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ARTICLE

Potential for Naturalization of Nonidigenous Tilapia *Orechromis sp.* in Coastal Louisiana Marshes Based on Integrating Thermal Tolerance and Field Data

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Abstract In the spring of 2009, tilapia *Orechromis sp.* were discovered in waterways near Port Sulphur, Louisiana. The potential naturalization of this population is of great concern as this invasive species can drastically disrupt fish community structure. Identification as an *Oreochromis* hybrid occurred through allozyme analysis, and our project investigated tilapia thermal tolerances at environmentally relevant salinities to determine lethal limits. In the laboratory, tilapia were acclimated for 1 month at salinities of 0.8, 7.0, or 15.0 ppt (g/L). Initial water temperatures of 26 C were decreased 2 C per day for all three acclimation salinities and 1 C per day for a cohort acclimated at 7 ppt until 100 % mortality of tilapia was observed. Tilapia acclimated to salinities of 0.8 and 7 ppt lost equilibrium at 10 C, while 15.0 ppt-acclimated individuals lost equilibrium at 8 C. Mortality within all acclimation salinities occurred between 8 and 6 C, with median lower lethal temperatures for all acclimations calculated between 6.0 and 6.6 C. Salinity acclimation prior to the thermal tolerance challenge did not alter cold tolerance. Experimental data were statistically modeled with field temperature data, and our results suggest low potential for tilapia naturalization, except in developed areas.

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Introduction

In the spring of 2009, a population of invasive, potentially naturalized tilapia (*Oreochromis* sp.) was detected in marsh canals located in the vicinity of Port Sulphur, Louisiana, USA (Louisiana Department of Wildlife and Fisheries Press Release, 5 May 2009 http://www.wlf.louisiana.gov/news/30211; Marshall 2009). The fish apparently originated from private ponds in the area that had been stocked for unknown reasons, as there were no recirculating-system aquaculture or research facilities within 65 km that had been permitted by the Louisiana Department of Wildlife and Fisheries (LDWF) for tilapia production. Potential establishment of this population was indicated by numerous tilapia nests at the site, as well as a large number of collected individuals that were carrying either eggs or fry in their mouths. Concerns about the expansion of this population and potential impacts on native fishes resulted in an extensive eradication effort by LDWF personnel, the success of which is still being evaluated. Control of this population is dependent on a number of factors, including the success of management actions and the location of physical barriers to dispersal. Survival of these fish also depends on responses to physiological challenges from higher salinities and colder temperatures than are typically found in their native habitats. In this paper, we assess the risk of persistence of these fish in surrounding wetlands based on laboratory studies of tilapia salinity and cold tolerance, and analysis of temperature conditions in these marshes over the last 18 years.

Cold tolerance is an important mechanism that contributes to the establishment of non-native fish populations, and lower lethal temperatures in teleosts can vary widely due to genetic, abiotic, and temporal factors (Cnaani et al. 2000). Breeding programs centered on selection, crossbreeding and hybridization of tilapia species have demonstrated changes compared to original stock in a number of relevant characteristics, including growth (Bentsen et al. 1998), disease resistance, salinity tolerance (Likongwe et al. 1996; Tayamen et al. 2002), and temperature tolerance (Tave et al. 1990). Interactions between abiotic physiochemical properties such as temperature and oxygen are believed to play a large role in temperature-dependant aerobic limits in fish through projections of oxygen limitations (Pörtner and Knust 2007). Although tilapia species of the genus *Oreochromis* are generally not considered cold tolerant due to their origins in tropical and subtropical regions (Trewavas 1982; Hargreaves 2000), juvenile blue tilapia (*Oreochromis aureus*) have demonstrated small (1 C) increases in thermal cold tolerance at isotonic salinity conditions of approximately 11.5 ppt (Zale and Gregory 1989). Tilapia have been introduced worldwide for a variety of purposes, such as foodfish culture, forage fish enhancement, live bait for recreational angling, and biological control of aquatic vegetation (FAO 2004). Conventional wisdom suggests that intolerance to temperatures under 10 C should limit tilapia survival and spread from points of introduction in temperate areas, however, populations have naturalized in Arizona, Alabama, California, Florida, Georgia, Mississippi, and Texas through intentional and unintentional releases (Costa-Pierce and Riedel 2000; Hargreaves 2000; Peterson et al. 2005).

Species preferred by aquaculturists are generally adapted to a wide range of stressful conditions such as salinity, temperature, confinement, and handling. In poikilothermic animals, rapid changes in body temperature cause a number of physiological problems, and aquaculture species often experience rapid temperature changes due to a number of industry practices, which may increase their adaptability to rapid changes over time (i.e., inherent evolved characteristics may allow tolerance to rapid changes in environmental conditions). Thermal tolerances of Nile tilapia (*O. niloticus*) have been reported between 7 and 12 C, with many tilapia species known to seek thermal refugia as temperatures decline (McBay 1961). Teleosts display critical thermal minima that are characterized as a pre-death thermal point in which individuals are immobilized during laboratory conducted thermal tolerance experiments and is often referred to as a loss of equilibrium (LOE; Beitinger et al. 2000). The ability to recover from a thermally induced LOE and the duration at which an individual could survive at this temperature would be critical in addressing the potential persistence of an invasive species. There is a lag between incapacitation and death as temperature decreases past the LOE and this window leaves fish vulnerable to predation as they are immobilized (Bennett et al. 1997). Moderate salinities can increase cold tolerance (Watanabe et al. 1989) and warmer temperatures can increase salinity tolerance (Schofield et al. 2011). The potential availability of thermal refugia and access to saline waters could aid in the persistence of an invasive population, as documented in coastal Mississippi (Peterson et al. 2005). There is often a separation in temperature between incapacitation and death, which might cause researchers to overlook the potential recovery of an incapacitated individual or population that is exposed to infrequent periods near the LOE temperatures. Therefore observations among incapacitation, time course to mortality at LOE, and median lower lethal temperature could be important predictors for survival.

Surveillance programs to detect the escape, invasion, and naturalization of tilapia in wetland systems are prohibitively expensive. However, inaction is unacceptable because tilapia are well documented to compete with and prey on native fish species (Schoenherr 1988; Courtenay 1991; Crutchfield 1995). Quantification of the risk of invasion and naturalization of specific habitats would greatly enhance the efficiency of surveillance programs and reduce the possibility of invasion and naturalization. Thermal and salinity tolerances of the Port Sulphur tilapia population must be documented to better evaluate potential naturalization. As part of the assessment and management response to the discovery of this species near Port Sulphur, we examined the minimum tolerable temperature for this population with respect to environmental salinity to determine whether salinity had any moderating effect on thermal mortality, and then used these data to predict naturalization potential. These experiments also indirectly provided data on potential thermal refugia based upon thermal tolerances exhibited by fish in the laboratory. The objectives of this study were to: (1) characterize minimal thermal tolerance of individuals acclimated to relevant regional salinity conditions, (2) determine the effect of time on mortality from LOE temperatures, and (3) integrate field temperature data with experimentally derived models to predict mortality to offer insights into the risk of dispersal, persistence, and potential naturalization.

Materials and Methods

Species Collection and Identification

Tilapia used for thermal tolerance testing were collected by Louisiana Department of Wildlife and Fisheries personnel in hoop nets set overnight in waterways near Port Sulphur, Louisiana (UTM zone 15, 820129 Easting, 3260374 Northing; Fig. 1). Fish were delivered to the LSU AgCenter, Aquaculture Research Station in Baton Rouge, Louisiana in May 2009 (Tilapia Importation, Culture, and Possession Permit # 1352). These fish were held in a closed recirculation system prior to thermal testing and were fed a commercially available 35 %

protein, 7 % fat, extruded 5-mm diameter feed (Burris Mill and Feed, Franklinton, LA, USA) once daily at a rate of approximately 3.5 % of initial body weight per day.

Recently euthanized tilapia (N=44) from Port Sulphur and reference individuals (N=5) of *O. niloticus* provided from a local tilapia hatchery (Til-Tech Aquafarm, Robert, LA, USA), which was 161 km from Port Sulphur, were kept on ice and delivered to the LSU AgCenter, School of Renewable Natural Resources for electrophoretic species identification in an effort to rule out local sources of tilapia from a known Louisiana aquaculture facility. Several individuals from Port Sulphur had deteriorated hepatic tissue, and these individuals were not used for species identification. Hepatic and muscle tissue from 22 wild-caught and 5 reference individuals were individually homogenized, placed in horizontal starch gels, stained, and scored for isocitrate dehydrogenase (IDH, EC 1.1.1.42) and adenosine deaminase (ADA, *EC 3.5.4.4*). Meristic counts of dorsal and anal fin spines and rays were recorded for all individuals utilized in thermal tolerance and recovery examinations following Trewavas (1982).

Thermal Tolerance and Recovery Experiments

Thermal tolerance testing was conducted in recirculation systems with groups of fish acclimated to salinities of 0.8, 7.0, or 15.0 ppt. Each salinity group was acclimated for 3–4 weeks at a temperature of 26 C and fed daily as previously described. Individuals (n=8) were held in triplicate tanks designated for mortality observations. Each 100-L tank was wrapped in black plastic and 1-cm thick insulation sheeting to visually isolate and insulate each tank. Each salinity treatment was fitted with a Dickson SK100 data logger (Dickson Inc., Addison, IL, USA) to record temperature hourly during the acclimation period and tolerance testing.

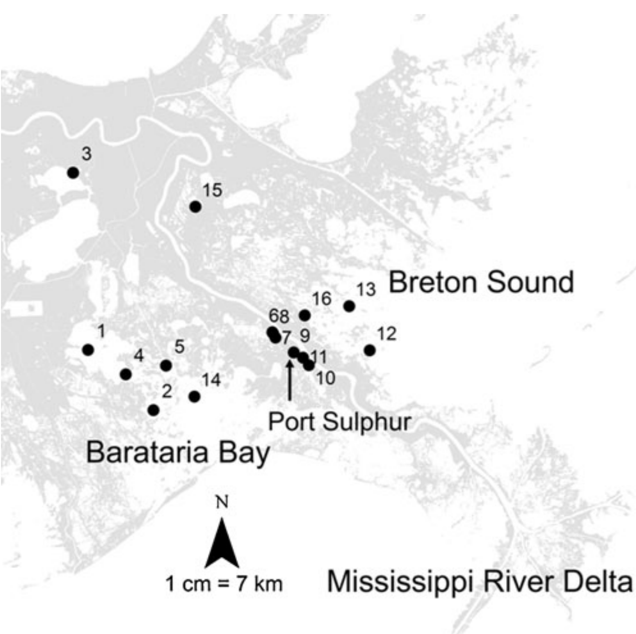

Fig. 1 Figure depicts six sites sampled in and near Port Sulphur (sites 6–11) and ten Coastwide Reference Monitoring System sites (sites 1–5, 7–16)

Treatment water was mixed to salinities of 0.8, 7.0, and 15.0 ppt with Coralife Salt Mix (Energy Savers Unlimited Inc., Carson, CA, USA), and was measured with a YSI 30 salinity/conductivity meter (YSI Inc., Yellow Springs, OH, USA). Hach water quality testing kits (Hach Co., Loveland, CO USA) were used to test alkalinity and hardness of the treatment water by titration (Hach methods 8203 and 8213, respectively). An Accumet® Basic AB15 pH meter (Fisher Scientific, Pittsburgh, PA USA) was used to determine pH. Total ammonia nitrogen (TAN) and nitrite were determined with a Hach DR 4000 Spectrophotometer based on the salicylate and diazotization methods, respectively (Hach methods 8155 and 8324, respectively). Alkalinity and hardness were determined at the beginning of each salinity trial, whereas salinity, pH, TAN, and nitrite were measured daily to insure similarity among tanks and acceptable water quality parameters during trials.

After the acclimation period the temperature in each recirculation system was reduced with digitally controlled water chillers. Temperature was decreased at a stepwise rate of 1 C per day at 24±1 h intervals for a group of fish acclimated to 7.0 ppt. Three other groups of fish acclimated to salinities of 0.8, 7.0, and 15.0 were subjected to a stepwise decrease in temperature of 2 C per day at 24-h intervals. Behavior related to temperature decreases were observed daily. These observations included: LOE, opercular movement, opercular flaring, and response to probing. Loss of equilibrium was observed and recorded as the temperature at which ≥90 % of the individuals in a replicate could no longer maintain a constant position in the water column and lay horizontally at the bottom the aquarium. Each tank was checked for mortalities prior to the daily temperature reduction. Mortality was determined by LOE, lack of opercular movement, and failure to respond to gentle probing (Behrends et al. 1990).

Recovery of fish from a temperature-induced LOE was investigated with individuals acclimated to a salinity of 7 ppt. A recirculation system comprised of six 100-L tanks was stocked with 12 tilapia per tank and maintained at 26 C. The temperature was decreased at a rate of 2 C per day as previously described. Once a temperature that induced LOE was reached (9.5±0.2 C, Mean±SD), two tanks each were returned to 26 C over periods of 48, 96, and 144 h at a rate of 0.6 C per hour. The number of individuals that recovered and died at 48, 96, and 144 h were recorded based on the criteria previously described.

Coastal Water Data Acquisition

To facilitate the use of the laboratory results for wetland and fisheries management, we integrated the laboratory data with field temperature measurements. Field temperature and salinity measurements were collected at six sites within and adjacent to Port Sulphur, LA monthly by the Louisiana Department of Wildlife and Fisheries (LDWF) during control efforts in fall and winter of 2009 (sites 6–11; Fig. 1). These sites provided data on the temperature regimes of developed, urbanized marshes and canals. Rather than rely solely on concurrently collected field measurements that only describe a brief temporal window, we elected to add data from the Coastwide Reference Monitoring System (CRMS; http://www.lacoast.gov/crms2/Home.aspx) for the 10 closest (range 8–45 km) CRMS sites in fresh, brackish, or salt marshes adjacent to Port Sulphur, LA for the period 3/10/1992 through 7/25/2010 (sites 1–5, 12–16; Fig. 1). These 10 sites were selected because they are in less developed marshes directly accessible to Port Sulphur from connecting waterways. Because escape from developed marshes and canals into less developed marshes is a concern, combining LDWF sites with CRMS sites allowed assessment of tilapia mortality contrasting warmer, modified temperature regimes at developed and urbanized LDWF sites with relatively undeveloped CRMS sites. Further distant CRMS sites in Louisiana marshes were not used because these sites have physical barriers (e.g., leveed canals or large landmasses) that would prevent tilapia dispersal from Port Sulphur (i.e., the fish were highly likely to be restricted by barriers to these marshes adjacent to Breton Sound and Barataria Bay, if escape from Port Sulphur occurred). These data would allow the estimation of the likelihood of over-winter survival of tilapia across a range of mild to severe winters. Although these sites are spatially distributed over a wide area, they are considered representative of marshes adjacent to Breton Sound and Barataria Bay by the Louisiana Coastal Wetlands Protection and Restoration Authority, and accordingly, we also considered these sites as representative of these marshes as well. Daily data included temperate maximum, minimum, and mean. Because winters have both colder temperatures and often higher salinities than spring and summer months from reduced river freshwater inputs and precipitation, winter (November through February) minima, mean temperature, maximum number of hours below 9 C and 10 C, and mean number of hours below 9 C and 10 C were calculated for each CRMS station. The calculation of maximum and mean number of hours below 9 C and 10 C were restricted to consecutive hours (e.g., 408 consecutive hours from 1/4/2001 to 1/22/2001 at CRMS site 7374527).

Data Analysis

Mortality trials were analyzed separately by acclimation salinity (0.8, 7.0, and 15.0 ppt), with logistic or log-binomial regressions (PROC GENMOD, SAS vers. 9.2, SAS Institute, Inc., Cary, NC) performed between temperature and tilapia mortality for each salinity level. Data on tilapia acclimated and subsequently held at constant temperature to examine mortality over time were analyzed by logistic regression (PROC GENMOD) of hours of exposure and mortality. Logistic regression was also used to analyze the relationship between hours post-LOE and mortality. With the exception of the log-binomial regression for the tilapia acclimated at 0.8 ppt, a logit link and binomial error distribution were used for all of these models (i.e., logistic regression). In the model for tilapia acclimated at 0.8 ppt, a log link and binomial error term (i.e., log-binomial regression) were used to more appropriately model the infrequency of mortality events (Spiegelman and Hertzmark 2005).

Models derived from logistic and log-binomial regressions were used to estimate hypothetical mortality of tilapia at each CRMS site in a functionally similar method to the estimation of presence-absence of round goby by Korni and Vander Zanden (2010). Using logistic functions derived hours of exposure and mortality experiments, exposure hours were input into each function to estimate a probability of mortality for each salinity level at each CRMS and LDWF site. Mortality was estimated for tilapia acclimated to salinities of 0.8, 7.0, and 15.0 ppt based on exposure hours to winter minima and winter mean temperatures. Mortality was also estimated for tilapia exposed to 10 C or lower for winter maxima and mean number of hours to predict mortality after LOE. All mortality estimations based on exposure hours were conducted in SAS, and estimated mortalities were imported into ARCMAP (vers. 9.3.1, ESRI, Inc., Redlands, CA, USA) and plotted for each CRMS and LDWF site based on winter minima hours, winter mean temperature hours, maximum number of hours below 9 C and 10 C, and mean number of hours below 9 C and 10 C.

Results

Species Identification

Based on hepatic tissue IDH, all reference fish (*O. niloticus*) scored a single slow moving band that was designated B1B1, signifying all fish had the same allele at each locus. The fish collected from Port Sulphur were scored B2B2, indicating all fish had the same allele at each locus, but were different from the reference fish. Scores of ADA from muscle tissue of reference fish yielded a single band that was designated B2B2. Of the 22 fish from Port Sulphur, 19 had a single band (B3B3), and 3 hybrid fish exhibited two bands. *O. niloticus* and *O. aureus* have one fixed allele each for the isozyme IDH, and based on the reference material, results indicate that the Port Sulphur fish were not *O. niloticus*.

Scoring ADA is more complex, as *O. niloticus* has two alleles, *O. aureus* has 3 alleles, and *O. mossambicus* has one allele (McAndrew and Majumdar 1983). Although all alleles are "fixed" to the specific species, having a standard with only one of these alleles made scoring the others more difficult. Specifically, 12 % of the Port Sulphur tilapia scored an allele that was anodally distinct from the allele in the *O. niloticus* standards. Based on the position of this allele relative to the *O. niloticus* standards, 3 of the 22 fish could be hybrids. Based on muscle tissue ADA, the remaining 19 fish were not *O. niloticus* based upon the *O. niloticus* standards received from LDWF. Modal dorsal fin ray counts for all the Port Sulphur tilapia used in thermal tolerance and recovery experiments was 28 with a mean of 28.1±0.05, while *O. niloticus* and *O. aureus* have reported modes of 30 and 28, respectively (Trewavas 1982).

Thermal Tolerance

The median lower lethal temperature for tilapia detected at Port Sulphur acclimated to a salinity of 7 ppt and exposed to temperature decreases of 1 and 2 C per day was 6.0±0.14 and 6.5±0.10, respectively. The median lower lethal temperatures for tilapia acclimated to salinities of 0.8 and 15.0 ppt and exposed to a temperature decrease of 2 C per day were 6.6±0.11 and 6.6±0.12, respectively. For all thermal tolerance trials a LOE was observed for all individuals at approximately 10 C, with the exception of tilapia acclimated to 15.0 ppt, which demonstrated a LOE between 10 and 11 C (Table 1).

Odds of Mortality

Tilapia acclimated to 0.8 ppt exhibited a significant increase in mortality (Likelihood ratio χ^2, $_{1df}$=136.8, p<0.01) with decreasing temperature. For these fish, a 2 C decrease in temperature per day increased the odds of mortality by 2.25 (1.1–4.43 95 % confidence interval, CI). Tilapia acclimated at 7.0 ppt and 15.0 ppt and exposed to a 2 C decrease in temperature also exhibited a significant increase in mortality (Likelihood ratio χ^2, $_{1df}$=102.5, p<0.01 and Likelihood ratio χ^2, $_{1df}$=115.2, p<0.01, respectively) with decreasing temperature. Fish acclimated at 7.0 ppt had increased odds of mortality of 3.7 (0.13–6.69 95 % CI) per 2 C temperature decrease per day, and fish acclimated at 15.0 ppt had increased odds of mortality of 2.29 (1.15–4.52 95 % CI) per 2 C temperature decrease per day. Increasing hours of exposure to 9 C significantly increased mortality (Likelihood ratio χ^2, $_{1df}$=36.2, p<0.01), with increased odds of 1.07 (1.03–1.12 95 % CI) per hour of exposure. Lastly, mortality following LOE significantly increased with increasing hours of exposure (Likelihood ratio χ^2, $_{1df}$=11.5, p<0.01), with increased odds of mortality of 1.07 (1.00–1.15 95 % CI) per hour.

Coastal Water Data and Estimated Mortality

Regardless of acclimation salinity, mortality estimated at all 10 CRMS sites based on acute exposure to most extreme daily winter temperature minima for the period 1992–2010 were all 99 % or 100 % (all 99.9–100 %, 95 % CI) and were all 99 % or 100 % (all 99.9–100 %, 95 % CI) at the LDWF tilapia collection locations in and around Port Sulphur (Table 2). However, these extreme temperature minima were uncommon, ranging from 0 days in 1992 to 41 days in 2004 (mean 21.9 days±2.8 standard error), and mortality estimates based on acute exposure to daily average temperature ranged from less than 1 % at all 10 CRMS and LDWF sites up to 29.4 % (95 % CIs all extend to 0 and between 24 % and 52 % on the upper end). Tilapia mortality based on acute exposure at temperature minima (1–99 % mortality) were highly dependent on whether extreme or average minima occurred.

Although short-term exposure to cold temperature does cause tilapia mortality, mortality increases substantially with increased hours of exposure. Therefore, the consecutive number of hours below 9 C and 10 C in a given winter may be better predictors of tilapia mortality. Number of hours of winter daily averages and maximum temperatures below 9 C and 10 C were very similar during each year of CRMS recording (1992–2010) and will be reported together. Winter daily temperatures were below 9 C and 10 C for a minimum of 24 h (2005 and 2009) and a maximum of 408 h (2001). Mean number of hours below 9 C and 10 C for winters 1992–2010 was 182.4 h (142–256 h 95 % CI) at CRMS sites. Estimated tilapia mortality at 24 h during the two warmest winters in the record was 3.3 % (0.3–23.0 % 95 % CI; Fig. 2; Table 2). Additionally, estimated mortalities for the coldest winter on record and for the average winter 1992–2010 were both 99.9 % (coldest 99.9–100 % and average 94.7–99.9 % 95 % CI; Table 2). Estimated mortality following LOE for the mildest winters of 2005 and 2009 were 1.5 % (0.01–33.0 % 95 % CI), and for the coldest and average winter 1992–2010

Table 1 Rate of temperature decline, mean weight (±SD), loss of equilibrium and median lower lethal temperature (±SE) for Port Sulphur tilapia acclimated to salinities of 0.8, 7.0, and 15.0 ppt

Acclimation salinity (ppt)	Temperature decline (°C/day)	Mean weight ±SD (g)	Mean total length ±SD (mm)	Loss of equilibrium temperature (°C)	Median lower lethal temperature (°C)
0.8	2	87±25	168±17	9.9±0.19	6.6±0.11
7.0	1	105±35	175±35	9.4±0.16	6.0±0.14
7.0	2	83±32	170±20	10.0±0.11	6.5±0.10
15.0	2	95±33	176±19	10.5±0.10	6.6±0.12

Table 2 Estimated over-winter mortality from logistic regressions varied based point temperature measurement observations in 2009 for developed Port Sulphur sites (exposure hours unavailable) and exposure hours <9 C 1996–2010 for undeveloped Coastwide Reference Monitoring System (CRMS) sites. Because estimates were similar across acclimations, estimates from 7 ppt acclimation shown for Port Sulphur sites

Port sulphur sites	2009 Data (estimate; 95 % C.I.)	Port sulphur sites	2009 Data (estimate; 95 % C.I.)
6	86 %; 66–95 %	9	47 %; 31–64 %
7	80 %; 59–91 %	10	92 %; 74–98 %
8	51 %; 66–95 %	11	<1 %; 0–1 %
CRMS sites	Warmest (estimate; 95 % C.I.)	Average (estimate; 95 % C.I.)	Coldest (estimate; 95 % C.I.)
1	47 %; 23–73 %	99 %; 96–100 %	100 %; 99–100 %
2	15 %; 4–42 %	99 %; 94–100 %	100 %; 99–100 %
3	82 %; 52–95 %	99 %; 94–100 %	100 %; 99–100 %
4	47 %; 22–73 %	99 %; 91–100 %	100 %; 99–100 %
5	3 %; 0–23 %	99 %; 85–100 %	100 %; 99–100 %
12	3 %; 0–23 %	99 %; 96–100 %	100 %; 99–100 %
13	15 %; 4–43 %	99 %; 98–100 %	100 %; 99–100 %
14	3 %; 0–23 %	99 %; 84–100 %	100 %; 99–100 %
15	15 %; 4–43 %	100 %; 99–100 %	100 %; 99–100 %
16	47 %; 22–73 %	99 %; 91–100 %	100 %; 99–100 %

were again both 99.9 % (coldest 41.7–100 % and average 34.2–99.9 % 95 % CI).

Discussion

The median lethal lower thermal limits determined for *Oreochromis sp.* in the current study confirm previous studies demonstrating values between 6 and 7 C for *O. aureus* and other *Oreochromis sp.* (Shafland and Pestrak 1982). Although salinity and the rate of temperature decrease (1 versus 2 C) were observed to modify the lower thermal tolerances observed from the fish in the current study, the effects were minimal with a maximum change in lower tolerance of 0.7 C. It is important to understand the relationship between immobilization through LOE and the resulting time course to mortality that was observed to occur at temperatures at or below 10–9 C. A dose response relationship between recovery and mortality across time appears when water temperatures induce a loss of equilibrium below 9 C. The ability for *O. niloticus* to persist within coastal waters of Mississippi was determined to be dependent on thermal effluents and the resulting refugia formed in the winter months (Peterson et al. 2005; Schofield et al. 2011). The situation in Mississippi is unique because of the presence of artificial and natural thermal effluents in the Pascagoula River and historical differences in permitting aquaculture species, conditions that do not apply to the *Oreochromis* hybrids that were discovered in Louisiana. As such, if present at all, the Port Sulphur tilapia should remain both limited in abundance and localized to developed reaches (sites 6–11 in Fig. 1) that provide thermal refugia.

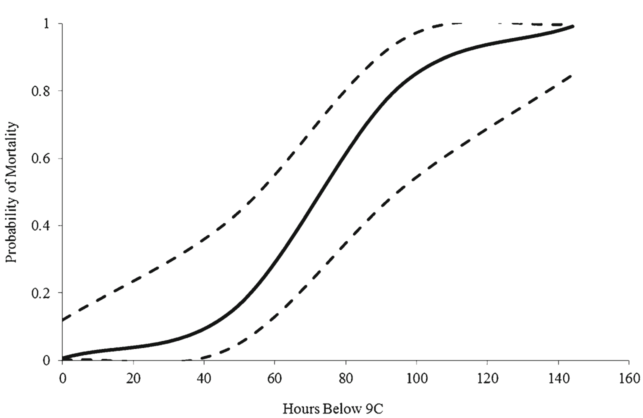

Fig. 2 During the mildest winters experiencing only 24 consecutive hours below 9 C, tilapia mortality is very low. Over-winter mortality increases rapidly when consecutive hours below 9 C increase past 60 h, which occurred frequently 1996–2010

Our results suggest that the tilapia collected at Port Sulphur, LA, represent an unnaturalized, hybrid population of *Oreochromis sp.* that is unlikely to establish in the open marsh outside of developed areas (e.g, sites 1–5, 12–16 in Fig. 1). Although over-winter survival may be possible in warmer than average winters, it is highly unlikely that tilapia will survive multiple winters in the open marsh because of the high probability of mortality due to a sufficient number of consecutive hours (>80 h) of water temperatures below 9 C. Unlike coastal Mississippi, where aquaculture and other warm effluents have created thermal refugia for overwintering tilapia (Peterson et al. 2005; Schofield et al. 2011), the spatial disconnection between the open marsh and developed areas in Louisiana probably limit the potential for tilapia being able to access thermal refugia. Further, because tilapia exhibited swimming difficulty at temperatures below 9 C, substantial mortality from alligators, birds, and piscivorous fishes is expected during winter.

Unlike California, Florida, Georgia, or Alabama where tilapia have naturalized in ponds and rivers essentially closed from surrounding wetlands and waterbodies (Hargreaves 2000), the situation in coastal Louisiana and Mississippi is quite different because of the connected nature of coastal wetlands. Similar to Mississippi (Peterson et al. 2005), the discovery of tilapia in Port Sulphur suggests that isolated populations could persist for periods of time with access to thermal refugia. However, our data indicate that widespread naturalization in Louisiana coastal wetlands in highly unlikely, unlike the predictions for coastal Mississippi by Schofield et al. (2011). We suggest that sampling in these areas should be continued to determine whether individuals have located such thermal refuges. Based on our analyses, we suggest that monitoring in the open marsh where average or colder winters will prohibit naturalization may not be necessary. Rather, efforts should be made to identify potential areas via GIS and sampling that maintain water temperatures above 9 C when surrounding marshes and wetlands are colder. Finally, invasion risks should be minimized by the application of aquaculture industry best practices and prudent management actions.

References

Behrends LL, Kingsley JB, Bulls M (1990) Cold tolerance in maternal mouthbrooding tilapias: phenotypic variation among species and hybrids. Aquaculture 85:271–280

Beitinger TL, Bennett WA, McCauley RW (2000) Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. Environ Biol Fishes 58:237–275

Bennett WA, Currie R, Wagner PF, Beitinger TL (1997) Cold tolerance and potential overwintering of redbodied piranha, *Pygocentrus nattereri*, in the United States. Trans Am Fish Soc 126:841–849

Bentsen HB, Eknath AE, Palada-de Vera MS, Danting JC, Bolivar HL, Reyes RA, Dioniso EE, Longalong FM, Circa AV, Tayamen MM, Gjerde B (1998) Genetic improvement of farmed tilapias: growth performance in a complete diallel cross experiment with eight strains of Oreochromis niloticus. Aquaculture 160:145–173

Costa-Pierce BA, Riedel R (2000) Fisheries ecology of the tilapias in subtropical lakes of the United States. In: Costa-Pierce BA, Rakocy JE (eds) Tilapia aquaculture of the Americas, vol 2. World Aquaculture Society, Baton Rouge, pp 1–20

Courtenay WR Jr (1991) Biological pollution through fish introductions. In: McKnight BN (ed) BN biological pollution: the control and impact of invasive exotic species. Indiana Academy of Science, Indianapolis, pp 35–61

Cnaani A, Hulata G, Gall1 GAE, Kashi Y, Indelman M, Hallerman EM (2000) Detection of putative QTLs for body weight and cold tolerance in interspecific tilapia hybrids. In: Genetics in Aquaculture VII, Townsville, Australia, 15–22 July 2000

Crutchfield JU Jr (1995) Establishment and expansion of redbelly tilapia and blue tilapia in a power plant cooling reservoir. In: Schramm HL, Piper RG (eds) Uses and effects of cultured fishes in aquatic ecosystems. American Fisheries Society, Bethesda, pp 452–461

FAO (Food and Agriculture Organization) (2004) State of World Fisheries and Aquaculture (SOFIA). FAO, Rome

Hargreaves JA (2000) Tilapia culture in the southeast United States. In: Costa-Pierce BA, Rakocy JE (eds) Tilapia aquaculture in the Americas, vol 2. World Aquaculture Society, Baton Rouge, pp 60–81

Korni MS, Vander Zanden MJ (2010) Forecasting the distribution of the invasive round goby (Neogobius melanostomus) in Wisconsin tributaries to Lake Michigan. Canadian Journal of Fisheries and Aquatic Sciences 67:553–562

Likongwe JS, Stecko TD, Stauffer JR, Carline RF (1996) Combined effect of water temperature and salinity on growth and feed utilization of juvenile Nile tilapia Oreochromis niloticus (Linneaus). Aquaculture 146:37–46

Marshall B (2009) Section of Plaquemines waterways to be poisoned to kill foreign fill. The New Orleans Times-Picayune. 6 May 2009. http://www.nola.com/news/index.ssf/2009/05/section_of_plaquemines_waterwa.html

McBay LG (1961) The biology of tilapia nilotica linneaus. Proceed Annu Conf Southeastern Assoc Game Fish Commission 15:208–218

Peterson MS, Slack WT, Woodley (2005) The occurrence of non-indigenous Nile tilapia, *Oreochromis niloticus* (Linnaeus) in coastal Mississippi, USA: ties to aquaculture and thermal effluent. Wetlands 25:112–121

Pörtner HO, Knust R (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 5:95–97

Schoenherr A (1988) A review of the life history of the dessert pupfish, *Cyprinodon macularius*. Bull South Calif Acad Sci 87:104–134

Schofield PJ, Peterson MS, Lowe MR, Brown-Peterson NJ, Slack WT (2011) Survival, growth and reproduction of non-indigenous Nile tilapia, Oreochromis niloticu (Linneaus 1758). I. Physiological capabilities in various temperatures and salinities. Mar Freshw Res 62:439–449

Shafland PL, Pestrak JM (1982) Lower lethal temperatures for fourteen nonnative fishes in Florida. Environ Biol Fishes 7:149–156

Spiegelman D, Hertzmark E (2005) Easy SAS calculations for risk or prevalence ratios and differences. Am J Epidemiol 162:199–200

Tayamen MM, Reyes RA, Danting MJ, Emdoza AM, Marquez EB, Salguet AC, Gonzales RC, Abella TA, Vera-Cruz EM (2002) Tilapia broodstock development for saline waters in the Philippines. Naga, The ICLARM Quaterly 25:1

Tave D, Jayaprakas V, Smitherman RO (1990) Effects of intra-specific hybridization in Tilapia nilotica on survival under ambient winter temperature in Alabama. J World Aquaculture Soc 21:201–204

Trewavas E (1982) Tilapias: taxonomy and speciation. In: Pullin RSV, Lowe-McConnell RH (eds) The biology and culture of tilapias, ICLARM conference proceedings 7. International center for living aquatic resources management, Manila, pp 3–15

Watanabe WO, Burnett KM, Olla BL, Wicklund RI (1989) The effects of salinity on reproductive performance in Florida red tilapia. J World Aquaculture Soc 20:223–229

Zale AV, Gregory RC (1989) Effect of salinity on cold tolerance of juvenile blue tilpias. Trans Am Fish Soc 118:718–720