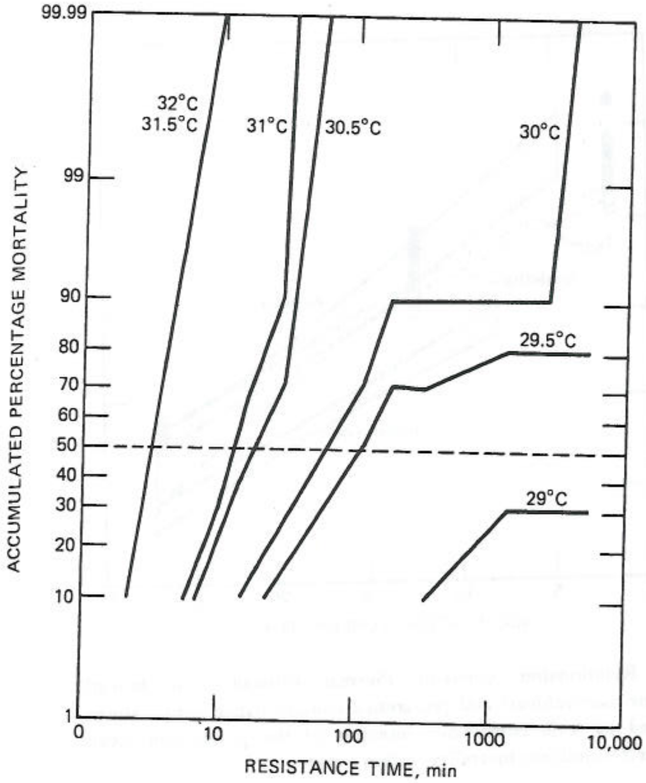


FIG. 1 Resistance times of bluegill (*Lepomis macrochirus*) acclimated to 13°C and exposed to lethal temperatures from 29 to 32°C. Fish exposed to 27 to 28°C survived the 96-hr exposure.

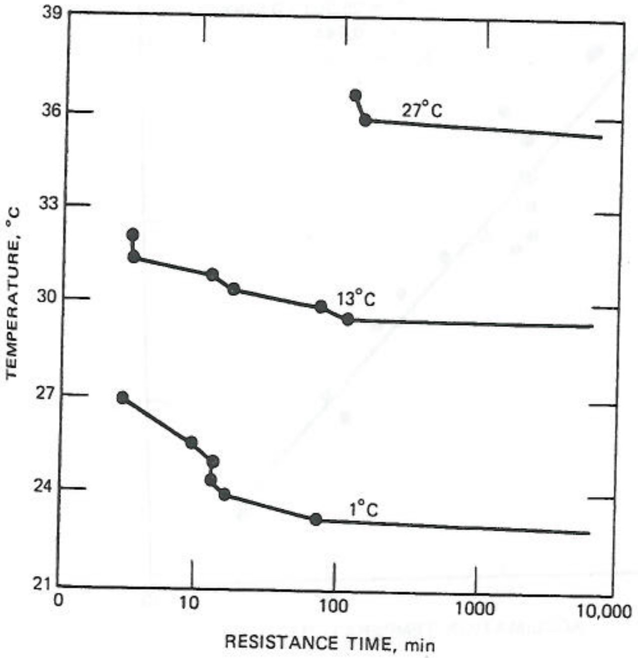
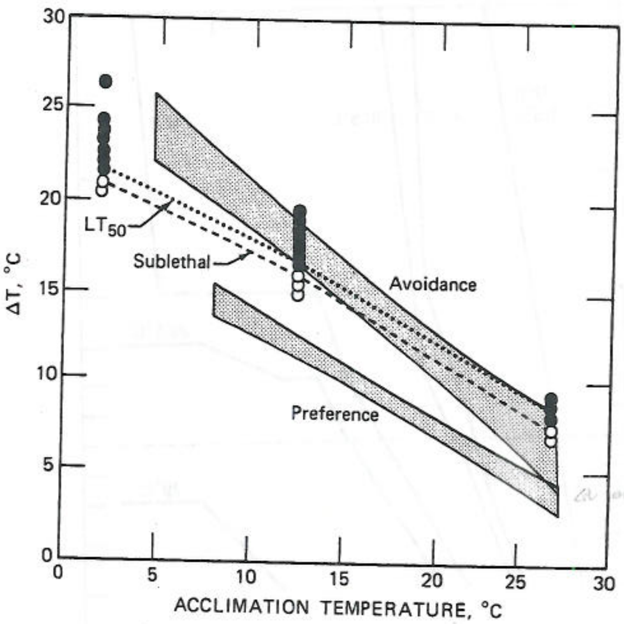


FIG. 2 Median resistance times of bluegill (*Lepomis macrochirus*) acclimated to 1, 13, and 27°C.



20 ot 16 to 10

33.5

FIG. 3 Relationship between thermal resistance of bluegill (*Lepomis macrochirus*) and preference and avoidance temperatures, expressed as 95% confidence intervals of the population mean. Black circles indicate mortality within a test.

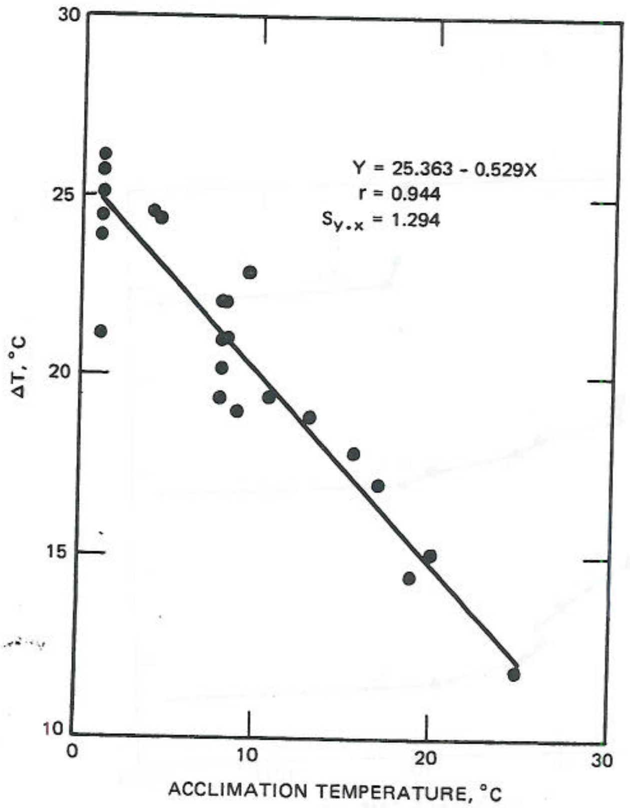
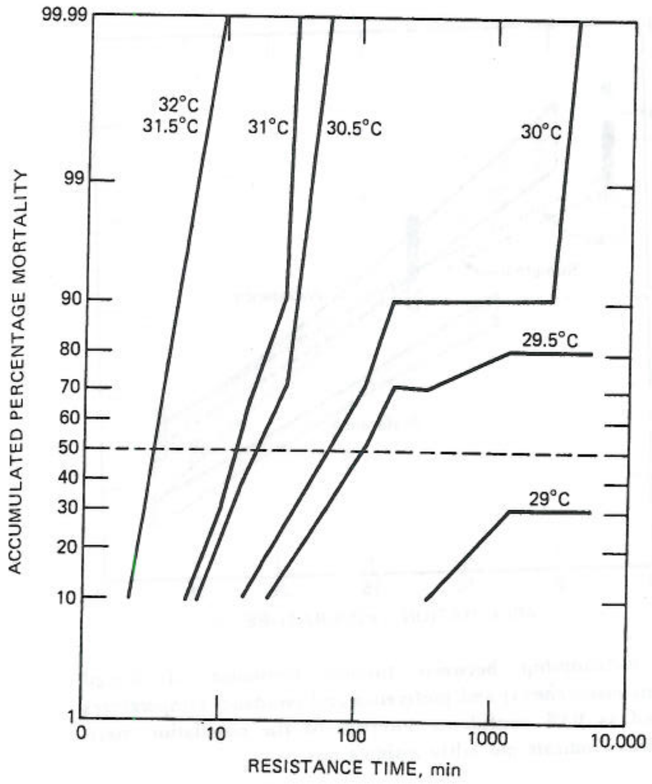


FIG. 4 Calculated regression line showing the relationship of low thermal responsiveness in bluegill (*Lepomis macrochirus*) to acclimation temperature and temperature differential (°C).



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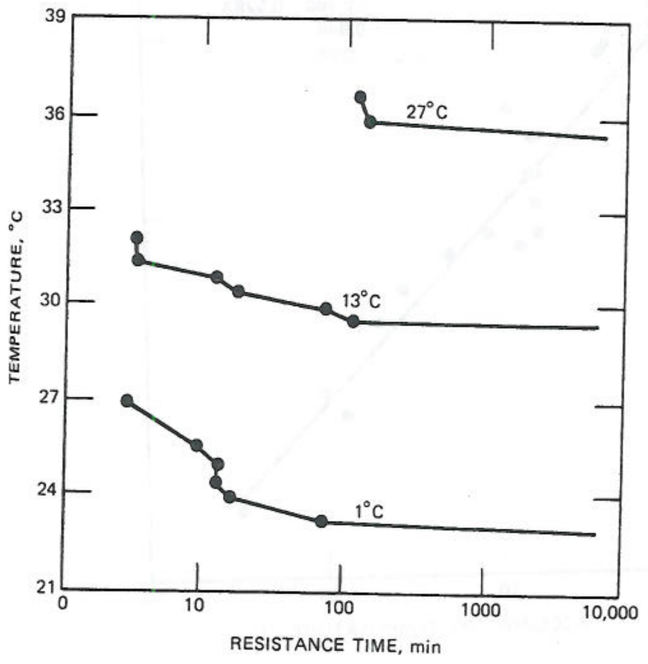

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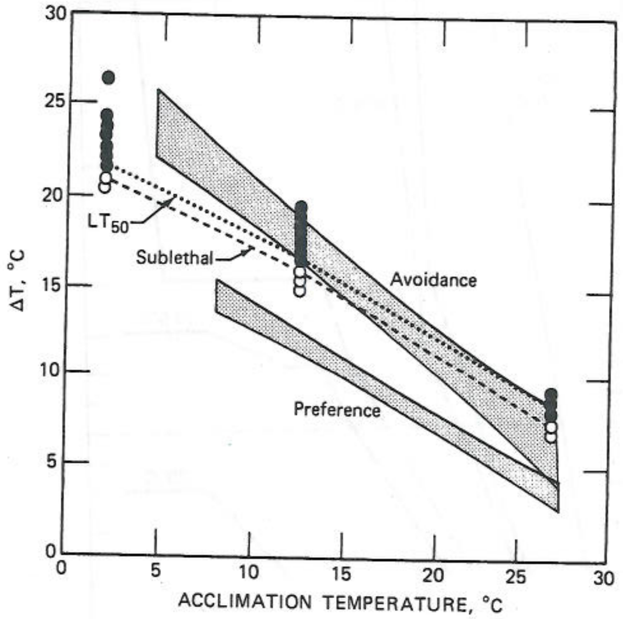

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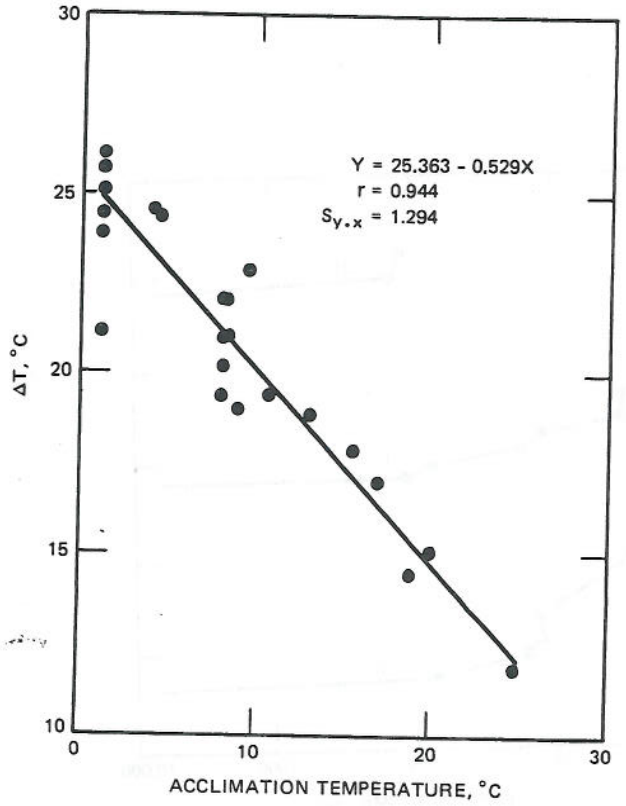

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TABLE 1 Effect of Acclimation Temperature on Upper Temperature Tolerance of Bluegill (*Lepomis macrochirus*)

Acclimation temperature, °C	Upper temperature tolerance, °C			Calculated temperature, °C		
	Sublethal	Upper incipient lethal	Ultimate upper lethal	Preference	Avoidance	Low thermal responsiveness
80.6 27.0	35.0 95	35.8 96.44	36.0 96.8	30.7 87.26	33.5 92.3	
55 13.0	28.0 82.4	29.3 84.74	30.0 86	24.6 76.28	30.3 86.54	31.5
33.8 1.0	22.0 71.6	23.3 73.94	23.5 74.3		27.6 81.68	25.8

↑ no preference due to LTR

pumpkinseed (*Lepomis gibbosus*), smallmouth bass (*Micropterus dolomieui*), and largemouth bass (*Micropterus salmoides*). It also occurred in avoidance tests where fish showed LTR prior to or coincidental with significant avoidance.

The relationship between thermal resistance of bluegill and preference and avoidance temperatures is presented in Fig. 3. Preference and avoidance temperatures were expressed as the 95% confidence intervals of the population mean. The median resistance temperature (LT_{50}) paralleled (1 ± 0.3°C) and sublethal temperature and was within the 95% confidence intervals for avoidance temperature when acclimation temperatures were ≥13°C. Under this condition, the avoidance temperatures were an estimate of the upper incipient lethal temperatures for bluegill. Avoidance temperatures were not a reliable estimate of upper incipient lethal temperatures for bluegill acclimated to temperatures <13°C.

At acclimation temperatures <13°C, upper lethal temperatures can be estimated by a regression analysis of LTR. This analysis was performed, comparing the temperature differential above acclimation within a preference or avoidance test at which LTR first occurred (Y) with the acclimation temperature of the specimen (X). A high correlation (r = 0.94) was found between the two variables, with the following equation best describing the relationship:

$$\begin{aligned} Y &= 25.363 - 0.529X \\ R^2 &= 0.891 \\ s_{y \cdot x} &= 1.294 \end{aligned} \qquad (3)$$

Estimates of LTR temperatures based on this relationship were determined for two acclimation temperatures (Table 1 and Fig. 4). The LTR temperatures varied directly with acclimation temperature, increased 0.5°C for each 1°C increase in acclimation temperature, and were 3.5 to 3.8°C above sublethal, 2.2 to 2.5°C above upper incipient lethal, and 1.5 to 2.3°C above ultimate upper lethal temperatures.

If certain ranges of avoidance temperatures are reliable estimates for LT_{50}, then LTR temperatures must exceed the avoidance temperatures in that range. Indeed, at acclimation temperatures ≥13°C, where avoidance temperatures are estimates of LT_{50}, the LTR temperatures exceed the avoidance temperatures by at least 1.2°C. In contrast, when avoidance temperatures are not reliable estimates of LT_{50}, LTR temperatures are less than avoidance temperatures. At an acclimation temperature of 1°C, where avoidance temperatures are not a reliable estimate of LT_{50}, the LTR temperature is 1.8°C less than the avoidance temperature. Despite the increased frequency of LTR and the apparent lack of thermal tolerance at low acclimation temperature, the estimated temperature differential to elicit an LTR response with bluegill acclimated to 1°C is 24.8°C. Thus temperatures that elicit LTR and avoidance are closely related.

In conclusion, bluegill actively avoided lethal temperatures when acclimation was ≥13°C. At acclimation temperatures ≥13°C, the LT_{50} could be estimated from the avoidance temperature ($Y_A = 27.392 + 0.226X$). Sublethal and ultimate upper lethal temperatures were within the boundaries of the 95% confidence intervals of the population mean of avoidance temperature.

At acclimation temperatures <13°C avoidance temperatures were not reliable estimates of LT_{50}. However, LT_{50} could be estimated from analysis of temperatures required to elicit LTR. A potential problem, however unlikely, could exist in a field situation if the instantaneous temperature differential experienced by the bluegill approached or exceeded 18.5°C. The maximum discharge temperature change predicted at Peach Bottom Atomic Power Station Units 2 and 3 is 11.7°C. Thus, even when acclimation temperatures indicate the potential for LTR, the temperature differential would not be sufficient to induce such a response.

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13.0	28.0	29.3	30.0	24.6	30.3	31.5
1.0	22.0	23.3	23.5		27.6	25.8

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At acclimation temperatures $<13°C$, upper lethal temperatures can be estimated by a regression analysis of LTR. This analysis was performed, comparing the temperature differential above acclimation within a preference or avoidance test at which LTR first occurred (Y) with the acclimation temperature of the specimen (X). A high correlation (r = 0.94) was found between the two variables, with the following equation best describing the relationship:

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REFERENCES

Banner, A., and J. A. Van Arman, 1973, Thermal Effects on Eggs, Larvae and Juveniles of Bluegill Sunfish, Report EPA-R3-73-041, Environmental Protection Agency, Washington, D. C.

Beltz, J. R., J. E. Johnson, D. L. Cohen, and F. B. Pratt, 1974, An Annotated Bibliography of the Effects of Temperature on Fish with Special Reference to the Freshwater and Anadromous Fish Species of New England, Massachusetts Agricultural Experiment Station Research Bulletin No. 605, Amherst.

Brett, J. R., 1952, Temperature Tolerance in Young Pacific Salmon, Genus *Oncorhynchus, J. Fish. Res. Board Can.,* 11(6): 265-323.

Christianson, A. G., and B. A. Tichenor, 1968, Industrial Waste Guide on Thermal Pollution, Federal Water Pollution Control Administration, Washington, D. C.

Coutant, C. C., E. E. Huber, and H. A. Pfuderer, 1972, Thermal Effects on Aquatic Organisms: Annotated Bibliography of 1971 Literature, USAEC Report ORNL-EIS-72-28, Oak Ridge National Laboratory.

—, and H. A. Pfuderer, 1973, Thermal Effects on Aquatic Organisms: Annotated Bibliography of 1972 Literature, USAEC Report ORNL-EIS-73-28, Oak Ridge National Laboratory.

—, H. A. Pfuderer, and B. N. Collier, 1974, Thermal Effects on Aquatic Organisms: Annotated Bibliography of 1973 Literature, USAEC Report ORNL-EIS-74-28, Oak Ridge National Laboratory.

Edsall, T. A., and P. J. Colby, 1970, Temperature Tolerance of Young-of-the-Year Cisco, *Coregonus artedii, Trans. Amer. Fish. Soc.,* 99: 526-531.

—, D. U. Rottiers, and E. H. Brown, 1970, Temperature Tolerance of Bloater, *Coregonus hoyi, J. Fish. Res. Board Can.,* 27: 2047-2052.

Fahmy, F. K., 1972, Heterogeneous Acclimation of Fish to Temperature, *Can. J. Zool.,* 50: 1035-1037.

Fry, F. E. J., J. S. Hart, and K. F. Walker, 1946, *Lethal Temperature Relations for a Sample of Young Speckled Trout, Salvelinus fontinalis,* University of Toronto Studies, Biological Series, No. 55, Ontario Fisheries Research Laboratory Publication 68, pp. 1-62.

Hart, J. S., 1952, *Geographic Variations of Some Physiological and Morphological Characters in Certain Freshwater Fish,* University of Toronto Studies, Biological Series No. 60, Ontario Fisheries Research Laboratory Publication 72, pp. 1-79.

Hathaway, E. S., 1927, Quantitative Study of the Changes Produced by Acclimation in the Tolerance of High Temperatures by Fish and Amphibians, *Bull. U. S. Bur. Fish.,* 43: 169-192.

Hickman, G. D., and M. R. Dewey, 1973, Notes on the Upper Lethal Temperature of the Duskystripe Shiner, *Notropis pilsbryi,* and the Bluegill, *Lepomis macrochirus, Trans. Amer. Fish. Soc.,* 102: 838-840.

Holland, W. E., M. H. Smith, J. W. Gibbons, and B. H. Brown, 1974, Thermal Tolerances of Fish from a Reservoir Receiving Heated Effluent from a Nuclear Reactor, *Physiol. Zool.,* 47: 110-118.

Jensen, A. L., 1972a, Standard Error of LC_{50} and Sample Size in Fish Bioassays, *Water Res.,* 6: 85-89.

—, 1972b, Variation in Results of Identical Bioassays of Minnows Subjected to Instant Temperature Increase, *Trans. Amer. Fish. Soc.,* 101: 403-407.

Meldrim, J. W., and J. J. Gift, 1971, Temperature Preference, Avoidance and Shock Experiments with Estuarine Fishes, Bulletin No. 7, Ichthyological Associates, Inc., Middletown, Del.

Peterson, S. E., and R. M. Schutsky, 1975, Temperature Experiments with Fishes, in Peach Bottom Atomic Power Station Postoperational Report No. 3 on the Ecology of Conowingo Pond for Units No. 2 and 3, T. W. Robbins and D. Mathur (Eds.), pp. 5-1–5-33, Ichthyological Associates, Inc., Drumore, Pa.

—, R. M. Schutsky, and S. E. Allison, 1974a, Temperature Experiments with Fishes, in Peach Bottom Atomic Power Station Preoperational Report on the Ecology of Conowingo Pond for Units No. 2 and 3, T. W. Robbins and D. Mathur (Eds.), pp 5-1–5-32, Ichthyological Associates, Inc., Drumore, Pa.

—, R. M. Schutsky, and S. E. Allison, 1974b, Temperature Experiments with Fishes, in Peach Bottom Atomic Power Station Postoperational Report No. 2 on the Ecology of Conowingo Pond for Units No. 2 and 3, T. W. Robbins and D. Mathur (Eds.), pp. 5.1–5-19, Ichthyological Associates, Inc., Drumore, Pa.

Raney, E. C., and B. W. Menzel, 1969, Heated Effluents and Effects on Aquatic Life with Emphasis on Fishes—A Bibliography, Bulletin No. 2, Ichthyological Associates, Inc.

—, B. W. Menzel, and E. C. Weller, 1974, Heated Effluents and Effects on Aquatic Life with Emphasis on Fishes—A Bibliography, Bulletin No. 9, Ichthyological Associates, Inc.

Tyler, A. V., 1966, Some Lethal Temperature Relations of Two Minnows of the Genus *Chrosomus, Can. J. Zool.,* 44: 349-361.

REFERENCES

Banner, A., and J. A. Van Arman, 1973, Thermal Effects on Eggs, Larvae and Juveniles of Bluegill Sunfish, Report EPA-R3-73-041, Environmental Protection Agency, Washington, D. C.

Beltz, J. R., J. E. Johnson, D. L. Cohen, and F. B. Pratt, 1974, An Annotated Bibliography of the Effects of Temperature on Fish with Special Reference to the Freshwater and Anadromous Fish Species of New England, Massachusetts Agricultural Experiment Station Research Bulletin No. 605, Amherst.

Brett, J. R., 1952, Temperature Tolerance in Young Pacific Salmon, Genus *Oncorhynchus, J. Fish. Res. Board Can.,* 11(6): 265-323.

Christianson, A. G., and B. A. Tichenor, 1968, Industrial Waste Guide on Thermal Pollution, Federal Water Pollution Control Administration, Washington, D. C.

Coutant, C. C., E. E. Huber, and H. A. Pfuderer, 1972, Thermal Effects on Aquatic Organisms: Annotated Bibliography of 1971 Literature, USAEC Report ORNL-EIS-72-28, Oak Ridge National Laboratory.

—, and H. A. Pfuderer, 1973, Thermal Effects on Aquatic Organisms: Annotated Bibliography of 1972 Literature, USAEC Report ORNL-EIS-73-28, Oak Ridge National Laboratory.

—, H. A. Pfuderer, and B. N. Collier, 1974, Thermal Effects on Aquatic Organisms: Annotated Bibliography of 1973 Literature, USAEC Report ORNL-EIS-74-28, Oak Ridge National Laboratory.

Edsall, T. A., and P. J. Colby, 1970, Temperature Tolerance of Young-of-the-Year Cisco, *Coregonus artedii, Trans. Amer. Fish. Soc.,* 99: 526-531.

—, D. U. Rottiers, and E. H. Brown, 1970, Temperature Tolerance of Bloater, *Coregonus hoyi, J. Fish. Res. Board Can.,* 27: 2047-2052.

Fahmy, F. K., 1972, Heterogeneous Acclimation of Fish to Temperature, *Can. J. Zool.,* 50: 1035-1037.

Fry, F. E. J., J. S. Hart, and K. F. Walker, 1946, *Lethal Temperature Relations for a Sample of Young Speckled Trout, Salvelinus fontinalis,* University of Toronto Studies, Biological Series, No. 55, Ontario Fisheries Research Laboratory Publication 68, pp. 1-62.

Hart, J. S., 1952, *Geographic Variations of Some Physiological and Morphological Characters in Certain Freshwater Fish,* University of Toronto Studies, Biological Series No. 60, Ontario Fisheries Research Laboratory Publication 72, pp. 1-79.

Hathaway, E. S., 1927, Quantitative Study of the Changes Produced by Acclimation in the Tolerance of High Temperatures by Fish and Amphibians, *Bull. U. S. Bur. Fish.,* 43: 169-192.

Hickman, G. D., and M. R. Dewey, 1973, Notes on the Upper Lethal Temperature of the Duskystripe Shiner, *Notropis pilsbryi,* and the Bluegill, *Lepomis macrochirus, Trans. Amer. Fish. Soc.,* 102: 838-840.

Holland, W. E., M. H. Smith, J. W. Gibbons, and B. H. Brown, 1974, Thermal Tolerances of Fish from a Reservoir Receiving Heated Effluent from a Nuclear Reactor, *Physiol. Zool.,* 47: 110-118.

Jensen, A. L., 1972a, Standard Error of LC_{50} and Sample Size in Fish Bioassays, *Water Res.,* 6: 85-89.

—, 1972b, Variation in Results of Identical Bioassays of Minnows Subjected to Instant Temperature Increase, *Trans. Amer. Fish. Soc.,* 101: 403-407.

Meldrim, J. W., and J. J. Gift, 1971, Temperature Preference, Avoidance and Shock Experiments with Estuarine Fishes, Bulletin No. 7, Ichthyological Associates, Inc., Middletown, Del.

Peterson, S. E., and R. M. Schutsky, 1975, Temperature Experiments with Fishes, in Peach Bottom Atomic Power Station Postoperational Report No. 3 on the Ecology of Conowingo Pond for Units No. 2 and 3, T. W. Robbins and D. Mathur (Eds.), pp. 5-1–5-33, Ichthyological Associates, Inc., Drumore, Pa.

—, R. M. Schutsky, and S. E. Allison, 1974a, Temperature Experiments with Fishes, in Peach Bottom Atomic Power Station Preoperational Report on the Ecology of Conowingo Pond for Units No. 2 and 3, T. W. Robbins and D. Mathur (Eds.), pp 5-1–5-32, Ichthyological Associates, Inc., Drumore, Pa.

—, R. M. Schutsky, and S. E. Allison, 1974b, Temperature Experiments with Fishes, in Peach Bottom Atomic Power Station Postoperational Report No. 2 on the Ecology of Conowingo Pond for Units No. 2 and 3, T. W. Robbins and D. Mathur (Eds.), pp. 5.1–5-19, Ichthyological Associates, Inc., Drumore, Pa.

Raney, E. C., and B. W. Menzel, 1969, Heated Effluents and Effects on Aquatic Life with Emphasis on Fishes—A Bibliography, Bulletin No. 2, Ichthyological Associates, Inc.

—, B. W. Menzel, and E. C. Weller, 1974, Heated Effluents and Effects on Aquatic Life with Emphasis on Fishes—A Bibliography, Bulletin No. 9, Ichthyological Associates, Inc.

Tyler, A. V., 1966, Some Lethal Temperature Relations of Two Minnows of the Genus *Chrosomus, Can. J. Zool.,* 44: 349-361.