A GEOGRAPHIC ASSESSMENT OF ESTABLISHMENT RISK FOR TILAPIA, A GROUP OF POTENTIALLY INVASIVE AQUATIC SPECIES

by

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(Under the Direction of Nathan Nibbelink)

ABSTRACT

As invasive species pose increasing threats to native species and their habitat, quantitative assessments of risk are needed to aid management decision-making regarding transport and culture of non-natives. We produced a spatially-explicit risk assessment model for tilapia establishment in Georgia based on water temperature. Thermal tolerance experiments coupled with Mayfield hierarchical logistic regression showed that daily probability of survival for tilapia is dependent upon temperature drop rate, average sustained temperature, tilapia strain, and weight-length ratio. Model results were directly applied to stochastically-simulated statewide water temperature predictions. Simulation results indicated that while long-term survival probabilities are low in Georgia, much of the state remains at high risk, indicating that survival is likely to occur once every three years. Survival probabilities are significantly lower in the northern areas than the coastal plain. This quantitative, geographically explicit risk assessment can inform regional management decisions by balancing economic benefits and potential for ecological damage.

INDEX WORDS: Risk assessment, tilapia, thermal regime, establishment probabilities, hierarchical logistic regression, geographic model

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CHAPTER 1

INTRODUCTION

Introduction of invasive species has become a leading cause of biodiversity loss, especially in freshwater systems (Kolar and Lodge, 2001; Kolar and Lodge, 2002; Marchetti, Moyle and Levine, 2004). Tilapia are a group of warm-water fishes that are established nuisance species in many areas of the world, indicating that caution should be taken before releasing them into new environments. To assess the risk of potential invasions before populations are released, we investigated the probability of tilapia establishment in Georgia using a combination of laboratory experiments and geographic modeling techniques. The literature review introduces a four stage risk assessment model that has been used extensively to identify potential invasive species (Kolar and Lodge, 2002). Establishment, a single stage in the model, is investigated in Chapters 2 (submitted to Freshwater Biology) and 3 (to be submitted to Biological Conservation). We developed a hierarchical logistic regression model based on Mayfield logistic regression to predict the survival of tilapia under different thermal regimes (Chapter 2). The model was then used in conjunction with spatially explicit GIS-based water temperature predictions to identify a probability of survival for each of Georgia's streams. The combination of experimental and GIS techniques allowed for real-world predictions that may be used by aquatic managers throughout the state. Because spread and risk are subsequent to establishment in the risk framework, the risk of establishment can highlight areas where ecological impacts of tilapia may be limited, allowing managers to balance ecological integrity with economic benefits.

LITERATURE REVIEW

Intentional or unintentional introduction of non-indigenous species is known to have negative impacts on native biodiversity around the globe (Taylor, Courtenay and McCann, 1984; Courtenay 1993; Bartley and Marttin, 2002). Many biological invasions are irrevocable as removal of non-indigenous species is expensive if not impossible (Simberloff, 2005). Non-indigenous species can impact native species through predation, competition for space or resources, hybridization with native species, introduction of parasites or disease (Shafland, 1979; Courtenay 1997) or by altering ecosystem or water quality conditions (Peterson, Slack and Woodley, 2005). Conversely, some non-indigenous species can provide extensive economic benefits (Pimentel *et al.*, 2000). As awareness of the threats and benefits of non-indigenous species increases, scientists have focused on producing models to predict which species are most likely to have large impacts on native populations and ecosystems (Kolar and Lodge, 2001; Kolar and Lodge, 2002; Marchetti *et al.*, 2004). Kolar and Lodge (2002) evaluate the invasion of non-indigenous fish species as a four stage process consisting of transport (introduction), establishment, spread and impact (Figure 1.1). Invasion success in each stage can be linked to certain life history characteristics (Kolar and Lodge, 2002; Marchetti *et al.*, 2004); the evaluation of these characteristics can therefore lead to a prediction of risk. We used this framework to evaluate the risk of establishment for tilapiine species in Georgia.

Tilapia is the common name for a variety of warm fresh-water fishes in the genera *Oreochromis*, *Saratherodon* and *Tilapia*. Tilapia have become important throughout the world as a aquaculture species, but are also commonly used for insect or aquatic plant control and as forage species for common sport fish such as largemouth bass (*Micropterus salmoides)*. High tolerance to poor water quality, fast growth potential, omnivorous eating habits, ease of spawning and resistance to disease make tilapia a popular species for aquaculture (Chervinski, 1982). According to the Census of Aquaculture (USDA, 2006), tilapia sales in the United States reached a total of $31 million in 2005 and are growing. Economies of 29 different states have benefitted from tilapia sales (USDA, 2006). A variety of tilapiine species and strains from all three genera are economically important in the United States, but the genus *Oreochromis* contains the species most important to aquaculture, including Nile tilapia (*O. nilotica*), blue tilapia (*O. aureus*), Mossambique tilapia (*O. mossambiqus*), *O. urolepis hornorum* and a multitude of hybrids (Watanabe *et al.*, 2002). As with most non-indigenous fishes, the characteristics that make tilapia attractive also give them the potential to alter aquatic communities into which they are introduced (Courtenay 1997). Various strains of tilapia can handle salinity values from 0 ppt to 35 ppt (full strength seawater), pH values from 5 – 9, dissolved oxygen levels of less than 2 mg/L and ammonia values of up to 50 mg/L (Fitzsimmons, 2005).

Tilapia were initially introduced into U.S. waters in 1954 for aquaculture experimentation in Alabama (Courtenay 1997). Since then, populations have become established in areas of Colorado, Texas, California, Arizona, Mississippi and Florida (Courtenay 1997; Fuller, Nico and Williams, 1999; Peterson *et al.*, 2004; Peterson *et al.*, 2005). Tilapia currently account for 90% of the biomass in some areas of the Colorado River (Fitzsimmons, 2005) and as a result are restricted in many southwestern states (Courtenay 1997). Tilapia are considered the most widespread foreign species in the state of Florida (Courtenay 1997), but other southeastern states have not experienced the rapid spread of the species (Fuller *et al.*, 1999). Populations in Alabama have rarely survived winters and there are no known wild populations in the state (Fuller *et al.*, 1999). The only known record of tilapia populations in North Carolina exists in a cooling reservoir, but would not be expected to survive without the thermal pollution (Fuller *et al.*, 1999). Records indicate that individuals introduced to Georgia in the 1980's survived for several years, but did not survive the winter of 1989 (Fuller *et al.*, 1999; Benson, personal communication). The last known records of tilapia within Georgia's borders were in Lake Seminole in 1992 (Benson, personal communication). Because tilapia are desirable both as game-fish forage and as a food fish, human-aided transport and introduction of the species is attractive. To balance the benefits and potential risks of tilapia introductions, state and federal agencies may benefit from regulating introductions based on risk assessments using Kolar & Lodge's (2002) four stages of invasion (transport, establishment, spread, impact).

Our analysis focused on the establishment phase of the invasion process. Kolar and Lodge (2002) use four life history characteristics to predict establishment success, one of which is the breadth temperature tolerances. Because tilapia are warm water species, we used acute minimum lethal temperatures and geographic information systems (GIS) to produce a risk of establishment model for streams occurring in Georgia. Our initial objective was to evaluate the effects of thermal and individual parameters on survival of tilapia. We hypothesized that the thermal experience of the fish, including drop rate and sustained temperature, would affect survival and that the effects would be mitigated by individual characteristics such as body size and type of tilapia. Our second objective was to transfer the results to a geographic extent using geographic conditions to vary water temperature. Theoretically, if we determine that establishment is unlikely, the impacts of releasing tilapia will likely be minimal regardless of characteristics leading to spread and impact.

Thermal Limits

A variety of experiments have been conducted over the past forty years to determine the acute minimum lethal temperature limits for tilapia and the results are as varied as the experiments. For Nile tilapia, cold water induced mortality begins somewhere between 13.6° C (Charo-Karisa *et al.*, 2005) and 10.6° C (Sifa *et al.*, 2002). One-hundred percent mortality occurs between 8.6° C (Charo-Karisa *et al.*, 2005) and 6.8° C (Atwood *et al.*, 2003). Fish size significantly affects mortality (larger fish are more tolerant to low temperatures); type of food does not (Atwood *et al.*, 2003). Isosmotic salinities, or salinities similar to the fish’s internal salinity, increase cold tolerance in some studies (Zale and Gregory, 1989; Hargreaves, 2000) but not in others (Atwood *et al.*, 2003).

Additionally, tilapia are able to adjust to gradual decreases in temperature (Sifa *et al.*, 2002). In 2003, Paz conducted intensive studies of cold tolerance in Nile tilapia, blue tilapia, and two hybrids referred to as MCS or silver tilapia and Florida red tilapia (Paz, 2003). Paz shows that the rate of temperature drop has a significant effect on minimum lethal temperature. Fish exposed to fast rates of change (-0.5° C per 5 hours) have acute lower lethal temperature limits ranging from 5.3° C to 5.6° C depending on the species. In contrast, fish exposed to a more gradual reduction in temperature (-1° C per 48 hours) have lethal limits ranging from 7.5° C for blue tilapia to 11.0° C for Red tilapia (Paz, 2003).

The studies mentioned above report a range of cold tolerances from 5.3° C to 13.6° C, with blue tilapia having the highest cold tolerance, followed by blue tilapia hybrids. Most of these studies test tolerances by lowering temperatures at a selected rate (e.g. -1° C per 24 hours). As an alternative to assessing cold tolerances, Cnanni et al. (2000) use Cooling Degree Days (CDD). Instead of observing final temperature at death, CDD results are a mathematical calculation of the number of days at each temperature the fish survived, thereby calculating a measure of how much thermal stress the fish can withstand. CDD are calculated as the sum of days the fish survived multiplied by the difference between daily temperature and the initial temperature (Cnaani, Gall and Hulata, 2000).

$$CDD = \sum_{i=1}^{k} (t_0 - t_1) \qquad (1.1)$$

Where: i = days, t_o = 16° C, the initial temperature, t_1 = temperature at the i^{th} day and k=day of mortality.

The cooling degree day approach is an important step in predicting survival of tilapia as it is the first approach that predicts mortality using both intensity and duration of exposure. Lethal temperatures for *O. mossambiqus* and *O. aureus* in this study range from 8° C to 11° C which is similar to other experiments, but CDDs for these species range from 49.7 to 98.5 (Cnaani *et al.*, 2000; Paz, 2003). The utility of these results are limited as a CDD cannot be directly translated to environmental conditions in a lake or stream. The number of CDDs changes depending on drop rates in the experiments and could represent a variety of environmental conditions. To account for intensity and duration of exposure and apply results to environmental conditions, we used Mayfield logistic regression (Hosmer and Lemeshow, 2000; Hazler, 2004) in a hierarchical framework (Raudenbush and Bryk, 2002). The approach allowed us to calculate survival probabilities in any given surface water based on the thermal regime of the site and characteristics of a given individual. Our survival probability model, based on parameters of individual thermal experience, provided a quantitative basis to address an important component (initial survival) of the risk of establishment of a potentially invasive species (Chapter 2). By adding stochastic simulation to a proven water temperature model (Chapter 3) and applying the survival model results, we produced a risk assessment for the establishment of tilapia throughout the state of Georgia. Each stream was given a probability of annual survival given cutoff values which can be determined by managers. Probabilities can then be aggregated to a coarser level such as county boundaries or watershed boundaries to facilitate management decisions.

Because our study addressed only one component of risk of invasion (potential establishment due to thermal tolerance), we offer a brief discussion here of other criteria potentially affecting establishment as well as the remaining components of invasion risk, spread and impact (Kolar & Lodge 2002). Based on water temperature alone, establishment of tilapia on an annual basis is unlikely in most of Georgia’s natural waters. However, other characteristics of invasive species have been shown to be related to probability of establishment. Species with a high probability of establishment have been shown to have a wide range of salinity tolerance, rapid growth rates, a history of invasiveness in other ecosystems (Kolar and Lodge, 2002), high propagule pressure, high parental care, long life spans, and small native ranges (Marchetti *et al.*, 2004). Tilapiine species do hold a variety of these characteristics including rapid growth rates, high parental care (Popma and Masser, 1999) and a history of invasiveness (Fuller *et al.*, 1999; Canonico *et al.*, 2005). Propagule pressure would be directly related to management decisions; large purposeful releases would likely cause different outcomes than an occasional accidental release. Risk assessment results were based on probabilities, and the more individuals in the ecosystem, the higher probability of at least minimal survival.

Spread

As previously noted, tilapia have developed feral populations almost everywhere with a suitable climate (Courtenay 1997) and the possibilities of eradication are limited should introduction and establishment of the species occur (Pimentel *et al.*, 2000). The third stage of the invasion model, spread, relates to the species' ability to move from one area to another (Kolar and Lodge, 2002). Characteristics related to rapid spread include slow growth rate, poor survival in high water temperatures, a wide temperature tolerance range (Kolar and Lodge, 2002), long life spans, proximity to a native source and a non-herbivorous trophic status (Canonico *et al.*, 2005). Based on these characteristics, tilapia may not qualify as quickly spreading species.

As a lacustrine species, the movement potential of tilapia has not yet been highly documented in the literature. Tilapia have been found to migrate between multiple sites in environments with thermal refugia and seasonal temperature changes (Scordella *et al.*, 2003; Peterson *et al.*, 2005). The presence of groundwater inputs, power plants or thermal effluent during winter months could drastically improve tilapia's ability to establish populations by providing refuge to escape cold water temperatures. The potential for thermal refugia should be evaluated in Georgia's river systems.

Impact

Canonico *et al.* (2005) produced a review article on the effects of tilapia on biodiversity, but empirical studies which measure the impacts of introduction have not yet been developed. Several aspects of tilapiine ecology demonstrate the ability to compete strongly with other species for food, habitat and spawning sites in a variety of aquatic habitats (Canonico *et al.*, 2005).

Tilapia are omnivorous eaters and demonstrate a wide variety of food preference during different life stages (Beveridge and Baird, 2000). Generally, juveniles prefer zooplankton until a shift occurs from particulate feeding to filter feeding, leading to a diet mainly consisting of plankton, plant material and detritus. Tilapia are highly opportunistic and have been shown to ingest a wide array of prey including insect larvae, fish eggs and embryos (Beveridge and Baird, 2000; Canonico *et al.*, 2005). Studies have shown that tilapia can adjust feeding techniques to reflect prey availability in their current environment (Gu, Schelske and Hoyer, 1997).

The ability to adapt to available prey has led to direct competition with other species. The presence of tilapia has been shown to reduce recruitment of largemouth bass (Zale, 1987). Largemouth bass grown with tilapia have stunted growth compared to populations grown without the influence of tilapia; tilapia grown with largemouth bass show the opposite relationship indicating high competition effects on both species (Traxler and Murphy, 1995). Foraging by adult largemouth bass may reduce intraspecific competition among tilapia leading to larger sizes in remaining individuals (Traxler and Murphy, 1995).

Several facets of reproductive strategy make tilapia a very competitive species. First, tilapia have high parental care and larger size at the initiation of exogenous feeding which lead to competitive advantages and faster growth potential (Zale, 1987). Tilapia have outcompeted indigenous fish for spawning grounds in India and Nicaragua (McKaye *et al.*, 1995; Lowe-McConnell, 2000). Secondly, reproductive maturity is a function of age, size and environmental conditions but can be reached within 3-6 months of age and at a weight of 20 grams (Popma and Masser, 1999). Reproduction occurs year-round often peaking with rising temperatures and rain events (Turner and Robinson, 2000). Given Georgia's hot summers and consistent rains, this may trigger spawning within the first year of release which would substantially increase propagule pressure in the environment.

Tilapia can also be plastic in habitat selection and water quality conditions which may lead to occupation in a variety of systems. Tilapia live in a wide-range of ecosystems including slow-moving sections of rivers, small shallow lakes, reservoirs, crater lakes, soda lakes, estuaries, thermal springs and coastal brackish lagoons (Lowe-McConnell, 2000; Canonico *et al.*, 2005). Small fast flowing streams are not optimal for tilapia, yet may serve as a mechanism for transport to a lentic system. In many systems, tilapia can serve the important limnological role of circulating nutrients by consuming abundant algae and detritus and improving water clarity (Lowe-McConnell, 2000). Conversely, in some ecosystems the consumption of algae releases nutrients back into the system causing eutrophication (Canonico *et al.*, 2005).

As juveniles, tilapia can serve as a food base for piscivorous fish and birds. Tilapia also control mosquito and midge populations, occupying a carnivorous niche. Depending on the environment, tilapia have been shown to occupy a niche without harm to indigenous populations which has created financial benefits to local fisheries (Lowe-McConnell, 2000). In other environments, they have out-competed indigenous populations and caused a collapse of native fisheries (Lowe-McConnell, 2000). After being introduced to lakes Victoria and Kyoga in the late 1950's and early 1960's, the Nile tilapia (*O. nilotica*), with the help of the Nile perch, replaced all native cichlid populations. *O. nilotica* grew to a larger size, with faster growth rates, higher fecundity, longer life span, a wider food spectrum and wider habitat criteria than any of the native species (Ogutu-Ohwayo, 1990). Overall, the largest impacts to Georgia's native fauna may come from competition with incoming invaders and the relinquishment of niches available in the habitat.

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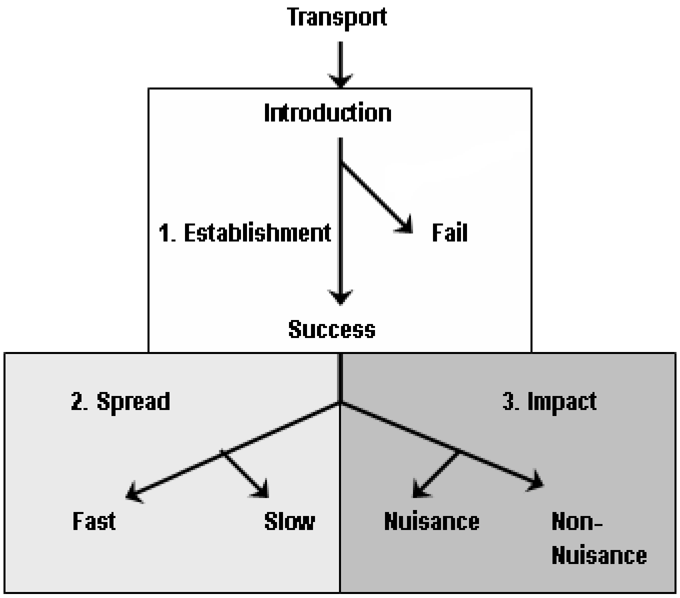

Figure 1.1: Risk assessment model developed by Kolar and Lodge (2002).

CHAPTER 2

THERMAL TOLERANCE EXPERIMENTS HELP ESTABLISH SURVIVAL PROBABILITIES FOR TILAPIA, A GROUP OF POTENTIALLY INVASIVE AQUATIC SPECIES[1]

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ABSTRACT

Estimating risk of establishment is an important step toward managing ecological risk posed by natural or intentional introductions. Species introductions in certain geographic areas may pose less of a threat when environmental conditions are unlikely to promote long-term establishment. Tilapia sp. have become widespread in certain areas of the southeastern United States (e.g. Florida), yet concerns remain regarding potential for spread or accidental introduction because of aquaculture in other areas. We created a model to predict survival of tilapia in Georgia (USA) based on individual "thermal experience". Lab experiments were conducted to measure survival rates of two strains of tilapia to duration and intensity of exposure using three minimum temperatures and two temperature drop rates. We used Mayfield hierarchical logistic regression (MHLR) to describe daily probability of survival as a function of temperature drop rate, average sustained temperature, tilapia strain, and weight-length ratio. Use of MHLR in this way is a novel approach for analyzing experimental data, uniquely suited to calculate survival probability based on experimental variables. Tilapia generally survived sustained temperatures over 12° C but survival rate was mediated by drop rate, weight-length ratio and strain. For every one degree increase in average sustained temperature, tilapia were 2.76 times more likely to survive. For every 0.1 increase in the slope of the drop rate, tilapia were 1.41 times less likely to survive. Healthier fish were more likely to survive (higher weight-length ratio), and strain had a minimal effect despite being supported by the best MHLR model (Akaike weight = 0.91). Our model

can be used with known surface waters temperature regimes to estimate survival probability for tilapia. Using MLHR in conjunction with experimental tolerance data may be useful for estimating susceptibility of natural systems to establishment of potentially invasive species.

INTRODUCTION

Introduced fish species have the ability to alter aquatic ecosystems in a variety of ways including direct competition with native species, decreases in water quality or aquatic vegetation and increases in turbidity (Pimentel *et al.*, 2000). Conversely, if an introduced species is unable to establish a population because of physiological constraints, the likely impact of that species is minimal. Water temperature has a significant impact on the metabolic rates of fish (Diana, 1995) and can therefore be considered as an environmental constraint to the establishment of a species (Kolar and Lodge, 2002).

Tilapia have become important as a cultured species throughout the world for human consumption, insect or aquatic plant control and as forage for sport fish such as largemouth bass (Fuller, Nico and Williams, 1999). Tilapia are particularly successful because of their high tolerance of poor water quality, rapid growth potential, omnivorous diets, ease of spawning, and resistance to disease (Chervinski, 1982). As a tropical species, tilapia are intolerant of cold water temperatures (Chervinski, 1982). To effectively quantify the impacts of cold water temperatures on the physiology of tilapia, the intensity and duration of exposure must be evaluated (Newman, 1995). We will refer to this combination as the “thermal experience” of the individual fish.

Hargraeves (2000) reviews studies conducted over the past fifty years regarding the cold temperature tolerances of several tilapiine species. Most studies find an acute minimum lethal temperature, but fail to evaluate the effects over time (Dan and Little, 2000; Sifa *et al.*, 2002; Atwood *et al.*, 2003, P. Paz, unpubl. data). The studies which do evaluate effects over time create “cooling degree” terms to represent thermal exposure before mortality occurred (Cnaani, Gall and Hulata, 2000; Charo-Karisa *et al.*, 2005). The results of these studies are difficult to apply to a given stream or lake because one “cooling degree” could represent a variety of environmental conditions. For example, a value of 60 cooling degree days could represent 15 days at 12° C or 6 days at 6° C, both of which would affect the fish differently. Starling *et al.* (1995) use a logistic model to predict duration of survival at different temperatures, which is similar to the methodologies used here, but they examine temperature drop rates not common in surface waters.

We used Mayfield logistic regression (Hosmer and Lemeshow, 2000; Hazler, 2004) in a hierarchical framework (Raudenbush and Bryk, 2002) to evaluate both intensity and duration of exposure and to quantify the relative contribution of each variable in the study. Additionally, the Mayfield approach allowed us to calculate survival probabilities in any given surface water based on the thermal regime of the site and characteristics of an individual fish. Our survival probability model, based on parameters of individual thermal experience, provided a quantitative basis to address an important component (initial survival) of the risk of establishment of a potentially invasive species (Kolar and Lodge, 2002).

METHODS

Thermal Experiments

We conducted laboratory experiments at the University of Georgia's Whitehall Fisheries Research Laboratory to determine cold temperature tolerance for two tilapiine strains: Nile tilapia (*Oreochromis nilotica*), and a blue tilapia, Nile tilapia hybrid (*Oreochromis nilotica* x *Oreochromis aureus*), under various temperature drop rates and final temperatures. Individuals of the two strains were obtained at the beginning of each experimental phase. Juveniles (29 mm to 95 mm in length) were used because previous studies of cold tolerance have used juveniles (Zale and Gregory, 1989; Likongwe *et al.*, 1996; Charo-Karisa *et al.*, 2005), and juveniles are more likely to escape aquaculture operations through preventative measures such as screens or to be released as forage fish in surface waters. Upon arrival, fish were maintained in a large storage tank at a temperature of 28° C for seven days to allow for acclimation to the new environment.

Experiments were conducted in 175-L culture tanks capable of 100% recirculation complete with biological filters. Temperature was controlled using six Pacific Coast CW-500 Chiller/Heaters (Woodburn, OR) accurate to 1.11° C. Every Chiller/Heater was attached to two replicate tanks, each of which held one of the strains tested (Figure 2.1). Two additional tanks were maintained at room temperature (18.5° C to 32.9° C) as controls. Each Chiller/Heater was randomly assigned a daily temperature drop rate (°C/day) and minimum final temperature. All experimental tanks were raised to 28° C before the fish were transferred to avoid shock. Twenty-five to thirty individuals were randomly selected and placed in each experimental tank. All tanks were dropped 2° C per day until they reached 18° C. Eighteen degrees is within the thermally neutral range of tilapia, indicating that they suffer no stress at that temperature (Hargreaves, 2000). Once the tanks reached 18° C, temperatures were dropped at their randomly assigned rates until they reached their assigned minimum temperature. The temperatures were then held constant until the end of the experiment. The experiment was replicated three times. Because the actual temperatures in each tank may have varied slightly because of slight variations in ambient room temperatures, Onset Hobo temperature loggers (Pocasset, MA) were installed in each head tank to record continuous experimental temperatures. Timers were set to maintain a daily photoperiod of 12 h.

Fish were fed once daily with a low protein diet of Wardley Premium Cichlid Floating Pellets. Water quality was monitored and maintained throughout the experiment at levels well above the known tolerances of tilapia (Popma and Masser, 1999). Dissolved oxygen values ranged from 4.21 to 9.7 mg/L, pH ranged from 6.78 to 8.22 and nitrite and ammonia levels were at or below 20 µg/l.

Mortality was monitored every 12 hours throughout the 30-60-d experiments. Experiments continued until 100% mortality in experimental tanks or a maximum of 60 days. Fish surviving after 60 days were counted, removed from the experimental tanks, and euthanized.

Statistical Analysis

We used Mayfield logistic regression (Hosmer and Lemeshow, 2000; Hazler, 2004) in a hierarchical framework (Raudenbush and Bryk, 2002) to model the relationship between tilapia survival and variables relating to the ‘thermal experience’ of the fish and the attributes of individuals (Table 2.1) (SAS Institute Inc., 2003). Experimental variables included tilapia strain, weight-length ratio, average sustained temperature and the slope of the temperature reduction (drop rate in °C/day). The Mayfield technique was developed to reduce bias in survival estimates calculated over a prolonged period. It has been used previously to quantify breeding success in nesting birds (Mayfield, 1961). Instead of using a typical logistic response of survival (1) or death (0), the Mayfield technique predicts daily survival probabilities by dividing the number of days survived by the total number of days in the experiment (Hazler, 2004). To separate effects of acclimation from sustained temperatures, the number of days survived and the total number of days in the experiment were calculated from the time temperatures fell within 1°C of the randomly assigned final temperature. The Mayfield method also allowed us to censor data for individual fish that died from unknown causes (Hazler, 2004). Hence, mortalities caused by external factors such as aggressive behavior were removed from the study. One assumption of Mayfield logistic regression is the independence of samples (Hazler, 2004). Because multiple fish were tested in each tank, independence could not be assumed and had to be accounted for using hierarchical regression. By adding a random effect for each tank, we prevented model over-dispersion and estimated the true effects of experimental variables (Bayley, 1993). Random effects represented the estimates of variability of tilapia survival within each tank because of unknown factors. The fixed effects were estimates of the effect of each variable. Pearson's correlations were used to determine multicollinearity in the data. Only variables with a Pearson correlation of $r < 0.7$ were included in candidate models (Tamayo, Grue and Hamel, 2000).

We used an information-theoretic approach (Burnham and Anderson, 2002) to examine the relationships between experimental variables and tilapia survival. Eight models were created *a priori* (Burnham and Anderson, 2001), including a global model which contained all variables tested (Table 2.2). Akaike's Information Criteria (AIC) was then used to assess the relative fit of each candidate model using a combination of Kullback-Leibler information and maximum likelihood methods (Burnham and Anderson, 2001) to identify the model best supported by the data (Anderson, Burnham and Thompson, 2000). We also used AICc which is a recommended modification that accounts for small sample size (Anderson *et al.*, 2000). AICc, ΔAIC and Akaike weights (w_i) were calculated for each model, to identify the best-fitting candidate model.

Odds ratios were calculated to interpret effects of each parameter. These values, along with estimates of the coefficients, standard errors and 95% confidence intervals were used to assess parameter precision. Confidence intervals were calculated using a t-statistic with n-1 degrees of freedom. For each predictor variable, we calculated scaled model-averaged log odds to allow for better interpretation of the effect size based on biologically relevant changes in each variable.

We evaluated the best model for out-of-sample predictive ability using ten-fold cross validation (Stone, 1974; Thurow, Peterson and Guzevich, 2006). The root mean square error (RMSE) was estimated using predicted and measured values for all data, thereby providing an unbiased estimator of out-of-sample model performance as described by Thurow *et al* (2006).

RESULTS

Tilapia generally survived sustained temperatures over 12° C; however, survival rate was mediated by temperature drop rate, weight-length ratio and tilapia strain (Table 2.1). Eight candidate models were considered for estimating survival probability using the experimental variables listed in Table 2.2. All eight models included the average sustained temperature of exposure and a combination of the remaining three variables: the drop rate, the weight-length ratio and the strain of tilapia tested. The most plausible model was the global model which contained all variables (Table 2.2). This model was nearly 11 times more likely than the next most plausible model which included all variables except tilapia strain. Model averaging was considered, but the high Akaike weight of the global model indicated no need for model averaging (Thompson and Lee, 2000). The fixed estimates in Table 2.3 represent the overall effects of each variable while the random effects represent variability present in each tank setup.

Using scaled log odds, the average sustained temperature and the slope of the temperature drop had the greatest effect on tilapia survival (Table 2.4). For every one degree increase in average sustained temperature, tilapia were 2.76 times more likely to survive. For every 0.1 increase in the slope of the drop rate, tilapia were 1.41 times less likely to survive, indicating that fish in tanks with fast drop rates died more quickly than those declining in temperature at slower rates. Fish with higher weight-length ratios were more likely to survive (1.29 times more likely for every 0.02 gram/mm increase). Nile tilapia were slightly less cold tolerant than the hybrid strain, but the difference was slight and may not be biologically significant. The ten-fold cross validation indicated that the global model had a RMSE of 0.178, meaning the predicted survival probability for any given day, on average, is accurate plus or minus 17.8%.

Based on the model, we calculated daily probabilities of survival for each species over a range of different conditions. Figure 2(a-d) shows the effect of each variable on the daily survival probability, while maintaining average values for all other variables. For example, an individual Nile tilapia with a weight-length ratio of 0.049, experiencing a decline in temperature with an average slope of -0.435 and a final temperature of 8° C, had a daily probability of survival of 21.4%. As indicated by the scaled odds ratios, sustained temperature had the largest effect (Figure 2.2a) and the effects of strain were minimal, despite support for the global model which included tilapiine strain. Using the model, survival probabilities can be predicted for any combination of the variables over any length of time (Figure 2.3). Probabilities of survival for extended periods are calculated by raising survival probability to the number of days exposed ($[\text{ProbSurvival}]^{\text{days}}$).

DISCUSSION

Numerous experiments have assessed environmental tolerances of tilapia. Accurate tolerance levels are critical to tilapia production as well as to evaluate the potential for establishment of this potentially invasive non-native species. By using an approach combining Mayfield techniques with a hierarchical model, we were able to not only assess the incipient cold thermal limits of tilapia, but also to determine a daily probability of survival under conditions that may not be acutely lethal. Our results are similar to those of other studies which place the incipient cold lethal temperature between 6 and 12º C, depending on the species and other environmental conditions (Hargreaves, 2000). Unlike previous studies, our daily survival probabilities also allowed me to model conditions that chronically stressed fish over time. These analyses provide a novel approach to predicting thermal tolerances of tilapia in the wild.

Using average daily temperatures in surface waters, these predictions can be used to assess establishment risk. Our results suggest that surface water temperatures can be used to predict survival of tilapia grown in different geographic regions, allowing managers to weigh the immediate advantages of rearing tilapia with the potential for long-term ecological damage. Alternatively, consider a given lake stocked with tilapia as bass forage during the summer. The same information could be used to determine percent survival of the population without intensive sampling, which may lead to more effective stocking during the next warm season.

Although we believe this method is robust in evaluating effects of environmental stressors on tilapia, some limitations must be considered. First, the average sustained temperature portion of the experiment lasted approximately 30 d indicating that predictions should not exceed that time frame. Secondly, tilapia exposed to chronic stress may experience mortality in a non-linear fashion which may introduce error in shorter exposure times than those used in our trials. For example, if the same conditions are held constant for ten days, the model would predict a constant survival rate for each of the ten days. Because of individual tolerances, mortality is more likely to occur unevenly during those ten days. We introduced a quadratic term (sustained temperature squared) to attempt to account for this variability; however, the data did not support the model. Thirdly, we used juveniles in the study because of the probability of their release, but the tolerances of adult fish may be different.

Our modeling approach, which captured the “thermal experience” of tilapia, may be particularly useful for estimating the probability of survival in the wild if temperature regimes for particular streams and lakes are known. We suggest that using the Mayfield logistic regression technique in conjunction with experimental tolerance data may be useful for estimating effects of a stressor over a given period thereby estimating susceptibility of natural systems to the establishment of potentially invasive species.

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Table 2.1. Description of explanatory variables used in candidate models including mean, standard deviation, range and units.

Variable	Description	Mean (Std. Dev.)	Range	Units
trial	Experimental trial (1st, 2nd, or 3rd)	2.084 (0.8526)	1 - 3	
tank	A concatenation of the trial in which the fish were tested and the tank setup in which they were tested.	24.752 (8.7253)	11 - 37	
surdays / finaldays	Value predicted using the Mayfield technique. Indicates number of days survived over the number of days in the trial	0.537 (0.3876)	0 - 1	
strain	Categorical variable representing strain of tilapia tested. A value of 1 indicates *O. nilotica*, 0 indicates a hybrid between *O. nilotica* and *O. aureus*	0.504 (0.5003)	0 - 1	n/a
wlratio	Weight to length ratio at end of experiment which can signify health condition	0.049 (0.02340)	0.01 - 0.16	gram /mm
avgtemp	Average sustained temperature of the tank once the temperature stabilized	14.073 (6.5486)	6.59 - 32.86	° C
surdays	Number of days the fish survived during the experimental run	18.123 (16.9987)	0 - 60	days
finaldays	Number of days the fish survived at or below the final temperature	31.051 (10.4695)	16 - 60	days
droprate	Overall slope of temperature change from beginning of experiment to mortality or end of experiment	-0.435 (0.2463)	-0.8759 - 0	° C/day

Table 2.2. Candidate models, number of parameters (K), AICc, ΔAICc, Akaike weights (w) for the set of candidate models (i) for predicting tilapiine survival and plausibility of Akaike weights for each candidate model.

Candidate Model	K	AICc	ΔAICc	w_i	% max w_i
Global Model avgtemp + wlratio + droprate + strain	7	5771.1	0	0.9129	100
avgtemp + wlratio + droprate	6	5776	4.79	0.0831	9
avgtemp + wlratio + strain	6	5782.1	10.98	0.0038	0
avgtemp + wlratio	5	5787.2	16	0.0000	0
avgtemp + droprate + strain	6	5857.4	86.22	0.0000	0
avgtemp + droprate	5	5858	86.84	0.0000	0
avgtemp + strain	5	5864.1	92.9	0.0000	0
avgtemp	4	5864.7	93.54	0.0000	0

Table 2.3. Estimates (standard errors) of fixed and random effects for the global model.

Parameter Estimate	Estimate (SE)	95% Confidence Limits	
		Lower	Upper
Fixed Effects			
Intercept	-9.401 (1.6246)	-12.7895	-6.0119
avgtemp	1.015 (0.1383)	0.7265	1.3034
wlratio	12.976 (1.3877)	10.0813	15.8708
droprate	3.425 (1.2028)	0.9161	5.9343
strain	-0.142 (0.0395)	-0.2246	-0.0599
Random Effect	0.975 (0.2015)	0.5550	1.3957
Trial 1 - Tank 1	-0.782 (0.4412)	-1.7018	0.1387
Trial 1 - Tank 2	-0.462 (0.4633)	-1.4280	0.5047
Trial 1 - Tank 3	-0.626 (0.2919)	-1.2347	-0.0170
Trial 1 - Tank 4	3.127 (0.6428)	1.7863	4.4682
Trial 1 - Tank 5	-0.286 (0.4964)	-1.3210	0.7498
Trial 1 - Tank 6	0.005 (0.4536)	-0.9415	0.9510
Trial 1 - Tank 7	0 (0.9752)	-2.0340	2.0346
Trial 2 - Tank 1	0.193 (0.382)	-0.6038	0.9898
Trial 2 - Tank 2	1.475 (0.5439)	0.3409	2.6101
Trial 2 - Tank 3	-0.569 (0.5285)	-1.6718	0.5332
Trial 2 - Tank 4	-0.211 (0.2716)	-0.7772	0.3559
Trial 2 - Tank 5	-0.435 (0.2601)	-0.9774	0.1077
Trial 2 - Tank 6	-0.566 (0.2978)	-1.1871	0.0552
Trial 2 - Tank 7	0 (0.9754)	-2.0346	2.0346
Trial 3 - Tank 1	-0.258 (0.3498)	-0.9876	0.4717
Trial 3 - Tank 2	0.89 (0.5867)	-0.3334	2.1140
Trial 3 - Tank 3	0.238 (0.4813)	-0.7663	1.2416
Trial 3 - Tank 4	-0.759 (0.2615)	-1.3045	-0.2136
Trial 3 - Tank 5	-0.421 (0.3098)	-1.0670	0.2254
Trial 3 - Tank 6	-0.63 (0.2864)	-1.2270	-0.0323
Trial 3 - Tank 7	0 (0.9753)	-2.0343	2.0346

Table 2.4. Log-odds for the global model including scaled odds ratios to produce biologically relevant results.

Parameter	Estimate (Std. Error)	Odds Ratio	Unit Scalar	Scaled OR	Lower OR	Upper OR
Fixed Effects						
Intercept	-9.401 (1.6246)					
avgtemp	1.015 (0.1383)	2.7591	1	2.7591	2.0678	3.6818
wlratio	12.976 (1.3877)	431965	0.02	1.2963	23892.0250	7809097.8267
droprate	3.425 (1.2028)	30.7288	0.1	1.4085	2.4995	377.7755
strain	-0.142 (0.0395)	0.8674	1	0.8674	0.7988	0.9418
Random Effect	0.975 (0.2015)	2.6522	1	1.7419	1.7419	4.0378

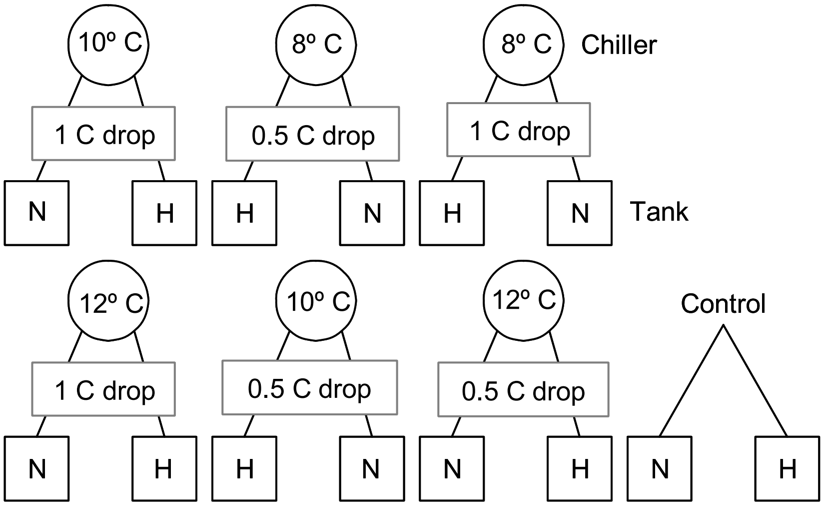

Figure 2.1. Example of experimental design. Before each of three replicates, the temperatures, drop rates and strains were randomly assigned to the tanks. H and N represent hybrid and Nile tilapia respectively.

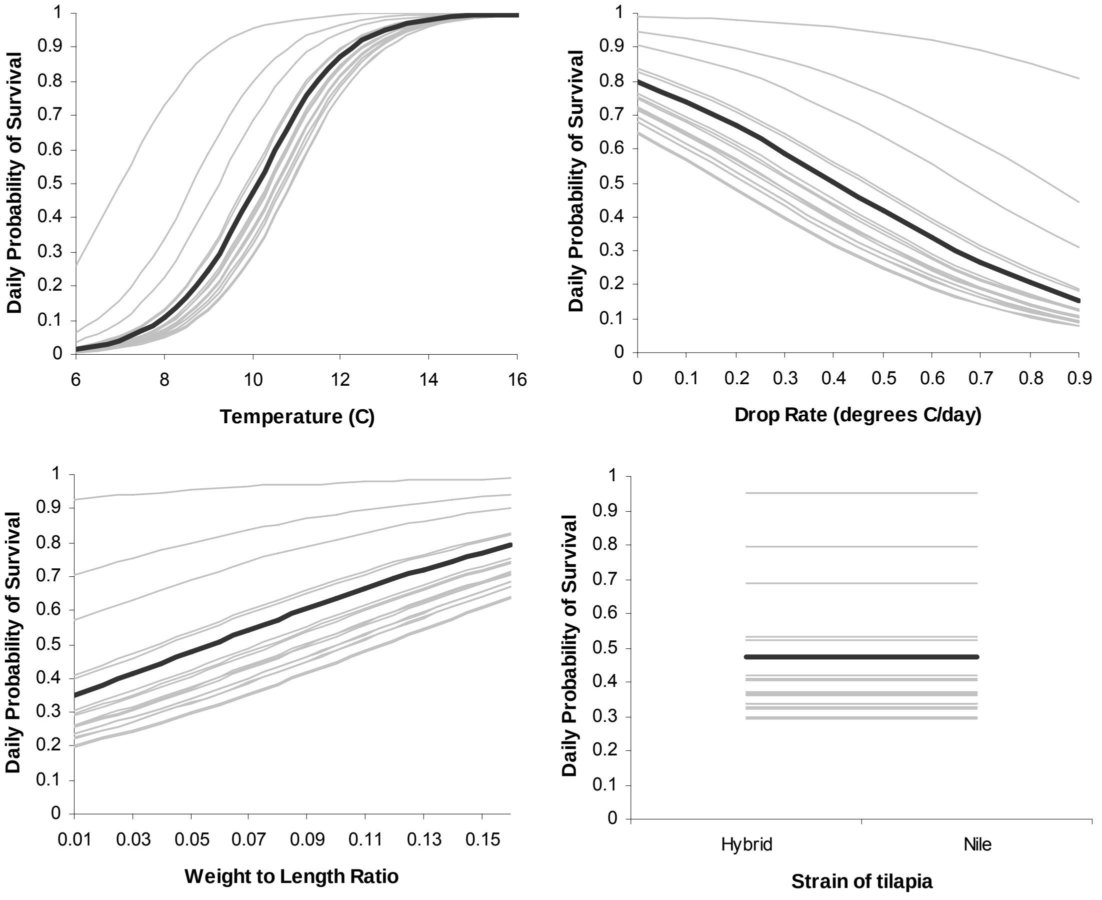

Figure 2.2. Empirical Bayes estimates of the relationship between daily probability of survival and (a) average sustained temperature (b) drop rate (c) health of the individual expressed by a weight to length ratio and (d) strain of tilapia. For each graph, all other variables are held constant at their average value so the effects of each variable can be seen clearly. The dark line indicates the fixed effect; the gray lines indicate the random effects seen in each tank. Average sustained temperature shows the largest effect on survival probabilities and is the single most important variable. The drop rate represents the acclimation of the fish to the lower water temperatures; faster drop rates negatively impacted the fish. The weight to length ratio shows health condition indicating that healthier fish can withstand slightly harsher conditions. Strain of tilapia shows little effect which casts doubt on biological significance of the term.

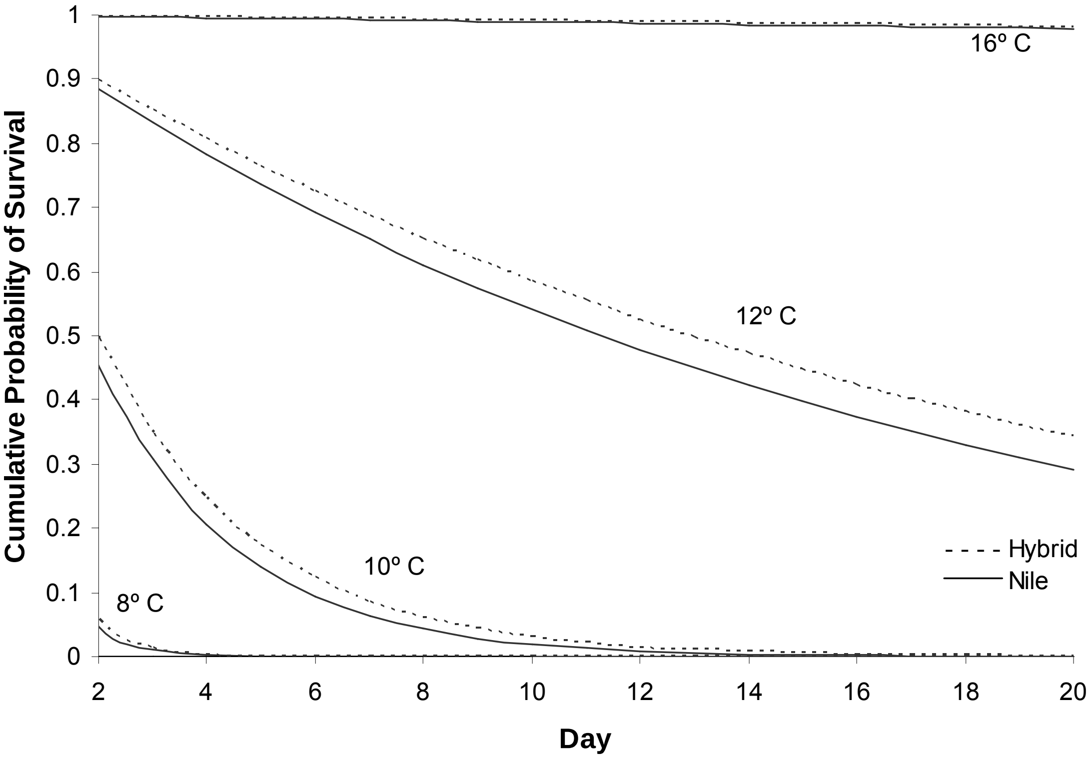

Figure 2.3. Cumulative probabilities of survival for both strains which could be directly translated to a given stream or lake. As the number of days exposed increases, the probability of survival decreases exponentially. The exception lies at 16º C which is known to be a temperature which does not negatively impact tilapiine species.

CHAPTER 3

ESTIMATING SPATIALLY EXPLICIT RISK OF ESTABLISHMENT OF TILAPIA: COMBINING THERMAL TOLERANCE AND WATER TEMPERATURE MODELS[2]

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[2] Wilson, J.C., Albeke, S., McAbee, K., Peterson, D., and Nibbelink, N.P. To be submitted to Biological Conservation.

ABSTRACT

Risk assessments are commonly used to evaluate risk posed by the introduction of nonindigenous species on local populations and ecosystems. An evaluation of establishment risk for tilapia in Georgia provides information regarding overall risk, assuming that areas unlikely to establish populations will also be unlikely to support the spread and ecosystem impact of the species. We used a combination of experimentally-derived thermal tolerance models and spatially-explicit stochastic simulations of stream temperature to estimate tilapia survival based on lethal cold water temperatures. Overall, tilapia populations are unlikely to persist for many years in most areas of Georgia. However, approximately 6% of streams were predicted to have tilapia annual survival probabilities greater than 50%. Further, most of the state is estimated to support populations through the winter once every three years or more. To complete the risk assessment for tilapia establishment, value decisions must be made as to levels of acceptable risk based on both establishment risk and damage potential during survival periods. By combining models of thermal tolerance and surface water temperature with stochastic simulations, we created a tool directly useful to management decision-making regarding transport and culture of a potentially invasive species.

INTRODUCTION

Invasive species have become one of the most significant threats to biodiversity around the globe (Wilson, 1992; Mack *et al.*, 2000; Canonico *et al.*, 2005; Simberloff, 2005). Humans have been the source of intentional and unintentional nonindigenous introductions for centuries (Mack *et al.*, 2000). Some nonindigenous species remain localized in their new environment and are not considered invasive, while other species become locally established and spread to become abundant, earning the status of an invasive species (Kolar and Lodge, 2001). Quantifying the threat of an invasive species may seem elusive because impacts may not be seen immediately after release or in the same location (Simberloff, 2005). Once impacts are seen, eradication can be expensive or even impossible (Pimentel *et al.*, 2000). Conversely, immediate economic benefits, as with sport fish or food fish, often outweigh concerns about future ecological damage. As knowledge of the adverse affects of invasive species grows, the use of risk assessments to determine potential effects of nonindigenous species before a species is introduced is becoming more common (Kolar and Lodge, 2002; Marchetti, Moyle and Levine, 2004).

With regards to invasive species, risk assessment is defined as a process that evaluates the likelihood of adverse ecological effects resulting from introduction (Simberloff, 2005). Many current invasive species risk assessments use a four stage structure including introduction, establishment, spread, and impact of the species considered (Kolar and Lodge, 2002). Species with a high likelihood of establishment, rapid potential for spread, and high impact potential are the most invasive in ecosystems into which they are introduced. Based on life-history characteristics and knowledge of invaders, previous studies have identified a variety of species that are likely to become invasive based on all four invasion stages (Kolar and Lodge, 2002; Marchetti *et al.*, 2004). Tilapia speciea fit this profile. In fact, feral tilapia populations currently exist in every country in which they have been introduced, as long as environmental conditions were acceptable (Canonico *et al.*, 2005). In this manuscript, we used thermal tolerance limits and water temperature data to create a spatially-explicit, quantitative assessment of risk of establishment for tilapiine species in Georgia.

Tilapia is the common name for a collective group of warm fresh-water fishes distributed among the three genera *Oreochromis*, *Saratherodon* and *Tilapia*. Tilapia have long been an important staple protein source around the world (Popma and Masser, 1999), but have only more recently increased in popularity in the US as both a food fish and as a forage fish to increase the growth of desirable sportfish (Fuller, Nico and Williams, 1999). As tilapia's popularity has increased, the economic benefit of farming or stocking the species has also increased. High tolerance to low water quality, fast growth potential, omnivorous diet, ease of propogation and resistance to disease are among the species most valued attributes (Chervinski, 1982). Unfortunately, as with most non-indigenous fishes, the characteristics that make tilapia attractive for aquaculture also give them the potential to alter aquatic communities where they are introduced (Courtenay 1997).

Because government agencies at all levels base regulatory decisions on risk assessments (Simberloff, 2005), the goal of this study was to provide establishment risk information on a population level to assist in the consideration of management decisions regarding farming or release. Two concerns regarding risk assessment are the inability to quantify the risk and the inability to imagine all possible risks associated with invasion (Simberloff, 2005). Many results are presented in the qualitative form of low, medium or high without corresponding quantitative values. By borrowing techniques from toxicological risk assessment (Suter, 1993), we can produce quantitative results. As noted above, it is impossible to imagine all forms of risk involved in introducing a nonindigenous species. Our study is limited to a single step in the invasion process: establishment threat as a function of predicted stream temperature.

Many risk studies focus on one or two stages of invasion relevant to the species being considered, such as introduction or establishment (Leung, Drake and Lodge, 2004; Meentemeyer *et al.*, 2004; Herborg *et al.*, 2007). Some of these studies consider population dynamics and allee effects (Leung *et al.*, 2004), while others use geographic data to identify potential sites for future establishment (Meentemeyer *et al.*, 2004). Establishment risk is based on the characteristics of the species as well as the vulnerability of the ecosystem where it is introduced (Mack *et al.*, 2000). To provide a quantitative risk model for the establishment of tilapia, we used cold water tolerances of tilapia to evaluate vulnerability of the ecosystem based on minimum surface water temperatures within Georgia.

Cold water tolerances of tilapia have been studied extensively under a variety of conditions (Hargreaves, 2000). In previous work, we used a Mayfield hierarchical logistic regression model to predict daily survival rates of tilapiine species in different thermal regimes (Wilson, Nibbelink and Peterson, in review).

By combining these results with predictions of water temperature in a Geographic Information Systems (GIS) framework, we used a spatially-explicit risk assessment approach, useful for decision-making regarding culture and transport of the species. Using GIS allows environmental data and species tolerances to be viewed and manipulated spatially, increasing the effectiveness of environmental management planning (Perez *et al.*, 2003; Meentemeyer *et al.*, 2004).

METHODS

Statewide Temperature Predictions

We used a model produced by Dyar and Alhadeff (1997) to simulate stochastic water temperatures for streams in Georgia based on geographic attributes. The model predicted stream temperature using the following sinusoidal curve equation:

$$\text{Temperature } (°\ \text{C}) = M + A\,[\sin\,(bt + c)] \qquad (3.1)$$

where M was the long-term mean stream temperature (harmonic mean coefficient), and A represented the annual cycling of temperature (amplitude coefficient). M and A were estimated using sub-models (3.2 and 3.3 below). Day of the year was represented by t, and b and c were estimated model coefficients. The model was developed using data from 78 stations around Georgia taken between 1955 and 1984 (Dyar and Alhadeff, 1997). Nested equations predicted M and A based on variations in latitude of the stream, drainage area and elevation (Dyar and Alhadeff, 1997):

$$M = 42.68 - 0.833*L + 0.743*\log D - 0.00133*E \quad (3.2)$$

$$A = -7.40 + 0.426*L + 0.947*\log D - 0.00075*E \quad (3.3)$$

where L was stream latitude in decimal degrees, D was stream drainage area in square miles and E was elevation in feet above sea level.

Stream temperatures were simulated for each of the 9772 stream segments that drain at least 40 sq miles in the state of Georgia. For each stream, the average values for M and A were calculated based on latitude, drainage area and elevation using the equations of Dyar and Alhadeff (1997) (Eq 3.2 and 3.3). We introduced stochasticity into the model by varying the harmonic-mean coefficient (M) and the amplitude coefficient (A) (Appendix 3.1). We added an error term which was randomly generated by multiplying the standard deviation of each coefficient by a normal random deviate. The attributes used to create predictions were derived from the 1:100,000 scale National Hydrography Dataset (NHD,USGS, 2007) and associated NHDPlus data (Horizon Systems Corp., 2007) for all streams in the state of Georgia. We stored and manipulated all datasets in ArcGIS Personal Geodatabases (ESRI, 2005). In the NHDPlus dataset, drainage area was represented by the variable CUMDRAINAGE, and elevation at the downstream terminus of each stream segment by the variable MINELEVSMO (Horizon Systems Corp., 2007). Latitude was calculated for the same point using a coordinate identification tool for vector line features (Scheitlin, 2003).

We simulated one hundred years for each stream segment. Because of the sinusoidal nature of the curve, predictions could be made for each day of the year (t). We produced a daily temperature prediction for 180 days starting November 1st for each year for each stream. Overall, we produced daily temperature predictions for 9772 stream segments, 180 days a year, over a 100 year time span or 175,896,000 daily temperature predictions. All predicted values less than 0 °C were adjusted to 0 °C because of the freezing properties of water.

We validated the model's predictive power by comparing predicted values with an independent dataset of stream temperature measurements from the STORET database (EPA, 2004). All stations in Georgia with data from 1999-2008 were downloaded and snapped to the NHD. To ensure stations were placed on the correct stream, the snapping was validated by comparing stream names. STORET stations with stream names that did not match the NHD were discarded. The stream temperature measurements were matched to predicted values based on their location and the month and day the measurement was collected.

We used 2326 temperature measurements in the STORET database to validate our predictions in two ways. First, the root mean squared error (RMSE) was produced for all temperature predictions. Secondly, to ensure that variation in the predictions was sufficient to account for the variability found in nature, we calculated the percentage of observations which fell within the 95% confidence intervals (95% CI) of the predictions. The RMSE and the 95% CI percentages were calculated statewide and for each Level III Ecoregion (Omernik, 2007).

Survival Model

To determine areas of highest vulnerability to tilapiine establishment, we combined the surface water temperature prediction with a survival model based on laboratory thermal tolerance experiments (Wilson *et al.*, in review). The model uses Mayfield hierarchical logistic regression to estimate daily survival probabilities based on average minimum temperatures, rate of temperature decline, tilapia weight-length ratio and tilapia strain:

$$\text{Probability of daily survival (\%)} = 1/(1+(\mathrm{EXP}(-9.4007 + 1.0149*A + 12.9761*W + 3.4252*D - 0.1423*S))) \tag{3.4}$$

where A represented average sustained minimum temperature, W was the weight-length ratio of an individual, D was the slope of the temperature drop, and S was tilapiine strain. To calculate average minimum temperature, the lowest predicted value for each year was found for each site. This temperature and temperatures for the previous ten days and following ten days were averaged, creating a 21-day average minimum temperature for the year (A) (Figure 3.1, Appendix 3.2). The properties of the sinusoidal curve ensured that this would be the coldest period of the year. We considered the 60 days occurring before the 21 day minimum as the acclimation period, and calculated the slope of the drop rate (S) for this time. The difference between the maximum and minimum temperatures was divided by 60 to calculate drop rate for each year at each stream segment. The numbers of days selected for both the average minimum and the drop rate were selected as they correspond to the experimental values used to create the model (Wilson *et al.*, in review). We calculated survival probabilities for each species by calculating a daily probability of survival using the average weight-length ratio of fish used in the experimental process: 0.049 grams/mm (W). To model population level effects, stochastic variation of individual response was included by adding the random error term from the original model. An error term for each simulation was randomly generated by multiplying the standard deviation of the random effect by a normal random deviate. Survival probabilities can be estimated for any combination of the variables over any length of time by raising daily survival probability to the number of days exposed ($[\text{ProbSurvival}]^{\text{days}}$). The daily probability of survival was raised to the 21st power, resulting in a cumulative probability of survival for that year (Wilson *et al.*, in review). By combining experimentally derived cold temperature tolerances and geographic predictions of daily water temperatures, our comprehensive model predicts average annual survival probabilities for both Nile tilapia (*O. nilotica*) and a hybrid of *O. nilotica* and *O. aureus* (S).

RESULTS

Our goal in producing stochastic temperature simulations was to capture realistic variability in winter temperatures. Results of simulating 100 sample years indicate a good balance between variability and accuracy. All temperature measurements from the validation data set (EPA STORET) fell within the range of temperatures simulated by the model for each stream segment. Statewide, the RMSE is 3.3 ° C and 74% of the STORET temperature measurements values fell within the 95% CI bounds of the simulated values. By evaluating the RMSE and the percentage for each Level III Ecoregion in the state, we found that simulations in the north were more accurate than those in the southern part of the state (Table 3.1). Overall, our simulations underestimated the magnitude of variability seen in recent years. Because historic data used to build the temperature model showed less variability, current temperatures fall closer to the tails of the normal curve used in simulations. Environmental variability could increase in the future requiring the addition of further variance in the model. On a stream to stream basis many extreme values appeared to support observations. For example, the highest predicted temperature for Spirit Creek, a tributary of the Savannah River, was 17.2° C. The highest measured temperature at the site, taken on January 31, 2002, was 16.9° C (EPA, 2004).

The comprehensive model estimated a probability of annual survival (%) for a population in a given stream segment. For Nile tilapia, the model predicts average probabilities of annual survival between 10.6% and 65.0%. Although all streams had at least one prediction of absolute mortality (<1%), results suggest that survival of the species is possible throughout Georgia. Of the 15,204 stream kilometers modeled in this analysis only 944.3 km were predicted to have an average annual survival rate of 50% or greater (Figure 3.2). In other words, approximately 6% percent of Georgia's streams have conditions where tilapia had a greater probability of survival than mortality in a given year. Results for the hybrid species are similar, but showed slightly less variation in annual survival probabilities. The hybrid models produced average probabilities of survival between 13.6% and 63.1%, with 1178.6 km of stream miles at 50% risk or greater (Figure 3.3).

The comprehensive model quantified risk on a stream segment to stream segment basis, but the model results can be aggregated to examine risk on a coarser scale, such as watersheds, basins or even counties. We examined risk at the 10-digit Hydrologic Unit Boundary (HUB) scale (USGS, 2007) as it may be useful to manage using watershed boundaries. In order effectively communicate results at a larger scale, an endpoint needed to be chosen to separate the probabilities into the binary response of survival or mortality. We examined four cutoffs (1%, 5%, 10% and 50%) to show the possible effects of this decision (Figure 3.4(a-d)). Changing the cutoff point between survival and mortality can bias the results conservatively or liberally depending on management goals. The binary cutoff value was the first of two sets of cutoff values used to group data into understandable results.

To summarize the data more easily, our second cutoff values classify risk frequency levels into areas of very high, high, moderate, low and very low risk (Table 3.2). We used a modified framework based on a logarithmic scale (Standards Australia/Standards New Zealand, 2004), a version of which has been previously used in non-native species risk identification (CABI Bioscience *et al.*, 2005). For this analysis we used a 50% cutoff of survival or mortality to create a percentage of years survived for the following analysis. For example, if survival was predicted 1 out of every 5 years, we display a survival risk of 20%. All watersheds in Georgia are considered to have high risk of tilapia establishment (Figure 3.5). Watersheds with a high risk of establishment predict that tilapia would survive at least one year out of every three. Notably, even if the survival/mortality cutoff were changed to 1%, the high risk designation for all watersheds would not change.

DISCUSSION

By combining two previously developed models, for water temperature and tilapia survival, with stochastic simulation, we modeled establishment risk for tilapia in Georgia. Although each individual model was evaluated and had an associated error term, we have yet to arrive at a way to validate overall model predictions without releasing tilapia into Georgian streams. Historical data suggests that tilapia have previously established populations on Georgia's eastern coast and in Lake Seminole, but likely perished during the winter of 1989 (Fuller *et al.*, 1999; Benson, personal communication). This result supports our model predictions, but little information exists regarding the extent of the prior establishment or confirmation of complete elimination. The model should therefore be used as a guide to help with management decisions, and not as proof that a population would or would not survive at a given point. We present our results as probabilities to acknowledge the uncertainty that exists in predicting future environmental states (Suter, 1993).

To classify quantitative results into useful categories, we chose a cutoff value that differentiated between survival and mortality (50% probability), and additional cutoffs to describe levels of risk (Table 3.2). According to Suter

(1993), cutoff values should be chosen based on the possible effects of an introduced species on its ambient environment or what is considered ‘acceptable risk’. Although multiple studies have examined effects of tilapia on native ecosystems (Ogutu-Ohwayo, 1990; Canonico *et al.*, 2005; Peterson *et al.*, 2006), none supply a quantitative measure of effects that could be directly applied to this model. As we are unaware of the comprehensive effects tilapia may have in Georgia, we made these determinations arbitrarily. As the body of literature regarding tilapia effects grows, we encourage new endpoints to be used and tested. To create effective endpoints, value judgments must be made as to what is considered an acceptable level of risk. For some, a 1% annual survival probability for an exotic would be reasonable, yet 5% would not be. For others, a guarantee of periodic mortality would justify the economic benefits received from the species. We presented the comprehensive model results in the five risk categories to make the results easier to understand, but acknowledge that this is only a sample framework that may not directly apply to management decisions depending on individual management goals.

Suter’s (1993) definition of risk assessment is “the process of assigning magnitudes and probabilities to the adverse effects of human activities”. True risk assessment should evaluate both the degree of risk, the potential cost of being incorrect and the risk involved in potential alternatives. We presented a single component of what could be expanded into a more complex risk model involving the other stages of invasions, spread and impact (Kolar and Lodge, 2002). Tilapia are invasive in other ecosystems and have caused environmental damage (Canonico *et al.*, 2005), indicating that being incorrect could have serious consequences.

Altering regulatory measures controlling tilapia culture and transport would be best served by investigating the third and fourth invasion stages of spread and impact. Little is currently known regarding the potential for spread of tilapia. Some movement within stream systems has been previously documented (Peterson, Slack and Woodley, 2005). Also, this study did not include thermal refugia which may be provided by groundwater inflows or industrial water use during the winter months. The impacts of tilapia on native ecosystems have been widely documented (Ogutu-Ohwayo, 1990; Canonico *et al.*, 2005; Peterson *et al.*, 2006). Tilapia are able to compete strongly with other species for resources including food, habitat and spawning sites because of their life history characteristics (Canonico *et al.*, 2005). The most common impact of introduced tilapia involves excluding native fish from adequate breeding grounds (Canonico *et al.*, 2005). Secondarily, tilapia are typically herbivores or detritivores, but can also consume eggs and larvae of other fish (Canonico *et al.*, 2005). Tilapia can cause eutrophication by consuming large amounts of benthic algae and releasing nutrients into the water (Canonico *et al.*, 2005). Tilapia are associated with this ‘ichthyoeutrophication’ because they can thrive at higher densities than many other species (Canonico *et al.*, 2005).

The effects listed above have been seen worldwide, but little is known regarding their potential impacts in the southeastern U.S. In a study examining the feeding habits of three Centrarchidae species native to the Mississippi river system and Nile tilapia, Peterson et al. (2006) do not find direct competition for food resources. However, Nile tilapia represent 66% of all individuals collected (Peterson *et al.*, 2006). The total potential for impact should be investigated, along with potential alternatives which could cause similar economic benefits without endangering native populations or ecosystems. The fact that biological invasions can be irrevocable should also be considered when decisions are made (Simberloff, 2005). We suggest the development of a decision network, such as a Bayesian Belief Network, to effectively quantify all aspects of potential invasion including alternatives (Varis, 1997; Marcot *et al.*, 2001; Rieman *et al.*, 2001; Nyberg, Marcot and Sulyma, 2006).

We predicted probabilities of annual survival for tilapiine species, by combining an experimentally-derived tilapia thermal experience model (Wilson *et al.*, in review) and simulations of annual water temperature regimes in streams throughout Georgia,. Although this study investigates one stage of the invasion process, it represents one key step towards determining total risk of tilapia establishment within Georgia’s boundaries. Our quantitative analysis provides probabilities of survival and risk maps which can be easily interpreted by aquatic managers around the state. As invasive species such as tilapia become increasingly pervasive, geographically explicit risk assessment tools like these can help us to understand environmental consequences before the species is impossible to eradicate.

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Table 3.1. Validation of predictions using STORET data.

Level III Ecoregion	RMSE	Percent of STORET measurements within 95% CI of simulated values
Blue Ridge	2.442	84.3%
Piedmont	3.172	75.0%
Ridge and Valley	3.048	70.6%
Southeastern Plains	3.416	74.4%
Southern Coastal Plain	3.610	73.6%
Statewide	3.306	74.7%

Table 3.2. Risk assessment cutoff values and definitions adapted from AS/NZS 4360.

Risk	Frequency of survival.
Very high	Once a year or more frequently
High	Once every three years
Moderate	Once every ten years
Low	Once every thirty years
Very low	Once every 100 years

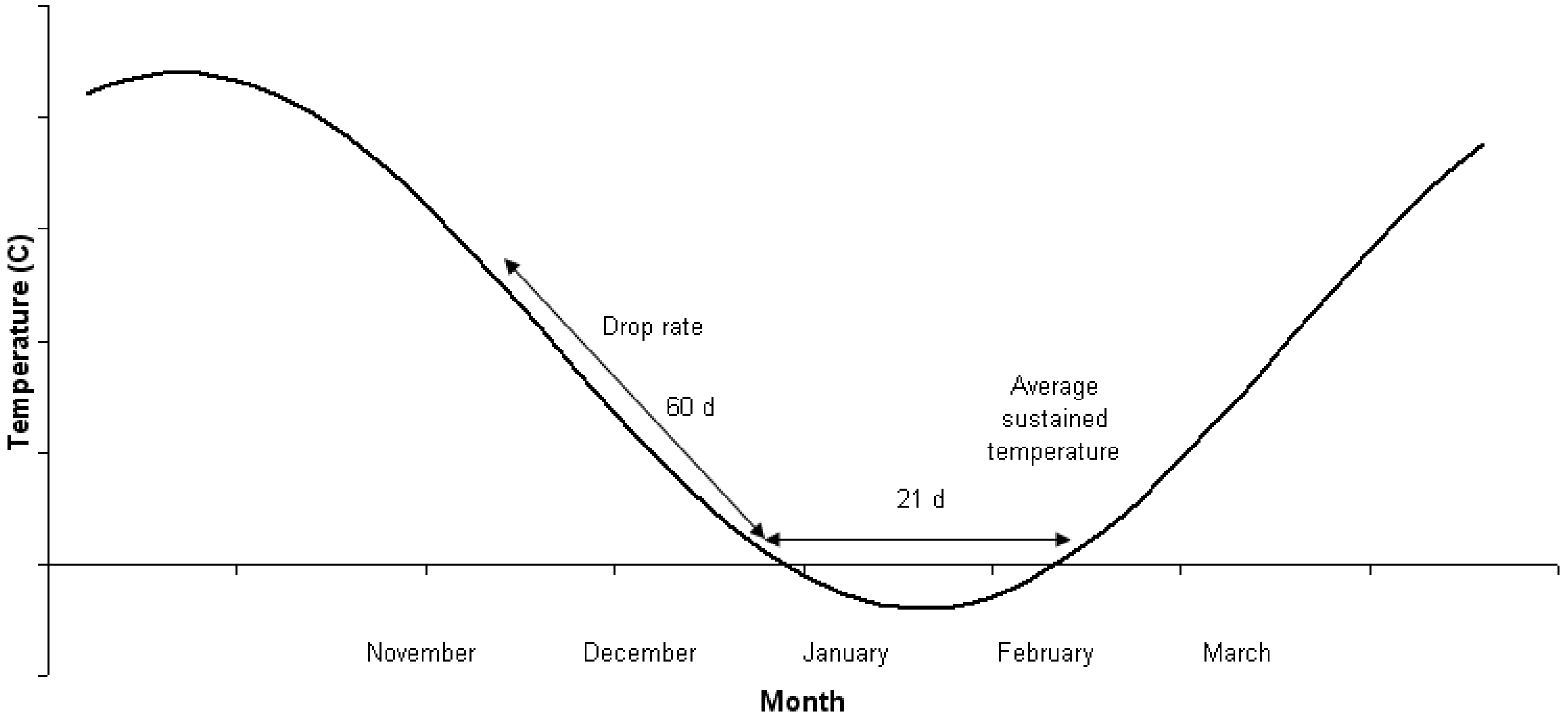

Figure 3.1. A graphical representation of the simulation model. Average sustained temperature values and drop rate were calculated for each year at each site. The values were then used in equation 3.4 to determine probability of annual survival.

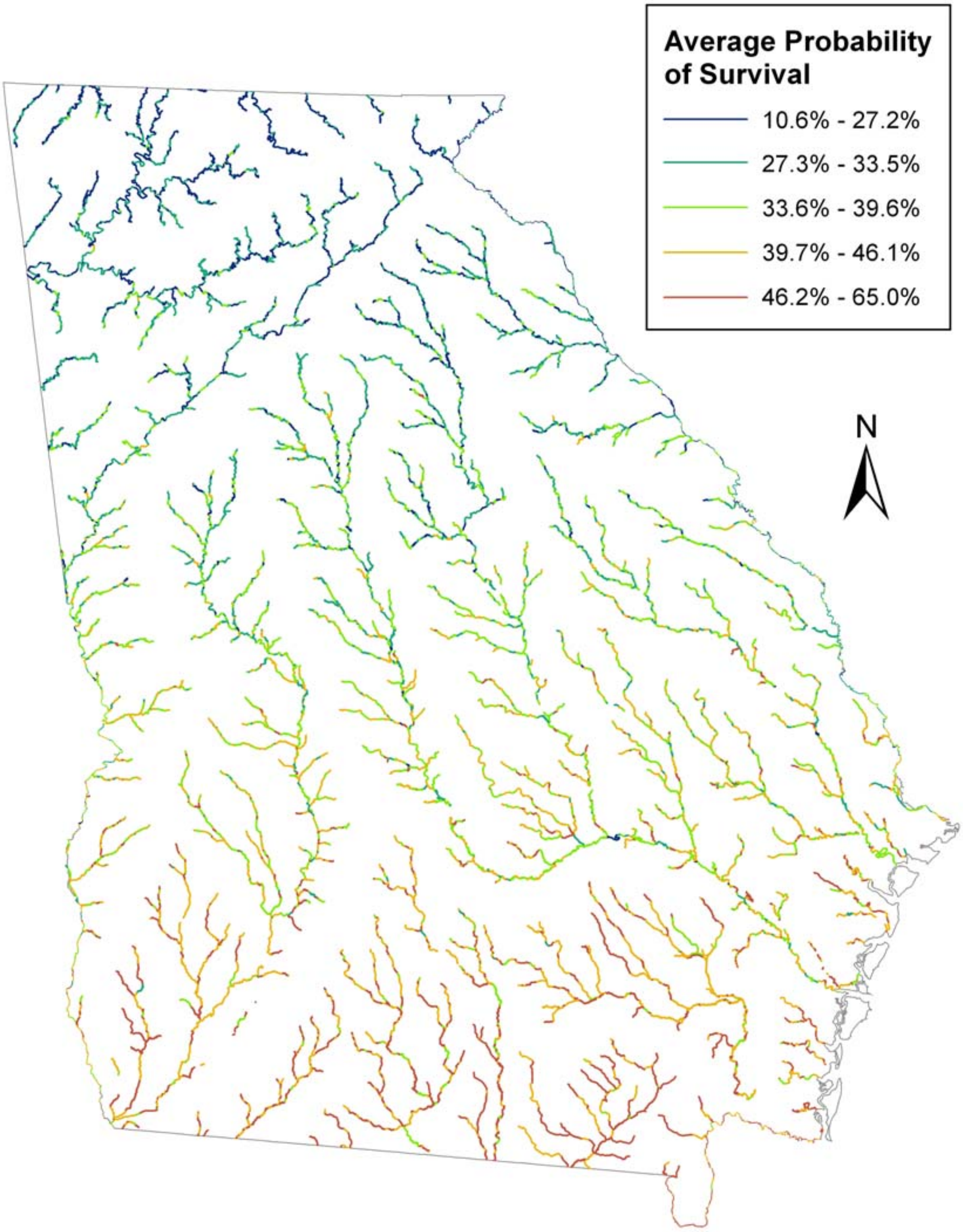

Figure 3.2. Average probability of annual population survival for Nile tilapia in Georgia streams. Areas shown in red indicate the highest probability of survival.

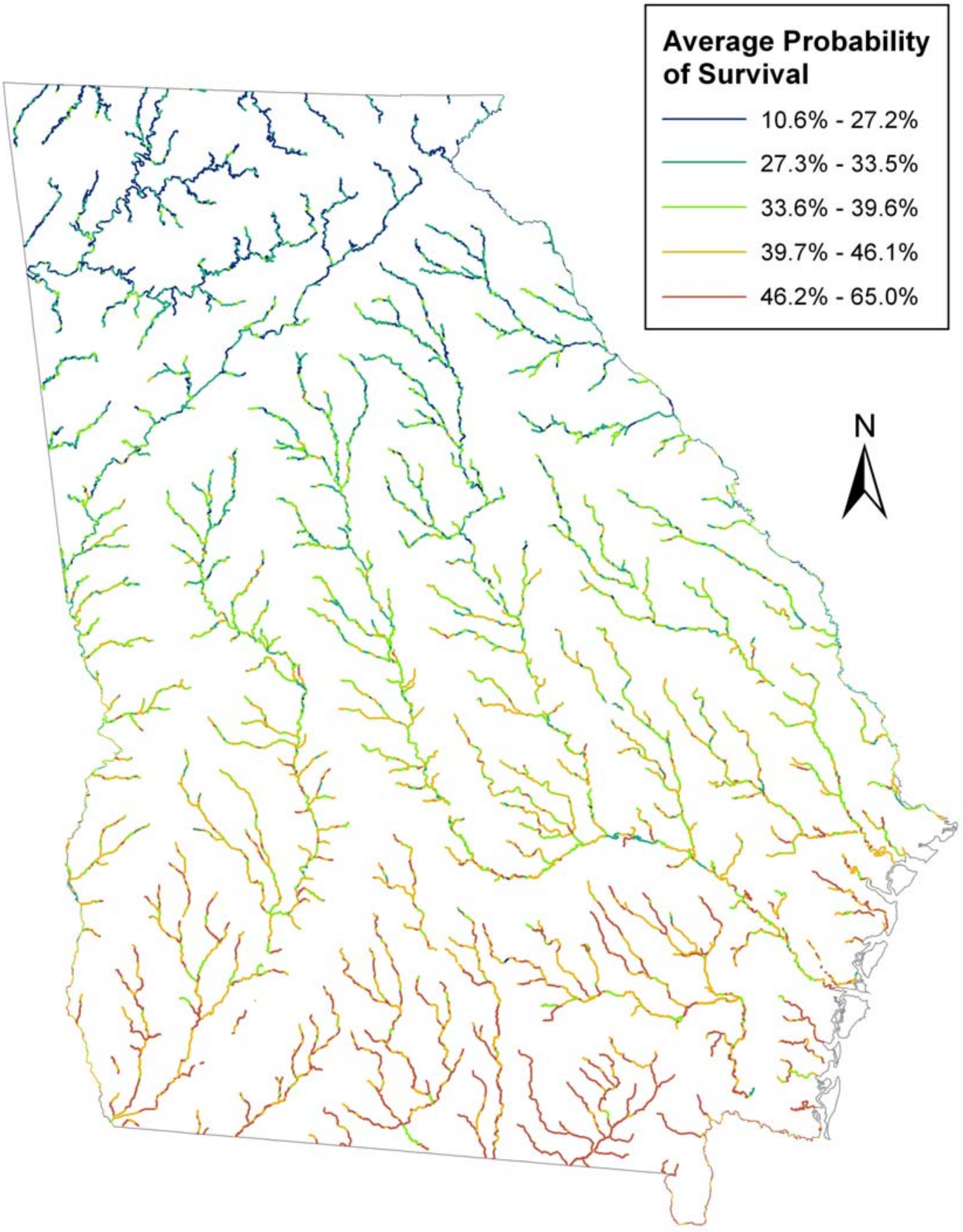

Figure 3.3. Average probability of annual population survival for Hybrid tilapia in Georgia streams. Areas shown in red indicate the highest probability of survival.

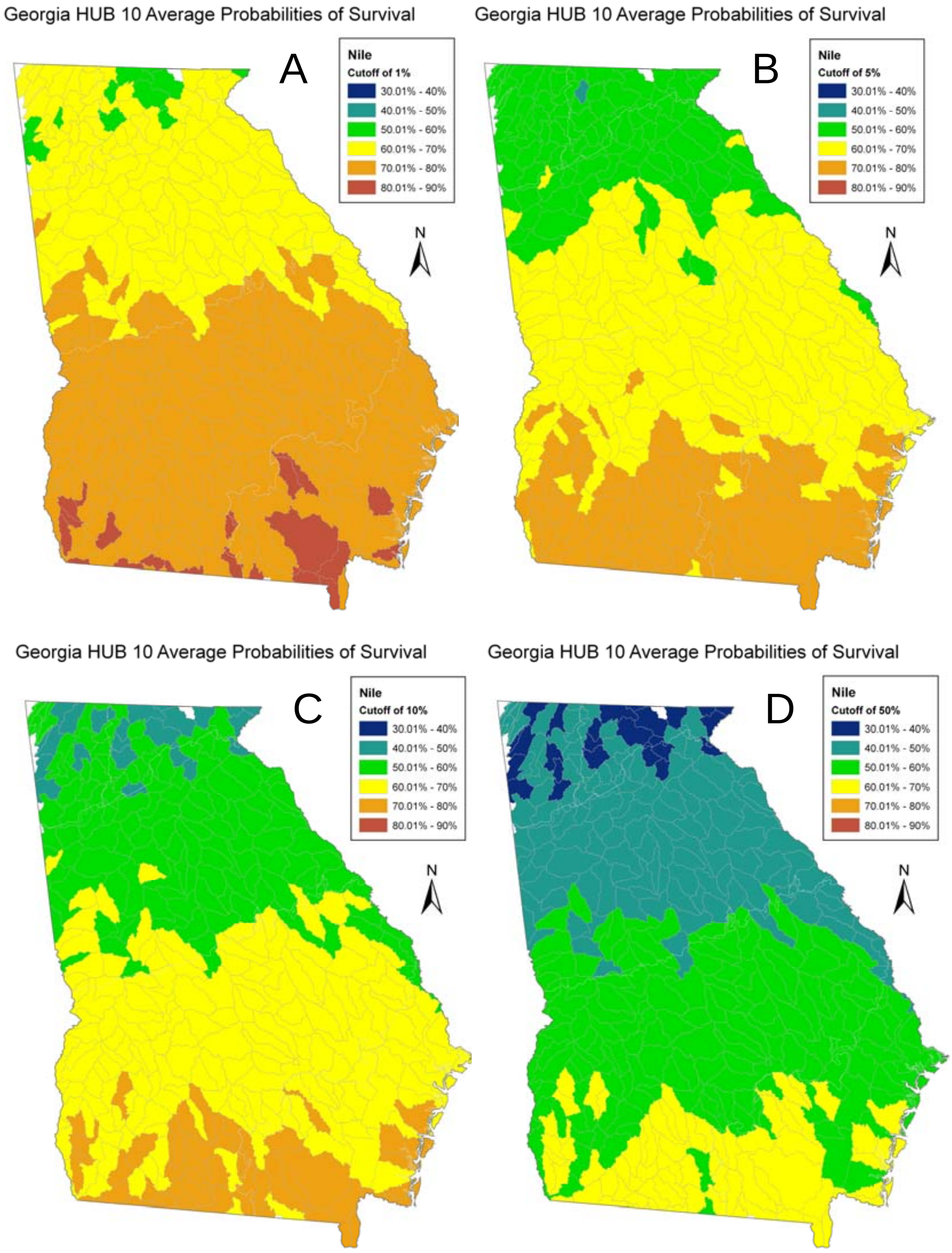

Figure 3.4. Probability of annual survival analyzed at a 10-digit HUB scale. Each map shows a different survival/mortality cutoff: A, 1%; B, 5%; C, 10%; D, 50%. As you increase the cutoff point, apparent probabilities decrease leading to a decrease in risk. The HUB values are an average of individual stream probabilities of survival, weighted by stream length.

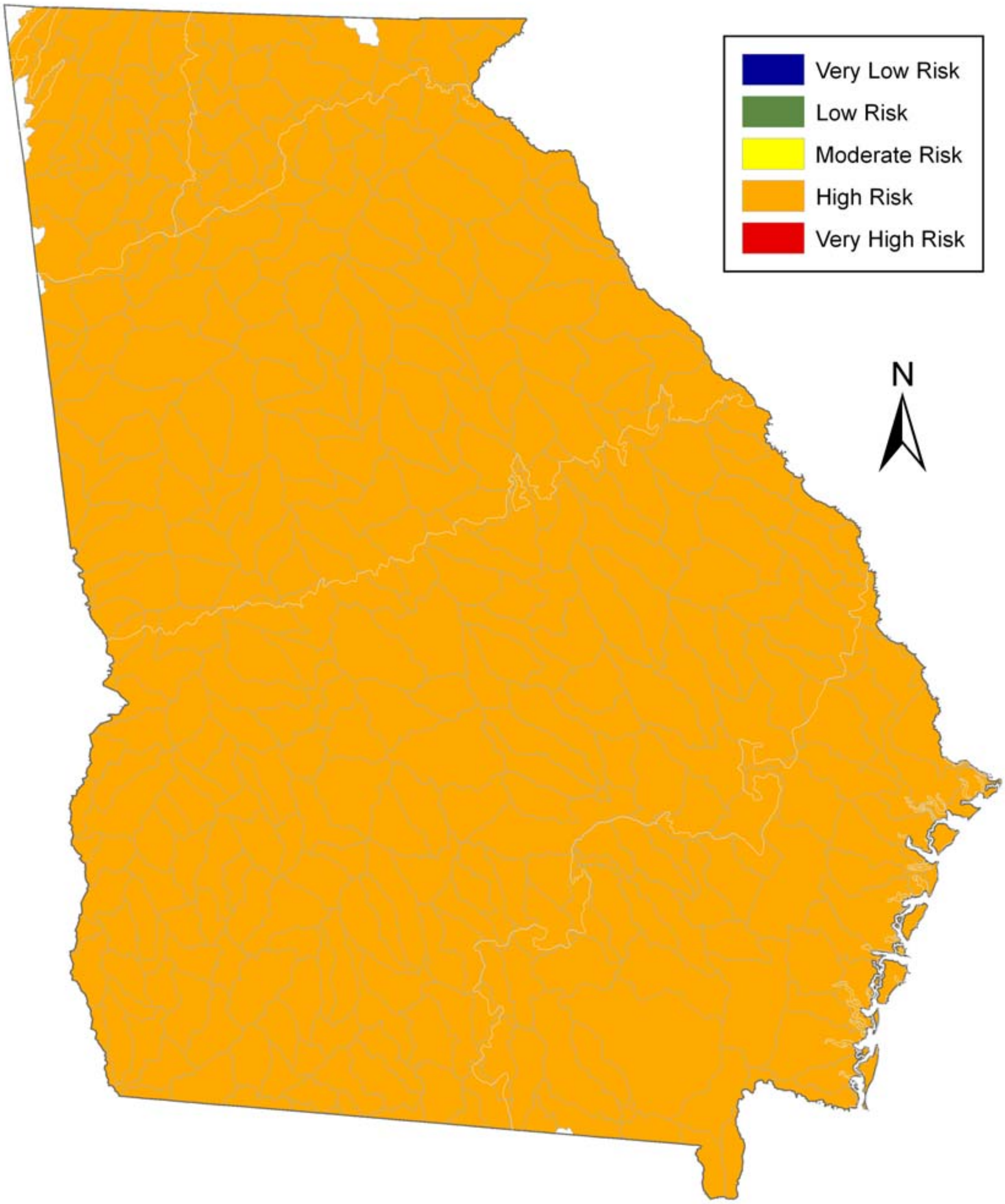

Figure 3.5. Probability of annual survival for Nile tilapia analyzed using a risk framework at a 10-digit HUB scale. All Georgia watersheds fell into the category of high risk, regardless of the survival/mortality cutoff chosen. The HUB values are an average of individual stream probabilities of survival, weighted by stream length.

CHAPTER 4

SUMMARY

Using a framework provided by a four stage invasion model (Kolar and Lodge, 2002), I have examined the risk of establishment of a non-native aquatic fish (tilapia species) in Georgia. In particular, a quantitative approach is presented to address the first stage of invasion, establishment, which can inform management decision-making regarding transport and culture of tilapia.. Probability of annual survival of tilapia was estimated for all streams in Georgia based on cold thermal tolerance levels and geographic temperature predictions. These survival probabilities can be used as a proxy for establishment when establishment is defined as survival through an entire year (making it through the winter).

Tilapia survival was dependent on average sustained temperature, drop rate, weight-length ratio and strain of tilapia (Wilson, Nibbelink and Peterson, in review). Average sustained temperature had the largest effect on survival. Model results were translated to geographic predictions using simulation techniques. Daily temperature values were simulated based on latitude, elevation and drainage area of a given stream (Dyar and Alhadeff, 1997). The temperature simulations were inserted into the survival model to create average probabilities of survival for a tilapia population in Georgia streams.

The second and third steps of the invasion risk assessment model (spread and impact) were examined with a literature review in chapter one. The potential for tilapia to spread from a stream to other water bodies or cause impacts once established has not been directly examined in Georgia waters. Further research is needed in this area before a comprehensive risk assessment can be completed. Effects of tilapia and other invasive species have been well documented in a variety of studies (Courtenay 1993; McKaye *et al.*, 1995; Courtenay 1997; Pimentel *et al.*, 2000; Canonico *et al.*, 2005; Peterson, Slack and Woodley, 2005). As noted in Peterson *et al.* (2005), allowing the release of individuals under the belief that establishment would not occur is problematic. The literature contains countless examples of unforeseen negative impacts of release and the expense or inability to remove problematic populations (Wilcove and Bean, 1994; McKaye *et al.*, 1995; Courtenay 1997; Lyons *et al.*, 1998; Wilcove *et al.*, 1998; Simberloff, 2003; Canonico *et al.*, 2005; Peterson *et al.*, 2005). Management decisions should be made with the awareness that great uncertainty remains in predicting the success or failure of potential invaders.

Management of nonindigenous species is complex and will continue to increase in complexity as globalization increases. Finding an appropriate balance between increasing economic benefits and preserving ecological resources is a difficult process requiring a variety of tools. Risk assessment is one such tool, despite its caveats. By combining experimental results with spatially-explicit geographic predictions, we provided a risk assessment for the establishment of tilapia in Georgia's streams. Combined with decisions about acceptable levels of risk, these models could be directly applied to management decision-making.

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APPENDIX A

SIMULATION CODE FOR WATER TEMPERATURES USING SAS

```
data stats10;

do streamid = 9001 to 9772;
set stream10s;    by streamid;

      M_hat = 42.68-(0.833*lat)+(0.743*log(drain))-(0.00133*elev);

        * M =   m_hat + m_var;

      A_hat = -7.40+(0.947*log(drain))+(0.426*lat)-(0.00075*elev);
            *y = A_hat(0,0.7,seed);

        * A =   A_hat + A_var;

      do year = 1 to 100;
         M = m_hat + 0.045291*normal(0);
         A = a_hat + 0.079259*normal(0);

            do day = 60 to 179;
                  b = (2*3.14159265)/365;
                  c = 2.69;
                  T = M + A*(sin(b*day+c));
                  output;
                  keep streamid comid lat drain elev m a t day year;
            end;
      end;
 end;
stop;
run;
```

APPENDIX B

CODE TO APPLY SIMULATED WATER TEMPERATURES TO THE SURVIVAL MODEL USING VISUAL BASIC

```
Option Compare Database
Public Sub Survival()

Dim rsStream As ADODB.Recordset
Dim rsYear As ADODB.Recordset
Dim rsMin As ADODB.Recordset
Dim rsDay As ADODB.Recordset
Dim rsDrop As ADODB.Recordset
Dim rsAvg As ADODB.Recordset
Dim rsFinal As ADODB.Recordset
Dim rsDrop2 As ADODB.Recordset

Set rsStream = New ADODB.Recordset
Set rsYear = New ADODB.Recordset
Set rsMin = New ADODB.Recordset
Set rsDay = New ADODB.Recordset
Set rsDrop = New ADODB.Recordset
Set rsAvg = New ADODB.Recordset
Set rsFinal = New ADODB.Recordset
Set rsDrop2 = New ADODB.Recordset

Dim dblStartTemp As Double
Dim dblEndTemp As Double
Dim intDay As Integer
Dim intSDrop As Integer
Dim intStart As Integer
Dim intStop As Integer
Dim dblDrop As Double
Dim dblNile As Double
Dim dblCross As Double
Dim dblAverage As Double
Dim dN1 As Double
Dim dN2 As Double
Dim dC1 As Double
Dim dC2 As Double
Dim RanNile As Double
Dim RanCross As Double

rsStream.Open "SELECT DISTINCT ComID FROM stats ORDER BY ComID",
CurrentProject.Connection, adOpenKeyset, adLockOptimistic
rsStream.MoveFirst

Do While Not rsStream.EOF

    rsYear.Open "SELECT DISTINCT YEAR FROM stats" _
```

```
      & " WHERE (ComID = " & rsStream("ComID") & ") ORDER BY YEAR",
      CurrentProject.Connection, adOpenKeyset, adLockOptimistic

    rsYear.MoveFirst
    Do While Not rsYear.EOF

        rsMin.Open "SELECT TOP 1 T, DAY FROM stats WHERE ((Year = " &
rsYear("Year") & ")and(ComID = " & rsStream("ComID") & "))" _
            & " ORDER BY T", CurrentProject.Connection, adOpenKeyset,
adLockOptimistic
        rsMin.MoveFirst

        intDay = rsMin("Day")
        intSDrop = intDay - 70
        intStart = intDay - 10
        intStop = intDay + 10

        rsAvg.Open "SELECT avg(T) as AvgofT FROM stats WHERE ((Year = "
& rsYear("Year") & ")and(ComID = " & rsStream("ComID") & ")" _
            & " AND (DAY BETWEEN " & intStart & " And " & intStop &
"))", CurrentProject.Connection, adOpenKeyset, adLockOptimistic
        rsAvg.MoveFirst

        rsDrop.Open "SELECT T FROM STATS WHERE ((Year = " &
rsYear("Year") & ")and(ComID = " & rsStream("ComID") & ")" _
            & " AND (DAY = " & intSDrop & "))",
CurrentProject.Connection, adOpenKeyset, adLockOptimistic
            dblStartTemp = rsDrop("T")
                'rsDrop.MoveNext
        rsDrop2.Open "SELECT T FROM STATS WHERE ((Year = " &
rsYear("Year") & ")and(ComID = " & rsStream("ComID") & ")" _
            & " AND (DAY = " & intStart & "))",
CurrentProject.Connection, adOpenKeyset, adLockOptimistic
            dblEndTemp = rsDrop2("T")
            dblDrop = (dblEndTemp - dblStartTemp) / 60

        RanNile = Excel.WorksheetFunction.NormInv(Rnd(), 0.975,
5.69215)
        RanCross = Excel.WorksheetFunction.NormInv(Rnd(), 0.975,
5.69215)

            dN1 = -1 * ((-9.4007) + (1.0149 * rsAvg("AvgofT")) +
(12.9761 * 0.049) + (3.4252 * dblDrop) + (-0.1423 * 1) + RanNile)
            dN2 = (1 + Exp(dN1))
            dblNile = (1 / dN2)
            dC1 = -1 * ((-9.4007) + (1.0149 * rsAvg("AvgofT")) +
(12.9761 * 0.049) + (3.4252 * dblDrop) + (-0.1423 * 0) + RanCross)
            dC2 = (1 + Exp(dC1))
            dblCross = (1 / dC2)

            rsFinal.Open "tblFinalNew", CurrentProject.Connection,
adOpenKeyset, adLockOptimistic, adCmdTable
                   rsFinal.AddNew
                    rsFinal![Comid] = rsStream("Comid")
                    rsFinal![Year] = rsYear("Year")
                    rsFinal![NileProb] = dblNile ^ 21
                    rsFinal![CrossProb] = dblCross ^ 21
```

```
                    rsFinal![Nile] = dblNile
                    rsFinal![Cross] = dblCross
            rsFinal.Update
        rsDrop.Close
        rsDrop2.Close
            rsFinal.Close
        rsAvg.Close
        rsMin.Close
    rsYear.MoveNext
    Loop
    rsYear.Close
rsStream.MoveNext
Loop
rsStream.Close

End Sub
```