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EFFECTS OF SALINITY ON PREFERRED AND LETHAL TEMPERATURES OF THE BLACKCHIN TILAPIA, SARATHERODON MELANOTHERON[1]

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ABSTRACT: Acclimation to varying salinities did not affect the preferred temperature of *Saratherodon melanotheron*, which had a final preferred temperature of 33.5 C. However, fish acclimated to 25 C and 0, 15, and 30 o/oo salinities had lower lethal temperatures of 15, 13, and 15 C, respectively, and an upper lethal temperature of 37 C. The thermal zone of tolerance for fish acclimated in freshwater was 17-35 C, and was not dependent upon acclimation temperatures. Data suggest this species could expand its range in North America.
(KEY TERMS: exotic species; salinity; temperature preference; lethal temperatures.)

INTRODUCTION

Temperature is one of the most important environmental parameters which affect aquatic poikilotherms (see Stauffer, 1980). Numerous studies show that fishes will select and avoid certain temperature regimes under both field and laboratory situations. Laboratory derived preferred temperatures appear to be reproducible, species specific (Reynolds, 1977), and consistent with temperatures selected *in situ* (Neill and Magnuson, 1974; Stauffer, *et al.*, 1976). Thus, temperature acts as a directive factor for aquatic poikilotherms, which rely almost exclusively on behavior to regulate body temperature (Fry, 1971; Stauffer, *et al.*, 1976). This behavior is manifested by locomotor responses to thermal stimuli (Reynolds, 1977), and initiates movement to new environments (Brett, 1956), thus allowing fishes to optimize physiological performance along a finite thermal gradient (Hokanson, 1977). Temperature can also act as a lethal factor for fishes (Fry, 1947). Physiological effects of low temperature which lead to death are reviewed by Umminger (1969) and Prosser, *et al.* (1970). Stauffer (1980) discusses causes of death at high temperatures.

Morgan, *et al.* (1981), indicated that temperature, salinity, and their interactions are the two most important environmental parameters which affect marine and estuarine teleosts. Salinity can increase, decrease, or shift temperature tolerance limits (Kinne, 1963). Kinne (1964) further notes that in open oceans, where salinities are relatively constant, temperature represents the master environmental parameter and in estuaries where both salinity and temperature vary to a greater extent, it is the combined effect which most influences distribution.

S. melanotheron is native to brackish water from central Liberia to southern Camaroon and has been introduced into Florida, where it occurs from the eastern estuarine waters of Tampa Bay from the Alafia River to Lithia Spring (Hensley and Courtenay, 1980).

The purpose of this study was to determine if acclimation to different salinities affected the preferred and lethal responses of the blackchin tilapia, *Sarotherodon melanotheron*, and to determine its upper and lower incipient lethal temperatures when acclimated to freshwater.

METHODS AND MATERIALS

All specimens of *S. melanotheron* which were used in these studies were raised at the Appalachian Environmental Laboratory (AEL) from brood stock collected in Florida. Fish were held on a 14:10/L:D photoperiod maintained by Vita-lites, which provided light of similar spectral quality to sunlight. Dissolved oxygen levels were maintained between 95-100 percent saturation, and pH ranged between 7.0-7.3.

Preference Tests

Fish for temperature preference trials were acclimated to 25 C at 0 o/oo salinity. After initial temperature acclimation, one -third of the specimens were acclimated to 15 o/oo salinity and one-third to 30 o/oo salinity at a rate which did not exceed 2 o/oo salinity per day. An attempt was made to acclimate fish at all three salinities to 15, 20, 25, 30, and 35 C. Fish were held at their respective temperature and salinity for a minimum of five days prior to testing.

Temperature preference trials were conducted in a trough (3.6 m x 0.203 m x 0.245 m) modeled after Meldrim and Gift (1971). The interior was painted with an epoxy paint. The exterior bottom was coated with a temperature resistant,

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flat, black paint which facilitated heating. Cool water was introduced at one end of the trough and heated by twelve 250-watt heat lamps, beneath the trough, thus creating a temperature gradient above and below the acclimation temperature. Preference tests were conducted in the same salinity water to which the fish were acclimated. Overhead vita-lites illuminated the test area. The sides of the trough were enclosed and test fish were viewed with overhead mirrors. Twenty-two thermistors were used to determine the selected temperature throughout the trough.

Eight fish at each acclimation temperature and salinity were individually placed in the experimental unit and allowed to orient for 40 minutes. Following their orientation period, the temperature at which the fish was located was recorded every 15 seconds for 20 minutes. Based on numerous other studies, these time periods appear to be optimal, since they are long enough to allow the fish to adjust to experimental conditions but short enough to prevent reacclimation to preferred temperatures. The mean was deemed the preferred temperature for that test. A two-way ANOVA was used to determine the influence of acclimation temperature and salinity on preferred temperature. Fish acclimated at 15 C at 15 o/oo salinity were eliminated from this analysis, so that there were an equal number of cells for all temperature classes.

Tolerance Tests

Holding and acclimation procedures followed those previously described. Upper and lower lethal temperatures were determined for fish acclimated to 25 C at 0, 15, and 30 o/oo salinity. Once acclimation was completed, 10 fish were immediately exposed to each of the following temperatures: 9, 11, 13, 15, 17, 19, 21, 23, 25*, 27, 29, 31, 33, 35, 37, 39, and 41 (* = control temperature). Fish acclimated to freshwater were tested in freshwater; those acclimated to 15 o/oo salinity were tested in 15 o/oo salinity; and those at 30 o/oo were tested at 30 o/oo salinity. Responses were monitored for 10,000 minutes at the following intervals: 1.0, 1.8, 3.2, 10, 18, 32, 56, 100, 180, 320, 560, 1,000; 1,800; 3,200; 5,600 and 10,000 minutes. Numbers of dead fish were recorded at each time interval in each test. Fish were considered dead when there was complete cessation of opercular movement and no response to mechanical stimuli. A control group was simultaneously observed with the test group to assess mortality due to handling and exposure to laboratory conditions.

In addition, lower lethal temperatures were determined for specimens acclimated to 17 C and upper lethal temperatures for specimens acclimated to 35 and 37 C at 0 o/oo salinity and resistance times recorded.

RESULTS

Temperature Preference

Mean preferred temperatures for each acclimation temperature and each salinity combination are in Table 1. There was a significant ($P \leq 0.05$) effect of acclimation temperature but not salinity on preferred temperature (Table 2). The lethal studies showed that, with the exception of those individuals acclimated to 15 o/oo salinity, all fish died when exposed to 15 C. When attempting to acclimate fish to 15 C for the preference tests, we lowered the temperature at a rate of 1° C/day. In no instance did fish at 0 o/oo and 30 o/oo survive for more than six days. Therefore, we concluded that the fish were under severe stress at this temperature.

TABLE 1. Mean Preferred Temperature and Standard Deviations for Each Acclimation Temperature and Salinity.

Acclimation Temperature (C)	Salinity (o/oo) 0			15			30		
	N	$\bar{X}$	S.D.	N	$\bar{X}$	S.D.	N	$\bar{X}$	S.D.
15	–	–	–	8	26.8	5.6	–	–	–
20	8	31.9	3.6	8	33.9	3.7	8	33.4	2.4
25	8	36.6	2.2	8	36.2	2.0	8	35.7	2.5
30	8	35.7	3.2	8	34.4	2.6	8	33.7	3.0
35	8	35.6	3.47	8	33.9	2.9	8	34.0	3.8

TABLE 2. Two-Way ANOVA on Preferred Temperature, with Acclimation Temperature (A) and Salinity Levels (S) as Classes.

Source of Variation	Sum of Squares	df	Mean Square	F
A	112.6	3	37.5	8.2*
S	6.9	2	3.4	0.76
A x S	32.4	6	5.4	1.2
Error	380.8	84	4.5	

*Significant at $P \leq 0.05$.

Lethal Temperatures

Table 3 summarizes the percent survival for the thermal bioassay for fish acclimated to 25 C. Due to the "all or none" response of the fish, no attempt was made to calculate median lethal levels (LT_{50}). It is interesting to note that 100 percent of the *S. melanotheron* survived at 15 C when acclimated to 15 o/oo salinity.

Table 4 summarizes the percent survival data for fish acclimated to 17, 35, and 37 C in freshwater. There was 100 percent survival in all controls, which were run simultaneously with the lethal experiment. Neither upper nor lower lethal temperature over the 10,000 minute period in freshwater varied with a change in acclimation temperature; however, resistance times did change with acclimation temperature (Table 4).

TABLE 3. Percent Survival for Fish Acclimated to 25 C and 0, 15, and 30 o/oo Salinity; and Exposed to Various Test Temperatures for 10,000 Minutes.

Test Temperature (C)	Salinity (o/oo)		
	0	15	30
9	0	0	0
11	0	0	0
13	0	0	0
15	0	100	0
17	100	100	100
19	100	100	100
21	100	100	100
23	100	100	100
25*	100	100	100
27	100	100	100
29	100	100	100
31	100	100	100
33	100	100	100
35	100	100	100
37	100	100	90
39	0	0	0
41	0	0	0

*Control.

DISCUSSION

The highest preferred temperatures for *S. melanotheron* occurred at 25 C at all salinities, as reported for *Tilapia* [= *Oreochromis*] *nilotica* (Beamish, 1970). Beamish (1970) also noted that the lowest preferred temperature was recorded at 15 o/oo for *T. nilotica*. However, our study indicated that salinity did not significantly influence the preferred temperature of *S. melanotheron* for acclimation temperatures between 20-35 C. However, fish could be acclimated at 15 C at 15 o/oo, at which the lowest preferred temperature was recorded for this study (Table 1).

Although salinity did not affect preferred temperatures of *S. melanotheron*, it did influence lethal temperatures. Fish survived at 15 C when acclimated to 15 o/oo salinity, but died at both 0 and 30 o/oo. We hypothesize that *S. melanotheron* is nearly isosmotic with its environment at 15 o/oo salinity. Farmer and Beamish (1969) reported the tonicity of *T. nilotica's* body fluids to be 11.6 o/oo. If this hypothesis is correct, then fish require less energy for osmoregulation at 15 o/oo than at 0 or 30 o/oo; thus, that energy could be used to combat thermal stress. This hypothesis is consistent with a corollary of Shelford's law of tolerance, i.e., that when an organism approaches its physiological tolerance limit for one factor, its tolerance changes for other factors. We hypothesize that *S. melanotheron* is under osmotic stress at both 0 and 30 o/oo salinity, thus it becomes less tolerant to temperature extremes.

TABLE 4. Number of *Saratherodon melanotheron* Surviving at Each Time Interval After Being Acclimated to 17, 25, 35, and 37 C and Subsequently Exposed to Various Test Temperatures in Freshwater. Fish acclimated to 25 C were exposed to all test temperatures; those acclimated to 17 were exposed to only temperatures at and below the acclimation temperature; and those acclimated to 35 and 37 C were exposed to temperatures at and above the acclimation temperatures. For those test temperatures not reported below (including controls) there was 100 percent survival through 10,000 minutes.

Test Temperatures (C)	9		11		13		15		39			41		
Acclimation Temperature (C)	17	25	17	25	17	25	17	25	25	35	37	25	35	37
Time of Observation (min)														
1.0	10	10	10	10	10	10	10	10	10	10	10	10	10	10
1.8	10	9	10	10	10	10	10	10	10	10	10	10	10	10
3.2	10	9	10	10	10	10	10	10	10	10	10	10	10	10
5.6	10	9	10	10	10	10	10	10	10	10	10	10	10	10
10	10	7	10	10	10	10	10	10	10	10	10	7	9	10
18	9	5	10	10	10	10	10	10	10	10	10	0	9	10
32	9	5	10	10	10	10	10	10	10	10	10		9	10
56	7	4	10	10	10	10	10	10	10	10	10		9	10
100	3	3	10	10	10	9	10	10	10	10	10		9	10
180	0	0	10	7	10	9	10	10	10	10	10		7	10
320			10	0	10	8	10	10	10	10	10		3	10
560			10		10	4	10	9	10	10	10		2	10
1000			10		10	0	10	9	10	10	10		0	10
1800			6		10		10	9	10	10	10			2
3200			0		8		10	6	10	10	10			0
5600					0		7	4	9	8	10			
10,000							0	0	0	0	2			

Shafland and Pestrak (1982) reported a lower lethal level of 10-11 C for *S. melanotheron.* They lowered the temperature 1 C/day and recorded survival. Our data indicated that fish acclimated to 17 C could survive 15 C temperatures for 4-7 days. Therefore, fish tested by Shafland and Pestrak (1982) may have died at 15 C if they were held at this temperature for a longer time period. This hypothesis is supported by Smit, *et al.* (1981), who found osmoregulatory collapse of *Sarotherodon* [= *Oreochromis*] *mossambicus* at 15 C. This observation is also consistent with the fact that *S. melanotheron* survived at 15 C when acclimated to 15 o/oo salinity. However, as shown by Shafland and Pestrak (1982) and suggested by data presented herein, *S. melanotheron* can survive at temperatures outside the zone of tolerance, although this is limited by the intensity of the stress and length of time. This conclusion is supported by observations by Springer and Finucane (1963), who misidentified *S. melanotheron* as *Tilapia heudeloti*, but observed specimens in Tampa Bay at 9.6 C. More research is needed to determine the lower incipient lethal temperatures of this species at a series of salinities.

CONCLUSIONS

Data presented herein is applicable to the assessment of the potential distribution of *S. melanotheron* in North America. Introduced cichlids such as *S. melanotheron* have successfully colonized areas inhabited by indigenous fishes in peninsular Florida (Courtenay and Hensley, 1980). Stauffer (1984) discusses those parameters which affect the dispersal and colonization of exotic fishes. It would appear that cool temperature is the most important parameter which limits the dispersal of these cichlids. *S. melanotheron* is euryhaline and can probably disperse further northward in brackish water than in freshwater or marine environs. Although relatively stenothermal in freshwater, it can withstand temperatures outside of its zone of tolerance for short time periods. Furthermore, when acclimated to cold temperatures, it does select temperatures well within its zone of tolerance. Shafland and Pestrak (1982) noted that Tilapia [= *Oreochromis*] *aurea* have been observed congregating in power plant effluents when ambient water temperatures were low (Buntz and Manooch, 1969; Nobel, *et al.*, 1976), and that they utilized other warm-water refuge areas when stressed by low temperatures. *S. melanotheron's* thermal tolerance zone may be expanded, depending upon salinity. Thus, it is postulated that *S. melanotheron* could expand its present range in North America.

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