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Effect of Salinity on the Temperature Preference and Tolerance of Age-0 Mayan Cichlids

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Abstract. — We measured the salinity tolerance, temperature tolerance, and preferred temperature of the Mayan cichlid *Cichlasoma urophthalmus* to determine if temperature and salinity would limit the range expansion of this introduced species in Florida. Salinity tolerance at 25°C exceeded 37‰. The upper and lower ultimate lethal temperatures in freshwater were 39 and 15°C, respectively. Acclimation to 15‰ salinity had no effect on the lower incipient lethal temperature (15°C). Thermal preference determined in a horizontal temperature gradient was 32.8°C in freshwater, 39.2°C at 15‰, and 26.9°C at 30‰ salinity. Our results indicated that temperature and salinity are not likely to limit range expansion of the Mayan cichlid from its present location in the southern portion of the Everglades National Park, but that the species' spread may be blocked by cold winter temperatures in north Florida.

The Mayan cichlid *Cichlasoma urophthalmus* is native to Central America (Miller 1966). In 1984, two reproducing populations were discovered in Everglades National Park, Florida (Loftus 1987), one in a freshwater pond and the other in a creek that alternates between fresh and brackish water seasonally. Since its discovery, the creek population has increased in numbers and its range has expanded across the southern region of the park (W. Loftus, Everglades National Park, personal communication). Because cichlid fishes have successfully colonized many habitats throughout the world and have been able to coexist with or cause the extirpation of portions of native ichthyofaunas (Stauffer 1984), there is concern over the Mayan cichlid's establishment, range expansion, and potential effect on native fishes. In its native range, the species occurs in freshwater (Loftus, personal communication), brackish, and marine habitats; it spawns primarily from May to September, although individuals in spawning condition have been found at other times of the year (Chavez et al. 1986).

To determine if temperature and salinity would limit the range expansion of the introduced population of the Mayan cichlid in southern Florida, we measured the species' upper ultimate lethal temperature in freshwater, its salinity tolerance at 25°C, its ultimate lower lethal temperature in freshwater and brackish water, and its preferred temperature in freshwater, brackish water, and seawater.

Methods

Holding facilities and procedures. — All fish used in this study were bred in the laboratory at The Pennsylvania State University, University Park campus. Brood stock was purchased from a fish dealer in Tampa, Florida. Progeny (F_1) of this stock were hatched and raised in freshwater at 25–27°C and were 12–30 weeks of age and 15–30 mm in total length at times of testing. Fish were fed flake food (Zeigler Brothers) ad libitum throughout the test. Observations in our laboratory have shown that tilapiine fishes can reach sexual maturity 6–8 weeks after being released from the parent's mouth. Because sexual maturity can affect temperature preference and tolerance in some species (Stauffer et al. 1985), fish exhibiting secondary sexual characteristics were not used. Experiments were conducted from September 1986 through February 1989, whenever fish were available. In order to avoid pseudoreplication caused by tank effect (Hurlbert 1984), two replicate test populations in separate acclimation tasks were used for each experiment. The two replicates were compared by analysis of variance to determine whether tank effect was significant ($P < 0.05$). Salinities were adjusted in a separate reservoir by mixing Instant Ocean with filtered water until the desired salinity was attained, as measured by a refractom-

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eter. Salinity levels in the test tanks were checked daily and saline water was added to the test tanks as needed. Water quality was maintained by partial water replacement twice per week and by a standard charcoal filter operated by an air supply. Tolerance tests were conducted in 38-L glass tanks with 10 fish per tank and two tanks per treatment. No fish was tested more than once.

Salinity tolerance.—All salinity tolerance tests were conducted with fish acclimated to 25°C. Previous work (Boltz 1989) showed that Mayan cichlids can survive salinities of 20‰ and higher, so we tested only salinities of 20‰ and higher. The rate of acclimation to salinity was 2‰/d and the acclimation period was 4 d (Boltz 1989). Fish were initially acclimated to 20‰ and exposed to higher salinities at 5‰ increments. Naive fish were acclimated to the highest salinity for which there was at least 80% survival for 10,000 min in the previous trial and tested. Responses were monitored every 1 min through the first 10 min, every 10 min through the first 100 min, every 100 min through the first 1,000 min, and then every 1,000 min through 10,000 min (Allanson and Noble 1964). The data from 0–10 and 10–100 min were summed for presentation. Testing continued until an increase in the acclimation salinity did not result in an increase of the upper lethal salinity.

Upper and lower lethal temperatures.—Fish were initially acclimated to 25°C in freshwater and reacclimated to the desired acclimation temperature at a rate that did not exceed 1°C/d. They were held at their acclimation temperature (±0.5°C) for a minimum of 5 d before testing. To assess low-temperature tolerance at 15‰ salinity, the fish were acclimated to 15‰ at a rate of 2‰ per day and then reacclimated to the lowest temperature for which survival had been 80% or higher for 10,000 min in freshwater. The fish were then exposed to a range of lower temperatures. Observations were made at the same intervals described for the salinity tolerance tests and dead fish were removed at the start of each observation period. The most extreme low temperature at which 80% or more fish survived was determined to be the new tolerance limit. To calculate the ultimate lower lethal temperature (Fry 1947), fish were then acclimated to this temperature and exposed to a range of even lower temperatures, until a change in acclimation temperature did not result in a lower lethal temperature. The same procedure, but with temperatures increasing above 25°C, was used to determine upper tolerances at various salinities. For each test temperature, a chi-square goodness-of-fit test was used to determine whether the two replicates were significantly different ($P < 0.05$).

Temperature preference.—Fish were acclimated as described above to the desired salinity (0, 15, or 30‰) and then to 15, 20, 25, 30, or 35°C. Eight fish (four from each replicate acclimation tank) were individually tested for each acclimation temperature at each salinity ($N = 120$). Tests were conducted in a 3.6 × 0.2 × 0.3-m aluminum trough (Meldrim and Gift 1971). The interior of the trough was covered with nontoxic white epoxy paint, and the exterior bottom was covered with a flat black paint to facilitate heating.

Freshwater entered at one end of the trough and exited through a standpipe at the opposite end. For saltwater experiments, the water flowed through the standpipe into a reservoir and was then pumped to a headbox and returned to the trough. The water in the reservoir was filtered and aerated with a standard charcoal filter and pump. Salinity was measured with a refractometer twice daily in the reservoir and trough. The water depth in the trough was 2.0–3.5 cm.

The trough was illuminated from above with Vitalites (Durotest, Inc.), which emitted light similar in spectral quality to sunlight. The trough was enclosed by high walls to minimize the effects of extraneous visual stimuli on the fish. Overhead mirrors were used to view the fish. Cool water was introduced at one end of the trough and heated by twelve 250-W heat lamps that were evenly spaced beneath the trough. A thermal gradient was established such that the acclimation temperature of the fish to be tested occurred near the middle of the trough. Temperature was monitored with 22 evenly spaced thermistors placed 0.75 cm above the bottom of the trough. Fish were tested individually to eliminate confounding effects from aggressive behavior (Richards et al. 1977); methods described by Stauffer (1981) were used. Each fish was placed in the gradient at its acclimation temperature and was allowed to adjust to the test conditions for 40 min. Thereafter, the temperature at the location of the fish was recorded every 15 s for 20 min (80 observations/fish).

The responses of each fish were analyzed by stepwise regression procedures with preferred temperature as the dependent variable and acclimation temperature and acclimation temperature squared as the independent variables. The final preferred temperature (Fry 1947) was estimated by solving the equation for the point where preferred temperature equaled acclimation temperature (Stauffer et al. 1976).

TABLE 1.—Salinity tolerance of age-0 Mayan cichlids acclimated at 25°C and salinities of 20 and 30‰ and exposed to higher salinities.

Acclimation salinity (‰)	Test group[a]	Survival and survival time (min)				
		100%	90%	80%	10%	0%
20[b]	20b	4,000	6,000	7,000[c]		
	35a	100			1,000[c]	
	35b	100				1,000
30[d]	37a	8,000	9,000[c]			
	37b	5,000	6,000[c]			

[a] Numerals indicate the test salinity.
[b] No mortality at 10,000 min in test groups 20a; 25a,b; 30a,b.
[c] No additional mortality in 10,000 min.
[d] No mortality at 10,000 min in test groups 30a,b; 33a,b; 35a,b.

Results

Salinity Tolerance

Of the fish acclimated to 25°C and 20‰ salinity and exposed to 20‰ (control), 80% survived for 10,000 min in one replicate and 100% survived in the other (Table 1). There was no mortality after 10,000 min among fish acclimated to 20‰ and exposed to either 25 or 30‰. When exposed to 35‰, no fish survived for 1,000 min in one replicate and 10% survived for 1,000 min in the other; only one fish survived for 10,000 min. All fish acclimated to 25°C and 30‰ salinity survived 10,000 min when exposed to 30, 33, and 35‰, and 90% survived at 37‰ (Table 1).

Lower Lethal Temperature

Among fish acclimated to 25°C in freshwater, exposure to 25°C produced no mortality in either replicate (Table 2). Seventy and 80% of the fish exposed to 17°C survived for 10,000 min. Fish exposed to 14°C all died after 3,000 min, whereas fish exposed to 11 and 8°C all died with 10 min. Among fish acclimated to 17°C, there was 100% survival at 17°C and 95% survival for fish exposed to 13°C. Exposure to 15°C resulted in 100% survival in one task; however, the heater failed in the second tank and the temperature decreased to 10°C, killing all the fish. All fish exposed to 11°C died within 2,000 min. We attempted to acclimate fish to 14°C; however, when we exposed these fish

TABLE 2.—Lower lethal temperature of age-0 Mayan cichlids in freshwater (0‰ salinity) and 15‰ salinity. Each trial consisted of two replicates (a and b) of 10 fish each.

Acclimation temperature (°C)	Test group[a]	Survival and survival times (min)									
		100%	90%	80%	70%	60%	40%	30%	20%	10%	0%
					Acclimation salinity 0‰						
25[b]	17a	5,000		6,000[c]							
	17b	7,000	8,000	9,000	10,000						
	14a	100						2,000			3,000
	14b								10		100
	11a,b										10
	8a,b										10
17[d]	15b										6,000[e]
	13b	6,000	7,000[c]								
	11a				10					100	1,000
	11b	10						100		1,000	2,000
					Acclimation salinity 15‰						
17[f]	15b	3,000	4,000	5,000[c]							
	13a	10		100		4,000		6,000	7,000[c]		
	13b	1,000	2,000		3,000	4,000	5,000[c]				
15[g]	15b	4,000	5,000[c]								
	13a	4,000		5,000[c]							
	13b	1,000	3,000	4,000[c]							

[a] Numerals indicate the test salinity.
[b] No mortality in test groups 25a,b.
[c] No additional mortality in 10,000 min.
[d] No mortality in test groups 17a,b; 15a; 13a.
[e] Heater malfunctioned causing tank temperature to drop rapidly.
[f] No mortality in test groups 17a,b; 15a.
[g] No mortality in test group 15a.

TABLE 3.—Upper lethal temperatures of age-0 Mayan cichlids in freshwater. Each trial consisted of two replicates (a and b) of 10 fish each.

Test group[a]	Survival and survival times (min)										
	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	0%
	Acclimation temperature 25°C[b]										
33a	5,000				6,000[c]						
39a	100									1,000	2,000
39b				10							100
42a											10
42b			10								100
	Acclimation temperature 36°C[d]										
38b	5,000	7,000	8,000[c]								
40a			1,000	2,000[c]							
40b	10	100			1,000		2,000	3,000[c]			
	Acclimation temperature 38°C										
38a	1,000	2,000	9,000[c]								
38b	1,000	2,000[c]									
38c	1,000	4,000	5,000[c]								
38d	100	1,000[c]									
39a	7,000		8,000								9,000
39b	5,000	6,000				7,000	8,000				9,000
40a	100		2,000						3,000	5,000	6,000
40b	100					1,000	2,000				3,000

[a] Numerals indicate the test temperature.
[b] No mortality at 10,000 min in test groups 25a,b; 33b; 36a,b.
[c] No additional mortality in 10,000 min.
[d] No mortality at 10,000 min in test groups 36a,b; 38a.

to 14, 13, and 12°C none survived for 10,000 min. The ultimate lower lethal temperature in freshwater was therefore considered to be between 14 and 15°C.

For fish acclimated to 17°C and 15‰ salinity, there was 100% survival at 17°C, 95% survival at 15°C, and 30% survival at 13°C (Table 2). For fish acclimated to 15°C and 15‰ salinity, there was 80% survival at 13°C. Of the 50 fish we attempted to acclimate to 13°C, only 20 survived the acclimation period, and all had died within the exposure period; thus, the ultimate lower lethal temperature at 15‰ salinity was judged to be between 13 and 15°C.

Upper Lethal Temperatures

Fish acclimated to 25°C in freshwater had 100% survival at 25 and 36°C and 100% and 60% survival at 33°C (Table 3). No fish exposed to 39 and 42°C survived for 2,000 min. For fish acclimated to 36°C in freshwater there was 100% survival at 36°C, 90% survival at 38°C, and 50% survival at 40°C. Eighty-five percent of fish acclimated to 38°C survived at 38°C; however, none survived for 10,000 min at 39°C or for 6,000 min at 40°C (Table 3). Therefore, the ultimate upper lethal temperature in freshwater was considered to be between 38 and 39°C.

Temperature Preference

Stepwise linear regression of preferred temperatures against acclimation temperatures (Figure 1) confirmed that quadratic equations best described the data. The final preferred temperatures (95% confidence intervals in parentheses) for fish acclimated to 0, 15, and 30‰ salinity were 32.8°C (±3.5°C), 39.2°C (±7.2°C), and 26.9°C (±3.5°C), respectively.

Discussion

We did not test the effect of salinity on the ultimate upper lethal temperature of the Mayan cichlid, but fish acclimated to 25°C tolerated a salinity of 37‰, which exceeds salinities in the coastal waters of Florida. Thus, the coastal waters could serve as a migratory pathway between freshwater drainage systems for this species.

Although a temperature–salinity interaction had been previously reported for two tilapiine fishes (Stauffer et al. 1984; Stauffer 1986), the ultimate lower lethal temperature of the Mayan cichlid in 15‰ salinity (13–15°C) was not appreciably different from that measured in freshwater (14–15°C). Thus, the low-temperature tolerance of the Mayan cichlid would not be different in brackish water. Therefore, fish that colonized the northern portion

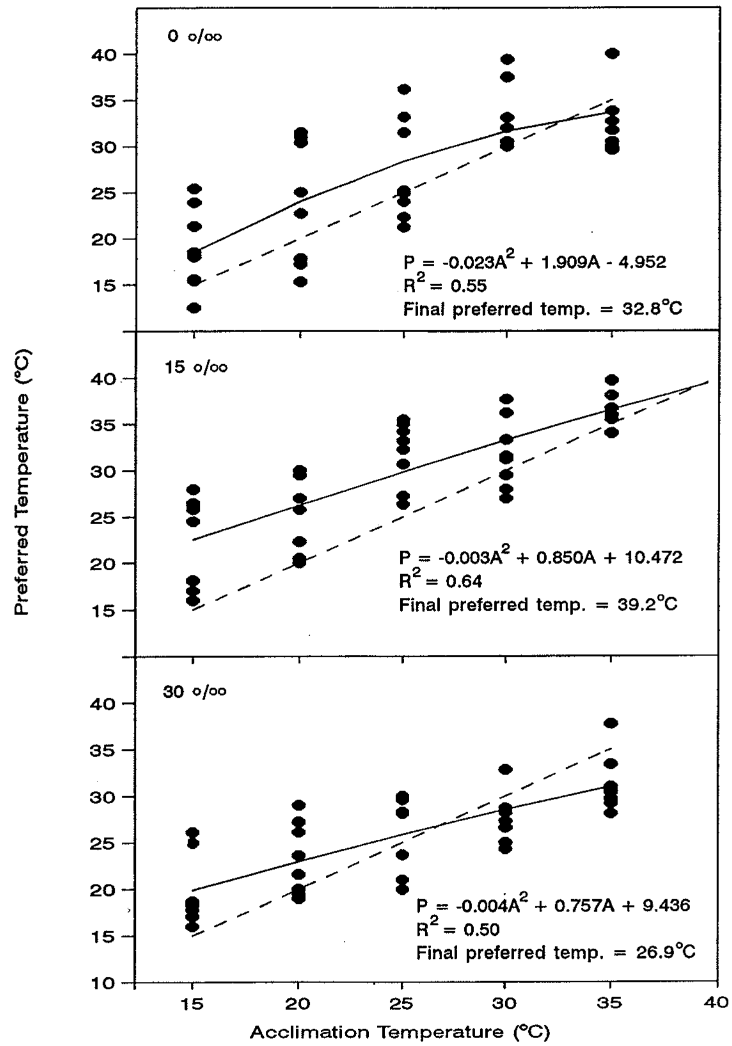

FIGURE 1.—Relationship between acclimation temperature (A) and preferred temperature (P) for age-0 Mayan cichlids acclimated to 0, 15, and 30‰ salinity. Solid lines are fitted to the data; dashed lines represent A = P. The final preferred temperature is the temperature at which the solid and dashed lines intersect.

of the state would not be able to effectively use estuarine waters for refugia during the winter, as postulated for the spotted tilapia *Tilapia mariae* (Siemien and Stauffer 1989). It may be possible, however, for the Mayan cichlid to use the freshwater springs in the west-central to the panhandle part of the state (Figure 2), which produce 20–25°C water throughout the year (Raisz 1964), as thermal refugia. Stauffer et al. (1988) showed that the blue tilapia *Oreochromis aureus*[2] overwintered in refugia created by artificial thermal outfalls in the Susquehanna River, Pennsylvania.

The final preferred temperature, which is an accurate indicator of the optimum temperature for growth (Gift 1977) was highest at 15‰ (39.2°C) salinity, lowest at 30‰ (26.9°C), and intermediate in freshwater (32.8°C). If the fundamental thermal niche (Magnuson et al. 1979) of the Mayan cichlid is taken as 39 ± 2°C in brackish water and 33 ± 2°C in freshwater, then the coastal areas south of St. Augustine and Miami and between Tampa and Pensacola probably would best support Mayan cichlids during the growing season (Figure 2).

The results of this study show that Mayan cich-

[2] The name preferred by the American Fisheries Society for this species is *Tilapia aurea*.

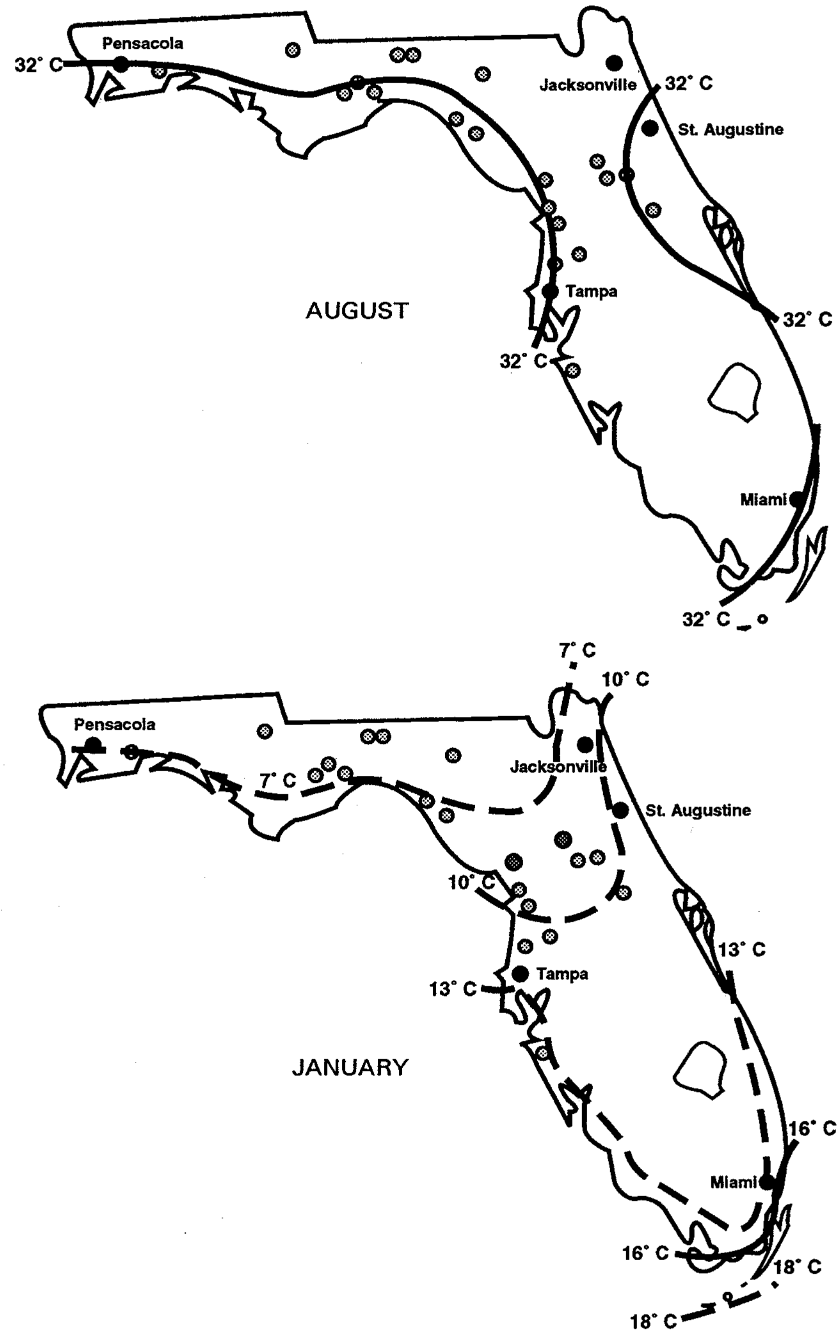

FIGURE 2.—Normal daily maximum temperatures in August (top) and minimum temperatures in January (bottom) for Florida (USDOC 1977). Shaded circles represent warmwater springs (Raisz 1964), which may serve as thermal refugia.

lids in freshwater can tolerate temperatures as high as 38–39°C and as low as 14–15°C. Because temperatures in the 38–39°C range rarely, if ever, occur in Florida, it is doubtful that high temperatures would limit the range of Mayan cichlids in the state. However, minimum daily winter temperatures of 14°C occur along the coast from just south of Tampa northward along the west coast and north of Cape Canaveral along the east coast (Figure 2). Thus, overwinter mortality could limit permanent expansion of the Mayan cichlid to north Florida unless the fish can use natural warmwater springs or artificial thermal outfalls as refugia.

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