Interrelationships among preferred, avoided, and lethal temperatures of three fish species

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Abstract

Temperature preference, avoidance and lethal experiments were conducted on *Salmo gairdneri*, *Notropis hudsonius* and *Pimephales notatus*. Acclimation temperature did not influence the preference responses of *S. gairdneri*, but did affect the selected temperatures of the two cyprinids. All three species had avoidance temperatures which were within their thermal zone of tolerance at low acclimation temperatures. At the high acclimation temperature, the avoidance temperature was above the ultimate incipient lethal temperature.

Introduction

Numerous studies have been conducted on the effect of temperature on the distribution, physiology, biology, and behavior of aquatic poikilotherms (e.g. Raney & Menzel 1967, 1969; Raney et al. 1973; Beltz 1974; Richards et al. 1977; Stauffer 1980, among others). Recent studies have shown that although fishes are poikilotherms, they are able to seek out thermal regimes which optimize their physiological process (Brett 1944; Beitinger 1977; Hohansen 1977; Reynolds 1977), and thus can maintain body temperatures which fluctuate less than ambient water temperatures. The purpose of this paper is to: 1) determine preference and avoidance temperatures of *Salmo gairdneri*, *Notropis hudsonius* and *Pimephales notatus*; 2) determine if these species can avoid lethal temperatures; and 3) determine the relationship among preferred temperatures avoided and lethal temperatures.

Methods and materials

Young-of-the-year rainbow trout were obtained from a Maryland Department of Natural Resources' trout hatchery in Boonsboro, Maryland. One to three year old *Pimephales notatus* and *Notropis hudsonius* were collected from tributaries of the Potomac River and the Susquehanna River, respectively. All specimens were maintained within 1 °C of collection temperatures while being transported to the Appalachian Environmental Laboratory (AEL). In the laboratory fish were acclimated to desired temperatures at a rate which did not exceed 1 °C/day (Brett 1944). All fish were held at their acclimation temperature (±0.5 °C) for at least five days prior to any tests. Seasonal photoperiods were maintained with electric timers and Vita-lites, which emitted light

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Table 2. Median resistance times (MRT) of rainbow trout, spottail shiners, and blunt-nose minnows subjected to abrupt increases in temperatures.

Species	Acclimation temperature (°C)	Test temperature (°C)	MRT (min)
Salmo gairdneri rainbow trout	6	30.0 ± 0.1	2.74 (2.72 – 2.78)*
	12	30.0 ± 0.1	8.6 (7.6 – 8.7)
	18	30.0 ± 0.1	25.9 (19.1 – 35.1)
Notropis hudsonius spottail shiner	6	30.0 ± 0.1	1.0 (not calculated)
	12	30.0 ± 0.1	18.6 (8.3 – 41.6)
	18	30.0 ± 0.1	187.7 (57.9 – 608.8)
	24	36.0 ± 0.1	4.6 (1.3 – 15.9)
	30	36.0 ± 0.1	243.8 (179.9 – 350.4)
Pimephales notatus bluntnose minnow	6	36.0 ± 0.1**	1.0 (not calculated)
	12	30.1 ± 0.1	36.1 (11.4 – 87.7)
	12	36.0 ± 0.1	1.0 (not calculated)
	18	36.0 ± 0.1	1.0 (not calculated)
	24	36.0 ± 0.1	40.7 (55.5 – 62.5)
	30	36.0 ± 0.1	428.9 (258.0 – 711.9)

* 95% confidence limits.

** Only 35% mortality was recorded when fish were exposed to 30 °C.

atures at which there was 100% mortality. As evidenced by data presented in Table 2, median resistance time at a particular test temperature, increased with increasing acclimation temperatures.

The estimated ultimate upper incipient lethal temperature for S. gairdneri agrees with the seven-day lethal temperature (i.e., 25 °C) of CHERRY et al. (1977), as does our estimate for P. notatus with results reported by HART

Interrelationships among tests

The final preferred temperature for each species was well below the estimates of lethal levels. At low acclimation temperatures all three species had avoidance temperatures within their zone of tolerance; however, at high acclimation temperatures, avoidance temperature was above the ultimate incipient lethal temperature. This relationship is more dramatic since our lethal tests were designed so that the fish experienced a 6 °C thermal shock. Higher upper lethal temperatures probably would have been observed if acclimation and test temperatures were closer to each other; thus our estimates of upper lethal temperatures are conservative. This contention is supported by the fact that P. notatus could be acclimated to 36 °C which was higher than the estimate of the lethal temperature.

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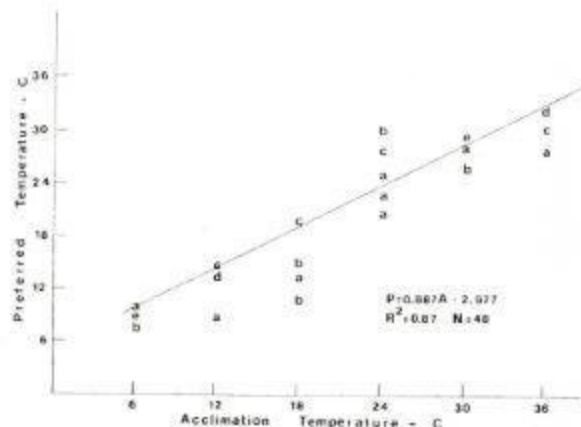
Fig. 3. Preferred temperature versus acclimation temperature for *Pimephales notatus*. A = 1 observation, B = 2 observations, etc.

The relationship between preferred temperature and acclimation temperature for *P. notatus* was best explained by the following model: $P = 0.887A + 2.977$ (Fig. 3). This model explained 87% of the variation in the data, and the addition of other terms into the model did not significantly improve the R^2 term. Solving the above equation for the point at which preference temperature equalled acclimation temperature yielded a final preferred temperature of 26.3 °C. CHERRY et al. (1977) found a final preferred temperature of 28.4 °C for populations from Virginia.

Temperature avoidance

Table 1 summarizes avoidance data for all three species acclimated to various temperatures. *S. gairdneri* avoided 18, 24 and 27 °C when acclimated to 6, 18 and 24 °C, respectively. Fish acclimated to 12 °C did not avoid lethal temperatures. Similar responses were observed for *N. hudsonii* acclimated to 6 °C and *Pimephales notatus* acclimated to 12 °C. This inability of fishes to avoid lethal temperatures has been observed by other authors (MELDRIM & GIFF 1971) and termed "low thermal responsiveness". Fish which exhibited this phenomenon swam into the warm water, became immediately stressed, were unable to escape from the lethal temperatures, and died.

Table 1. Avoidance and upper incipient lethal temperatures (UILT) of rainbow trout, spottail shiners, and bluntnost minnows acclimated to various temperatures.

Species	Acclimation temperature (°C)	Avoidance temperature (°C)	UILT (°C)	Final preferred temperature (°C)
Salmo gairdneri rainbow trout	6	18	24.6	
	12	[a]	25.9	
	18	24	26.7	14.7
	24	27	26.0[b]	
Notropis hudsonius spottail shiner	6	[c]	26.9	
	12	27	27.0	
	18	21	26.7	29.0
	24	33	33.1	
	30	36	33.1	
Pimephales notatus bluntnose minnow	6	15	31.9	
	12	[d]	27.0	
	18	33	33.1	
	24	30	33.1	26.3
	30	36	33.1	
	36	39	32.0	
				[e]

[a] = no avoidance reaction shown, 100% mortality at 24 °C.
[b] = this value is an estimate based on data of BRETT (1952), EDSALL & COLBY (1970); and CHERRY et al. (1977).
[c] = no avoidance reaction shown, 100% mortality at 27 °C.
[d] = no avoidance reaction shown, 100% mortality at 35 °C.
[e] = no test.

Lethal temperatures

Table 1 summarizes the upper incipient levels which were calculated for each species at each acclimation temperature. FRY (1947) defined the ultimate upper incipient lethal temperature as "...the temperature beyond which no further increase in lethal temperature results from further increase in acclimation temperature." Therefore, based on the data presented herein (Table 1), we estimated the ultimate upper incipient temperatures of *S. gairdneri*, *N. hudsonii*, and *P. notatus* to be 26, 33 and 33 °C, respectively.

According to FRY (1947) organisms have a zone of tolerance to a variety of environmental parameters. Theoretically, an organism can live indefinitely when it is within the boundaries of its zones of tolerance. Outside of this zone, survival is a function of both time and intensity of the stress. Therefore, we calculated median resistance times (i.e., the amount of time an organism can live at a particular temperature which falls outside of the zone of tolerance), for each species at all combinations of acclimation and test temper-

To assess the results of the upper incipient lethal temperature bioassays, methods for calculation of dosage-mortality curves developed by Bliss (1935) were initially employed. These methods employ probits and required the construction of aprovisional regression line to predict probits of 0% and 100% mortality. Many bioassays exhibited an "all or nothing" response; therefore, the required probit values for 0% and 100% mortalities could not be obtained because points for constructing the provisional line were not available. Because probit analysis could not be utilized, upper incipient lethal temperatures were determined graphically (Fry et al. 1942, 1946; Brett 1944). Plots of percent mortality after 10,000 min vs. test temperature were constructed and temperatures producing 50% mortality were interpolated. In bioassays where 0% and 100% mortality occurred, the highest temperature producing 0% mortality and the lowest temperature producing 100% mortality were plotted.

Results and discussion

Temperature preference

None of the models utilized could significantly explain the relationship between preferred and acclimation temperatures for *S. gairdneri* (Fig. 1). Therefore, the overall mean of 14.7 °C was used as an estimate of the final preferred temperature, as suggested by McCauley et al. (1977).

The lack of relationship between preferred and acclimated temperatures for *S. gairdneri* reported herein has been observed in other salmonid genera
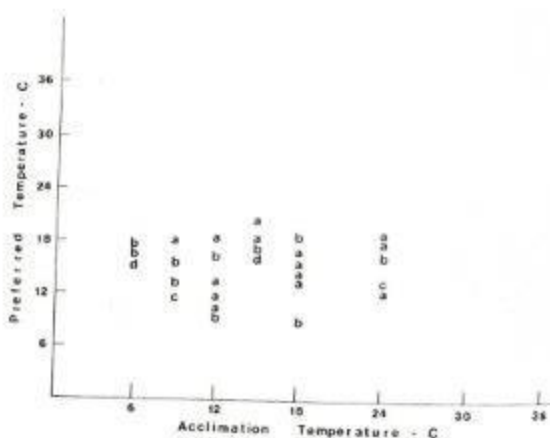

Fig. 1. Preferred temperature versus acclimation temperature for *Salmo gairdneri*. A = 1 observation, B = 2 observations, etc.

$$P: 0.042 A^2 - 2.618A - 5.502$$
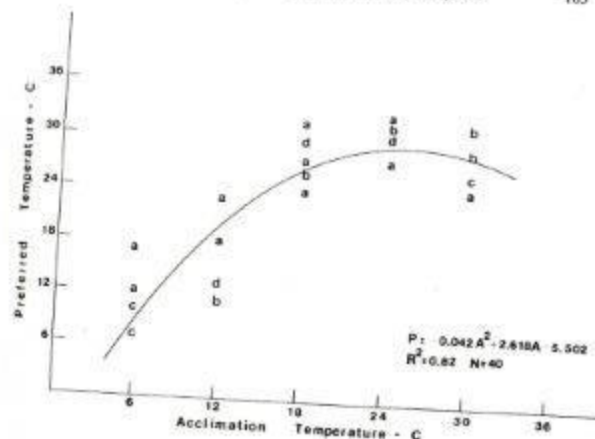$$R^2 = 0.82 \quad N = 40$$

Fig. 2. Preferred temperature versus acclimation temperature for *Notropis hudsonius*. A = 1 observation, B = 2 observations, etc.

by Brett (1952), Garside & Tait (1958), McCauley & Tait (1970) and McCauley et al. (1977). However, studies on different populations of *S. gairdneri* by Cherry et al. (1975) indicated that acclimation temperature did influence preferred temperature. The final preferred temperatures reported for this species by Garside & Tait, Fast (1973), and McCauley et al. (1977) are comparable to our estimate. Estimates for Cherry et al. (1975) and Javaid & Anderson (1967) were considerably higher.

The relationship between preferred temperature and acclimation temperature for *N. hudsonius* was best explained by the following model: $P = -0.042A^2 + 2.618A - 5.502$ (N = 40), where P = preferred temperature, A = acclimation temperature, and N = number of observations (Fig. 2). This model explained 82% of the variation of these data. Fish acclimated to 6, 12, 18 and 24 °C generally selected temperatures above their acclimation temperature, while those acclimated to 30 °C selected a temperature below the acclimation temperature. A final preferred temperature of 29.0 °C was determined by solving the above model for the point at which preferred temperature equalled acclimation temperature. Ecological Analysts (1978) reported that adult *N. hudsonius* collected from the Hudson River had a preferred temperature of 20.1–24.8 °C.

similar in spectral quality to sunlight. Fishes were fed a commercial trout diet between 15.00 and 17.00 h. Organisms which exhibited fungal or bacterial infections were treated with 500 mg of tetracycline HCl, 130 mg of sulfathiazole sodium and 44 mg of acriflavine per 37 l water. Fishes not fully recovered after 48 h were discarded.

Temperature preference experiments

Temperature preference trials were conducted in a horizontal trough (3.6 × 0.203 × 0.254 m) patterned after the one described by MELDRIM & GIFT (1971). The interior of the trough was coated with a non-toxic epoxy paint to prevent the introduction of dissolved metals through oxidation; and the exterior was painted with a flat black paint to facilitate heat conduction. Twelve 250 watt heat lamps, set at varying intensities and located underneath the trough, established the desired temperature gradient. Twenty-three thermistors evenly spaced throughout the trough monitored the thermal gradient. Water was not recycled and was maintained at a depth of 1–2.5 cm depending upon the body depth of the test organisms. Overhead Vita-lites luminated the test area. The trough was enclosed and test organisms observed via overhead mirrors.

Eight fish of each species at each acclimation temperature were individually tested. S. gairdneri were tested at acclimation temperatures of 6, 9, 12, 15, 18 and 24 °C; N. hudsonius were tested at 6, 12, 18, 24 and 30 °C; and P. notatus at 6, 12, 18, 24, 30 and 36 °C. Fish were placed into the thermal gradient at their acclimation temperature and were allowed to orient to the test conditions for one hour. Following this orientation period, the temperature at which the fish was located was recorded every minute for 20 minutes. The mean of these observations was deemed the preferred temperature for that individual.

A simple linear regression model, a quadratic, and third and fourth degree polynomials were used to test the relationship among preferred and acclimation temperatures. The model which best explained this relationship was determined via stepwise regression procedures. Final preferred temperature was determined by solving the selected model for the point at which preferred temperature equalled acclimation temperature.

Temperature avoidance experiments

The avoidance apparatus consisted of two subtroughs and was described by McINSKY et al. (1979). Water flowed from opposite ends of each subtrough and drained at the center. Water temperatures were controlled by two circulating water baths. Water from each water bath controlled the water temperature at opposite ends of each subtrough. The unit was enclosed and fish movement monitored with one-way mirrors. Vita-lites illuminated the test area.

Eight fish were individually tested at each acclimation temperature, except at the 36 °C acclimation temperature only six P. notatus were tested. S. gairdneri were acclimated to 6, 12, 18 and 24 °C. Acclimation temperatures for the other two species were the same as for the preference tests. At the initiation of the experiment, one fish was placed in each subtrough. Water from both ends of both subtroughs was maintained at the acclimation temperature. One half of one subtrough and the opposite half of the other subtrough was designated the "acclimation side" and the other half the "test side". After a one hour orientation period, the amount of time each fish spent in the acclimation half of each subtrough was recorded for a 10-minute period. Following

the initial 10-minute period, the water in the test side of each subtrough was raised 1 °C, and the amount of time spent on the acclimation side was again recorded for a 10-minute period. The test was continued by increasing both sides of each subtrough by 1 °C increments and recording the amount of time each fish spent on the acclimation side. The experiment was terminated when a set of temperatures was reached at which fish in each subtrough spent 500 of the possible 600 seconds in the acclimation side.

A two-way factorial ANOVA, using the fish tested and the temperature of the test side as classes (STAUFFER et al. 1976), was used to analyze these data. The time each fish spent on the acclimation side was recorded in each block of the table. Because the temperature intervals were a "fixed-effects" and the fish "random effects", a mixed effect model of ANOVA was used; thus the temperature interval was tested by the interaction term "fish group x temperature interval". The mean square and degrees of freedom for the interaction term were used to conduct Duncan's multiple range tests at various levels of the fixed effects. The temperature at which significantly (P ≤ .05) more time was spent in the acclimation side during an experimental run than in the control run (i. e., when temperatures in both halves of both subtroughs equalled the acclimation temperature) was deemed the avoidance temperature.

Upper incipient lethal temperatures

S. gairdneri were acclimated to 6, 12, 18 and 24 °C and both cyprinids were acclimated to 6, 12, 18, 24 and 30 °C. Responses of fish exposed to abrupt temperature increases of 6, 12, 18, 24 and 30 °C were recorded for 10,000 minutes. Two control groups (i.e., fish tested at their acclimation temperature) were simultaneously observed with the test groups. Maximum test temperatures for S. gairdneri, N. hudsonius and P. notatus were 30, 42, and 36 °C. Two groups of 10 fish were tested at each treatment level, and the number of dead fish in each test aquarium was recorded at logarithmic time intervals. Cessation of opercular movement and lack of response to mechanical stimulation (BECKER et al. 1977) were used as criteria for death. Observations during dark hours were made with a dim flashlight covered with a red filter to minimize stress caused by interruption of the photoperiod regime. Specimens were fed a commercial trout diet once per day. Water was filtered to remove excess food and feces. Fishes were inspected for fungal and bacterial infections daily.

In order to alleviate bias in mortality rates which resulted from handling fishes, adjustments to percent mortalities were made prior to analysis of all mortality data. The following equations were used to calculate survival probabilities and to adjust mortality rates (Ecological Analysts 1978):

$$P_{sc} = \frac{\text{no. of fish surviving control temp.}}{\text{sample size}}$$

$$P_{st} = \frac{\text{no. of fish surviving test temp.}}{\text{sample size}}$$

$$P_{cs} = \frac{P_{st}}{P_{sc}}$$

$$\text{adj. mort.} = 1 - P_{cs}$$

where P_{sc} = probability of surviving control,
 P_{st} = probability of surviving experiment,
 P_{cs} = probability of surviving particular test temperature,
adj. mort. = adjusted rate of mortality.