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COLD TOLERANCE OF BLUE TILAPIA (OREOCHROMIS AUREUS) STOCKS IN SOUTH CAROLINA

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ABSTRACT

Blue tilapia (*Oreochromis aureus* Steindachner) have been stocked in water bodies for control of nuisance aquatic vegetation and as food for predators. Overwintering and reproducing blue tilapia pose potential risks for aquatic systems through alteration of nesting habitats, food resources, and aquatic plant communities. To evaluate the potential for overwintering, the overall objective of this study was to measure the cold tolerance of blue tilapia stocks currently cultured in South Carolina. Experiments were conducted with fingerling fish (38 – 54 mm) exposed to declining water temperatures, where water temperatures were reduced by 2°C at 24 hour intervals. Temperature decreases continued until 100% mortality was observed. Trials indicated the lethal temperature for 50% of the population (LT_{50}) was approximately 8.8° C. Complete mortality of fingerling tilapia were observed at water temperatures of 6° C. Mean water temperatures (8.9 - 20.6° C) in coastal South Carolina during the winter of 2014 – 2015 indicate there is potential for naturalization of blue tilapia. Pond managers and government agencies are encouraged to thoroughly evaluate the potential risk for tilapia escapes when selecting blue tilapia for use in their coastal impoundments, as the potential for naturalization is evident.

INTRODUCTION

Overwintering blue tilapia in the southeastern U.S. pose potential risks to aquatic ecosystems through alteration of nesting habitats, food resources, and aquatic plant communities (Knight & Devi, 2009; Schwartz et al., 1986). Blue tilapia (*Oreochromis aureus)* are members of the *Cichlidae* family, with native ranges extending through the Middle East and Northern Africa (ISSG, 2006). Blue tilapia are a commonly cultured species throughout much of the world, and are a commercially and economically important species in North America (Holland et al., 1999). Blue tilapia exhibit the greatest cold tolerance of commercially important tilapia species (Armas-Rosales, 2006). Production of tilapia in cooler climates outside their tropical native range is challenging under winter conditions (Lutz, 1998). Blue tilapia have been extensively used in development of commercial strains of tilapia in the U.S. (Watanabe et al., 2002). Investigations of the improvement of cold tolerance in commercial crosses when blue tilapia genetics have been added indicate that cold tolerance can be passed to offspring (Cnaani et al., 2000; Kamel et al., 2008; Paz, 2004), with blue tilapia passing “cold-tolerant” phenotypic traits (i.e. allele[s]) to other species of tilapia following generational cross breeding (Cnaani et al., 2000). Clearly, potential overwintering of blue tilapia is of ecological importance, and unconfounded temperature exposure-response data are needed.

Lower lethal temperatures for tilapia have been the focus of numerous studies (Armas-Rosales, 2006; Cnaani et al., 2000; Kamel et al., 2008; McBay, 1961; Paz, 2004; Starling et al., 1995; Zale & Gregory, 1989). The objective of prior studies was to improve cold tolerance for commercial tilapia production, and results indicate considerable variability in cold tolerance exists within stocks of blue tilapia. Armas-Rosales (2006) compiled cold tolerance data from several studies, and reported lethal temperatures for blue tilapia ranged from 3˚-13˚ C. Cnaani et al. (2000) attributed the variation of lower lethal temperatures to environmental and genetic effects; however, they also indicated that genetic control of cold tolerance was poorly understood. Zale and Gregory (1989) stated that improved resistance to cold would expand the potential range of blue tilapia. Based on the results and conclusions of prior cold tolerance studies, cold tolerance of specific stocks of blue tilapia need to be evaluated on a local or population basis.

Risk of naturalization of tilapia in the southern U.S. is a serious threat (Hale et al., 1995; Nico et al., 2013). Naturalized populations of tilapia are currently estimated to exist in ten U.S. States (Nico et al., 2013). The Florida Fish and Wildlife Conservation Commission (2017) reports that 41 non-native freshwater fish species currently reside within Florida waters, and 14 other non-native fish species have been eliminated. The blue tilapia is the most widely distributed non-native fish species found in Florida (Hale et al., 1995, Nico et al., 2013). Established tilapia populations in the U.S. compete with native species for nesting habitat, food resources, and can alter the aquatic plant communities in areas where they occur (Nico et al., 2013).

The purpose of this study was to measure the cold tolerance of blue tilapia to improve our ability to assess the risk potential of naturalization in the southeastern U.S. The overall objective of this study was to investigate the cold tolerance of blue tilapia stocks in South Carolina. To achieve this, the specific objectives were to: 1) measure lethal responses of blue tilapia to decreasing water temperatures (range 20 - 2°C) in experiments; 2) estimate median lethal effects of temperature (LT_{50}s) with Kaplan-Meier regression analyses; 3) compare and contrast tilapia responses with other cold tolerance tilapia studies. It is our hope that this study will allow extension professionals to provide clientele with sound ecological information needed to promote responsible utilization of blue tilapia.

MATERIALS AND METHODS

This study was conducted at Clemson University's Aquatic Animal Research Laboratory (AARL) under Animal Use Protocol No. 2014-087. Cold tolerance trials were conducted in Living Stream® 720 liter flow-through aquaculture tanks (Frigid Units, Inc., Toledo, OH, USA). Water temperatures were regulated with thermostatically controlled chilling units designed specifically for use with Living Stream System® aquaculture tanks. Tanks (n=2) were assigned to either control or temperature reduction treatments prior to initiation of trials. Each tank was divided into three sections with screen dividers supplied by Frigid Units, Inc. Tank sections were used for pseudo-replicates (3 treatment, 2 control). Water temperatures were monitored with Fisher Scientific Traceable digital thermometers (Fisher Scientific, Pittsburgh, PA, USA).

The animal use protocol allowed for 50 fish to be used in this trial. Blue tilapia fingerlings (38 - 54 mm total length; n=50) were obtained from Orangeburg Aquaculture in spring 2015 and transported to the AARL in aerated tanks. Upon arrival, tilapia were inspected to assess general health of fish. Following health assessment, fish were randomly assigned to either treatment or control tanks. Fish were then randomly assigned to a section within the corresponding tanks. Thirty tilapia fingerlings were placed in the temperature reduction treatment tank (10 per section, 3 sections). Twenty fish were placed in the control tank (10 per section, 2 sections). Cold tolerance trials were conducted one time with 3 pseudo-replications measured simultaneously.

Water temperature was adjusted to 20°C prior to initiation of trials. Water temperature was held at 20°C for 24 hours to allow for acclimation of fish. The control tank was held at 20°C for the duration of the study. In the treatment tank, temperature was reduced by 2°C every 24 hours. Temperature decreases continued until 100% mortality was observed. Mortality evaluations were conducted prior to each temperature reduction period. Mortality was defined as loss of equilibrium or lack of opercular movement. Mortalities were removed from tanks daily and recorded along with corresponding water temperature. Fish total length (TL) was recorded to the closest mm. Water quality parameters (i.e. pH, alkalinity, hardness, dissolved oxygen (D.O.), and conductivity) were measured and recorded daily. *In situ* dissolved oxygen, pH, and conductivity of water were measured using a YSI® Model 52 dissolved oxygen meter (YSI,Inc. Yellow Springs, OH, USA), Orion® Model 250A pH meter (Thermo Electron Corp., Beverly, MA, USA), and Triode® electrode (Thought Technology Ltd., Montreal, Quebec H4X 1N1, Canada), and Orion® Model 142 conductivity meter (Thermo Electron Corp., Beverly, MA, USA), respectively (Table 1). Alkalinity and hardness of aqueous samples were determined according to Standard Methods for the Examination of Water and Waste Water (APHA, AWWA, and WEF 2005).

Table 1. Water quality measurements from blue tilapia cold tolerance trials. Parameter measurements were recorded daily within each section (n = 3) of each tank (n = 2).

Parameter	Control		Treatment	
	Mean (n=10)	**Range**	**Mean (n=10)**	**Range**
pH	8.02	7.90 - 8.12	8.05	7.90 - 8.12
Dissolved Oxygen (mg/L)	7.8	6.8 – 8.10	8.2	7.80 – 8.5
Alkalinity (mg $CaCO_3$/L)	76	72 - 80	76	72 - 80
Hardness (mg $CaCO_3$/L)	90.5	88 - 92	92	88 - 94
Conductivity (µmhos/cm)	258	252 - 265	256	253 - 265

Lethal temperature effects for 50% of the population (LT_{50}) and time of exposure effects for 50% of the population (TL_{50}) were calculated with logistic and Kaplan-Meier (Kaplan and Meier, 1958) survivorship curve regression analyses (JMP v11; SAS Institute Inc., Cary, NC, USA). Possible relationships among fish size and temperatures were determined with one-way analysis of variance (ANOVA) with follow-up pairwise comparisons and contrasts with linear models. Differences were considered significant at $P \leq 0.05$.

Mean winter coastal water temperatures for 2014 – 2015 from Wilmington, NC south to Savannah, GA were obtained from the National Oceanographic and Atmospheric Administration (NOAA).

RESULTS

Fingerlings ranged from 38 – 54 mm TL with mean TL of 46.6 mm (SD = 4.32). There was no difference in fish length between pseudo-replicates (α = 0.05: P = 0.6237). Initial mortalities (n = 3; 10%) were observed at 12° C. Seven fingerlings (23.3%) expired at 10° C; 18 (60%) mortalities at 8° C; and total mortality was observed at 6° C (n = 2; 6.7%). Mortality temperatures differed among pseudo-replicates (n = 3) (α = 0.5: P = 0.0162). Means for fish mortality in pseudo-replicates were 8.4° C, 8.0° C, and 9.8° C, respectively. No mortalities were experienced in the control groups (n = 2). Distribution of fingerling mortality in relation to water temperature can be seen in Figure 1. Survivorship curves (Figure 2) indicated LT_{50} was approximately 8.8° C (95% confidence interval 8.2 – 9.4).

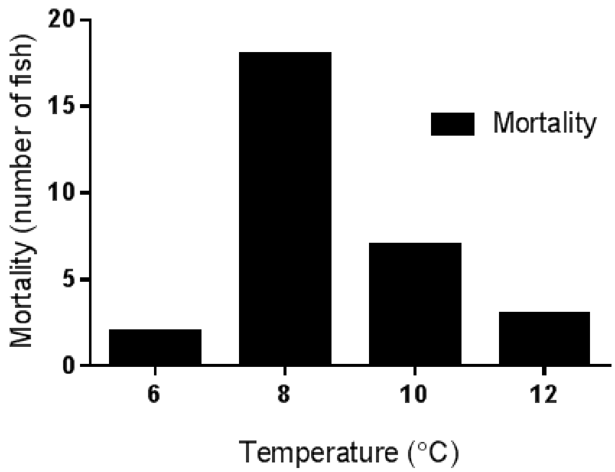

Figure 1. Distribution of tilapia mortalities observed in cold tolerance trials where juvenile blue tilapia (n = 30) were exposed to temperature reduction beginning at 20° C and decreasing at -2° C per 24 hours in order to determine LT_{50} and LT_{100}.

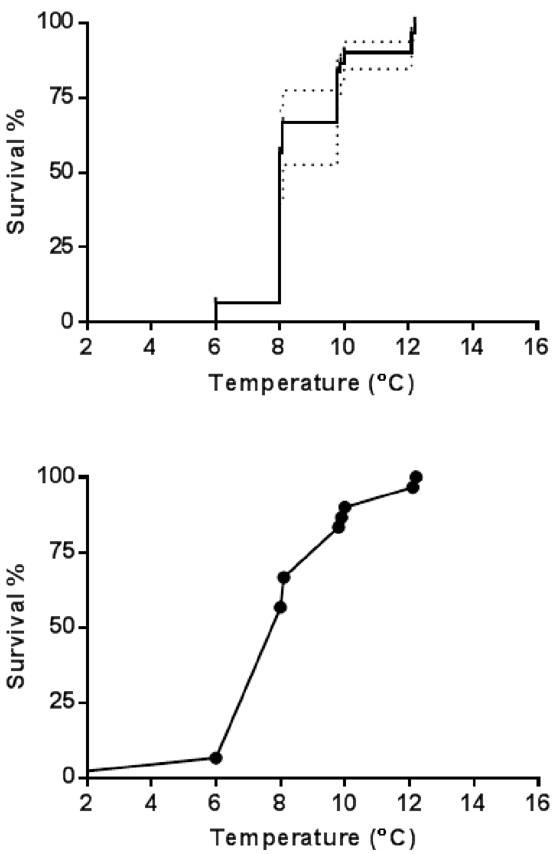

Figure 2. Survival regression using the Kaplan-Meier non-parametric method (top = step-down graphic; bottom = “smoothed”) for survival estimation of blue tilapia juveniles (n =30) exposed to temperature reduction trials beginning at 20° C and decreasing at a rate of -2° per 24 hours.

The relationship of fish length and lethal temperature was analyzed by creating 3 fish length groups. Groups were defined as follows: A = 38 - 44 mm (n = 8); B = 45 - 49 mm (n = 11); and C = 50 - 54 mm (n = 11). Mean lethal temperature for the groups were A = 10° C (SD = 1.51); B = 8.73° C (SD = 1.35); and C = 7.82° C (SD = 1.08). Analysis of variance indicated that lethal temperatures were different among groups ($\alpha = 0.05$: $P = 0.0050$).

Analysis of variance for water quality parameters indicated no significant differences were observed within or among treatments for any parameter measured (α = 0.05: *P* > 0.05). Mean water quality parameters were as follows: pH = 8.02 ± 0.06, D.O. = 7.8 mg/L ± 0.2, Alkalinity = 76.4 mg $CaCO_3$/L ± 1.2, Hardness = 90.5 mg $CaCO_3$/L ± 1.4, and Conductivity = 258.4 µS/cm ± 3.1. Water quality parameters were within acceptable ranges based on USEPA testing guidelines for sentinel aquatic organisms (United States Environmental Protection Agency, 2002).

Winter coastal water temperatures from Wilmington, NC south to Savannah, GA ranged from 8.9 – 20.6° C (November – March). Mean coastal water temperatures were lowest in January (10.98° C) for the area analyzed. Myrtle Beach, SC reported the lowest mean water temperature (11.9° C) during the 2014 – 2015 winter (Table 2).

Table 2. Mean coastal water temperatures for the winter months of 2014 – 2015 from Wilmington, NC south to Savannah, GA. Temperatures are reported as mean of monthly temperature measurements collected by the National Oceanic and Atmospheric Administration (NOAA).

Location	November	December	January	February	March
Wilmington, NC	20.6	16.7	14.4	14.4	16.7
Myrtle Beach, SC	16.1	11.7	8.9	10	12.8
Charleston, SC	17.2	12.2	10	10	13.9
Savannah, GA	17.8	12.2	10.6	11.1	15

DISCUSSION

Minimal mortality of blue tilapia above 10° C is in agreement with the findings of Paz (2004) and Starling et al. (1995), both of whom reported significant mortality initially occurred at 9.5° C. Likewise, in our study significant mortality (n = 18, 60%) was observed during the 8° C period and complete mortality was observed during the 6° C period (n = 2, 6.7%). Lethal temperatures in this study were in accordance with the range of lethal temperatures reported in the literature (Armas-Rosales, 2006; Kamel et al., 2008; Paz, 2004; Starling et al., 1995; Wilson et al., 2009; Zale and Gregory, 1989). Mean lethal temperatures related to fish length groups observed in this study indicated cold tolerance was greater in larger fingerlings. Similarly, Cnaani et al. (2000) and Behrends et al. (1990) indicate a positive relationship with increasing fish size (length and mass) and lethal temperature. It should be noted that total lengths among fish our study ranged from 38 – 54 mm, which may minimize the probability of discerning relationships. The ability of larger fingerlings to tolerate lower temperatures could be important to aquaculturists attempting to overwinter tilapia fingerlings. Additionally, this ability could be important in predicting the potential of tilapia escapes to naturalize in waters of the southeastern U.S.

State game and fish agencies across the southern U.S. have implemented various strategies to reduce the potential of tilapia introductions (Popma and Masser, 1999). Some states have restricted the use of blue tilapia due to the potential for naturalization. South Carolina currently has no restriction on the use of tilapia, except that one must obtain a “Possession of Non-Indigenous Fish Species” permit to possess (South Carolina Department of Natural Resources, 2015). Research has shown that variation in lethal temperature exists among blue tilapia stocks in the U.S. (Cnaani et al., 2000; Zale & Gregory, 1989; McBay, 1961; Kamel et al., 2008; Starling et al., 1995; Armas-Rosales, 2006; Paz, 2004), indicating that to properly understand the potential risk of naturalization, lethal temperatures need to be evaluated on a local or state by state basis (Table 3).

Table 3. Reported lethal temperatures (LT_{100}) of blue tilapia from this study and others in the literature.

Author	Year	Location	Lethal Temperature (Celsius)
Armas-Rosales	2006	Louisiana	4
Chervinsky & Lahov	1976	Israel	9
El-Gamal	1987	Alabama	7.3
Paz	2004	Louisiana	5.5
Starling et al.	1995	Texas	10.9
Zale & Gregory	1989	Florida	6
Heaton	2015	South Carolina	6

Reported mean water temperatures from Wilmington, NC, Myrtle Beach, SC, Charleston, SC, and Savannah, GA suggest that based on water temperatures, blue tilapia could have overwintered in coastal areas of Georgia, North Carolina and South Carolina (Table 2). Reported mean coastal water temperature only approached the blue tilapia LT_{50} (8.8° C) found in this study at Myrtle Beach during January 2015 (8.9° C). This was the only site and only month where mean coastal water temperatures dropped into the single digits. Based on the observed tilapia mortalities in this study, in context of coastal water temperatures, it is possible that tilapia overwintered along the coast during the 2014 - 2015 winter. This assumption was verified in Charleston, SC in May of 2015 (W. C. Heaton, 2015). Blue tilapia and blue tilapia crosses overwintered in numerous impoundments on Kiawah Island in Charleston County, where tilapia were present in both fresh and brackish water impoundments. Based on results from this study, it is clear that naturalized populations would likely suffer from unusually cold winters (water temperatures consistently below 8.8° C), but the rate and extent of blue tilapia loss is currently unknown.

It is imperative that we address the cold tolerance of stocks of blue tilapia in the southeastern U.S. Blue tilapia are frequently stocked into private waters for aquatic weed control and fisheries improvement. In many situations tilapia are stocked into impoundments that are directly connected to waters of the state. Blue tilapia have been observed to overwinter in impoundments within multiple states in the U.S. (Nico et al., 2013). This conservative experiment indicates blue tilapia are relatively tolerant (LT_{50} = 8.8 °C) to coastal winter water temperatures common of southern Atlantic States. Based on multiple lines of evidence, including field observations (W.C. Heaton, 2015), cold tolerance experiments, and historical coastal water temperature data, there is reasonable potential that blue tilapia are overwintering in portions of the Southeastern U.S.

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