ABSTRACT

HENSON, MARY NELL. Assessing the Influence of Tilapia on Sport Species in North Carolina Reservoirs (Under the direction of Dr. D. Derek Aday and Dr. James A. Rice).

Redbelly Tilapia *Tilapia zilli*, Blue Tilapia *Oreochromus aureus* and Nile Tilapia *Oreochromis niloticus* are indigenous to Africa and the Middle East, but now are globally popular in aquaculture and for private and public stocking as a forage fish for sport species and as a biological control for nuisance vegetation. It is fairly well understood that foraging flexibility and wide salinity, oxygen and temperature tolerances have led to general invasion success for most tilapia species. The invasive nature of these species at a global scale and the lack of recent and applicably tested thermal tolerance methodology requires research to assess the current potential for invasion risk in North Carolina and elsewhere. In addition, conflicting empirical information about the influence of tilapia on native sport species leaves natural resource managers unable to anticipate potential risks to native sport species associated with possible additional introductions. This investigation focuses on assessing thermal cold tolerance of popular tilapia species, thought to be one of the only factors limiting their expansion, as well as investigating effects of tilapia on sport species in two NC reservoirs with extant tilapia populations: Hyco Lake, a large reservoir in northcentral NC and Lake Julian, a small impoundment in western NC. These reservoirs receive heated effluent from power-generating plants, which is assumed to provide winter refuge for tilapia at a time when temperatures would otherwise lead to mortality. We assessed abundances of tilapia and native sport species (Bluegill *Lepomis macrochirus*, black basses and Black Crappie *Pomoxis nigromaculatus*) in these systems and examined their trophic interactions via diet and stable isotope analyses in both lakes. In Hyco Lake we assessed potential spatial interactions and influences on abundance using *a priori* candidate models and model selection via Akaike's Information Criterion (AIC). These analyses indicate little evidence of tilapia influencing the abundances of the sport species in Hyco Lake. In both lakes, however, we did find substantial diet overlap between the tilapia and Bluegill along with isotopic niche overlap between Blue Tilapia and Bluegill in Lake Julian and Redbelly Tilapia and Bluegill in Hyco Lake. Due to the omnivorous and flexible diet of all three species these interactions were not surprising, but the ultimate influence on Bluegill growth and size structure is unknown. Overall, our results suggest that tilapia are exploiting relatively open niches in these two very different reservoir systems and that their direct impact on sport species as competitors or prey is not substantial. In the thermal tolerance assessment we focused on a moderate declination rate (1°C/3d) and a slow declination rate (1°C/week) in water temperature to assess the thermal minima of Blue Tilapia and 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 water temperature decline in North Carolina. As lineage and breeding history may vary by supplier, fish from multiple suppliers of each experimental group were tested. This study determined that in general, these tilapia cannot survive below 8°C. The moderate declination rate did not show significant differences in temperature at death or survival curves between species. In the slow declination rate, there were significant differences in the mean temperatures at death of Blue Tilapia (9.5°C) and the 'White' strain of Nile Tilapia (9.3°C) when compared to the less cold tolerant, Common Nile Tilapia (10.8°C), which were also reflected in statistically different survival curves. This suggests that breeding of White Nile Tilapia may have selected for increased thermal tolerance while developing a color variation.

While there was some variation of temperature at death by supplier (0.0-0.6°C) within a species, all fish perished at ≥ 8°C. Average temperature at death ranged from 0.5-1.6°C above the final lethal temperature of any species group. This difference should be considered when using experimental results to evaluate invasion risk. The minimum lethal temperature of 8°C may explain why these tilapia species have been restricted to reservoirs with heated effluent in North Carolina.

Assessing the Influence of Tilapia on Sport Species in North Carolina Reservoirs

by
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BIOGRAPHY

I was born in Massachusetts but grew up all over. In the US, my family moved to Connecticut, Illinois and Minnesota. We had the opportunity to live in Mexico and England as well. After high school I attended college in North Carolina and Colorado then returned to North Carolina for graduate school. Both sides of my large extended family are very close; no matter where we lived at the time we always came back to spend the summers in Connecticut and Maine with family.

My family is not outdoorsy what-so-ever. I had not even tried camping until I was in my early twenties. I caught up quickly. Backpacking became an obsession while pursuing my B.S. in Fish, Wildlife and Conservation Biology. I surprised family and myself when I developed an interest in fisheries science and applied statistics. I thoroughly enjoyed my technician experience during school and after graduation. I was fortunate to work for the Rocky Mountain Bird Observatory for summers backpacking and completing bird surveys in Colorado, Wyoming, Nebraska and South Dakota. In between, I was able to camp in Colorado and Utah while surveying and removing invasive species for the Larval Fish Laboratory at Colorado State University. Motivated to make a difference in the field of conservation, I began preparing for graduate school.

In 2013 I began my journey at North Carolina State University to earn a master's degree in Fisheries, Wildlife and Conservation Biology working with Drs. James A. Rice and D. Derek Aday. I have enjoyed living here and made lifelong friends who, along with wonderful family and wise advisors, have been nothing but encouraging and supportive.

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Most importantly, I would like to thank my family. Without their constant cheering and reassurance, I would have given up in my first graduate level statistics class. My wonderful parents passionately support my wildest dreams and are always there for all of the moments of struggle and all of the accomplishments. My awesome brother and his terrific fiancé are always there to listen to the latest news on tilapia. My inspiring grandparents never fail to provide valuable life advice to remind me of what is most important. My Raleigh aunts, uncle and cousins continuously cheer me on and so thoughtfully provide love and care. My great new in-laws have warmly welcomed me into their wonderful family. My delightful husband, Mike, has been so loyal and reassuring through the entire experience. I could not have done it without everyone. Thank you so much everyone for all your support.

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INTRODUCTION

Freshwater organisms are strongly affected at a global scale by anthropogenic factors such as invasive species introductions, habitat alteration, exploitation, and pollution (Dextrase and Mandrak 2006). Invasive species in freshwater systems are a leading cause of native species endangerment and extinction, second only to habitat loss (Canonico et al. 2005). Quantification of the impact of invasive species on freshwater native species is essential because freshwater flora and fauna are among the most threatened in the world (Dudgeon et al. 2006). Biologists and managers must understand the potential for range expansions and the influence they may have on native species for the development of effective management and mitigation strategies (Moyle and Light 1996, Vander Zanden et al. 2004).

Most established invasive species are characterized as highly tolerant and exhibit plasticity in life-history traits that allow occupancy of a variety of habitats (Russell et al. 2012). Members of a tribe in the Cichlidae family, collectively known as tilapia, flourish globally due to their wide salinity, oxygen, temperature, and population density tolerances (Stickney 1986, McKaye et al. 1995; Coward and Little 2001). These fishes are reproductively prolific, able to feed at multiple trophic levels, and can significantly alter the aquatic community (Canonico et al. 2005), which further enables them to thrive in foreign water bodies.

Tilapia are indigenous to Africa and the Middle East (Canonico et al. 2005). Historically, accidental and purposeful introductions of tilapia were generally the result of aquarium releases and live-transportation of food sources for the Japanese during World War II (Canonico et al 2005). More recently tilapia have become popular for stocking in private and public waters as biological control for nuisance vegetation and as forage fish for sport species (Mallin 1985, Canonico et al. 2005). They are also one of the most popular and widespread groups of species in the aquaculture trade (El-Sayed 2006), and selective breeding for more tolerant and productive strains of tilapia in the aquaculture environment strengthens their invasion potential (Dey et al. 2000). In fact, researchers suspect that the only factor limiting the spread of Blue Tilapia *Oreochromis aureus* in the United States is temperature (Shafland and Pestrak 1982, Zale 1984).

In North Carolina there are presently two tilapia species in some freshwater systems across the state, Blue Tilapia and Redbelly Tilapia *Tilapia zillii*. The U.S. Geological Survey

(Nico et al. 2016) reported that with the exception of Virginia, these two species have established populations in every state in the southeastern region of the United States. These invasions are a cause for concern because of the potential influence on co-occurring sport species (e.g., Largemouth Bass *Micropterus salmoides* and Spotted Bass *Micropterus punctulatus,* Bluegill *Lepomis macrochirus,* and Black Crappie *Pomoxis nigromaculatus).* Relatively few studies have considered possible interactions among invasive tilapia and sport species, and literature that is available has produced equivocal outcomes; research has indicated positive, negative and no influence of tilapia on various sport species. For example, Martin et al. (2010) determined that invasive tilapia have negative impacts on Redspotted Sunfish *Lepomis miniatus*, as the Redspotted Sunfish spend less time in their preferred habitats when tilapia are present. In addition, tilapia can negatively influence growth and survival of Largemouth Bass (Zale 1987, Traxler and Murphy 1995). Conversely, studies have indicated that Bluegill will consume tilapia (Bickerstaff et al. 1984) and small tilapia can serve as the optimal and preferred prey (over Bluegill) of Largemouth Bass (Schram and Zale 1985), although somewhat contradictory data suggest that Largemouth Bass will choose Redspotted Sunfish over tilapia as prey (in structured habitats) (Martin et al. 2010) and that Largemouth Bass and Black Crappie do not consume invasive tilapia (Gu et al. 1996). Finally, previous studies have found both that tilapia do compete (Crutchfield 1995) and do not compete (Mallin 1985, Peterson et al. 2006) with native fishes for resources. This mix of results prevents natural resource managers from developing effective policy and mitigation strategies.

In this investigation we assessed interactions between tilapia and sport species in two very different freshwater reservoirs through quantification of diets, stable isotope analyses, and abundance analyses. Gut content analyses (GCA) can be used in combination with stable isotope analyses (SIA) to understand trophic niche use and potential resource-based overlap between tilapia and sport species (Montaña and Winemiller 2013). Stable isotope analysis provides a more integrated signature of food habits than GCA and can be used to explore longer-term food web interactions (Vander Zanden et al. 1997; Rybczynski et al 2008). The stable isotope $\delta^{13}C$ can be used to determine original sources of carbon in the diet of an individual, and $\delta^{15}N$ is indicative of their trophic position (Layman et al. 2012). Although a variety of biotic and abiotic factors can be drivers of fish abundance in particular systems (Middaugh et al. 2013), it is unclear how the presence of tilapia might be influencing the abundance of sport species

following invasion of our study systems. In the present study we use multimodel inference in the form of Akaike's Information Criterion (AIC; Burnham and Anderson 2002) to assess the influence of tilapia relative to other biotic and abiotic factors on abundance of co-occurring sport species. Our goal was to collect fundamental information about tilapia-sport species interactions to address concerns about how these common invaders might be affecting sport species in our study reservoirs and similar systems.

METHODS

Study Area

There are currently two lakes in North Carolina, Hyco Lake (Roxboro, NC) and Lake Julian (Arden, NC), that host established populations of tilapia. Both lakes are cooling reservoirs for steam electrical power plants, which provide heated effluent to the lakes. The heated effluent is suspected to allow tilapia to persist and overwinter in an otherwise intolerable North Carolina winter climate (Crutchfield 1995). In 1984, both Redbelly Tilapia and Blue Tilapia were accidentally introduced to Hyco Lake during an onsite experiment (Crutchfield 1995). In 1965, Blue Tilapia were purposefully introduced to Lake Julian to serve as aquatic plant control and as forage fish for black bass populations (Corey Oakley, NCWRC, personal communication). The lakes differ dramatically in size, structure, and use and are not offered as a comparison in the present investigation, but rather as an opportunity to examine tilapia-sport fish interactions in two very different study systems.

Hyco Lake (Figure 1.1) is 1,520 ha with an average depth of 6 m and a maximum depth of 9 m and was constructed on the Hyco River at the confluence of three main tributaries (North Hyco Creek, South Hyco Creek and Cobbs Creek). The single heated-effluent source is centrally located and creates a heated pool that cools as distance from the effluent source increases. Water quality is heterogeneous across the lake with variable temperatures. Ongoing fisheries assessments suggest that the dominant fish species are Bluegill, Largemouth Bass, Black

Crappie, Gizzard Shad *Dorosoma cepedianum,* and the two tilapia species. Lake Julian (Figure 1.2) is a bowl-shaped lake of 87 ha, with an average depth of nine m and maximum depth of 33 m. Dissolved oxygen and temperature are largely homogenous within season. Much like the species found in Hyco Lake, Centrarchids dominate the fish community (in this case Bluegill, Largemouth Bass and Spotted Bass).

Field Sites and Fish Collection

Hyco Lake experiences strong heterogeneity in temperature due to lake morphology and the heated effluent, so we classified the lake into three different zones in an effort to understand fish distributions and potential interactions. Using preliminary temperature data from a pilot study we delineated three temperature zones: 'hot', which extends roughly 0.8 km from the heated effluent outfall; 'intermediate', from the edge of the hot zone extending outward approximately 3 km; and 'cold', which includes all areas of the lake beyond the intermediate zone (Figure 1.1). Preliminary data collection indicated that the cold zone is outside the influence of the heated effluent and experiences ambient temperatures. We established three replicate study sites within each zone for a total of nine throughout the lake (Figure 1.1). Sampling was conducted bimonthly from July 2014 through November 2015. At each site (N = 9) each sampling occasion consisted of one trap net set for approximately 24 h, followed by a 10-min boat electrofishing sample. Trap nets were 2.5-cm knotted bar mesh with two rectangular frames (182 cm width x 92 cm height), a center upright crosspiece and six round hoops (76 cm diameter). Nets were set perpendicular to shore at depths of less than 3 m with a 15-m leader extending from the shoreline. Electrofishing was conducted along the shoreline using pulsed DC current, standardized to 3,000 Watt power transfer to fish (Burkhardt and Gutreuter 1995; Fischer and Quist 2014), typically covering about 250 m of shoreline, samples were defined by

exactly 10-min of applied pedal time. Electrofishing samples and trap net locations within each site were separated by 300-500 m to ensure no interference between gears. Each site also had a string of temperature loggers (Onset HOBO 8K Pendant® Pendant UA-001-08; accuracy: ± 0.14°C) to collect temperature every 30 min at depths of 0.5 m, 1.5 m, 2.5 m and 3.5 m. Fish collected at each site were weighed (g) and measured (TL, mm) and immediately released on site after processing, except for a subset collected in September 2014, March 2015, and September 2015 that were euthanized and frozen for later GCA and SIA analyses. To explore interactions between sport species and tilapia we focused on Largemouth Bass, Bluegill, Black Crappie and both tilapia species in Hyco Lake for GCA, SIA and abundance analyses. Due to bimodal size distributions in catch and possible differential influences on sport species, each tilapia species was divided into two size classes (Blue Tilapia; small: ≤120 mm TL, large: >120 mm TL, Redbelly Tilapia; small: ≤150 mm TL, large: >150 mm TL). The remaining sport species were also split into size classes to account for size-based ontogenetic diet shifts (Bluegill small: ≤100 mm TL, large: >100 mm TL, Largemouth Bass (and Spotted Bass in Lake Julian) small: ≤130 mm TL, large: >130 mm TL). To comprehensively assess GCA and SIA in each lake we aimed to capture a minimum of seven individuals of each size class of our study species during each sampling occasion.

Lake Julian is much smaller and more abiotically homogenous, so we sampled three replicate sites representing the entire lake (Figure 1.2). Fish were collected using the methodology described above in September 2014, March 2015 and September 2015. We conducted supplemental night electrofishing transects to meet sample size goals due to the low turbidity of the lake and associated challenges of electrofishing during the day. In Lake Julian we conducted GCA and SIA on the same size classes of black bass (Largemouth Bass and

Spotted Bass), Bluegill and Blue Tilapia (Redbelly Tilapia are not present in Lake Julian), also attempting to collect at least seven individuals of each size class.

Sport Species Abundance

We constructed *a priori* Poisson regression models and used Akaike's Information Criterion (AIC; Burnham and Anderson 2002) to assess abundance of sport species based on the influence of tilapia and other potential drivers in Hyco Lake. Although not a primary goal of this investigation, we were able to use the same data and approach to assess drivers of tilapia abundance in Hyco Lake. Temperature in Hyco Lake was assessed in two ways: categorically (hot, intermediate, cold zones) and numerically (direct quantification of temperature at each sample site calculated using the average temperature recorded by HOBO loggers at surface, 1.5 m, 2.5 m and 3.5 m depths during each week of sampling). It was necessary to include both temperature variables as they convey different aspects of thermal history; temperature differences were relatively constant among zones throughout the year, but there was substantial within- and among-season variation within zones (Figure 1.3). The modeling approach that we used allowed us to include correlated variables as we assessed functional relationships between abiotic factors and abundance of each fish species (McDonald 2014). We did not conduct abundance analyses on Lake Julian because of the small size and homogenous nature of abiotic conditions in that system.

For model construction we used abundance estimates separated by our two collection methods (trap nets and electrofishing), as it would be inappropriate to combine them. Trap net data and electrofishing data were used separately for Redbelly Tilapia, Blue Tilapia and Bluegill; only trap net data were used for Black Crappie abundance estimation (electrofishing for Black Crappie was inefficient and produced very small sample sizes). Largemouth Bass were primarily

collected via electrofishing. Small and large individuals may have different roles in the food web, so we separated Largemouth Bass into small (≤130 mm TL) and large (>130 mm TL) size classes based on approximate sizes associated with ontogenetic diet shifts (Garcia-Berthou 2002). Trap net data were not included for Largemouth Bass due to insufficient sample size.

During model development we considered two potential ways in which tilapia might influence abundance of sport species: as possible competitors or as prey of possible predators. We identified three sport species or life stage abundance variables that might be affected by competitive interactions with tilapia for food resources: Bluegill trap net Catch-per-unit-effort (CPUE), Bluegill electrofishing CPUE and small Largemouth Bass electrofishing CPUE (Zale 1987, Crutchfield 1995, Traxler and Murphy 1995). Because tilapia may compete with each other as well as with sport species, four additional species variables were also considered in the competitor category: Redbelly Tilapia trap net CPUE, Redbelly Tilapia electrofishing CPUE, Blue Tilapia trap net CPUE and Blue Tilapia electrofishing CPUE. We identified two variables that may indicate if tilapia availability as prey is important to piscivorous predators: large Largemouth Bass electrofishing CPUE and Black Crappie trap net CPUE.

We developed sets of *a priori* candidate models predicting abundance indices of sport fishes that may be potential competitors with tilapia (Bluegill, small Largemouth Bass). Each set included models based on abundances of tilapia only, all possible competitors (native sport species and tilapia), possible predators, abiotic factors only (zone, temperature), and various combinations of these groups (Table 1.1). For potential predators on tilapia (large largemouth bass, black crappie) our approach was to build candidate models using abundances of tilapia only, the native species community (native sport species), abiotic factors only, and various combinations of these groups (Table 1.2).

We used Akaike's information criterion corrected for small sample size (AICc) to assess all *a priori* candidate models within each set simultaneously. A Poisson distribution was used for abundances in the models. The best model was determined by the lowest AICc value and the highest model weight (W_i; Burnham and Anderson 2002).

Stable Isotope and Diet Processing

Processing for SIA followed the procedure of Vander Zanden et al. 2006. We removed a tissue sample (approximately 2 g) from the dorso-lateral region of all collected fish. Each tissue sample was dried at 60°C for at least 72 h and then ground to a fine powder using a mortar and pestle. Subsamples of the dried, ground powder were placed into tin capsules (Costech). We sent the samples to the Stable Isotope Ecology Laboratory in the Center for Applied Isotope Studies at the University of Georgia, where $\delta^{13}C$ and $\delta^{15}N$ isotopes were measured with an isotope ratio mass spectrometer.

Fish collected for SIA were also used for GCA. We preserved stomach contents of each fish in 95% ethanol. Using a dissection microscope (10X magnification), we examined and identified all food items present to the lowest feasible taxonomic level (Winemiller 1990, Montaña and Winemiller 2013). These lowest taxonomic units of food items were then simplified into eight categories: terrestrial insects (Coleoptera, Hemiptera, Hymenoptera), benthic macroinvertebrates (Ephemeroptera, Odonata, Trichoptera, Diptera, Molluska, Hydrachanidia), small zooplankton (includes Cladocera, 0.55 ± 0.3 mm SD; Rotifera, 0.46 ± 0.3 mm SD; Ostracoda, 0.21 ± 0.05 mm SD), large zooplankton (Copepoda, 1.04 ± 0.4 mm SD), detritus, fish, eggs, and crayfish. The volumetric proportion of each category was estimated using water displacement in a graduated cylinder (Winemiller 1990).

Analysis of Stable Isotope and Diet Overlap

To assess isotopic niche overlap between tilapia and sport species for each lake independently we used Stable Isotope Bayesian Ellipses in R (SIBER). This approach allowed us to plot calculated Bayesian standard ellipse areas that represent the isotopic niche area of the species of interest (Jackson et al. 2011). We used the $\delta^{13}C$ and $\delta^{15}N$ data for each species to inform the standard ellipse area for small sample size (SEAc). The ellipse created is an estimate containing approximately 40% of the data based on bivariate normal distributions (Jackson et al. 2011). These estimated ellipses were then plotted and used to visually identify trophic niche position and patterns, as well as to calculate the overlap between species of interest in each lake. For Hyco Lake we compared Blue Tilapia, Redbelly Tilapia, Bluegill, Largemouth Bass and Black Crappie. For Lake Julian we compared Blue Tilapia, Bluegill, Largemouth Bass and Spotted Bass. As we were interested in general trophic niche patterns and there were no noteworthy differences across seasonal sampling occasions, we compared pooled data across the three sampling occasions (September 2014, March 2015, September 2015) in each lake.

To evaluate diet overlap between tilapia and sport species for each lake independently, we calculated Pianka's (1973) index of symmetrical niche overlap using the volumetric proportions of the food items consumed by each species of interest. Any individuals with empty stomachs were excluded from analysis. This method of analysis is an index ranging from 0 (no overlap) to 1 (absolute overlap) where 0.6 or higher represents meaningful overlap (Montaña and Winemiller 2013). Gut content analyses provide an immediate snapshot of food consumption while SIA delivers a more integrated assessment of niche overlap. Because of the finer resolution of GCA, we were able to compare species using the size classes delineated in our collection methods. Each size class of a sport species category was compared to the available tilapia species size classes in that lake by season.

RESULTS

Abundance

In Hyco Lake, the only system for which distribution and abundance analyses were conducted, we captured 810 fish across the five species of interest over nine bimonthly sampling occasions (Appendix A). Within each set of candidate models we developed for predicting the abundance of sport species with which tilapia may potentially compete, i.e., Bluegill (trap net and electrofishing CPUE) and small Largemouth Bass (electrofishing CPUE), abundance were best fit by the global model (Table 1.1). For both Bluegill abundance metrics only the global model had any model weight; no submodels with only tilapia or tilapia in combination with abiotic factors had any model weight. The global model also best fit the abundance of small Largemouth Bass, and had 75% of the weight. The only other model with substantial weight contained competitors, which included both tilapia and Bluegill (Table 1.1).

Tilapia abundance was also not a prominent component of models that best fit the abundance of their possible predators, Black Crappie and large Largemouth Bass. Black Crappie abundance was best fit by the global model, which had all of the model weight (Table 1.2). For large Largemouth Bass abundance, two models including only abiotic factors had 94% of the model weight. The best model contained only abiotic factors (temperature and zone), and the second best model contained only temperature. A model that contained tilapia and abiotic factors had substantially lower model weight (0.03) than the model with abiotic factors alone (0.49), indicating that the presence of tilapia has little or no value in terms of describing Largemouth Bass abundance (Table 1.2).

Stable Isotopes and Diet Overlap

We collected 406 individuals of the five species of interest in Hyco Lake for SIA; of those, 335 with non-empty stomachs were analyzed in GCA. Bayesian standard ellipse areas estimated using $\delta^{13}C$ and $\delta^{15}N$ concentrations exhibited some overlap in isotopic niche space among species (Figure 1.4). However, the only ellipse overlap between tilapia and sport species occurred between Redbelly Tilapia and Bluegill. Gut content analyses indicated that none of the sport species except small Bluegill ever exhibited any significant diet overlap with Redbelly Tilapia or Blue Tilapia in Hyco Lake (Table 1.3). Small Bluegill diets overlapped substantially with diets of both small and large Blue Tilapia and Redbelly Tilapia in spring of 2015, but not in fall of either year. The considerable overlap is caused by the high proportion in the detritus diet

category in the small Bluegill and both sizes of Blue Tilapia and Redbelly Tilapia (Figure 1.5). Redbelly Tilapia and Blue Tilapia of all sizes had high diet overlap with each other during all seasons (Table 1.3, Figure 1.5).

We collected 274 individuals of the three species of interest in Lake Julian for SIA; of those, 253 with non-empty stomachs were used for GCA. The Bayesian standard ellipse area estimated for Blue Tilapia overlapped with that of Bluegill, but not that of Largemouth Bass (Figure 1.6). In the 2015 sampling periods (spring and fall) we did not collect enough small Blue Tilapia for GCA so diet overlap was only evaluated between large Blue Tilapia and small black bass, large black bass, small Bluegill and large Bluegill. In both years and seasons there was almost no diet overlap between small or large Largemouth Bass with either size of Blue Tilapia (Table 1.4). In contrast, in both fall and spring 2015 small Bluegill exhibited strong diet overlap with large Blue Tilapia, as did large Bluegill in fall 2015. The considerable overlap is caused by the high proportion of detritus in the diets of Bluegill and large Blue Tilapia (Figure 1.5).

DISCUSSION

Our results indicate that tilapia are likely not affecting the abundance of sport species in Hyco Lake and that the potential for diet and niche overlap between tilapia and native sport species is limited. In Hyco Lake we found that the abundance of important sport species – both as potential predators of, and competitors with, tilapias – was driven by a complex combination biotic and abiotic interactions rather than by the presence of tilapia in the system. While previous research addressing similar relationships between native sport species and invasive tilapia has provided conflicting results (e.g., Traxler and Murphy 1995, Martin et al. 2010), our findings are consistent with those that indicate relatively minor tilapia influence (e.g., Gu et al. 1996, Peterson et al. 2006). This is an important result given the general assumption that invaders are typically strong interactors with native species (Canonico et al. 2005).

We found no evidence for strong influence of tilapia on piscivorous sport species in measures of abundance or food habits. Rather, our results indicate that abundance of large Largemouth Bass is primarily a function of abiotic parameters including temperature and lake zone, a correlate of temperature. This result is not particularly surprising given the large body of literature describing the general effect of temperature on fish abundance, including Largemouth Bass (e.g., Hasler et al. 2009, Peat et al. 2016), but it is important to note that the

presence of tilapia, a potential prey, had no meaningful influence on abundance of large Largemouth Bass, which are typically those most sought by anglers (Barhart 1989, Noble 2002). Although we are not aware of other published research linking the presence of tilapia with Largemouth Bass abundance, our results are consistent with previous investigations that have indicated limited effects of interactions among these species (e.g., Gu et al. 1996, Peterson et al. 2006). Likewise, our results indicate that tilapia have no substantial effect on the abundance of Black Crappie; no submodel containing only tilapia or tilapia plus abiotic factors had any model weight. The only model with weight was the global model, indicating that a complex mixture of biotic and abiotic factors is driving Black Crappie abundance. This is the first study that we are aware of to examine impacts on abundance between these species.

Our ranked abundance models for the piscivores were supported by diet and SIA analyses, which indicated no influence of tilapia on trophic niche use of the piscivorous sport species in our study. We anticipated that diet-associated effects of tilapia on piscivorous species would be primarily through consumption of tilapia, but this pattern was not apparent in our data. Interpretation of the SIA ellipses suggests that piscivorous Black Crappie and black basses in both study lakes are consuming few, if any, tilapia, a pattern confirmed by our diet analyses. Each trophic transfer of $\delta^{15}N$ increases by ~3 ‰ and of $\delta^{13}C$ by ~1 ‰, the lack of any tilapia species reflecting that area of the SIA graphic space compared to the piscivores does not suggest a predator-prey relationship. These results are consistent with previous research that has suggested tilapia are rarely consumed by these apex predators (Gu et al. 1996). Conversely, the patterns we observed are inconsistent with studies that have indicated that tilapia can, in fact, serve as an important forage species for black basses, depending on prey size and habitat characteristics (Schram and Zale 1985). In sum, the combination of abundance analyses and trophic niche data from two very different reservoir systems reveal only minor, if any, influence of tilapia on Largemouth Bass and Black Crappie.

We also found little influence of tilapia on abundance of the sport species that could potentially compete with them. The best model for predicting abundance of Bluegill and small Largemouth Bass was the global model, and in the case of Bluegill (collected both by electrofishing and trap nets), the global model had all of the model weight. In the case of small Largemouth Bass, there was substantial weight in a submodel that included all potential competitors (Blue Tilapia, Redbelly Tilapia and Bluegill). However, as none of the submodels

that included only tilapia or tilapia plus abiotic factors had any weight, it is reasonable to conclude that the model weight of this competitor submodel was driven primarily by the inclusion of Bluegill. As in the case with Black Crappie, this pattern suggests that the abundance of Bluegill and small Largemouth Bass in our study system is best described by interactions among multiple biotic and abiotic factors, and there is no evidence for strong influence of tilapia presence on abundance of either species. This lack of influence conflicts with some of the previous research suggesting negative influence of tilapia on growth and survival of young Largemouth Bass (Zale 1987) or on sunfish habitat use and survival in the presence of piscivores (Martin et al. 2010).

Although we found little evidence of tilapia affecting abundance of potential competitor sport species, we did find substantial diet and niche overlap between tilapia and Bluegill in both study systems; there was considerable diet overlap between Bluegill (especially small bluegill) and both species of tilapia in Hyco Lake and between Bluegill and Blue Tilapia in Lake Julian. In addition, these overlap patterns were apparent in all of our stable isotope Bayesian ellipse analyses with the exception of Blue Tilapia and Bluegill in Hyco Lake. Although assessing consequences of this interaction is beyond the scope of this investigation, our results do indicate the potential for competitive interactions between Bluegill and invasive tilapia that might lead to decreases in Bluegill growth or population size structure, as observed in other cases (Garvey and Stein 1997, Wolfe et al. 2009, Kaemingk et al. 2012).

Despite evidence suggesting that Bluegill may opportunistically forage on tilapia (Bickerstaff et al. 1984), we found no evidence of that in our diet and SIA data. In addition, we expected based on previous work (Zale, 1987, Traxler and Murphy 1995) that tilapia would overlap with small Largemouth Bass in terms of diet and trophic niche, but once again we found no evidence for that in our study.

While our study provides useful information about the influence of tilapia relative to other common drivers of sport fish abundance, it is important to note that abundance of all of our target species is clearly a function of many factors, some of which we likely did not capture in our analyses. The contribution of our study, however, is in the assessment of relative rather than absolute importance of an increasingly common invasive species in warm-water reservoir systems. Further, the general lack of strong tilapia effects on sport species abundance was supported by our trophic niche analyses, which indicated no significant diet or SIA overlap

except in the case of Bluegill. It is worth pointing out that tilapia could be influencing sport species in ways that we did not assess in this investigation. For example, there might be competition at earlier life stages or for access to spawning sites (Zale 1987, Canonico et al. 2005). Despite little evidence in our study of strong interactions between tilapia and sport species, the wide variety of habitats suitable for tilapia invasion – due to the temperate climate in the southeastern region of the US, plethora of heated reservoirs and popularity of tilapia as a biological control and forage fish for sport species (Mallin 1985, Canonico et al. 2005) – suggests that continued monitoring and assessment will be necessary to prevent further invasion risk and threat to native biodiversity.

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TABLES

Table 1.1. Sets of *a priori* candidate models estimating abundance of sport species (Bluegill trap net CPUE, Bluegill electrofishing CPUE, small Largemouth Bass electrofishing CPUE) potentially affected by competition with tilapias, and their corresponding number of covariates (K), AICc value, ΔAICc, and model weight. Gear type for each covariate CPUE is indicated by (T) for trap net or (E) for electrofishing, species are defined as follows: Blue Tilapia (BT), Redbelly Tilapia (RT), Bluegill (BLG), Largemouth Bass (LMB), and Black Crappie (BC). Covariates used in each candidate model are indicated by X, abiotic factors (AF) are Zone, which represents hot, intermediate or cold, and Temp., which is the average temperature (°C) during the week of sampling. The top ranking model is indicated by bold font.

	Covariates Included in Candidate Model											**AICc Output**			
***a priori* candidate models**	Zone	Temp.	BT (T)	BT (E)	RT (T)	RT (E)	BLG (T)	BLG (E)	Sm. LMB (E)	Lg. LMB (E)	BC (T)	K	AICc	ΔAICc	ωi
Bluegill Trap Abundance															
Global	**X**	**X**	**X**	**X**	**X**	**X**			**X**	**X**	**X**	**11**	**1041.5**	**0**	**1**
Competitors + AF	X	X	X	X	X	X			X			9	1077.4	35.89	0
Tilapias + AF	X	X	X	X	X	X						6	1119.23	77.73	0
Competitors			X	X	X	X			X			6	1155.97	114.47	0
Tilapias			X	X	X	X						3	1231.27	189.76	0
Predators + AF	X	X								X	X	6	1373.54	333.03	0
AF	X	X										4	1427.18	385.67	0
Temperature		X										3	1483.06	441.56	0
Predators										X	X	3	1542.21	500.71	0
Zone	X											2	1594.93	553.43	0

Table 1.1 Continued

Bluegill Electrofishing Abundance														
Global	**X**	**X**	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**11**	**2009.6**	**0**	**1**
Competitors + AF	X	X	X	X	X	X		X			9	2038.64	29.04	0
Tilapias + AF	X	X	X	X	X	X					8	2156.46	146.86	0
Competitors			X	X	X	X		X			6	2284.55	274.95	0
Tilapias			X	X	X	X					*5*	2379.65	370.05	0
Predators + AF	X	X							X	X	*6*	2612.28	602.68	0
AF	X	X									*4*	2689.78	680.18	0
Temperature		X									*3*	2701.28	691.68	0
Predators									X	X	*3*	2860.86	851.26	0
Zone	X										*2*	2936.54	926.94	0
Small Largemouth Bass Electrofishing Abundance														
Global	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**12**	**250.31**	**0**	**0.75**
Competitors			X	X	X	X	X	X			7	253.01	2.7	0.19
Predators + AF	X	X							X	X	6	256.06	5.76	0.04
AF	X	X									4	259.55	9.24	0.01
Tilapias + AF	X	X	X	X	X	X					*8*	260.99	10.68	0
Competitors + AF	X	X	X	X	X	X	X	X			*8*	260.99	10.68	0
Zone	X										*3*	261.07	10.76	0
Tilapias			X	X	X	X					*5*	263.74	13.43	0
Temperature		X									*2*	263.79	13.48	0
Predators									X	X	*2*	457.73	207.43	0

Table 1.2. Sets of *a priori* candidate models estimating abundance of sport species that are potential predators on tilapia (large Largemouth Bass electrofishing CPUE, Black Crappie trap netting CPUE), and their corresponding number of covariates (K), AICc value, ΔAICc, and model weight. Gear type for each covariate CPUE is indicated by (T) for trap net or (E) for electrofishing, species are defined as follows: Blue Tilapia (BT), Redbelly Tilapia (RT), Bluegill (BLG), Largemouth Bass (LMB), and Black Crappie (BC). Covariates used in each candidate model are indicated by X, abiotic factors (AF) are Zone, which represents hot, intermediate or cold, and Temp., which is the average temperature (°C) during the week of sampling. The top ranking model is indicated by bold font.

	Covariates Included in Candidate Model											**AICc Output**			
a priori **candidate models**	Zone	Temp.	BT (T)	BT (E)	RT (T)	RT (E)	BLG (T)	BLG (E)	Sm. LMB (E)	Lg. LMB (E)	BC (T)	K	AICc	ΔAICc	ωi
Large Largemouth Bass Electrofishing Abundance															
AF	X	X										4	262.68	0	0.49
Temperature		X										2	262.84	0.17	0.45
Tilapias + AF	X	X	X	X	X	X						8	268.17	5.5	0.03
Native Community + AF	X	X					X	X	X		X	8	268.6	5.92	0.03
Global	X	X	X	X	X	X	X	X	X		X	12	274.74	12.06	0
Native Community							X	X	X		X	5	303.45	40.77	0
Zone	X											3	307.86	45.18	0
Tilapias			X	X	X	X						5	309.46	46.78	0
Black Crappie Trap Abundance															
Global	X	X	X	X	X	X	X	X	X	X		12	759.17	0	1
Native Community + AF	X	X					X	X	X	X		8	796.06	36.9	0
Tilapias + AF	X	X	X	X	X	X						8	867.2	108.04	0
AF	X	X										4	997.32	238.15	0
Zone	X											3	1021.95	262.79	0
Native Community							X	X	X	X		5	1069.1	309.94	0
Tilapias			X	X	X	X						5	1078.82	319.66	0
Temperature		X										2	1137.93	378.76	0

Table 1.3. Estimates diet overlap quantified with Pianka's Index of Symmetrical Niche Overlap using the invasive tilapias in Hyco Lake (Blue Tilapia and Redbelly Tilapia) and the native sport species (Bluegill, Largemouth Bass and Black Crappie) separated by size class (Blue Tilapia; small: ≤120 mm TL, large: >121 mm TL, Redbelly Tilapia; small: ≤150 mm TL, large: >151 mm TL, Bluegill small: ≤ 100 mm TL, large: >100 mm TL, Largemouth Bass small: ≤ 130 mm TL, large: >130 mm TL). Relationships that show high overlap (>0.60) are indicated with bold values.

	Fall 2014			Fall 2015				Spring 2015			
	Redbelly Tilapia		Blue Tilapia	Redbelly Tilapia		Blue Tilapia		Redbelly Tilapia		Blue Tilapia	
Compared Species	Small	Large	Large	Small	Large	Small	Large	Small	Large	Small	Large
Small LMB	0.121	0.084	0.056	0.370	0.501	0.468	0.471	0.015	0.001	0.002	0.002
Large LMB	0.189	0.122	0.090	0.011	0.068	0.030	0.034	0.030	0.022	0.026	0.026
Black Crappie	0.258	0.191	0.160	0.444	0.045	0.046	0.044	0.199	0.187	0.188	0.187
Small Bluegill	0.568	0.546	0.523	0.306	0.352	0.358	0.350	**0.703**	**0.698**	**0.694**	**0.699**
Large Bluegill	0.304	0.122	0.220	0.372	0.497	0.466	0.468	0.442	0.402	0.397	0.399
Small Redbelly Tilapia			**0.995**			**0.816**	**0.819**			**0.979**	**0.986**
Large Redbelly Tilapia			**0.999**			**0.999**	**0.999**			**0.994**	**0.995**
Small Blue Tilapia				**0.816**	**0.999**			**0.999**	**0.999**		
Large Blue Tilapia	**0.995**	**0.999**		**0.819**	**0.999**			**0.999**	**0.999**		

Table 1.4. Estimates of diet overlap quantified with Pianka's Index of Symmetrical Niche Overlap between invasive Blue Tilapia in Lake Julian and the native sport species (Bluegill, black bass (Largemouth Bass and Spotted Bass)) separated by size class (Blue Tilapia; small: ≤ 120 mm TL, large: >120 mm TL, Bluegill small: ≤ 100 mm TL, large: >100 mm TL, black bass small: ≤ 130 mm TL, large: >130 mm TL). Relationships that show high overlap (>0.60) are indicated with bold values.

	Fall 2014 Blue Tilapia		Fall 2015 Blue Tilapia	Spring 2015 Blue Tilapia
Compared Species	Small	Large	Large	Large
Small black bass	0.000	0.000	0.021	0.051
Large black bass	0.024	0.023	0.101	0.019
Small Bluegill	0.000	0.001	**0.873**	**0.983**
Large Bluegill	0.251	0.554	**0.969**	0.554

FIGURES

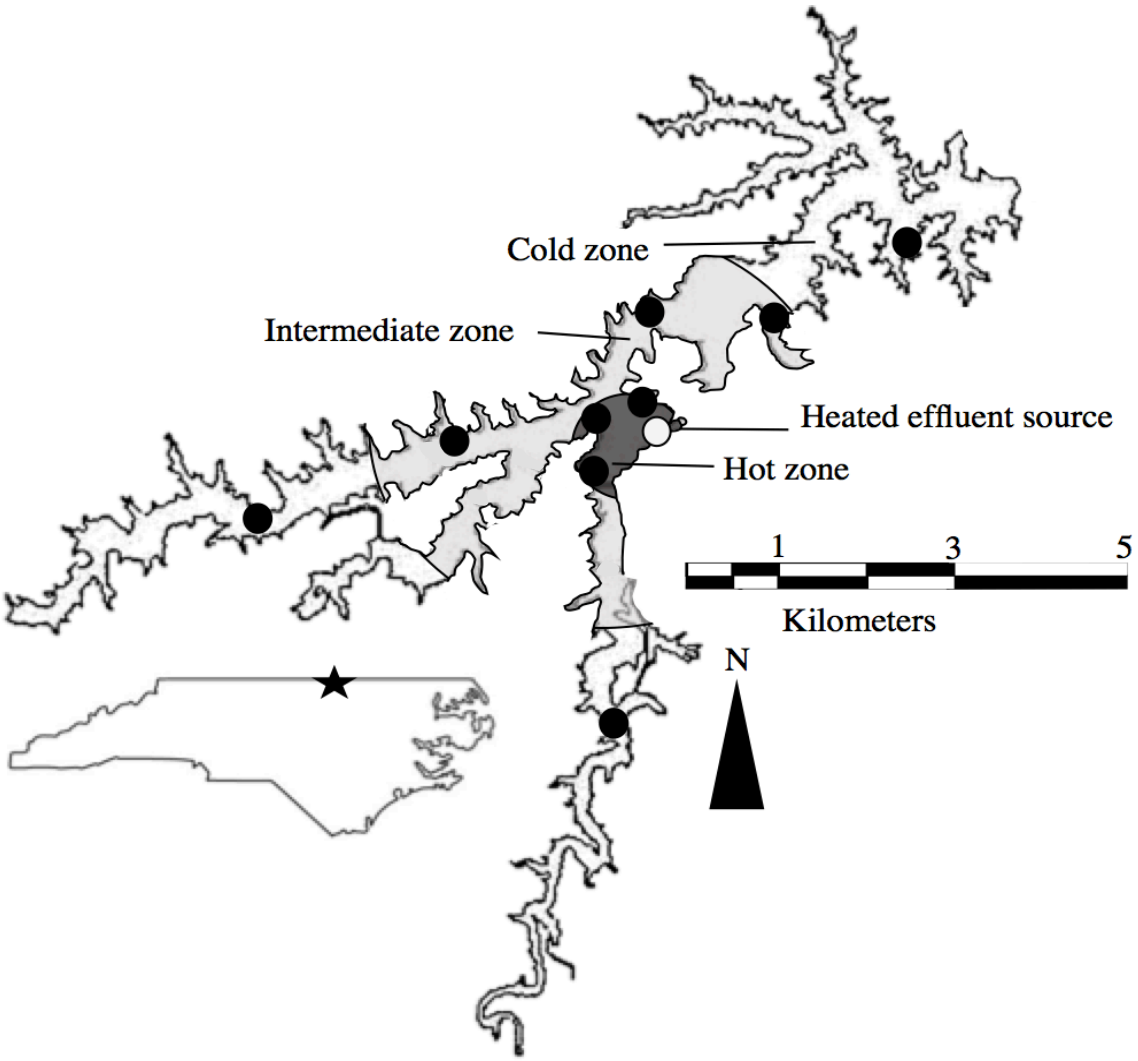

Figure 1.1. Map of Hyco Lake and its location in North Carolina. Heated effluent influence on lake temperature is delineated into 'hot,' 'intermediate,' and 'cold' zones (temperature trends shown in Figure 3). Hot zone is defined as the central thermal pool within 0.8 km of the heated effluent source, the intermediate zone extends 3 km beyond the edge of the hot zone, and the remainder of the lake is the cold zone and considered ambient. Zones were determined based on a pilot study. Three replicate sampling sites within each zone of the lake are indicated by black circles.

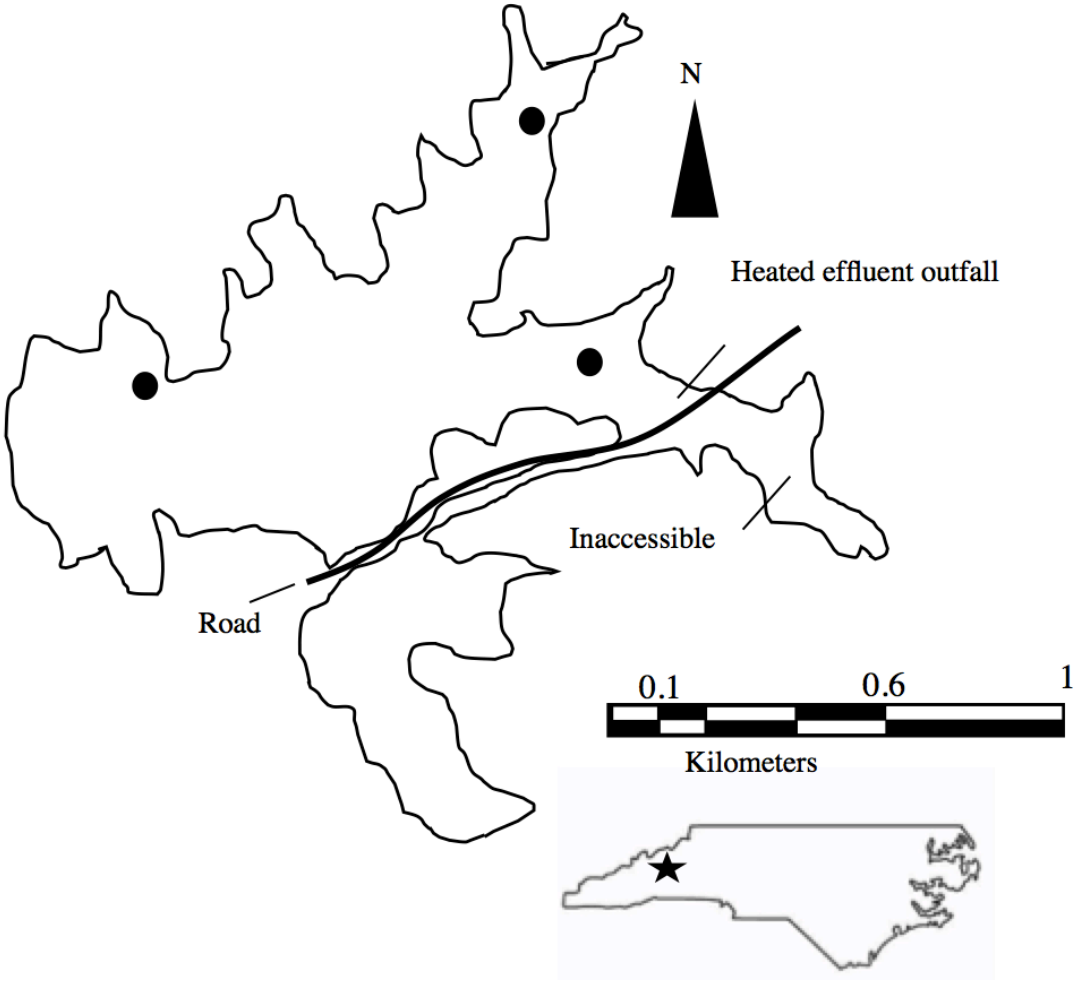

Figure 1.2. Depiction of Lake Julian and its location in North Carolina; black circles indicate three replicate sample sites.

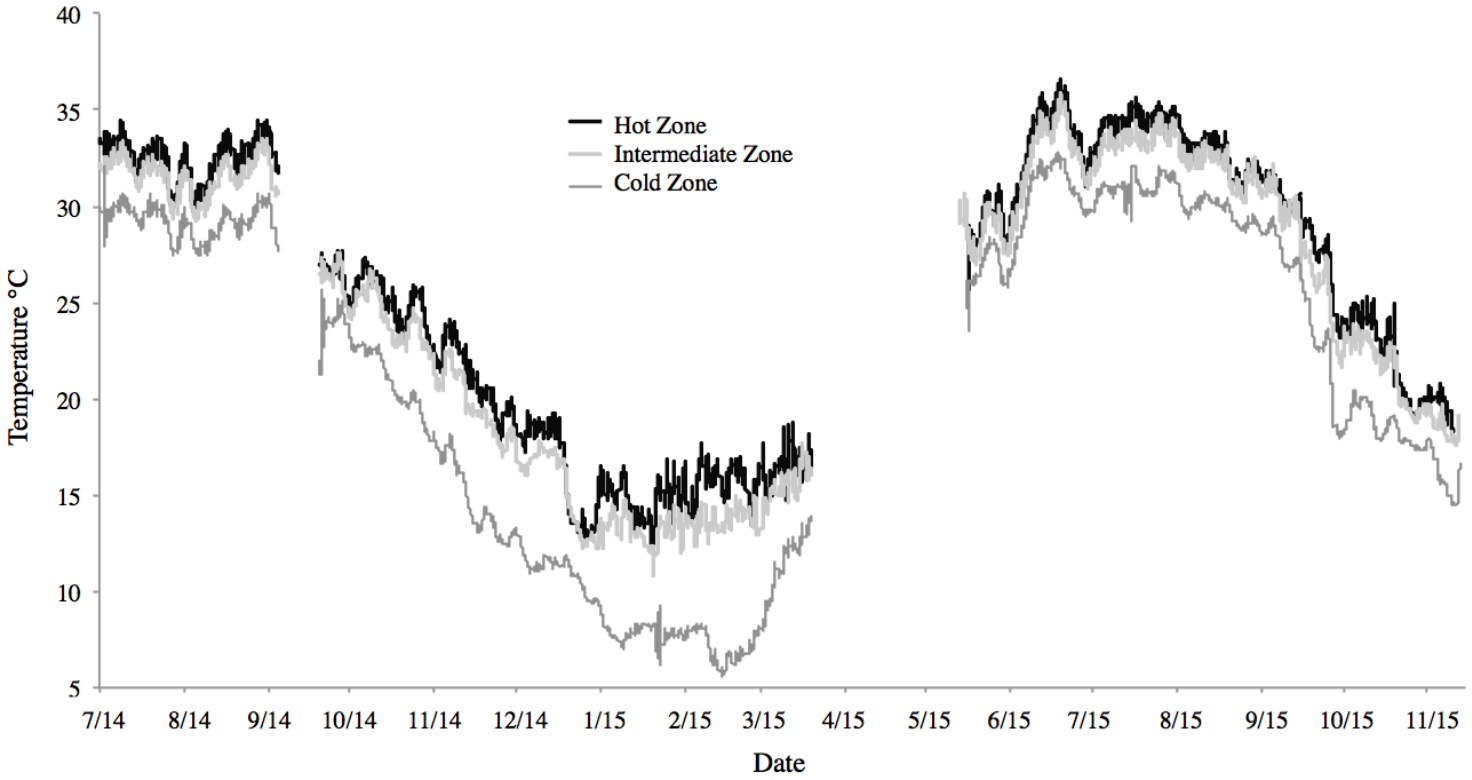

Figure 1.3. Temperature (°C) trends in Hyco Lake during the 2014 - 2015 sampling period. Temperatures were averaged from temperature logger data collected at the surface, 1.5 m, 2.5 m and 3.5 m depths at each of the three replicate sites in each zone (hot, intermediate, cold). Gaps in the data were due to equipment malfunctions.

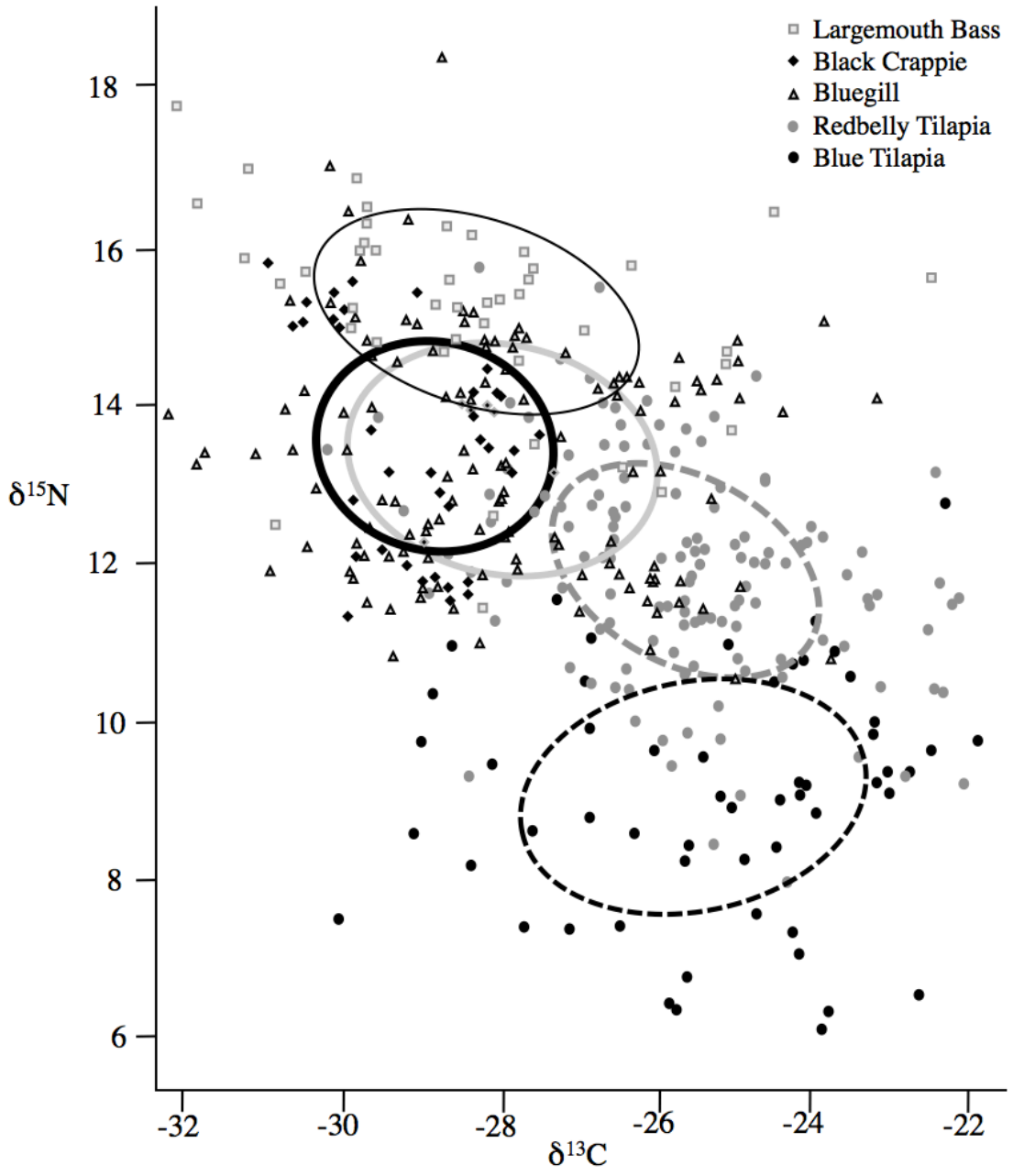

Figure 1.4. Bayesian standard ellipse areas calculated using δ13C and δ15N for invasive Blue Tilapia and Redbelly Tilapia and native sport species including Largemouth Bass, Bluegill and Black Crappie, from three collections in fall 2014, spring 2014 and spring 2015 in Hyco Lake. Black circles with a dashed black ellipse represent Blue Tilapia, grey circles with a dashed grey ellipse represent Redbelly Tilapia, grey triangles with a solid grey ellipse represent Bluegill, black diamonds with a solid thick black ellipse represent Black Crappie, and Largemouth Bass are represented by open grey squares and the thin solid black ellipse.

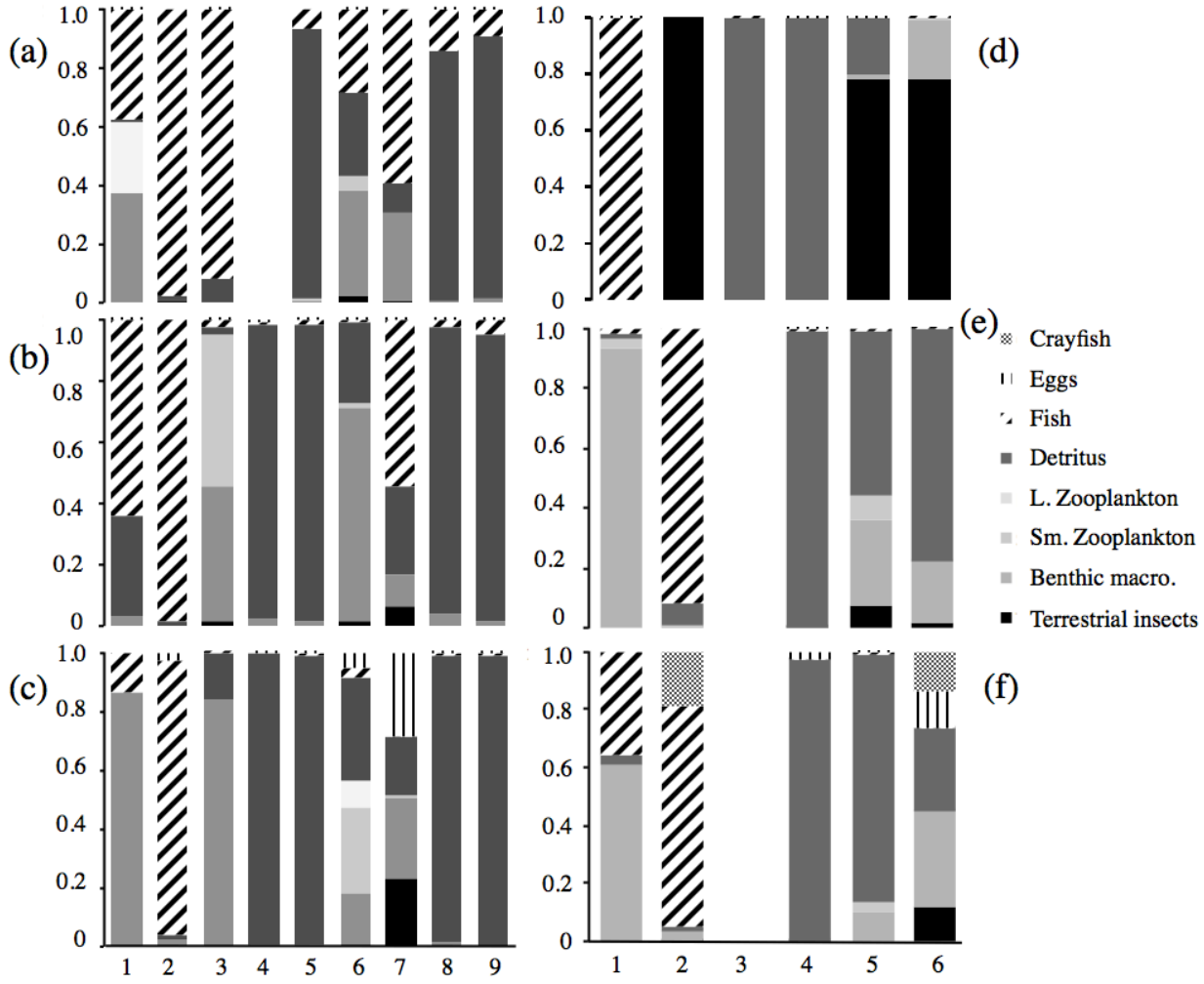

Figure 1.5. Proportion by volume of the eight prey categories used in the stomach content analyses. The left column (a-c) represents Hyco Lake samples from (a) September 2014, (b) September 2015 and (c) March 2015 while the right column (d-f) represents Lake Julian samples from (d) Fall 2014, (e) Fall 2015 and (f) Spring 2015. The species in (a-c) are represented using numbers: (1) small Largemouth Bass, (2) large Largemouth Bass, (3) Black Crappie, (4) small Blue Tilapia, (5) large Blue Tilapia, (6) small Bluegill, (7) large Bluegill, (8) small Redbelly Tilapia and (9) large Redbelly Tilapia. The species in (d-f) are represented using numbers: (1) small black bass, (2) large black bass, (3) small Blue Tilapia, (4) large Blue Tilapia, (5) small Bluegill, and (6) large Bluegill. Missing data indicate a lack of availability of that size class during sampling.

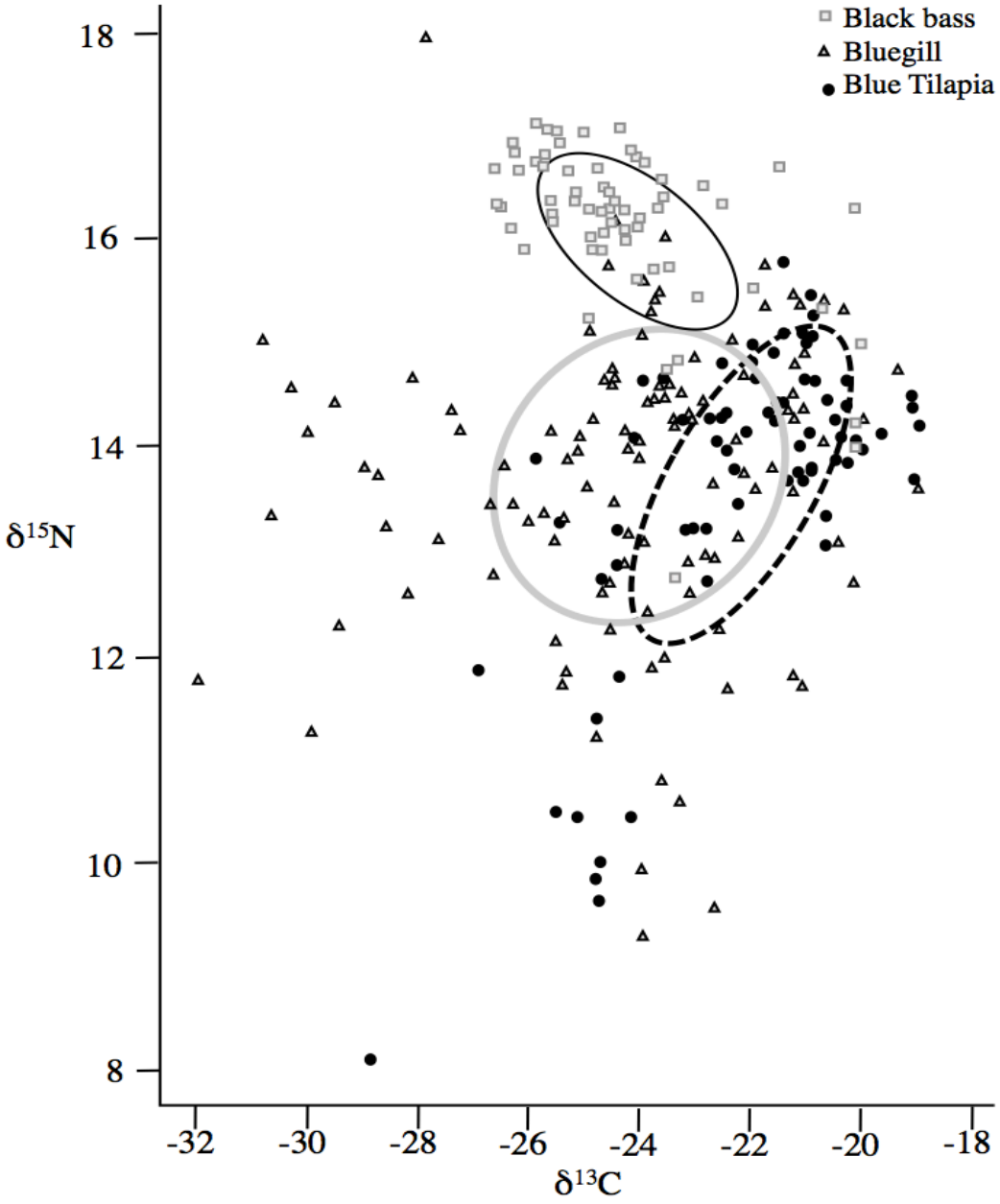

Figure 1.6. Bayesian standard ellipse areas calculated using δ13C and δ15N for invasive Blue Tilapia and native sport species, Bluegill and black basses (Largemouth Bass and Spotted Bass), summarized from three collections in September 2014, March 2014 and September 2015 in Lake Julian. Black circles with a dashed black ellipse represent Blue Tilapia, triangles and solid grey ellipse represent Bluegill, and black bass (Largemouth Bass and Spotted Bass) are represented by open grey squares and the thin solid black ellipse

EFFECT OF DECLINING WATER TEMPERATURE ON SURVIVAL OF NILE TILAPIA *OREOCHROMIS NILOTICUS* AND BLUE TILAPIA *OREOCHROMIS AUREUS*

INTRODUCTION

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 delineate the eventual distribution upon invasion (Zale 1984, Moyle and Light 1996, Vander Zanden et al. 2004). With predicted stream and river temperatures increasing 0.009 to 0.077°C per year in the United States due to climate change (Kaushal et al. 2010), it is essential to ascertain the thermal tolerance for threatening invaders to stay ahead of a growing risk.

Blue Tilapia *Oreochromis aureus* and Nile Tilapia *Oreochromis niloticus* are the principle commercial tilapia species that have a global impact today. Indigenous to Africa and the Middle East, these invasive species now exist under feral conditions in every nation in which they have been introduced (Cononico et al. 2005). Their high success upon introduction has been attributed to their life history traits including tolerance of variable salinity levels, hypoxia, overcrowding, temperature extremes and trophic flexibility (Trewavas 1983, McKaye et al. 1995; Coward and Little 2001). Their exceptional environmental tolerance (Cononico et al. 2005) and prolific nature (Stickney 1986) make both species highly popular farmed fish around the globe. Tilapia are bred and raised for use as supplemental forage fish for sport fishes, a biological control 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 U.S. (depending on local regulations) and receive fry by mail within a couple days. Private pond stocking of tilapia may

pose a serious threat to native water bodies, as there is risk of successful establishment upon accidental escapement or spread.

Blue Tilapia have invaded and become established in 10 states and Puerto Rico, and are possibly established in seven additional states (Nico et al. 2013). Nile Tilapia are established in one state and possibly established in five additional states (Nico et al. 2013, R. Stroud, SCDNR 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, NC) for vegetation control, and they were accidentally introduced to Hyco Lake (Roxboro, NC) during a study in 1984 on vegetation control (Crutchfield et al 1992). These reservoirs receive heated effluent from steam electrical power plants; tilapia are thought to be dependent upon the warm-water discharge to survive winter temperatures in the region (C. Oakley, NCWRC personal communication). Nile Tilapia of unknown origin were recently discovered (2015) in Lake Wylie (Belmont, NC), 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 tilapia and other fishes has used rapid drops in temperature ranging from a fraction of 1°C/minute (e.g., Currie et al. 1998, Prodocimo and Fiere 2001), 1°C/h (e.g., Cameron et al. 2012) up to 0.5°C/d (Wilson et al. 2009) or 1°C/d (e.g., Charo-Karisa et al. 2005, Bennett et al. 1997). The slowest temperature declination rate applied to Blue Tilapia was 1°C/3d (Zale 1984) and to Nile Tilapia was 0.5°C/d (Atwood et al. 2003, Wilson et al. 2009) and 1°C/2d (Paz 2003). While these methods allow for rapid testing and easy comparison of species' thermal minima, their 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 rate of decline (Paz 2003, Wilson et al. 2009). Zale (1984) and Paz (2003) determined that the estimated thermal minimum for tilapia was lower when temperatures dropped at a rapid rate (0.5°C/5 h and 1°C/4 h) than when they were dropped at a slower rate (1°C/d down to 1°C/3 d).

The range of thermal minima suggested by previous research on Blue Tilapia and Nile Tilapia using a variety of differing methodologies necessitates further investigation to determine the true risk of success and overwintering upon expansion. The thermal minima for Blue Tilapia determined using declination rates from 1°C/4 hr to 1°C/3 d range from 5.0-11.6°C (Zale 1984, Paz 2003) and for Nile Tilapia determined using declination rates from 0.5°C/5 hr to 1°C/d range from 6.8-12.6°C (Paz 2003). 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 it exceeds the rate that 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.

Selective breeding adds additional uncertainty regarding thermal tolerance of Blue Tilapia and Nile Tilapia. Selective breeding within the aquaculture industry for more tolerant tilapias may be changing the thermal minima of these species (Dey et al 2000, Sifa et al. 2002). 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). Two color-variant strains of Nile Tilapia of unknown origin have been developed and are widely

available on the market today: White Nile Tilapia and Red Nile Tilapia. These strains are considered the same species as the original Nile Tilapia (Common Nile Tilapia), but were selected for their colors for the food industry to meet the aesthetic preferences of different consumer demographics (Harry Daniels, NCSU, personal communication). Selective breeding for the desirable morphological traits may have altered thermal tolerance. Hybridization is also a tool commonly used in tilapia aquaculture to manipulate growth rate and sex of offspring (Pinto 1982, Guarduno-Lugo 2003). These hybrids are fertile (Pinto 1982) so the potential exists for fish that appear to be one species to have some genetic and phenotypic traits of another species. 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.

The methodological approaches used in prior studies and potential changes in the 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 tolerance of Blue Tilapia, Nile Tilapia (Common Nile Tilapia) and White Nile Tilapia under a relatively moderate temperature declination rate and a slow temperature declination rate approximating natural cooling rates in temperate systems. Because these species groups are bred all over the country with different lineages and selective breeding, we further tested for differences within each species or strain among fish from multiple suppliers. 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 (1.5ml/L for 1.5 h) and Praziquantel (Biltricide, 3.3mg/L (dissolved in 70% ethanol at 12.5mg/ml)) to ensure that any parasites, fungi, bacteria or disease pathogens were removed. All 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, 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

Our experiment consisted of two treatments and a control, each housed in a separate recirculating system. Temperature in the moderate declination treatment system was dropped 1°C/d until 10% of the experimental fish lost equilibrium, then the rate of decline slowed to 1°C/3 d. Temperature in the slow declination treatment system was dropped 1°C/week. The control system, which was used for both experiments, was maintained at 20°C for the duration of the experiments. The moderate declination treatment system and the slow declination treatment system each contained 180 1-L tanks, while the control system contained 60 1-L tanks. Temperature in each system was controlled by separate heat pumps (AquaLOGIC Delta Star

DSHP7 ¾ HP). HOBO Pendant Temperature Data Loggers (Onset HOBO 8K Pendant® UA-001-08; accuracy: ± 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 ppt. Temperature, salinity and dissolved oxygen of each system were monitored each morning; pH, alkalinity, hardness, ammonia and nitrites were tested bi-weekly. Algal growth was removed from the front of each tank in the slow declination treatment system and the control system once during the 14-week experiment, to maintain visibility for assessments of fish health. 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.

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 mm (TL) before it was placed in its assigned tank. Each of the two treatment systems (moderate and slow) received 23 individuals from each of the seven experimental groups (N = 161 per treatment). Eight individuals from each of the seven experimental groups were added to the control system (N = 56). Beginning 48 h after the fish were added to their assigned tanks the temperature in each system was brought down 1°C each day until a starting temperature of 20°C was reached. This secondary acclimation temperature was used to limit the duration of stress to the experimental fish, so deaths represented the reaction to temperature rather than extended experimental stress. This new acclimation temperature was maintained for one week before the experimental treatment temperature changes began.

Each fish was fed (Ziegler Brothers crumble feed, 55% protein, 15% fat) a maintenance ration of 1% (± 0.02%) body weight once per day in the morning for the entire experiment. Feeding began at a randomly assigned tank and proceeded sequentially through all three systems. Tilapia are known to lose equilibrium and cease feeding as water temperature drops below optimal range (Zale 1984). Therefore, immediately after feeding each fish we recorded its reaction to food (options: ate within 5 seconds, ate within 30 seconds, did not eat within 30 seconds) and activity/equilibrium status (active/swimming, lethargic/not moving, struggling/minor disequilibrium, full loss of equilibrium). Activity/equilibrium status was noted a second time each day at 1500 hours. If dead fish were encountered they were removed from the tank, weighed to the nearest 0.1 g and measured (TL) to the nearest mm.

Statistical Analyses

We used two methods of statistical analysis to investigate the difference in thermal tolerance among species groups (Blue Tilapia, Common Nile Tilapia and White Nile Tilapia, suppliers combined), within each temperature treatment. First, we performed a Kruskal-Wallis Rank Sum Test on temperature at time of death by species group. Second, we calculated Kaplan Meier Survival Curves using the daily survival of each species group and 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 time of death among suppliers within species groups, done separately for each temperature treatment. First, we ran Wilcoxon Rank Sum Tests for two independent samples to compare suppliers for the Common Nile Tilapia and White Nile Tilapia (two suppliers each). As there were three suppliers of Blue Tilapia we compared them using a Kruskal-Wallis Rank Sum Test, 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 suppliers within species group.

Covariates of length and growth for individual fish were included to test for any effects on individual survival. Starting length (mm TL) and specific growth rate (percent change in body weight per day) during the experiment (from start of experiment to 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 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 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 reliable indicators of imminent death. In all three systems water quality parameters remained within appropriate range throughout the experiment (Table 2.1). In both treatments neither starting length nor specific growth rate during the experiment had a significant effect on survival probability (X^2 = 0.1865, df = 1, p-value = 0.67; X^2 = 1.71, df = 1, p-value = 0.19; X^2 = 0.0692, df = 1, p-value = 0.79; X^2 = 0.1661, df = 1, p-value = 0.68). Therefore, all survival rates and temperatures at death were assumed to attributable to species and supplier.

Temperatures in both the moderate and slow declination treatments closely followed the intended treatment temperatures (Figure 2.1) with minimal variation. The average standard deviations of daily temperature were 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 (Table 2.2, 2.3). When a

change in temperature was applied to the system, the chillers generally reached the new temperature within 2 hours. Temperature would temporarily increase during daily filter backwashing (typically 0.5 - 2°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/d to 1°C/3 d was triggered at 13°C when 10% of one of the seven groups (Common Nile Tilapia, 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 (X^2 = 3.1, df = 2, p-value = 0.2) (Figure 2.2a). On average, temperature at death ranged from 9.3 (White Nile Tilapia) to 9.6 (Common Nile Tilapia) and the lowest temperature at death was 8.8 for all groups (Table 2.4). The survival curves for the three groups were statistically equivalent (X^2 = 4.3, df = 2, p-value = 0.115) (Figure 2.3a).

Temperature at death of the White Nile Tilapia from the two suppliers differed significantly (W = 268, p-value = 0.008), though 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, while fish from supplier 4 died at an average of 9.1°C (Table 2.4, Figure 2.4a). There were no significant differences in mean temperature at death between the Common Nile Tilapia from the two suppliers (W = 238, p-value = 0.9) or among the three suppliers of Blue Tilapia (X^2 = 5.6, df = 2, p-value = 0.06), and their range of temperatures at death were identical (8.8-10.5°C; Table 2.4, Figure 2.4b-c).

As for temperature at death, survival curves differed significantly with supplier for White Nile Tilapia (X^2=12.5, df = 1, p-value < 0.001), but not for Common Nile Tilapia or Blue Tilapia (X^2 = 0.2, df = 1, p-value = 0.62 and X^2 = 5.6, df = 2, p-value = 0.06 respectively) (Figure 2.5a-c).

Slow Declination Treatment

There was a significant difference in temperature at death among the three species groups (X^2 = 23.9, df = 2, p-value < 0.001). Temperature at death of White Nile Tilapia and Blue Tilapia differed from Common Nile Tilapia (p-value < 0.002), but not from each other (Figure 2.2b). White Nile Tilapia and Blue Tilapia died at average temperatures of 9.3°C and 9.5°C, while Common Nile Tilapia died at a warmer temperature of 10.6°C (Table 2.4). Survival curves of the three species groups were statistically different (X^2 = 14.6, df = 2, p-value < 0.001) (Figure 2.3b). The White Nile Tilapia survival curve was statistically different from both the Common Nile Tilapia survival curve (X^2 = 12.29, df = 1, p-value = 0.0005) and from the Blue Tilapia survival curve (X^2 = 5.76, df = 1, p-value = 0.016), but the Common Nile Tilapia and Blue Tilapia survival curves were not statistically different from each other (X^2 = 2.991, df = 1, p-value = 0.083) (Figure 2.3b).

We found a significant difference in temperature at death of White Nile Tilapia from the two suppliers (W = 134, p-value = 0.04). Fish from suppliers 1 and 2 died at an average temperature of 9.4°C and 9.0°C (Table 2.4). The supplier had no effect on the temperature at death of the Common Nile Tilapia (W = 280, p-value = 0.4). Common Nile Tilapia from both suppliers died at an average temperature of 10.8°C (Table 2.4). The temperature at death of the Blue Tilapia from the three suppliers was not statistically different (X^2 = 5.1, df = 2, p-value =

0.07) (Figure 2.4d-f). Average temperature at death ranged between 9.0°C and 9.6°C (Table 2.4).

The survival curves of fish from the two suppliers of White Nile Tilapia did not differ (X^2 = 3.8, df = 1, p-value = 0.05), but the survival curves of fish from the suppliers of Common Nile Tilapia and Blue Tilapia did (X^2 = 5.5, df = 1, p-value = 0.02 and X^2 = 9, df = 2, p-value = 0.01 respectively) (Figure 2.5d-f).

DISCUSSION

While there was some variation in mean temperature at death by declination rate, supplier and species, the lowest temperature any of the treatment fish reached before death was 8°C. Our assessment confirms the importance of a relatively slow declination rate when attempting to assess the invasion risk of an invasive species. The use of the rapid rates in previous treatments may be resulting in estimates that are colder than the true thermal minima that fish in feral conditions would exhibit, as the experimental fish may not have had time to acclimate adequately to successive temperature drops and treatment rate may exceed the speed at which responses to temperature become evident. 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 (Zale 1984, Paz 2003) (Figure 2.6). The declination rates used in this experiment, moderate (1°C/d slowed to 1°C/ 3 d) and slow (1°C/week), allow 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, Nile Tilapia and the White Nile Tilapia strain would exhibit upon introduction to new water bodies. Blue Tilapia and Nile Tilapia held at constant temperatures for 60 d exhibit even warmer thermal minima (Zale 1984, Wilson et al. 2009) (Figure 2.6), 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 on tilapias that indicated temperature at death is approximately 9-10°C when declination rates are ≤ 1°C/d (Atwood et al. 2003, Paz 2003, Charo-Karisa et al. 2005), but contrary to studies that have indicated death at colder temperatures (6-8.4°C) at the same rate (Shafland and Pestrak 1982, Zale 1984, Sifa et al. 2002, Paz 2003) (Figure 2.6). The effect of winter temperatures below the lethal temperatures uncovered in this study may explain why the invasive tilapia in North Carolina are limited to reservoirs that receive heated effluent.

Our study yielded mixed results regarding shifts in thermal minima due to selective breeding. White Nile Tilapia exhibited thermal minima virtually identical to those of Blue Tilapia, that were 1°C (median) and 1.5°C (mean) cooler than those of Common Nile Tilapia. The lower thermal minima of White Nile Tilapia support 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 selectively bred for aesthetics or to manipulate sex ratios (Harry Daniels, NCSU personal communication). This experiment highlights the possibility that the strain of White Nile Tilapia we randomly selected from public suppliers may be exhibiting indirect selection for thermal tolerance as a result of being bred for the white coloration. Although we also detected some differences in thermal minima among fish from different suppliers within strains, the differences in mean were only 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 mean, median and lowest temperature at death among the species and strains, averaged across suppliers, are more

substantial: 1.0-1.5°C. These differences suggest that Blue Tilapia and White Nile Tilapia are more tolerant to cold temperatures than Common Nile Tilapia and therefore potentially may pose more of threat of success upon introduction.

In this study we focused on Blue Tilapia and Nile Tilapia, the two tilapia species most commonly used in aquaculture and pond management in North America and also the two species thought to be most cold tolerant (Rakocy and McGinty 1989, El-Sayed 2002, Canonico et al. 2005). Our results suggest that 8°C is a reasonable and conservative thermal minimum to use when evaluating 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 and reproductive potential of tilapias, survival of a few individuals could lead to establishment and possible strong selection for a colder thermal minimum, so even with a slow rate of decline, the mean temperature may still overestimate the minimum temperature relevant to assessing invasion risk. As climate change is a concern and water bodies are expected to warm (Kaushal et al. 2010), managers need to continue to evaluate shifts in minimum winter temperatures relative to the tilapia thermal minima (by species and strain) to determine if invasion risk changes.

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TABLES

Table 2.1: Summary of average and range of water quality parameters in the moderate declination, slow declination and control systems.

Parameter		Moderate	Slow	Control
pH	Average	8.2	8.4	8.3
	Range	7.5-8.5	8-8.5	7.8-8.5
Alkalinity (mg/L)	Average	122	116	106
	Range	103-154	103-154	85-137
Hardness (mg/L)	Average	183	180	135
	Range	120-222	137-222	51-205
Ammonia(mg/L)	Range	0	0	0
Nitrite range (mg/L)	Range	0-0.4	0	0
Salinity avg. (ppt)	Average	0.05	0.05	0.05
Dissolved oxygen (mg/L)	Range	7.4-9.8	7.3-9.6	6.9-8.8

Table 2.2: Average temperatures (°C) during each temperature treatment and the associated standard deviations in the moderate declination treatment.

Experiment Day	Average Temp. °C	Standard Deviation
Last Day of Acclimation	20.5	0.1
1	19.8	0.2
2	18.7	0.2
3	17.6	0.2
4	16.5	0.2
5	15.4	0.2
6	14.8	0.2
7, 8, 9	13.7	0.2
10, 11, 12	12.4	0.3
13, 14, 15	11.5	0.3
16, 17, 18	10.5	0.4
19, 20, 21	9.7	0.6
22, 23, 24	8.8	0.7

Table 2.3: Average temperatures (°C) during each temperature treatment and the associated standard deviations in the slow declination treatment.

Experiment Day	Average Temp. °C	Standard Deviation
Last Day of Acclimation	20.4	0.2
1 - 7	19.8	0.2
8 - 14	18.7	0.2
15 - 21	17.6	0.2
22 - 28	16.4	0.2
29 - 35	15.6	0.4
36 - 42	14.7	0.2
43 - 49	13.6	0.2
50 - 56	12.5	0.2
57 - 63	11.3	0.2
67 - 70	10.2	0.3
71 - 77	9.2	0.3
78	8	0.3

Table 2.4: Sample size (N), average initial total length (mm) ± standard deviation, average initial weight (g) ± standard deviation, average percent change in body mass per day from the beginning of the experiment to death, and median, mean, standard deviation and range of temperatures at death, for each species group (suppliers combined) and each supplier within species group.

Species Group	Sup.	N	TL (mm) ±SD	Avg. Weight (g)±SD	Specific Growth Rate	Temperature at Death			
						Median	Mean	SD	Range
RAPID DECLINATION TREATMENT									
White Nile (*Oreochromis niloticus*)	All	39	72±9	7.7±3.1	8.2	9.7	9.3	0.5	8.8-9.7
White Nile	1	22	69±8	6.6±2.5	8.4	9.7	9.5	0.4	8.8-9.7
White Nile	4	17	76±9	8.9±3.2	8	8.8	9.1	0.5	8.8-9.7
Common Nile (*Oreochromis niloticus*)	All	44	55±6	3.4±1.1	4.8	9.7	9.6	0.5	8.8-10.5
Common Nile	2	23	56±6	3.4±1.1	6.2	9.7	9.6	0.4	8.8-10.5
Common Nile	3	21	54±6	3.3±1.2	3.3	9.7	9.6	0.6	8.8-10.5
Blue Tilapia *(Oreochromis aureus)*	All	54	64±9	5.5±2.7	5.2	9.7	9.4	0.6	8.8-10.5
Blue Tilapia	2	22	59±7	3.9±1.6	4.5	9.7	9.5	0.5	8.8-10.5
Blue Tilapia	3	17	62±6	4.7±1.2	5.1	8.8	9.1	0.5	8.8-10.5
Blue Tilapia	4	15	74±6	8.7±2.3	6.2	9.7	9.5	0.6	8.8-10.5

Table 2.4: Continued

	SLOW DECLINATION TREATMENT								
White Nile (*Oreochromis niloticus*)	All	31	71±8	7.4±2.5	5.4	9.2	9.3	0.5	8.0-10.2
White Nile	1	22	71±8	7.4±2.5	5.8	9.2	9.4	0.5	9.2-10.2
White Nile	4	9	71±9	7.3±2.8	4.4	9.2	9.0	0.4	8.0-9.2
Common Nile (*Oreochromis niloticus*)	All	44	52±7	2.8±1.3	2.3	10.2	10.8	1.9	9.2-16.4
Common Nile	2	22	56±7	3.5±1.4	3.1	10.2	10.8	1.5	9.2-16.4
Common Nile	3	22	48±6	2.3±0.8	1.6	9.2	10.8	2.3	9.2-16.4
Blue Tilapia *(Oreochromis aureus)*	All	49	64±7	5.1±1.9	3.2	9.2	9.5	0.6	8.0-10.2
Blue Tilapia	2	19	62±7	4.4±1.5	2.8	9.2	9.6	0.5	9.2-10.2
Blue Tilapia	3	22	64±5	5.0±1.2	3.5	9.2	9.5	0.6	8.0-10.2
Blue Tilapia	4	8	71±9	7.5±2.7	3.6	9.2	9.0	0.5	8.0-9.2

FIGURES

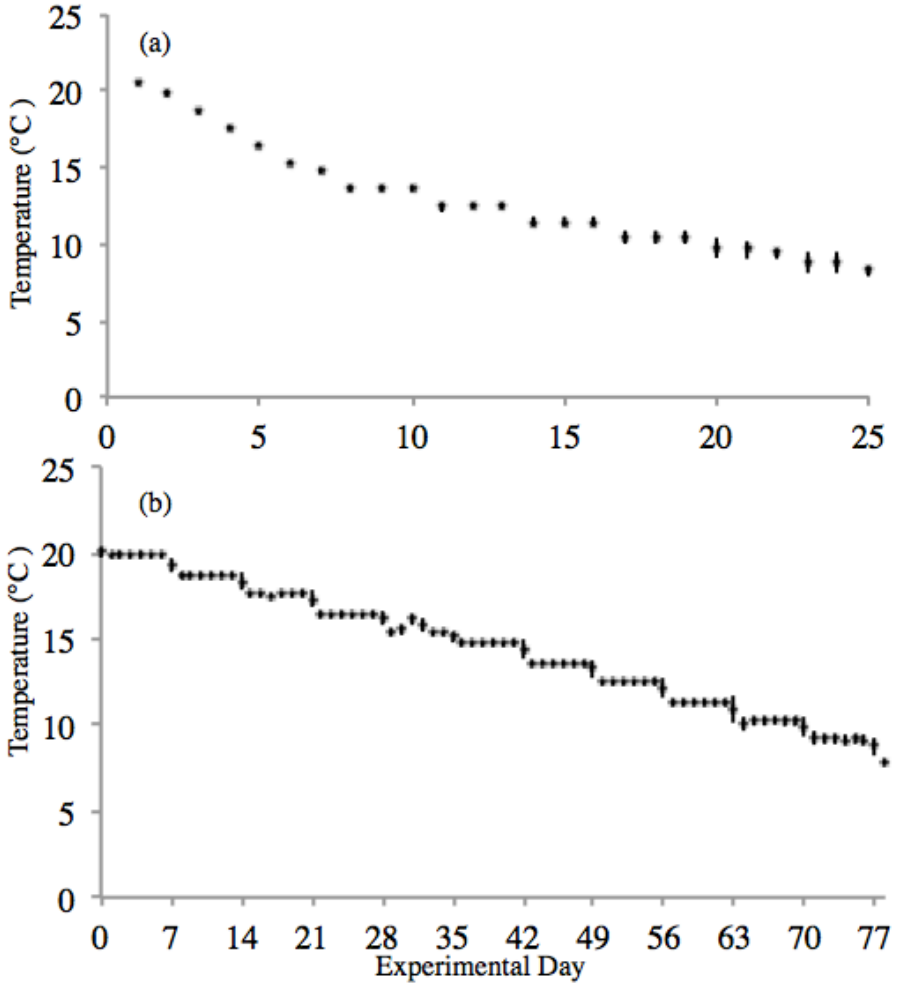

Figure 2.1: Average temperature and associated standard deviation each day of the experiment in a) the moderate declination treatment and b) the slow declination treatment.

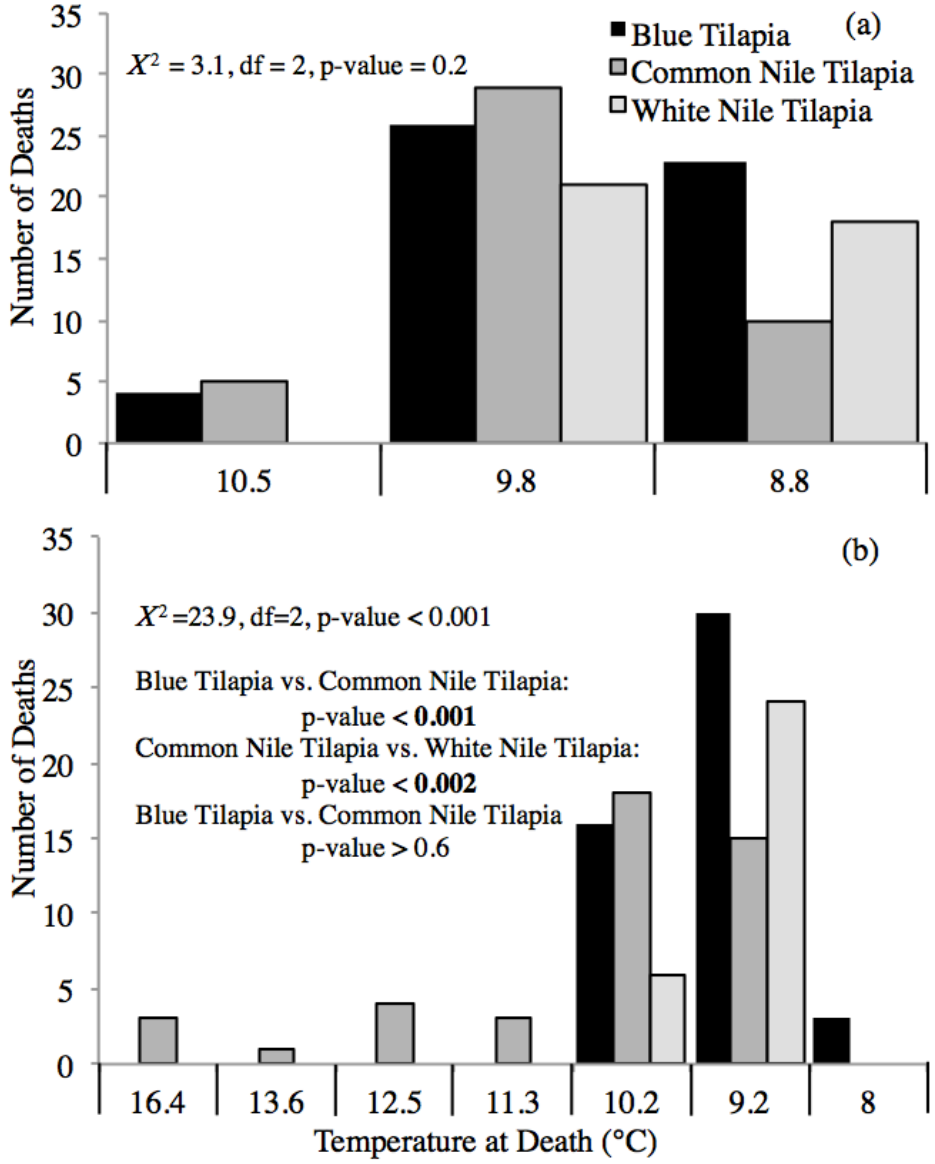

Figure 2.2: Number of deaths by species group (suppliers combined) at each of the final temperatures in the rapid declination treatment (a) and slow declination treatment (b). There were no differences among species groups in temperature at death in the moderate declination treatment (X^2 = 3.1, df = 2, p-value = 0.2). In the slow declination treatment, there was a significant difference in temperature at death (X^2 = 23.9, df = 2, p-value < 0.001). Significant differences in the pairwise comparisons are reflected in bold p-values; temperature at death of White Nile Tilapia and Blue Tilapia differed significantly from that of Common Nile Tilapia, but not from each other.

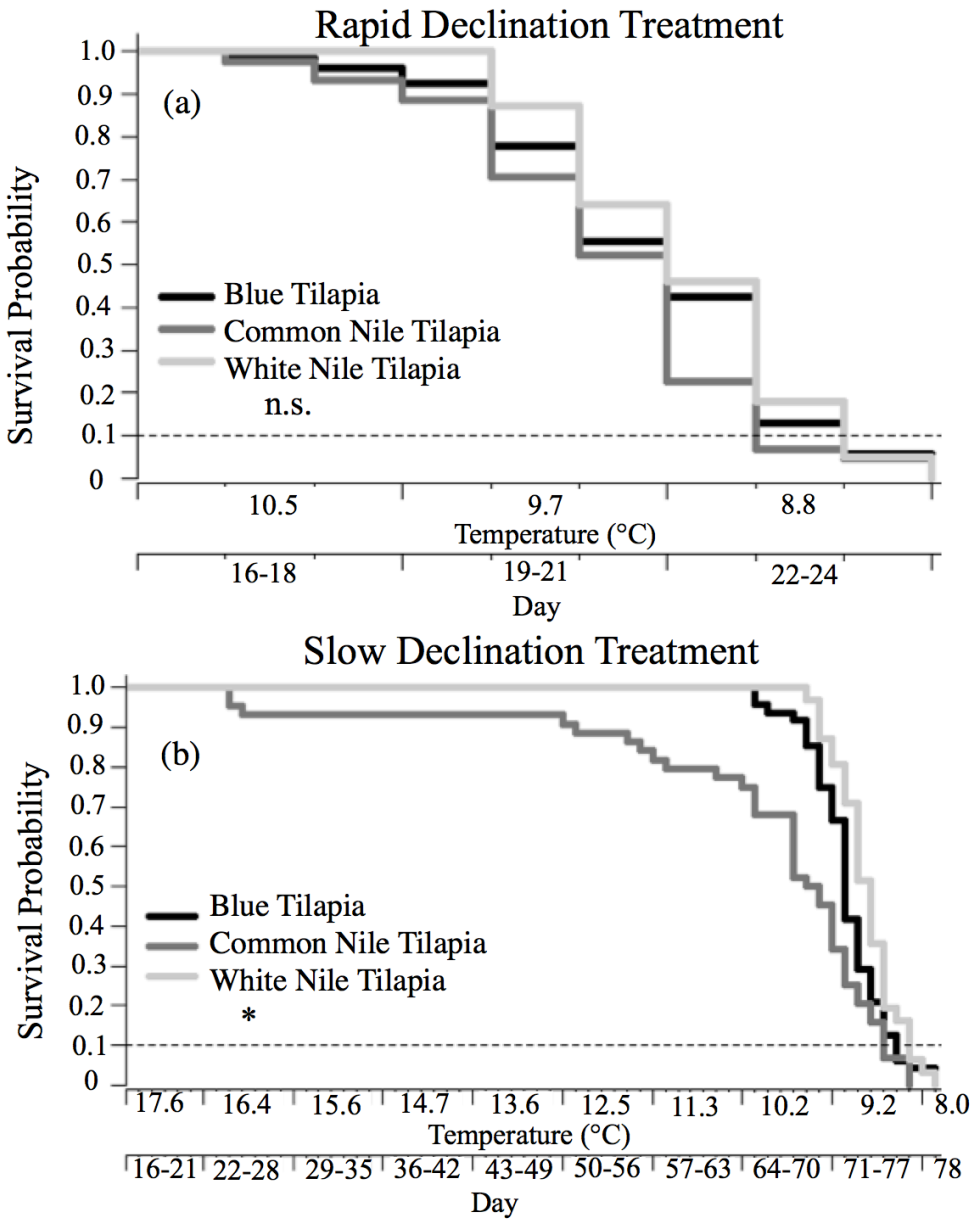

Figure 2.3: Survival curves by species group throughout the treatments in the moderate declination treatment (a) and slow declination treatment (b). There were no differences among species groups in the moderate declination treatment (n.s.). The asterisk denotes a significant difference among species groups in the slow declination treatment; The White Nile Tilapia survival curve was statistically different from both the Common Nile Tilapia survival curve and the Blue Tilapia survival curve, while the Common Nile Tilapia and Blue Tilapia survival curves were not significantly different from each other.

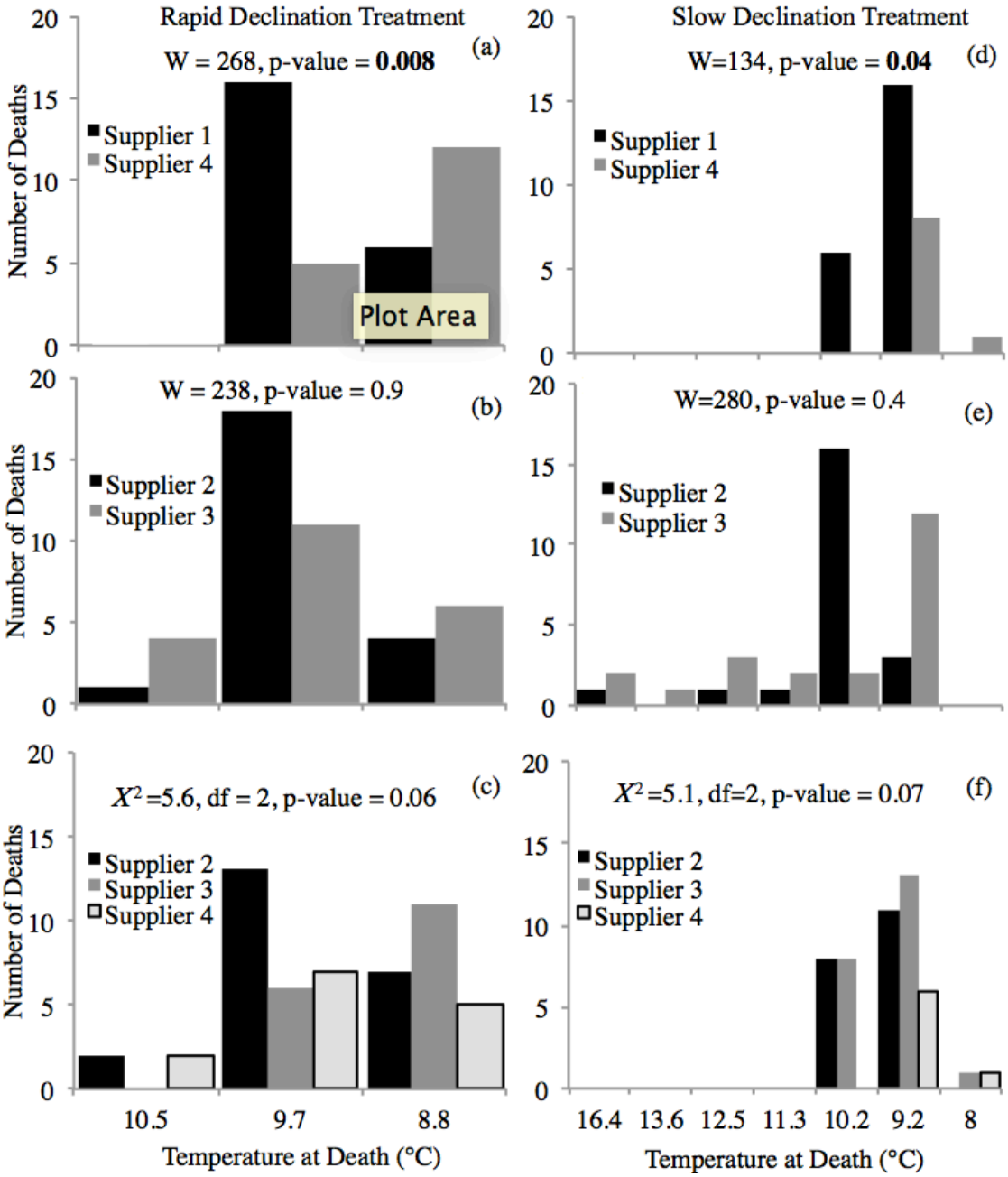

Figure 2.4: Number of deaths by supplier within species group at each of the final temperatures in the rapid declination treatment (a-c) and slow declination treatment (d-f). Species groups are organized by White Nile Tilapia (a, d), Common Nile Tilapia (b, e) and Blue Tilapia (c, f). Significant differences are indicated with bold p-values. The temperatures at death of White Nile Tilapia were significantly different between suppliers in both the rapid declination treatment (a) and the slow declination treatment (d).

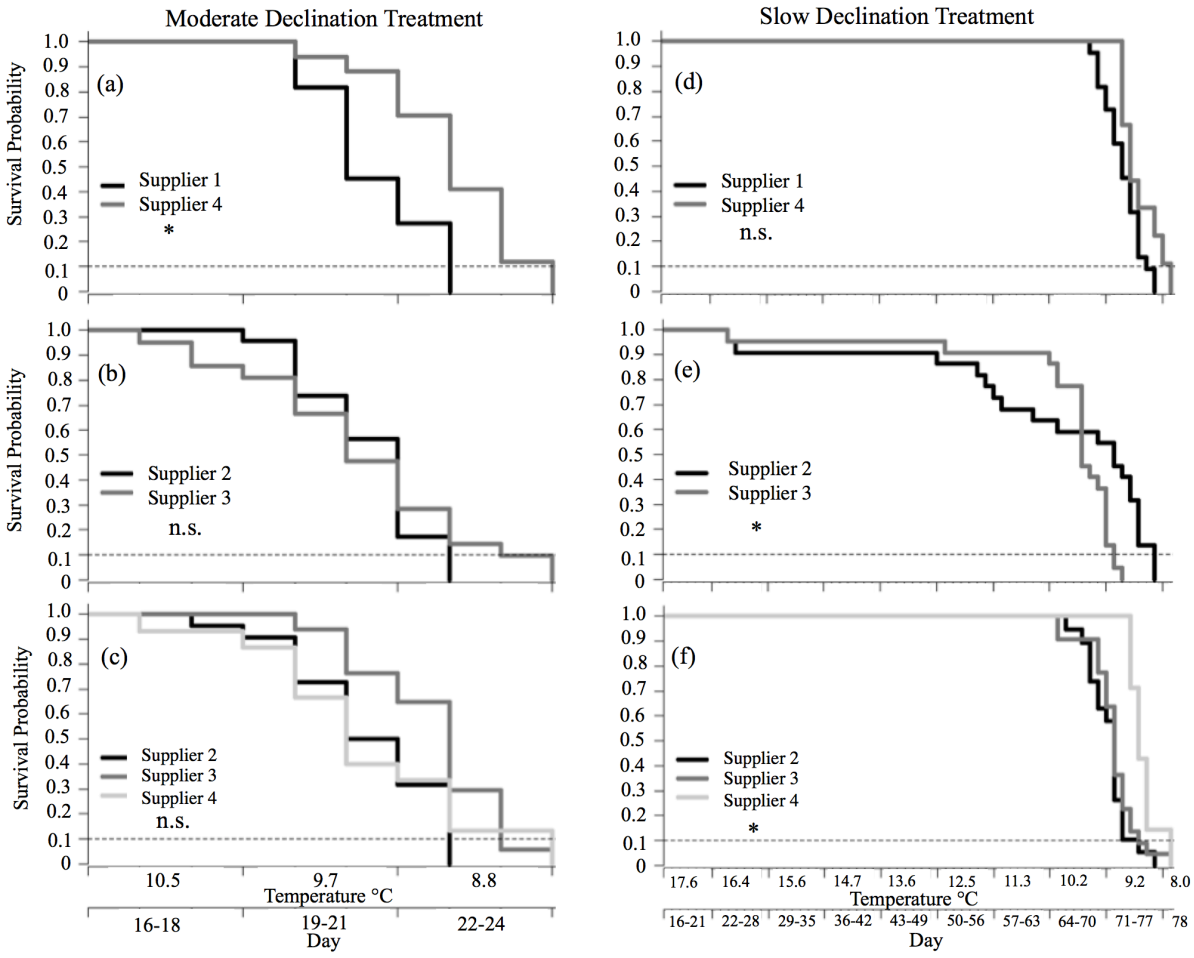

Figure 2.5: Survival curves by suppliers within species groups plotted versus both temperature and day in the rapid declination treatment (a-c) and slow declination treatment (d-f). Species groups are organized by White Nile Tilapia (a, d), Common Nile Tilapia (b, e) and Blue Tilapia (c, f). Asterisks denote significant differences between or among suppliers; n.s. indicates no significant difference.

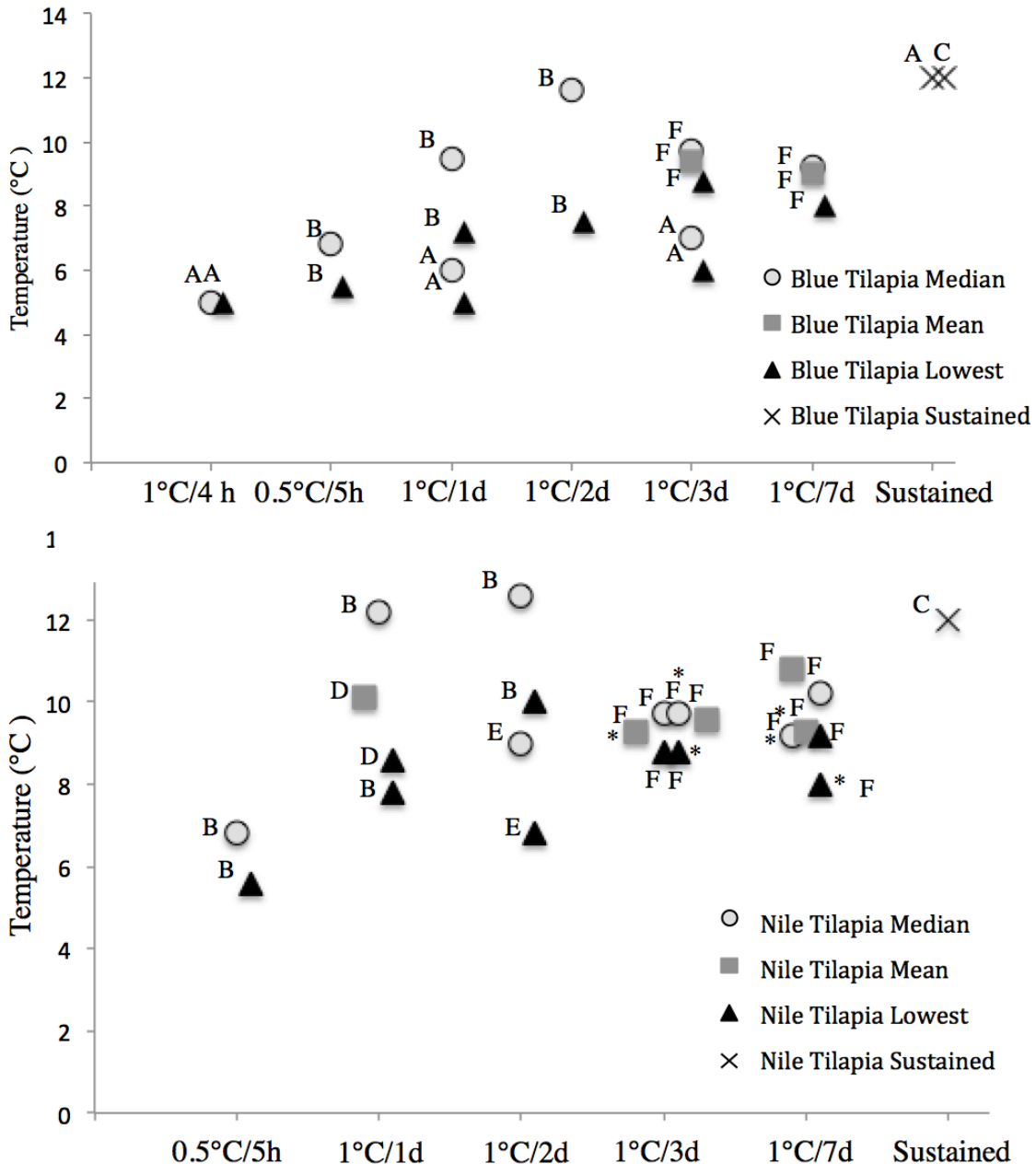

Figure 2.6: Estimates of median, mean and lowest and thermal minima reported in previous research for (a) Blue Tilapia and (b) Nile Tilapia. Letters indicate the source: (A) Zale 1984, (B) Paz 2003, (C) Wilson et al. 2009, (D) Charo-Karisa et al. 2005, (E) Atwood et al. 2003, (F) this study, Common Nile Tilapia strain and (F*) this study, White Nile Tilapia strain.

APPENDICES

Appendix A: Count data from field collections in Hyco Lake presented by number of each species caught at each site using electrofishing (E) or trap nets (T). Abiotic covariates are also included that represent the zone of the lake (hot, intermediate and cold) as well as the temperature during the week of sampling. Species are: Redbelly Tilapia (RBT), Blue Tilapia (BT), Bluegill (BLG), Black Crappie (BC), and Largemouth Bass (LMB), which is broken into two size classes small (S) and large (L). Although not included as a covariate in the analysis, month and year of sampling are indicated in the appendix to delineate sampling effort.

Site	Zone	Year	Month	Temp(°C)	RBT_T	RBT_E	BT_T	BT_E
1	Cold	2014	Nov	16.4	0	7	0	7
1	Cold	2014	Sept	23.1	0	0	1	1
1	Cold	2014	July	29.8	0	0	0	0
1	Cold	2015	May	25.9	0	0	1	0
1	Cold	2015	July	31	3	0	0	0
1	Cold	2015	March	13.8	0	0	0	0
1	Cold	2015	Nov	16.1	0	0	1	0
1	Cold	2015	Jan	6	0	0	0	0
1	Cold	2015	Sept	24.3	0	0	0	2
2	Inter	2014	Sept	26.6	0	2	0	1
2	Inter	2014	July	32	3	2	0	0
2	Inter	2014	Nov	21.5	8	14	5	9
2	Inter	2015	July	33	2	0	0	1
2	Inter	2015	Sept	27.6	2	0	0	2
2	Inter	2015	Jan	12.8	0	1	0	0
2	Inter	2015	Nov	18.1	0	14	0	28
2	Inter	2015	March	17.2	0	0	0	0
2	Inter	2015	May	28.6	0	0	0	0

Appendix A, continued.

Site	Zone	Year	Month	Temp(°C)	RBT_T	RBT_E	BT_T	BT_E
3	Hot	2014	Nov	22.5	51	29	29	6
3	Hot	2014	July	32.7	18	4	0	0
3	Hot	2014	Sept	26.5	46	0	2	0
3	Hot	2015	Nov	18.4	1	0	0	1
3	Hot	2015	March	17.6	7	0	0	0
3	Hot	2015	Jan	14.3	0	0	0	0
3	Hot	2015	Sept	28.6	3	2	0	0
3	Hot	2015	May	29.1	0	0	0	0
3	Hot	2015	July	34.5	5	0	0	0
4	Hot	2014	Nov	22.1	0	11	0	1
4	Hot	2014	July	32.7	0	0	0	0
4	Hot	2014	Sept	26.3	0	0	0	0
4	Hot	2015	Jan	14.1	0	0	0	0
4	Hot	2015	Sept	28.4	6	0	1	0
4	Hot	2015	Nov	21.4	0	0	0	0
4	Hot	2015	March	17.6	0	0	0	0
4	Hot	2015	May	29.1	0	0	1	0
4	Hot	2015	July	34.4	1	0	0	0
5	Hot	2014	Nov	23.1	10	19	6	6
5	Hot	2014	Sept	27	4	4	0	1
5	Hot	2014	July	33.8	9	1	0	0
5	Hot	2015	Nov	19	0	0	0	0
5	Hot	2015	May	27.7	1	1	0	1
5	Hot	2015	March	15.7	0	4	0	7
5	Hot	2015	Jan	15.7	0	0	0	1
5	Hot	2015	Sept	28.9	0	1	0	0
5	Hot	2015	July	25	0	0	0	0

Appendix A, continued.

Site	Zone	Year	Month	Temp(°C)	RBT_T	RBT_E	BT_T	BT_E
6	Cold	2014	July	18.4	2	0	0	0
6	Cold	2014	Nov	16	1	0	2	1
6	Cold	2014	Sept	22.6	1	0	0	0
6	Cold	2015	March	13.2	0	0	0	0
6	Cold	2015	Jan	5.6	0	0	0	0
6	Cold	2015	Nov	14.4	0	1	1	0
6	Cold	2015	Sept	23.7	0	0	1	0
6	Cold	2015	May	25.3	0	0	1	0
6	Cold	2015	July	29.8	0	0	0	0
7	Inter	2014	July	32.5	11	7	0	0
7	Inter	2014	Sept	26.5	0	0	2	13
7	Inter	2014	Nov	22.2	0	6	0	14
7	Inter	2015	Nov	17.8	0	0	1	0
7	Inter	2015	May	27.9	9	0	42	0
7	Inter	2015	Jan	13.4	0	0	0	0
7	Inter	2015	March	16.5	22	0	1	0
7	Inter	2015	Sept	27.2	1	0	1	0
7	Inter	2015	July	34.1	0	0	0	0
8	Cold	2014	Sept	25.1	1	0	1	0
8	Cold	2014	Nov	20.6	0	1	2	0
8	Cold	2014	July	31.1	1	0	0	0
8	Cold	2015	Jan	11.3	0	0	0	0
8	Cold	2015	May	27.4	1	0	0	0
8	Cold	2015	Nov	15.8	0	0	0	0
8	Cold	2015	July	32.2	3	0	0	0
8	Cold	2015	March	14.8	1	0	1	0
8	Cold	2015	Sept	24.9	5	0	3	0

Appendix A, continued.

Site	Zone	Year	Month	Temp(°C)	RBT_T	RBT_E	BT_T	BT_E
9	Inter	2014	Sept	26.1	0	1	0	0
9	Inter	2014	July	33.5	19	0	1	0
9	Inter	2014	Nov	21.6	2	2	3	0
9	Inter	2015	Nov	18.1	0	2	0	0
9	Inter	2015	Jan	12.6	0	0	0	0
9	Inter	2015	March	15.2	0	2	0	0
9	Inter	2015	May	27.7	0	0	0	0
9	Inter	2015	Sept	26.4	1	0	0	0
9	Inter	2015	July	33.5	0	0	0	0

Appendix A, continued.

BLG_T	BLG_E	LMB_T	LMB_E	LMB_E_S	LMB_E_L	BC_T	BC_E
0	44	0	2	1	1	0	0
3	14	0	2	0	2	1	0
1	42	0	1	1	0	38	0
2	0	0	1	0	1	0	0
3	8	0	1	0	1	0	0
8	20	0	4	0	4	12	2
11	5	0	6	1	5	0	0
1	1	0	8	0	8	2	0
2	18	0	0	0	0	7	0
1	36	1	2	1	1	0	0
11	54	1	1	0	1	0	0
6	159	0	4	2	2	5	0
5	20	1	1	0	1	0	0
1	49	0	3	1	2	1	0
0	158	0	4	1	3	0	0
1	115	0	4	0	4	1	0
1	74	0	9	0	9	6	0
14	32	0	1	1	0	0	0
10	35	0	5	1	4	8	0
101	60	1	1	1	0	0	0
52	10	0	0	0	0	0	0
1	2	0	3	2	1	0	0
1	6	0	1	0	1	1	0
22	3	0	1	1	0	3	0
2	20	0	2	2	0	0	0
2	2	1	0	0	0	0	0
17	0	0	0	0	0	2	0

Appendix A, continued.

BLG_T	BLG_E	LMB_T	LMB_E	LMB_E_S	LMB_E_L	BC_T	BC_E
0	31	0	3	1	2	0	0
14	28	0	2	2	0	0	0
0	10	0	0	0	0	0	0
5	7	0	3	1	2	8	0
22	15	0	5	3	2	0	0
0	0	0	2	0	2	0	0
1	2	0	10	1	9	0	0
2	16	0	0	0	0	0	0
21	0	2	0	0	0	0	0
0	29	0	1	0	1	50	0
1	11	1	1	0	1	5	0
40	51	0	19	16	3	0	0
0	2	0	1	0	1	1	0
0	5	0	2	0	2	0	0
0	9	0	4	1	3	4	0
4	2	0	5	0	5	24	0
9	4	0	1	1	0	1	0
0	0	0	0	0	0	0	0
67	90	0	1	0	1	0	0
1	8	0	2	0	2	60	0
1	11	0	0	0	0	23	0
3	2	0	1	0	1	25	0
1	1	0	1	0	1	1	0
0	12	0	1	0	1	1	0
0	11	0	2	0	2	14	0
0	5	0	0	0	0	1	0
2	1	0	0	0	0	1	0

Appendix A, continued.

BLG_T	BLG_E	LMB_T	LMB_E	LMB_E_S	LMB_E_L	BC_T	BC_E
8	11	0	3	2	1	0	0
4	11	0	2	0	2	0	0
0	9	0	0	0	0	0	0
0	8	0	5	4	1	1	0
8	1	0	2	0	2	0	0
0	0	0	4	1	3	0	0
13	4	0	4	0	4	1	0
3	10	0	5	0	0	0	0
2	2	0	0	0	0	0	0
28	28	0	2	1	1	0	0
5	66	0	3	2	1	11	0
8	0	0	0	0	0	0	0
0	4	0	5	4	1	0	0
0	3	0	2	0	2	0	0
0	0	0	2	0	2	0	0
18	4	0	2	0	2	0	0
20	5	0	3	0	3	3	0
13	1	0	1	1	0	0	0
5	27	0	4	3	1	1	0
50	23	0	7	6	1	0	0
2	19	0	2	0	2	3	0
0	0	0	3	1	2	0	0
12	0	0	5	3	2	0	0
0	3	0	3	0	3	0	0
3	0	0	0	0	0	0	0
2	9	0	0	0	0	0	0
1	1	0	0	0	0	0	0