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ARTICLE

Thermal Tolerance and Survival of Nile Tilapia and Blue Tilapia under Rapid and Natural Temperature Declination Rates

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Abstract

Blue Tilapia *Oreochromis aureus* and Nile Tilapia *O. niloticus* are indigenous to Africa and the Middle East but now are globally popular in aquaculture and for private and public stocking as forage fish for sport species and biological control for nuisance vegetation. The invasive nature of these species at a global scale and the lack of recent and applicably tested thermal tolerance methodology require research to assess the current potential for invasion risk in North Carolina and elsewhere. In this thermal tolerance experiment we focused on a moderate declination rate (1°C per 3 d) and a slow declination rate (1°C per week) in water temperature to assess the thermal minima of Blue Tilapia, common Nile Tilapia, and a "white" strain of Nile Tilapia. The moderate rate was used to compare results to previous literature, and the slow rate was used to mimic natural seasonal water temperature decline in North Carolina. As lineage and breeding history may vary by supplier, fish from multiple suppliers within each species group were tested. Our results indicate that in general, these tilapias cannot survive below 8°C. We did not observe differences in temperature at death or survival curves between species in the moderate rate treatment. Under the slow declination rate, we found significant differences in the mean temperatures at death of Blue Tilapia (9.5°C) and white Nile Tilapia (9.3°C) relative to the less-cold-tolerant common Nile Tilapia (10.8°C); this result was reflected in statistically different survival curves. In addition, although there was some variation in temperature at death among different supplier groups (0.0–0.6°C) within a species, all fish died at 8°C or above. Our results suggest that selective breeding of white Nile Tilapia may have resulted in increased thermal tolerance during the process of developing this color variant. Furthermore, the minimum lethal temperature of 8°C may explain why these tilapia species have been restricted to reservoirs with heated effluent in North Carolina.

Freshwater biodiversity is among the most threatened in the world (Dudgeon et al. 2006). Second to habitat destruction, invasive fishes are a leading cause of species endangerment and extinction in freshwater systems (Wilcove et al. 1998). Understanding the sensitivity of a potential invasive species to environmental factors such as temperature can help delineate its eventual distribution upon invasion (Zale 1984; Moyle and Light 1996; Vander Zanden et al. 2004). With predicted stream and river temperatures increasing 0.009–0.077°C per year in the United States due to climate change (Kaushal et al. 2010), it is essential to quantify thermal tolerance for threatening invaders to support efforts to stay ahead of potential invasions.

The Blue Tilapia *Oreochromis aureus* and Nile Tilapia *O. niloticus* are two of the principal commercial tilapia species that have a global impact today (Costa-Pierce 2003). Indigenous to Africa and the Middle East, these invasive species now exist under feral conditions in every nation in which they have been introduced (Canonico et al. 2005). Their high success upon introduction has been attributed to life history traits, including tolerance of variable salinity levels, hypoxia, overcrowding, and temperature extremes, as well as significant trophic flexibility (Trewavas 1983; McKaye et al. 1995; Coward and Little 2001). Their exceptional environmental tolerance (Canonico et al. 2005) and prolific nature (Stickney 1986) make

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both species highly popular farmed fish around the globe. Tilapia are bred and raised for use as supplemental forage for sport fishes; as a biological control agent for nuisance vegetation (Mallin 1985); and, most commonly, in aquaculture as food for human consumption (McGinn 1998). Growers and private pond owners can easily purchase tilapia fry from numerous suppliers throughout the USA (depending on local regulations) and can receive fry by mail within a couple of days. Private pond stocking of tilapias may pose a serious threat to native water bodies, as there is a risk of successful establishment upon accidental escapement or spread.

Blue Tilapia have invaded and become established in 10 U.S. states and Puerto Rico and are possibly established in seven additional states (Nico et al. 2013). Nile Tilapia are positively established in one U.S. state and are potentially established in five additional states (Nico et al. 2013; R. Stroud, South Carolina Department of Natural Resources, personal communication). In North Carolina, Blue Tilapia are established in two cooling reservoirs associated with electricity generation: in 1965, Blue Tilapia were deliberately introduced into Lake Julian (Arden, North Carolina) for vegetation control, and they were accidentally introduced into Hyco Lake (Roxboro, North Carolina) during a 1984 study on vegetation control (Crutchfield et al. 1992). These reservoirs receive heated effluent from steam electrical power plants; tilapias are thought to be dependent upon the warmwater discharge to survive winter temperatures in the region (C. Oakley, North Carolina Wildlife Resources Commission, personal communication). Nile Tilapia of unknown origin were recently discovered (i.e., 2015) in Lake Wylie (Belmont, North Carolina), another cooling reservoir.

Varying methodology in investigating thermal minima of fish species makes it challenging for managers to understand the possible invasion risk or further spread of some alien species. Historically, most research on thermal minima of tilapias and other fishes has used rapid declines in temperature, ranging from a fraction of 1°C per minute (e.g., Currie et al. 1998; Prodocimo and Freire 2001) to 1°C per hour (e.g., Cameron et al. 2012), and up to 0.5°C per day (Wilson et al. 2009) or 1°C per day (e.g., Bennett et al. 1997; Charo-Karisa et al. 2005; Green et al. 2012). The slowest temperature declination rate applied to Blue Tilapia was 1°C per 3 d (Zale 1984), and the slowest rates applied to Nile Tilapia were 0.5°C per d (Atwood et al. 2003; Wilson et al. 2009) and 1°C per 2 d (Paz 2004). Although these methods allow for rapid testing and easy comparison of species' thermal minima, the resulting findings may not apply to fish in water bodies experiencing slower seasonal temperature declines. Rate of decline is important when addressing invasion risk, as previous experiments have found significant differences in minimum lethal temperature depending on the rate of decline (Paz 2004; Wilson et al. 2009). Zale (1984) and Paz (2004) determined that the estimated thermal minimum for tilapias was lower when temperatures dropped at a rapid rate (0.5°C per 5 h and 1°C per 4 h) than when they were dropped at a slower rate (1°C per day down to 1°C per 3 d).

The range of thermal minima for Blue Tilapia and Nile Tilapia as suggested by previous research using a variety of differing methodologies necessitates further investigation to determine the true risk of success and overwintering upon range expansion. The thermal minima for Blue Tilapia determined using declination rates from 1°C per 4 h to 1°C per 3 d ranged from 5.0°C to 11.6°C (Zale 1984; Paz 2004); the minima for Nile Tilapia determined using declination rates from 0.5°C per 5 h to 1°C per day ranged from 6.8°C to 12.6°C (Paz 2004). The methodological approaches used in prior studies—and the potential changes in genotypic and phenotypic traits of Blue Tilapia and Nile Tilapia since then—suggest that further investigation is needed to gauge the likely cold tolerance of these fishes in feral conditions. The objectives of this investigation were to determine the minimum thermal tolerances of Blue Tilapia, common Nile Tilapia, and a selectively bred strain, the white Nile Tilapia, under a relatively moderate temperature declination rate and a slow declination rate approximating the natural cooling rates in temperate systems. Because these species groups are bred across the country with different lineages and selective breeding, we further tested for differences among fish obtained from multiple suppliers (hereafter, "supplier groups") within each species or strain. Understanding the thermal minima and survival rates of these groups at different rates of temperature decline will provide a more rigorous basis for determining how winter temperatures may influence the potential for invasion and establishment of these tilapias.

METHODS

Acclimation.— Juvenile Blue Tilapia were purchased from three suppliers, common Nile Tilapia were purchased from two suppliers, and white Nile Tilapia were purchased from two suppliers, totaling seven experimental groups. Upon arrival, experimental fish were immediately treated with formalin (1.5 mL/L for 1.5 h) and Praziquantel (Biltricide, 3.3 mg/L; dissolved in 70% ethanol at 12.5 mg/mL) to ensure that any parasites, fungi, bacteria, or disease pathogens were removed. Fish were held in 50-L holding tanks by group (species and supplier) in a recirculating system with a bubble bead filter for at least 48 h after treatments. Fish were initially acclimated to 24°C, which is within the temperature range of normal development for tilapias but below temperatures that are optimal for spawning (El-Sayed 2006). During acclimation and all experimental treatments, fish experienced a 12-h dark : 12-h light photoperiod, with a 15-min crepuscular period at the start and end of each light period. Filter backwashing (25% water change) was completed daily at midday.

Experiment.— Individual fish were randomly assigned to a system and a tank number. Each fish was weighed to the nearest 0.1 g and measured to the nearest millimeter TL before it was placed in its assigned tank. Eight individuals from each of the seven experimental groups ($N = 56$ fish) were placed randomly into the 60 available 1-L tanks in the control system. In each of the two treatment systems, 23 individuals from each of the seven experimental groups ($N = 161$ fish/treatment) were placed randomly into the available 180 1-L tanks in each system. Loading density ranged from 0.29 to 0.77 g/L among the three systems. Beginning 48 h after fish were added to assigned tanks, the temperature in each system was lowered by 1°C each day until reaching 20°C. This secondary acclimation temperature was used to limit the duration of the experiment (i.e., dropping from an initial temperature of 20°C rather than 24°C) in an effort to increase the likelihood that mortality could be accurately attributed to temperature rather than to extended experimental stress. This new acclimation temperature was maintained for 1 week before the treatment regime began. The control system was maintained at 20°C for the duration of the experiment.

Each fish was fed (Ziegler Brothers crumble feed; 55% protein, 15% fat) a maintenance ration of 1.00 ± 0.02% of body weight once per day in the morning for the entire experiment. The morning (including feeding) and evening status checks began at a randomly assigned tank and proceeded sequentially through all three systems. When a dead fish was encountered, it was removed from the tank, weighed to the nearest 0.1 g, and measured (TL) to the nearest millimeter.

Our experiment consisted of two treatments and a control, each housed in a separate recirculating system. Following the 1-week acclimation period at 20°C, the temperature in the moderate declination treatment was dropped by 1°C per day until 10% of the experimental fish in a particular species group lost equilibrium; the rate of decline was then slowed to 1°C per 3 d. Tilapias are known to lose equilibrium and cease feeding as water temperature drops below the optimal range (Zale 1984). The declination rate was slowed after the initiation of thermal stress to avoid overshooting the temperature at death. Temperature in the slow declination treatment was dropped by 1°C per week. The control system maintained constant temperature for the duration of the experiments. Temperature in each system was controlled by separate heat pumps (AquaLOGIC Delta Star DSHP7, 0.75 hp). HOBO Pendant temperature data loggers (Onset HOBO 8K Pendant UA-001-08; accuracy of ±0.14°C) that recorded water temperature every 15 min were placed in the sump of each system and in tanks randomly distributed throughout each system (10 in each treatment system; 4 in the control system). The salinity of all three systems was maintained at 0.05‰. Temperature, salinity, and dissolved oxygen in each system were monitored each morning; pH, alkalinity, hardness, ammonia, and nitrites were tested biweekly. To maintain visibility for assessments of fish health, algal growth was removed from the front of each tank in the slow declination treatment system and in the control system once during the 14-week experiment. Cleaning consisted of a quick wipe with an aquarium brush during the crepuscular light period in an effort to minimize stress to the fish. Due to the short duration of the moderate declination treatment, no cleaning was necessary in that system.

Statistical analyses.— We used two methods of statistical analysis to investigate the difference in thermal tolerance among the species groups (Blue Tilapia, common Nile Tilapia, and white Nile Tilapia; supplier groups combined) within each temperature treatment. First, we performed a Kruskal–Wallis rank-sum test on temperature at the time of death by species group. Second, we calculated Kaplan–Meier survival curves using the daily survival of each species group, and we used the log-rank test to examine heterogeneity among survival curves.

We also used two statistical analyses to test for potential differences in temperature at the time of death among supplier groups within species; these analyses were performed separately for each temperature treatment. First, we performed Wilcoxon rank-sum tests for two independent samples to compare suppliers for the common Nile Tilapia and white Nile Tilapia (two supplier groups each). Because there were three suppliers of Blue Tilapia, we compared the supplier groups by using a Kruskal–Wallis rank-sum test, which was followed by a pairwise post hoc Nemenyi test if any significant differences were found. Second, we used log-rank tests to examine heterogeneity in Kaplan–Meier survival curves among supplier groups within each species group.

Covariates of length and growth for individual fish were included to test for any effects on individual survival. The initial TL (mm) and the specific growth rate (percent change in body weight per day) during the experiment (from the start of the experiment until death) were tested for differences using log-rank tests.

RESULTS

Some initial deaths, likely due to handling and acclimation stress, occurred in all three systems early in the experiment and we assumed that these were not temperature related. The last death in the control system occurred on day 15 of the experiment, so all fish that died on or before day 15 in any system were censored. We observed that most experimental fish slowed their feeding days or weeks before death and showed signs of disequilibrium on the day of or day before death, but neither decreased feeding nor loss of equilibrium served as a reliable indicator of imminent death. In all three systems, water quality parameters remained within appropriate ranges throughout the experiment (Henson 2017). In both treatments neither initial TL nor specific growth rate during the experiment had a significant effect on survival probability ($\chi^2 = 0.07$–1.71, df = 1, $P = 0.19$–0.79). Therefore, all survival rates and temperatures at death were assumed to be attributable to species and supplier group.

Temperatures in both the moderate and slow declination treatments closely followed the intended treatment temperatures (Figure 1) with minimal variation. The average SD in daily temperature was only 0.3°C (range = 0.1–0.7°C) in the moderate declination treatment and 0.2°C (range = 0.1–0.5°C) in the slow declination treatment. When a change in temperature was applied to the system, the experimental system (and each associated tank) generally reached the new temperature within 2 h. Temperature would temporarily increase during daily filter backwashing (typically 0.5–2.0°C) until the heat pump could restore the intended temperature, normally within the hour. Some modest variation also occurred during occasional routine heat pump maintenance. In the moderate declination treatment, the shift in declination rate from 1°C per day to 1°C per 3 d was triggered at 13°C when 10% of one of the seven groups (common Nile Tilapia from supplier 2) lost equilibrium.

Moderate Declination Treatment

There was no significant difference in temperature at death among the three species groups in the moderate declination treatment ($\chi^2 = 3.1$, df = 2, $P = 0.20$; Figure 2). On average, temperature at death ranged from 9.3°C (white Nile Tilapia) to 9.6°C (common Nile Tilapia), and the lowest temperature at death was 8.8°C for all groups (Table 1). The survival curves for the three groups were not statistically different ($\chi^2 = 4.3$, df = 2, $P = 0.115$; Figure 3).

Average temperature at death of the white Nile Tilapia from the two suppliers differed significantly (Wilcoxon rank-sum test: $W = 268$, $P = 0.008$), although they had the same range of temperatures at death (8.8–9.7°C). White Nile Tilapia from supplier 1 died at an average temperature of 9.5°C, while fish from supplier 4 died at an average of 9.1°C (Table 1; Figure 4). There were no

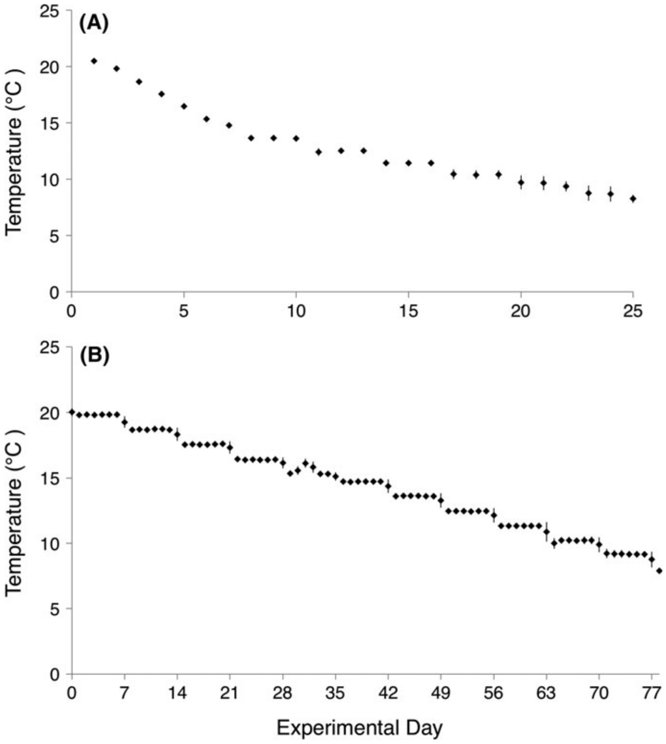

FIGURE 1. Average temperature (circles) and associated SD (bars) for each day of the experiment in **(A)** the moderate declination treatment and **(B)** the slow declination treatment.

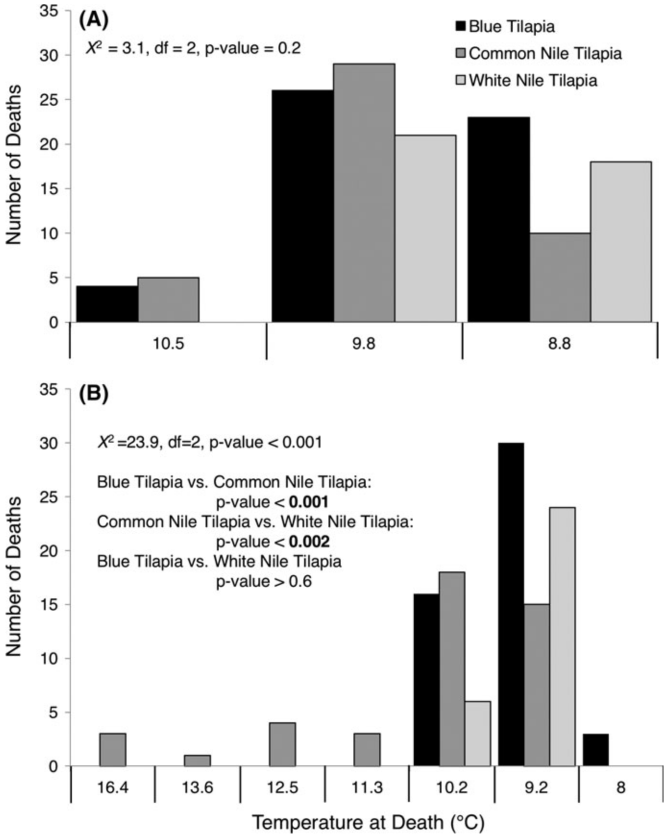

FIGURE 2. Number of deaths for each tilapia species group (suppliers combined) at each of the final temperatures in the **(A)** moderate declination treatment and **(B)** slow declination treatment. Significant differences in the pairwise comparisons are denoted by bold *P*-values.

TABLE 1. Sample size (N); average (±SD) initial TL (mm); average (±SD) initial weight (g); average percent change in body mass per day from the beginning of the experiment until death (growth rate); and median, mean, SD, and range of temperatures at death for each tilapia species group (suppliers combined) and for each supplier group within a given species group.

						Temperature (°C) at death			
Species group	Supplier	N	TL (mm)	Weight (g)	Growth rate	Median	Mean	SD	Range
				Moderate declination treatment					
White nile tilapia	All	39	72 ± 9	7.7 ± 3.1	8.2	9.7	9.3	0.5	8.8–9.7
	1	22	69 ± 8	6.6 ± 2.5	8.4	9.7	9.5	0.4	8.8–9.7
	4	17	76 ± 9	8.9 ± 3.2	8.0	8.8	9.1	0.5	8.8–9.7
Common nile tilapia	All	44	55 ± 6	3.4 ± 1.1	4.8	9.7	9.6	0.5	8.8–10.5
	2	23	56 ± 6	3.4 ± 1.1	6.2	9.7	9.6	0.4	8.8–10.5
	3	21	54 ± 6	3.3 ± 1.2	3.3	9.7	9.6	0.6	8.8–10.5
Blue tilapia	All	54	64 ± 9	5.5 ± 2.7	5.2	9.7	9.4	0.6	8.8–10.5
	2	22	59 ± 7	3.9 ± 1.6	4.5	9.7	9.5	0.5	8.8–10.5
	3	17	62 ± 6	4.7 ± 1.2	5.1	8.8	9.1	0.5	8.8–10.5
	4	15	74 ± 6	8.7 ± 2.3	6.2	9.7	9.5	0.6	8.8–10.5
				Slow declination treatment					
White nile tilapia	All	31	71 ± 8	7.4 ± 2.5	5.4	9.2	9.3	0.5	8.0–10.2
	1	22	71 ± 8	7.4 ± 2.5	5.8	9.2	9.4	0.5	9.2–10.2
	4	9	71 ± 9	7.3 ± 2.8	4.4	9.2	9.0	0.4	8.0–9.2
Common nile tilapia	All	44	52 ± 7	2.8 ± 1.3	2.3	10.2	10.8	1.9	9.2–16.4
	2	22	56 ± 7	3.5 ± 1.4	3.1	10.2	10.8	1.5	9.2–16.4
	3	22	48 ± 6	2.3 ± 0.8	1.6	9.2	10.8	2.3	9.2–16.4
Blue tilapia	All	49	64 ± 7	5.1 ± 1.9	3.2	9.2	9.5	0.6	8.0–10.2
	2	19	62 ± 7	4.4 ± 1.5	2.8	9.2	9.6	0.5	9.2–10.2
	3	22	64 ± 5	5.0 ± 1.2	3.5	9.2	9.5	0.6	8.0–10.2
	4	7	71 ± 9	7.5 ± 2.7	3.6	9.2	9.0	0.5	8.0–9.2

significant differences in mean temperature at death between the common Nile Tilapia from the two suppliers ($W = 238$, $P = 0.90$) or among the Blue Tilapia from the three suppliers ($\chi^2 = 5.6$, df = 2, $P = 0.06$), and their range of temperatures at death were identical (8.8–10.5°C; Table 1; Figure 4).

Survival curves differed significantly with supplier group for white Nile Tilapia ($\chi^2 = 12.5$, df = 1, $P < 0.001$) but not for common Nile Tilapia ($\chi^2 = 0.2$, df = 1, $P = 0.62$) or Blue Tilapia ($\chi^2 = 5.6$, df = 2, $P = 0.06$; Figure 5).

Slow Declination Treatment

There was a significant difference in temperature at death among the three species groups ($\chi^2 = 23.9$, df = 2, $P < 0.001$) in the slow declination treatment. Average temperature at death of white Nile Tilapia and Blue Tilapia differed from that of common Nile Tilapia ($P < 0.002$), but the averages for white Nile Tilapia and Blue Tilapia did not differ from each other (Figure 2). White Nile Tilapia and Blue Tilapia died at average temperatures of 9.3°C and 9.5°C, respectively, while common Nile Tilapia died at a warmer temperature of 10.6°C (Table 1). Survival curves of the three species groups were statistically different ($\chi^2 = 14.6$, df = 2, $P < 0.001$; Figure 3). The survival curve for white Nile Tilapia was statistically different from those of both the common Nile Tilapia ($\chi^2 = 12.29$, df = 1, $P = 0.0005$) and the Blue Tilapia ($\chi^2 = 5.76$, df = 1, $P = 0.016$), but the common Nile Tilapia and Blue Tilapia survival curves were not statistically different from each other ($\chi^2 = 2.991$, df = 1, $P = 0.083$; Figure 3).

We found a significant difference in temperature at death of white Nile Tilapia from the two suppliers (Wilcoxon rank-sum test: $W = 134$, $P = 0.04$). Fish from suppliers 1 and 2 died at average temperatures of 9.4°C and 9.0°C (Table 1). Conversely, supplier group had no effect on the temperature at death for common Nile Tilapia ($W = 280$, $P = 0.40$). Common Nile Tilapia from both suppliers died at an average temperature of 10.8°C (Table 1). The temperature at death of the Blue Tilapia from the three suppliers was not statistically different ($\chi^2 = 5.1$, df = 2, $P = 0.07$; Figure 4); average temperature at death ranged from 9.0°C to 9.6°C (Table 1).

The survival curves of white Nile Tilapia from the two suppliers did not differ ($\chi^2 = 3.8$, df = 1, $P = 0.05$) in the

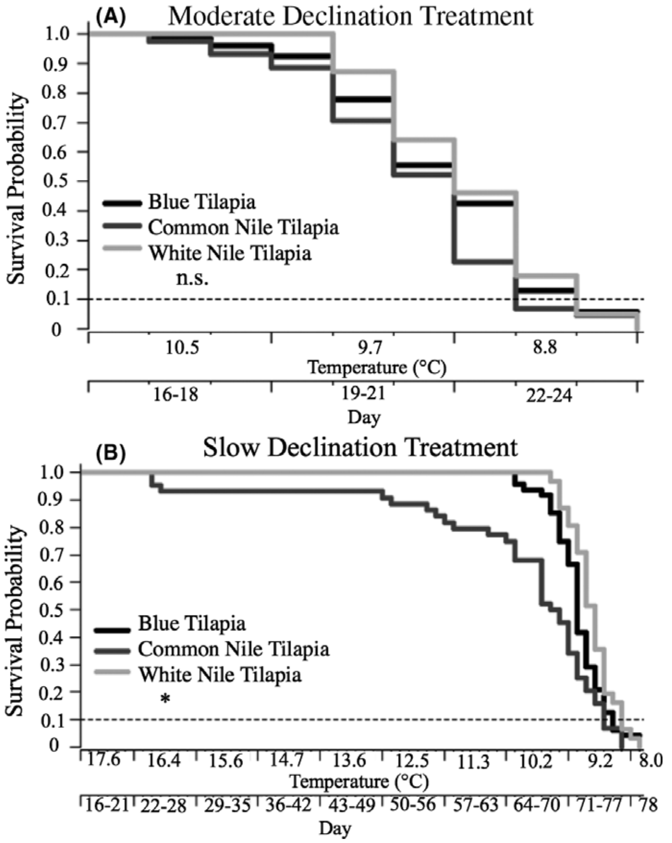

FIGURE 3. Survival curves for each tilapia species group throughout the experiment in the **(A)** moderate declination treatment and **(B)** slow declination treatment. The asterisk denotes a significant difference among species groups in the slow declination treatment; the survival curve for white Nile Tilapia was statistically different from those of both the common Nile Tilapia and the Blue Tilapia, while the latter two survival curves were not significantly different from each other.

slow declination treatment, but the survival curves of common Nile Tilapia ($\chi^2 = 5.5$, df = 1, $P = 0.02$) and Blue Tilapia ($\chi^2 = 9$, df = 2, $P = 0.01$; Figure 5) differed among supplier groups within each species.

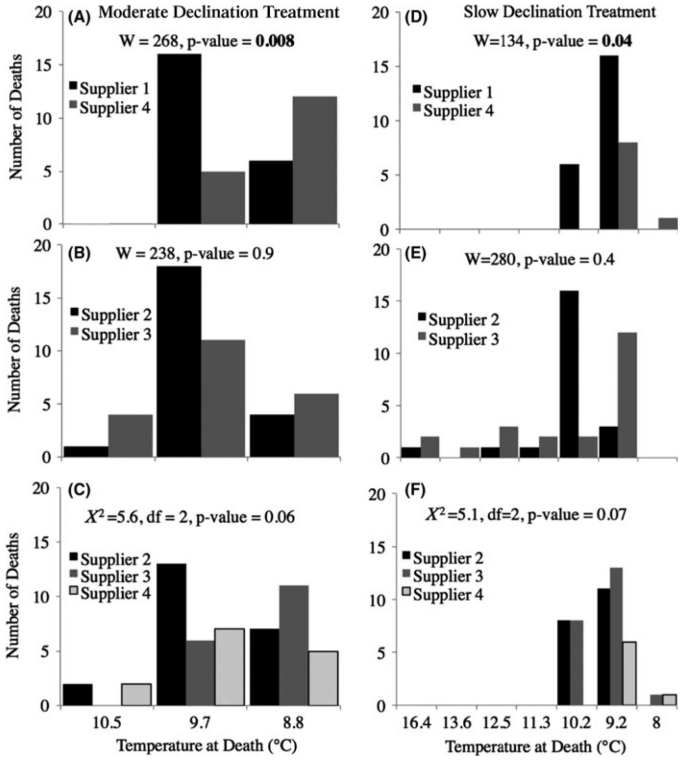

FIGURE 4. Number of deaths for tilapias from different suppliers within each species group at each of the final temperatures: **(A)** white Nile Tilapia, **(B)** common Nile Tilapia, and **(C)** Blue Tilapia in the moderate declination treatment; and **(D)** white Nile Tilapia, **(E)** common Nile Tilapia, and **(F)** Blue Tilapia in the slow declination treatment. Significant differences are indicated with bold *P*-values (W = Wilcoxon rank-sum test statistic).

DISCUSSION

Our results indicate that although there was some variation in mean temperature at death depending on the declination rate (moderate versus slow), supplier, and species, the lowest temperature any of the treatment fish reached before death was 8°C. Similar to previous experiments comparing multiple declination rates, we observed a trend of higher thermal minima when fish were exposed to a slow declination rate relative to a more rapid declination rate (Figure 6; Zale 1984; Paz 2004). Rapid declination rates may cause temperature to drop below the true thermal minimum so quickly that its lethal effect is not manifested before a colder temperature is reached, or decline more quickly than the rate at which fish can acclimate to the cooler temperatures, resulting in an erroneous estimate of the minimum temperatures that would prevent establishment of these species in the wild. The declination rates used in this experiment—moderate (1°C per day, slowed to 1°C per 3 d) and slow (1°C per week)—allowed for comparison between a rate similar to that used in previous research and an emulated autumn cooling rate in temperate natural systems, exposing the most likely thermal minima that Blue Tilapia, common Nile Tilapia, and white Nile Tilapia would exhibit upon introduction to new water bodies. Blue Tilapia and Nile Tilapia held at constant temperatures for 60 d have exhibited even warmer thermal minima (Figure 6; Zale 1984; Wilson et al. 2009), but such conditions are unlikely to be reliably sustained that long during winter in North Carolina and similar areas.

Our findings are similar to previous research indicating a temperature at death of approximately 9–10°C for tilapias when declination rates are ≤1°C per day (Atwood et al. 2003; Paz 2004; Charo-Karisa et al. 2005), but our results are contrary to those of studies that have indicated death at colder temperatures (6.0–8.4°C) at the same rate

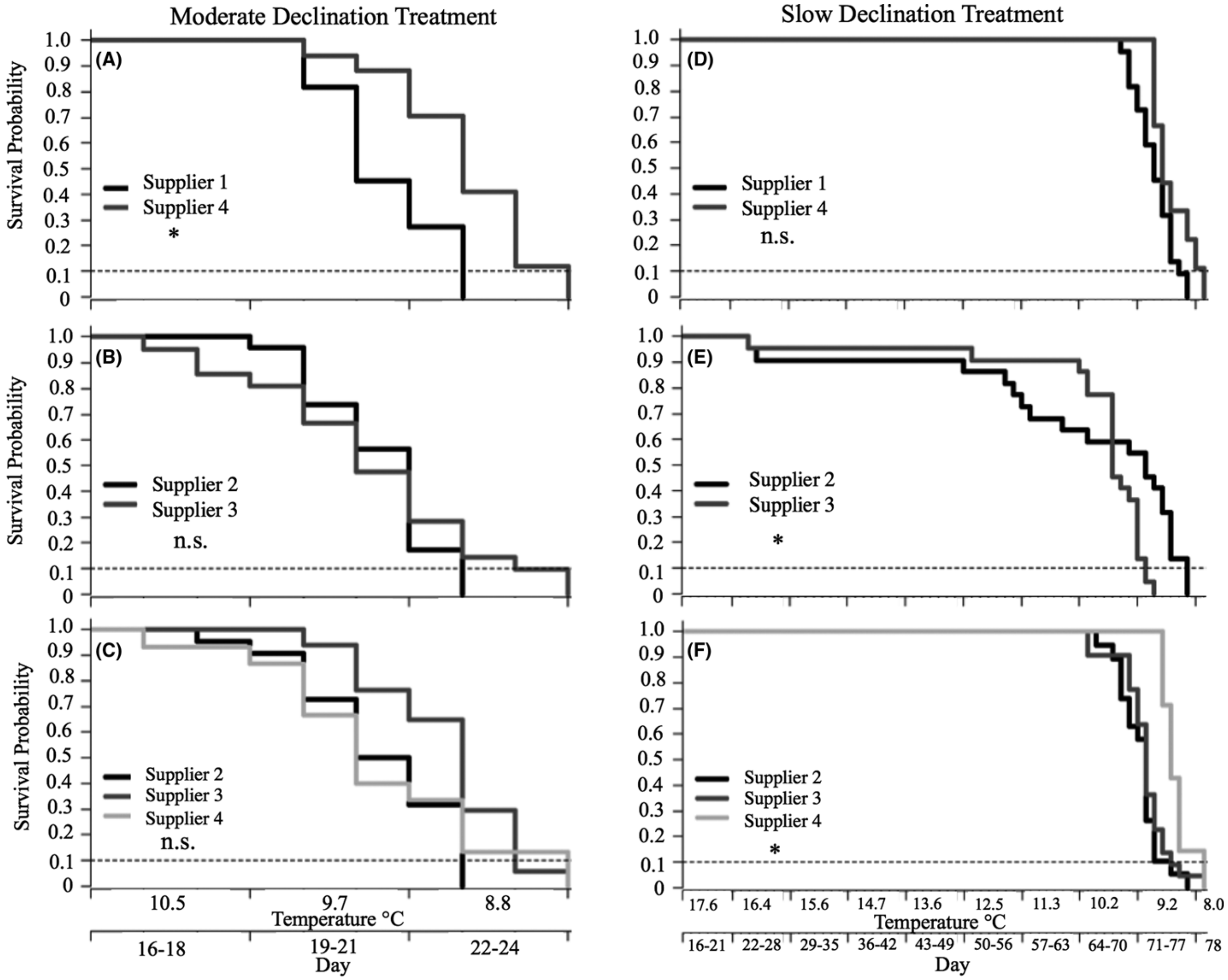

FIGURE 5. Survival curves for tilapias from different suppliers within each species group plotted versus both temperature and day: **(A)** white Nile Tilapia, **(B)** common Nile Tilapia, and **(C)** Blue Tilapia in the moderate declination treatment; and **(D)** white Nile Tilapia, **(E)** common Nile Tilapia, and **(F)** Blue Tilapia in the slow declination treatment. Asterisks denote significant differences between or among supplier groups (n.s. = indicates no significant difference).

(see literature summary, Figure 6; Shafland and Pestrak 1982; Zale 1984; Sifa et al. 2002; Paz 2004; Green et al. 2012). The effect of winter temperatures below the lethal levels revealed in this study may explain why invasive tilapias in North Carolina are limited to reservoirs that receive heated effluent. Tilapia populations in regions with milder winter temperatures or in regions where climate change may bring more suitable temperatures for tilapia establishment pose a higher threat of invasion success.

Selective breeding within the aquaculture industry for more tolerant tilapias may be changing the thermal minima of Blue Tilapia and Nile Tilapia (Dey et al. 2000; Sifa et al. 2002), generating additional uncertainty regarding the thermal tolerances of these species. Furthermore, several strains within each species have appeared over time due to geographical segregation and associated selective pressures; some of these strains have exhibited different cold tolerances (Khater and Smitherman 1988) due to genetic variation (Sifa et al. 2002). Within the aquaculture sector, there are many breeders and suppliers of these popular tilapia species; lineages from different suppliers within a species or strain may differ in thermal tolerance.

Our study yielded mixed results regarding shifts in thermal minima due to selective breeding. White Nile Tilapia exhibited thermal minima that were virtually identical to those of Blue Tilapia, which were 1°C (median) and 1.5°C

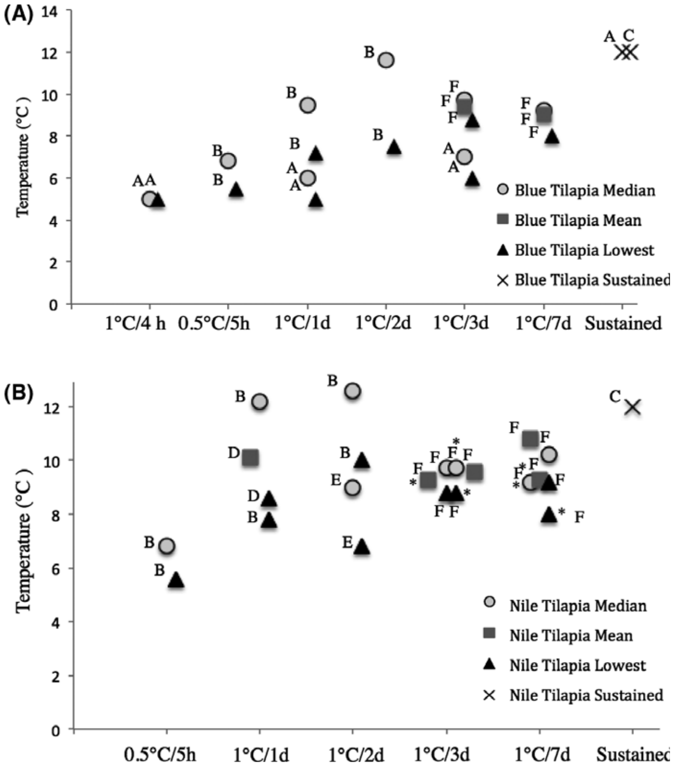

FIGURE 6. Estimates of median, mean, lowest, and sustained thermal minima reported in previous research for **(A)** Blue Tilapia and **(B)** Nile Tilapia (asterisk = white-strain Nile Tilapia; all others are common strain). Uppercase letters indicate the source (A = Zale 1984; B = Paz 2004; C = Wilson et al. 2009; D = Charo-Karisa et al. 2005; E = Atwood et al. 2003; F = present study).

(mean) cooler than those of common Nile Tilapia. The lower thermal minimum of white Nile Tilapia supports previous findings that selective pressure on strains of the same species may be causing divergence in thermal tolerance (Khater and Smitherman 1988; Sifa et al. 2002). There are many varieties of tilapia available on the market today, including hybrids and strains that have been selectively bred for aesthetics or to manipulate sex ratios (Harry Daniels, North Carolina State University [NCSU], personal communication). The present experiment highlights the possibility that the white Nile Tilapia strain we obtained randomly from public suppliers may be exhibiting indirect selection for thermal tolerance as a result of being bred for white coloration. Although we also detected some differences in thermal minima among fish from different suppliers within strains, the differences in means were only 0.0–0.6°C. Such a small difference in temperature at death among fish of the same species or strain from different suppliers may not be biologically meaningful in the wild. The differences in the mean, median, and lowest temperature at death among the species and strains, averaged across suppliers, were more substantial: 1.0–1.5°C. Initial mortality was particularly high among fish from supplier 4 relative to those from other suppliers, but it ultimately did not impact the lower lethal temperatures reached by individuals. In terms of a threat of invasion, the thermal minimum of few is as biologically relevant as that of many. These differences suggest that Blue Tilapia and white Nile Tilapia are more tolerant to cold temperatures than common Nile Tilapia and therefore potentially pose a greater threat of success upon introduction.

In this study, we focused on Blue Tilapia and Nile Tilapia, the two tilapia species that are most commonly used in aquaculture and pond management in North America and also thought to be the most cold tolerant (Rakocy and McGinty 1989; El-Sayed 2006; Canonico et al. 2005). Our results suggest that 8°C is a reasonable and conservative thermal minimum to use when evaluating the invasion potential of the tilapia species and strains we tested, as it is the lowest temperature any of the experimental fish experienced before death. Utilization of mean and median temperature at death allowed for comparison to relevant previous studies. However, given the prolific nature of tilapias, survival of a few individuals could lead to establishment of reproducing populations, so even with a slow rate of decline the mean temperature may still overestimate the minimum temperature that is relevant to assessing invasion risk in feral populations. Generations of established tilapia populations in water bodies without heated refuge could experience further selection for reduced minimum thermal tolerances. As climate change is a concern and water bodies are expected to warm (Kaushal et al. 2010), managers must continue to evaluate shifts in minimum winter temperatures relative to the thermal minima of tilapias (by species and strain) to determine whether the invasion risk changes.

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