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Salinity tolerance of juveniles of four varieties of tilapia

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SALINITY TOLERANCE OF JUVENILES OF FOUR VARIETIES OF TILAPIA

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ABSTRACT

Juvenile (4 g) tilapia of four varieties, Nile tilapia (*Oreochromis niloticus*), blue tilapia (*O. aureus*), Florida red tilapia (*O. urolepis hornorum* x *O. mossambicus*), and Mississippi commercial tilapia (*Oreochromis* spp.) were tested for salinity tolerance. This was accomplished by subjecting them to four salinity regimes during a 97-h period with as long as 63 h acclimation. Salinity regimes represented salinities found along coastal Louisiana. Each variety was challenged individually against every other variety and thus in triplicate. All four varieties are commercially produced in the southeastern United States. The Florida red tilapia and Mississippi commercial tilapia are hybrid-based varieties (distinct species were crossed to produce their lineages). Juvenile *O. aureus*, *O. niloticus*, and Florida red tilapia exhibited good survival (≥ 81%) in salinity regimes up to 20 ppt, with moderate survival of *O. aureus* (54%) and Florida red tilapia (33%) at 35 ppt salinity. Mississippi commercial tilapia survived salinity regimes up to 10 ppt and exhibited poor survival at 20 ppt (5%). The potential for acclimation and survival of juvenile tilapia in Louisiana's coastal aquatic habitats was evaluated based on a comparison of trial results with prevailing seasonal salinities in coastal waters. The study indicated *O. aureus*, *O. niloticus*, and Florida red tilapia could survive salinities throughout the year along coastal Louisiana. These results will allow Louisiana and other Gulf of Mexico states to more objectively evaluate risks associated with tilapia production in coastal watersheds by quantifying the salinity tolerances of two commercially important hybrid-based tilapia strains.

CHAPTER 1- INTRODUCTION

Tilapia have become well established in United States aquaculture, with 9 million kilograms produced in the United States annually (DeWandel, 2001). The rapid growth of tilapia, their resistance to poor water quality, ability to grow under sub-optimal nutritional conditions, and high fecundity all make them well suited for aquaculture. While all tilapia species exhibit these traits there can be broad variation among species in environmental tolerances such as temperature and salinity (Villegas, 1990). Nile tilapia (*Oreochromis niloticus*) and blue tilapia (*Oreochromis aureus*) are widely accepted in commercial production (Wohlfarth and Hulata, 1981). *Oreochromis aureus* and *O. mossambicus* survive salinities above 35 ppt (Lotan, 1960) (Whitefield and Blaber, 1979), *O. niloticus* is more moderate in its tolerance to saline conditions and dies at salinities >20 ppt (Kirk, 1972).

Development of tilapia hybrids has been pursued to incorporate desirable traits into hybrids and hybrid-based varieties. These efforts have had varying degrees of success (Rakocy et al., 1993). Some hybrid-based varieties may exhibit traits beneficial to commercial-scale farming such as cold or salinity tolerance, but their small terminal size or slow growth may discourage commercial development (Lutz, 2001a). Hybrid varieties may emerge purposely through research, or through accidental contamination of stocks. The development of hybrid-based tilapia varieties is an important step toward increasing production and marketability of tilapia (Lutz, 2001a). By combining the beneficial traits found in several tilapia species into hybrids scientists and producers create fish that grow faster and are less expensive to produce. Two hybrid-based varieties of tilapia that have already been commercialized in the southern United States are the "Mississippi commercial tilapia" (MCT) and Florida red tilapia (FRT).

Founding populations of the MCT variety (Fig. 1-1) were originally developed in Colorado and marketed as the "Rocky Mountain White" ® tilapia. Its ancestry is poorly defined, and it is distinguishable by its grey to white coloration. The MCT has become a commercially important tilapia with pond and recirculating system production throughout the southeastern United States (Lutz, 2001b). The FRT (Fig. 1-1) was commercially developed in the 1970s by

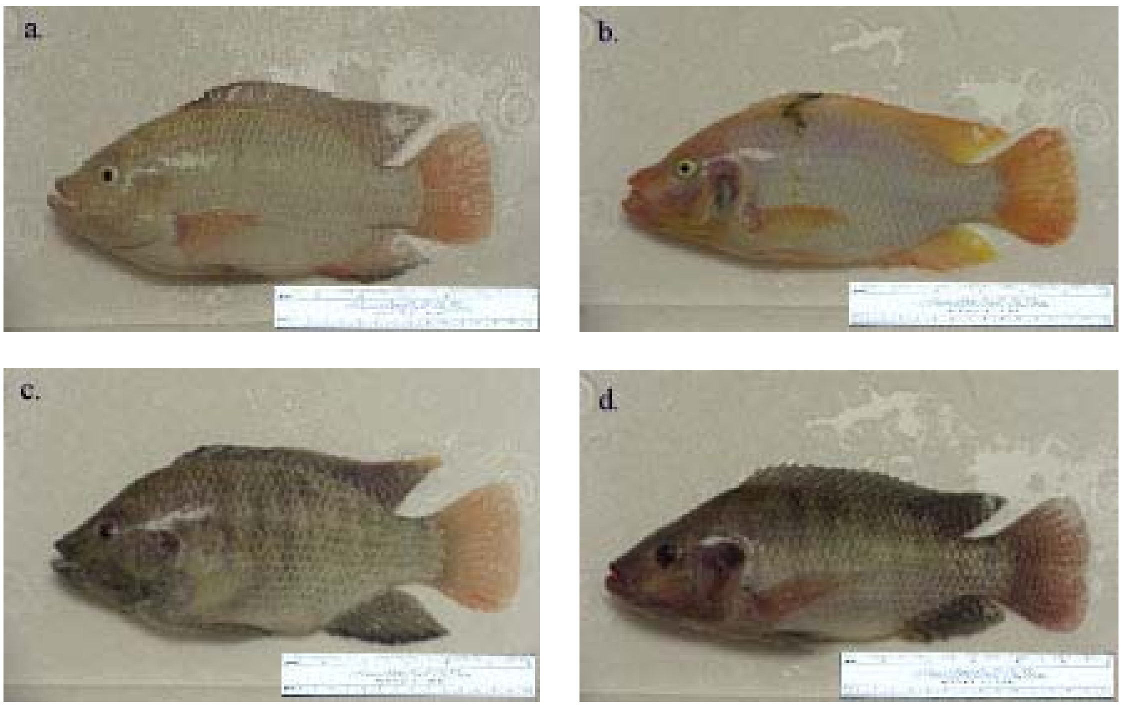

Figure 1-1. Photographs of adult Mississippi commercial tilapia (a), Florida red tilapia (b), blue tilapia (c), and Nile tilapia (d) , bar = 15 cm (Lutz, 2002).

crossing *O. urolepis hornorum* females and *O. mossambicus* males (Rakocy et al., 1993). The bright red coloration and fecundity of FRT allow them to be easily marketable as an inexpensive alternative to marine fish with similar coloration. This hybrid is cultured in the southeastern United States and the Caribbean basin (Watanabe et al., 1997; Head et al., 1994).

There are unanswered questions about the basic environmental requirements and tolerances of the MCT. Because the ancestry of the MCT is not completely understood it is

impossible to predict the tolerance of this variety to saline conditions based on the traits of the parental species. While the ancestry of the FRT is well documented, its basic environmental tolerances have not been well-characterized. The FRT has been shown to be tolerant to salinities as high as 35 ppt (Clark et al., 1990) presumably because of its *O. mossambicus* ancestry. *Oreochromis mossambicus* has been reported to survive salinities in excess of 35 ppt (Stickney, 1986) and is the most salt tolerant of the tilapia species (Wohlfarth and Hulata, 1981). Despite these poorly defined environmental parameters, MCT and FRT are both are commercially produced in the United States (Lutz, 2001b).

Although much research has been conducted on the environmental tolerances of major tilapia species such as *O. niloticus* (Fig. 1-1) and *O. aureus* (Fig. 1-1), there is little information available with respect to hybrid-based varieties (Wohlfarth and Hulata, 1981). Collection of baseline environmental data on commercial hybrid-based varieties has become important in light of concerns throughout the southeastern United States about establishment of non-indigenous and invasive aquatic species.

The primary goals of this research were to generate baseline data to assist in evaluating the potential environmental threat that MCT and FRT varieties might pose if released into Louisiana waters, and to review the salinity tolerance ranges of each tilapia variety in the context of salinities found in coastal Louisiana waters.

It has been reported that tilapia would succumb to low temperatures if released in much of the southeastern United States (Hargreaves, 2000) could be challenged because the coastal waters of Louisiana become increasingly less saline during winter months (National Oceanic and Atmospheric Administration, 1998) when tilapia would most need a thermal refuge, and where water temperatures might be high enough to allow survival. Tilapia could possibly become a

nuisance species and prove costly or impossible for Louisiana and other southern states to control. While Louisiana has strict regulations concerning the possession, sale, and production of tilapia (Appendix C) these regulations could be inadequate if tilapia were able to survive in adjacent coastal areas (e.g. Mississippi, Alabama).

References

Clark, A.E., Watanabe, W.O., Olla, B.L. and Wicklund, R.I. 1990. Growth, feed conversion and protein utilization of Florida red tilapia fed isocaloric diets with different protein levels in seawater pools. Aquaculture 88:75-85.

DeWandel, R. 2001. Year 2000 ATA tilapia situation and outlook report. American Tilapia Association, Arlington, VA.

Hargreaves, J.A. 2000. Tilapia culture in the southeast United States. Pages 60-81. in Costa-Pierce and Rakocy, J.E. Tilapia Aquaculture of the Americas, volume 2. The World Aquaculture Society, Baton Rouge, Louisiana.

Head, W.D., Zerbi, A. and Watanabe, W.O. 1994. Preliminary observations on the marketability of salt-water cultured Florida red tilapia in Puerto Rico. Journal of the World Aquaculture Society 25: 432-441.

Kirk, R.G. 1972. A review of recent developments in tilapia culture, with special reference to fish farming in the heated effluents of power stations. Aquaculture 1:45-60.

Lotan, R. 1960. Adaptability of *Tilapia nilotica* to various saline conditions. Bamidgeh 12:96-100.

Lutz, C.G. 2001a. Practical Genetics for Aquaculture. Fishing News Books, Blackwell Sciences, Oxford, United Kingdom.

Lutz, C.G. 2001b. Personal communication on Mississippi commercial tilapia ancestry. Louisiana State University Agricultural Center, Baton Rouge, Louisiana.

Lutz, C.G. 2002. Photographs of Mississippi commercial, Florida red, blue, Nile tilapias. Louisiana State University Agricultural Center, Baton Rouge, Louisiana.

National Oceanographic and Atmospheric Administration, 1998. Seasonal Salinity Isohaline Distribution Maps. http://biogeo.nos.noaa.gov/gom-efh/lma.htm.

Rakocy, J.E., Hargreaves, J.A. and Bailey, D.S. 1993. Comparison of tilapia species for cage culture in the Virgin Islands. UVI Research. 5:13-17.

Stickney, R.R. 1986. Tilapia tolerance to saline waters: a review. Progressive Fish Culturist 48:161-167.

Villegas, C.T. 1990. Evaluation of the salinity tolerance of *O. mossambicus*, *O. niloticus*, and their F1 hybrids. Aquaculture 85:281-292.

Watanabe, W.O., Olla, B.L., Wicklund, R.I. and Head, W.D. 1997. Saltwater culture of the Florida red tilapia and other saline-tolerant tilapias: a review. Pages 54-141 in B.A. Costa-Pierce and J.E. Rakocy, eds. Tilapia Aquaculture in the Americas, Volume 1. The World Aquaculture Society, Baton Rouge, Louisiana.

Whitefield, A.K. and Blaber, S.J.M. 1979. The distribution of the freshwater cichlid *Sarotherodon mossambicus* in estuarine systems. Environmental Biology of Fishes 4:77-81.

Wohlfarth, G.W and Hulata, G.I. 1981. Intraspecific Hybridization. Applied Genetics of Tilapias. International Center for Living Aquatic Resources Management Studies and Reviews. 2-18.

CHAPTER 2- JUVENILE SALINITY TOLERANCE OF FOUR TILAPIA VARIETIES

As tilapia have become more widely cultured, research into their genetics and environmental tolerances (for growth and survival) have followed suit. Occasionally, misidentification of species has resulted in incorrect assessments of environmental tolerances. This can be attributed to the diversity of tilapine species (77 genera), their common hybridization, and inadequate record keeping during collection in the wild (Wohlfarth and Hulata, 1981). Undoubtedly, a rush to production of new strains has also clouded hybrid-based tilapias' ancestries.

The Mississippi commercial tilapia (MCT) is an example of this trend. It was derived from a commercial variety originally developed in Colorado and marketed as "Rocky Mountain White" ® tilapia (Lutz, 2000). The MCT exhibits varied coloration, ranging from white to silvery-blue to occasional grey-black individuals. This variety is perceived by producers to tolerate low temperatures (< 20 C), but studies to confirm temperature tolerance are lacking. It has accounted for the bulk of pond and tank production in Arkansas and Mississippi, and is widely cultured among producers from Texas to Maryland.

Electrophoretic protein analysis of samples of MCT have confirmed genetic contributions from at least 3 distinct tilapia species: Nile tilapia, *O. niloticus*; blue tilapia, *O. aureus*; and mossambique tilapia, *O. mossambicus* (Lutz, 2001). These three species have differing salinity tolerances. The Nile tilapia exhibits a moderate tolerance to salinity with 60 d fish surviving direct transfer up to 25 ppt (Watanabe et al., 1985a), but its highest growth is achieved at 0-10 ppt (Villegas, 1990a). *Oreochromis aureus* and *O. mossambicus* both have higher tolerances for salinity with experimental production of blue tilapia occurring at 44 ppt (Chervinski and Yashouv, 1971) and 35-70 d old mossambique tilapia surviving exposure to 64 ppt salinity after

3 d acclimation (Potts et al 1967). Due to the complex ancestry of the MCT and its broad genetic base it is difficult to predict its tolerance to saline conditions.

There are no available data on adaptation of MCT to saltwater. *Oreochromis aureus* has been shown to reproduce in salinities as high as 19 ppt, grow in salinities of 36 ppt, and die at 53 ppt (Chervinski and Yashouv, 1971). *Oreochromis niloticus* is widely accepted as a culture species due to its rapid growth (Mair, 2000), but its salinity tolerance is comparatively low, about 20-25 ppt (Watanabe et al., 1985a).

The Florida red tilapia (FRT) is descended from a cross between *O. urolepis hornorum* females and *O. mossambicus* males, which was described in 1960 by C.F. Hickling at the Fish Culture Research Station in Malacca (Hickling, 1960) while conducting experiments on hybrid tilapia crosses. In the 1970s Natural Systems, a Florida based company, developed the hybrid cross into a commercially available variety (Rakocy et al., 1993). It is assumed that the salinity tolerance of FRT is directly related to its parental species' tolerances. *Oreochromis mossambicus* is among the most salt tolerant of tilapia species, with documented reproduction in experimental production ponds at 49 ppt and survival at 64 ppt (Popper and Lichatowich, 1975; Potts et al 1967). *Oreochromis urolepis hornorum* has been observed surviving salinities as high as 35 ppt in seawater ponds (Fryer and Iles, 1972). The FRT exhibits fecundity, that has been attributed its parental species sharing a close ancestry (Hickling, 1960). As in the case of MCT, FRT has become an important commercially produced hybrid, warranting a better understanding of its basic environmental parameters.

The salinity tolerance of FRT has been investigated at the Caribbean Marine Research Center at Lee Stocking Island in the Bahamas (Watanabe et al., 1989a; Watanabe et al., 1989b), but salinity tolerances of the FRT commercially produced in the southeastern United States could

differ from these results. Florida red tilapia used in previous work were fertilized, hatched, and held at 12-18 ppt salinity (Watanabe et al., 1997). Tilapia have been found to adapt while still developing within the egg if exposed to elevated salinity conditions (Watanabe, et al., 1990). It has also been suggested that with successive generation of FRT bred at elevated salinity, tolerance improves because of selection response based on individual genotypic differences (Watanabe et al., 1989b). Florida red tilapia commercially produced in the southeastern United States would not typically be exposed to salinity levels above 5 ppt and therefore may not completely reflect the high salinity tolerances observed at the Caribbean Marine Research Center.

It has been reported that influences of salinity tolerance within tilapia species or varieties is more effected by size rather than age (Watanabe et al., 1985a), but age also influences tolerance during abrupt salinity changes (Watanabe, 1990). The earlier eggs and fry are exposed to high salinity conditions, the higher the survival (Watanabe et al., 1985b). Prolonged acclimation over days or even weeks from freshwater to saltwater also increases survival (Al Asgah, 1984). It has been recognized that some tilapia may have higher survival, with higher growth, in saline conditions than in freshwater. The FRT is one tilapia variety that exhibits superior survival and growth at elevated salinities (Ernst et al., 1989). Natural selection can aid long-term population adaptation to saline waters (Watanabe et al., 1989) by allowing only tilapia that survive saline waters to reproduce.

Mechanisms that allow tilapia to tolerate elevated salinities have also been investigated. It is believed that tilapia developed from a marine teleost ancestry and that the mechanisms that allow for salt tolerance in some species are based in this ancestry (Allen, 1971). Chloride cell proliferation in gill filaments, along with increased Na^+/K^+ ATPase activity when fish are

subjected to elevated salinity levels, apparently enhances tilapia survival under saline conditions (Avella et al., 1993). It has been suggested that chloride cell counts and elevated blood levels of Na^{+}/K^{+} ATPase, an enzyme used in salt regulation, are indicators of salt tolerance in tilapia (Avella et al., 1993).

Other mechanisms that may aid in salt tolerance in *O. mossambicus* include a secondary hemoglobin type that develops 47 days after hatch (Perez and Maclean, 1976). This hemoglobin molecule had been reported to have a better affinity for oxygen at elevated osmotic and temperature levels than in the conventionally recognized hemoglobin. This secondary hemoglobin could transfer oxygen at elevated salt levels when conventional hemoglobin would be less effective.

The characteristic rapid growth to market size of Nile tilapia (Hulata et al, 1986) has made it a well-accepted fish with tilapia farmers. Hybridization between Nile tilapia and other varieties for research purposes is widely accepted (Villegas, 1990). The blue tilapia, while not as widely cultured as the Nile tilapia, is an accepted species for culture due to its tolerance of temperatures as low as 7 C (Perry and Avault, 1972) and a salinity tolerance as high as 54 ppt (Lotan, 1960). The blue tilapia has been shown to reproduce in salinities up to 19 ppt, grow in salinities of 36 ppt, and die at 53 ppt (Chervinski and Yashouv, 1971). These two tilapia species provide a frame of reference for comparison of the hybrid-based varieties MCT and FRT.

The state of Louisiana has been proactive in regulating the tilapia industry and has imposed a number of restrictions upon hatcheries and farmers. Tilapia are listed as exotic species by the Louisiana legislature and a permit is needed for possession (Louisiana Revised Statutes 56:319). Facilities that produce tilapia in Louisiana must be fully enclosed and above the 100-year flood plain (Louisiana Administrative Code 76:VII.903). These protections are in

response to the establishment of tilapia released into habitats in Florida, Georgia, and California (United States Congress, Office of Technology Assessment, 1993). Tilapia are tropical species that are assumed to be poorly adapted to survive long-term in Louisiana, but the possibility of commercially available strains of tilapia using costal watersheds as thermal refuges during extreme cold episodes ($\leq$ 15 C) or as over-wintering grounds has been raised repeatedly and must be further evaluated. Exposure to salinity has also been recognized to increase cold tolerance in tilapias (Watanabe et al., 1993).

This study was conducted to establish salinity tolerances of juvenile Mississippi commercial and Florida red tilapias raised in freshwater conditions and to compare these results to those of Nile and blue tilapia juveniles under identical conditions.

Methods

Experimental Design

To evaluate salinity tolerance of the varieties examined in this study, four treatments were used: 0, 10, 20, and 35 ppt salinity. Two of the four varieties were challenged at a time, and each challenge included 120 fish from each of the two varieties being evaluated. Each variety was divided into four groups (30 fish), these tilapia were then subdivided into three sets of ten fish each, with each salinity treatment including three independent replicates (10 fish) for each variety (Table 2-1). Each fish was randomly assigned by placing one fish at a time into each treatment container. Six challenges were conducted, to allow each variety to be tested independently with every other and to replicate salinity trials for each variety in triplicate (Table 2-2).

Table 2-1. Physical layout of the experimental design used to determine salinity tolerance of four tilapia strains. Twenty-four containers were divided into groups of three and fish of two varieties were stocked accordingly.

	Container number							
	1-3	4-6	7-9	10-12	13-15	16-18	19-21	22-24
Target treatment salinity	0	0	10	10	20	20	35	35
Variety	1	2	1	2	1	2	1	2

Table 2-2. Dates salinity challenges were initiated and completed. Challenges began in September 2001, with completion of two challenges per month through November 2001.

Date	Varieties challenged
September 16-20	blue tilapia and Mississippi commercial tilapia
September 24-28	Nile tilapia and Florida red tilapia
October 4-8	blue tilapia and Nile tilapia
October 18-22	Nile tilapia and Mississippi commercial tilapia
November 6-10	blue tilapia and Florida red tilapia
November 16-20, 2001	Mississippi commercial tilapia and Florida red tilapia

Physical Description of Trial Layout

Salinity trials were conducted at the Aquaculture Research Station (ARS) in Baton Rouge, Louisiana, a unit of the Louisiana State University Agricultural Center. Juveniles were maintained in the same laboratory where the trials occurred. A design was used in which two varieties of tilapia could be challenged simultaneously in order to rule out outside environmental influences, with each variety being challenged in triplicate. Triplication allowed each variety to be challenged with every other and allowed statistical comparisons with the facilities available. Accordingly, 24 containers were used to hold tilapia for the duration of each trial (Table 2.1).

Non-toxic 20-liter polyethylene buckets purchased from a local hardware store were used because of their low cost ($5 United States), adequate volume for the size and number of fish

stocked, and ease of handling. Each container was numbered for data collection and a specific fill line indicated the amount of water needed during water changes in the experiments.

Each container had 1 x 1 mm plastic mesh screen fitted over its top and held in place by an elastic band. Each container had an airstone supplied with compressed air from a .36 kW regenerative blower. A hole was made in the mesh to allow airline access to the container. Airstones had individual valves and airflow for each container was set to maintain $\geq$ 5.5 mg/L dissolved oxygen after fish were stocked. Oxygen levels throughout trials were determined using a dissolved oxygen meter (Yellow Springs Instrument Company, model 58).

Salts used in this study were Cargill brand livestock feed salt (Appendix A) mixed with commercial grade calcium chloride (Cal Chlor Corporation) in a 19 to 1 weight to weight ratio. Salts were dissolved and mixed with Baton Rouge municipal water and agitated in 120-liter plastic tanks with a sump pump until the water cleared. Saline water was held in 120-liter plastic tanks and was prepared 8 hours prior to use to allow for complete mixing and adjustment of water temperature to room temperature. The salinity of each mixing tank was adjusted prior to use, and treatment salinities were monitored using salinity/conductivity meters (Orion Research corporation, model 840 or Yellow Springs Instruments, model 33). Salinity/conductivity meters were calibrated against a hand refractometer (Spartan Refractometer Company, A 366 ATC; salinity).

During the initial trial, salinity in the 10 ppt containers was monitored every 9 hours for the first 63 hour. Upon all fish reaching their prescribed salinity (63 h) subsequent observations were made at 75 h, 87 h, and at the end of the experiment at 97 h. Water in the 10 ppt containers was changed the least and therefore evaporation effects would be expected to be the highest. Laboratory temperature conditions were held constant by a central heating ventilation and air

conditioning system that maintained an average room of temperature of 27 C as indicated by glass thermometer (Fisher Scientific Corporation, model 14-995-5B). Hot-water heated blowers (Modine, model HS86S01) were used on colder nights to maintain a temperature of 27 C.

Collection of Tilapia and Preparation for Treatments

Breeding stocks used to produce juveniles were obtained from Til-Tech Aquafarm in Robert, Louisiana. *Oreochromis niloticus* used in this experiment were of "Auburn-Egypt" variety origin and *O. aureus* used were of the "Manzala" variety. During June of 2001, broodstock were introduced into 900-liter above-ground outdoor pools located at the ARS. Five pools were used for each tilapia variety. Broodstock were fed daily and observations were made on fish health and presence of fry. Juveniles were collected from pools when their average total length reached approximately 3 cm. Juveniles used in salinity challenges ranged in size from 2.5-7.5 g each, with a mean weight of approximately 4 grams (Table 2-3), and were $\geq$ 6 weeks old. Juveniles were blotted dry and weighed individually immediately prior to each challenge using a laboratory gram scale (Denver Instruments Company, model XL-1810) with weights rounded to the nearest thousandth of a gram. Mean weights for each variety in each challenge were calculated (Table 2-3).

Juveniles were moved into four indoor closed recirculating systems and acclimated for at least 96 hours prior to being challenged. Each recirculating system included a 0.007 m^3 air-wash bead filter (Aquaculture Systems Technologies, Limited Liability Corporation, New Orleans, Louisiana) connected to four 100-liter tanks, and each variety was housed in its own system. Juveniles were equally distributed among tanks within each system. Filters on these holding systems were backwashed weekly, and salinities never exceeded 0.7 ppt. Juveniles were fed

Table 2-3. Mean weights ($\pm$ SD) of four varieties of tilapia acclimated for salinity challenges.

Date	Variety	Weight (g)
September 16	Mississippi commercial	4.35 ± 1.08
September 16	Blue	3.90 ± 0.41
September 24	Florida red	4.04 ± 3.09
September 24	Nile	3.10 ± 1.22
October 4	Nile	2.75 ± 1.80
October 4	Blue	4.03 ± 2.37
October 18	Nile	3.18 ± 1.89
October 18	Mississippi commercial	2.76 ± 1.43
November 6	Florida red	3.38 ± 1.24
November 6	Blue	3.24 ± 1.30
November 16	Florida red	4.92 ± 1.56
November 16	Mississippi commercial	3.28 ± 1.16

Cargill Aquafeed ® catfish diet (28% protein) daily. A twelve-hour light, twelve-hour dark photoperiod was maintained throughout the experiment.

A pilot study was performed prior to salinity challenges in order to assess ammonia accumulation and appropriate stocking densities for the containers to be used in the trials. In this preliminary study the four varieties of tilapia were stocked separately at three densities: 0.50, 0.75, and 1.25 fish/L. Ammonia and nitrite accumulation, and fish behavior were documented every 24 hours over 96 hours to determine the highest stocking density in which ammonia levels remained $\leq$ 10 mg/L. Based on these results a stocking density of 0.50 fish/L was chosen. The ratio of 1:19 $CaCl_2$ to NaCl was used to provide total hardness ($\geq$ 145 mg/L) during the trials. Feeding juveniles prior to challenge, at 24 h, and at 97 h did not result in elevated ammonia levels ($\geq$ 15 mg/L).

Description of Acclimation and Salinity Trials

All fish were held in freshwater (0 ppt salinity) for 8 hours. Subsequently, the salinity in each experimental treatment was raised by 5 ppt every 8 hours until target salinities were

reached. Salinity increases were accomplished over 1-hour periods between 8 hour acclimation periods (Fig. 2-1). Each container was removed from its position and placed over a floor drain. Water was drained from the bucket while gently straining the tilapia out of the container with a dip net. Tilapia were immediately placed in a transfer container which held a portion of the new, higher salinity water. The container the tilapia were strained from was filled with new water until its volume equaled the difference between the water in the transfer container and the final designated volume. The tilapia were gently replaced into their original container with the additional water, filling to the designated fill line. If additional water was needed to reach the fill line it was added and the container returned to its original position. While fish were in transfer containers they were observed for mortalities and exterior signs of aggression (ragged fins and scale loss). The water temperature throughout each trial was also recorded from a container located within the middle of all containers (container 12/24) with a glass thermometer (Fisher Scientific Corporation, model 14-995-5B). Mortalities were determined by three criteria: failure to maintain position in the water column, lack of opercular movement as would normally be observed during respiration, and lack of response to touch. This process was repeated every 8 hours until each of the containers reached target salinities. After all experimental salinities were reached (63 h), observations and temperature readings were made every 12 hours until the end of the challenge. The total duration of each challenge was 97 hours. Fish were offered feed at 24 hours into each challenge. At 40 hours and 97 hours ammonia was recorded in the 0 ppt salinity treatments, under the assumption that ammonia would reach the highest levels in those containers that did not receive a water exchange. The 0 ppt salinity treatments intentionally did not receive water exchanges to indicate highest ammonia levels possible among varieties over a 97 h period. All surviving fish were re-acclimated to fresh water (< 5 ppt) following each trial

and were maintained for future use in other research or as broodstock. All expired fish were photographed to document their size and then disposed of.

Data Analysis

Data sets containing the following information were created for all replicates in the trials: challenge number, replicate number, salinity, time interval, mortality, and variety. These data were analyzed using the programs: PROC ANOVA and PROC LIFETEST (Statistical Analysis Software system version 8 for Windows ™; SAS Institute Inc., Cary, North Carolina).

A two-way ANOVA was performed to test for significant differences among replicate containers, salinities, and the interaction between salinity and replicates. Differences were considered significant at $P \leq 0.05$. Minimum, maximum, and mean were also calculated during the analysis for mortalities from all replicates for each variety. Survival means for each challenge salinity, from the three replicates, were recorded and summed across the three trials each variety was evaluated in. This final mean was designated the "cumulative survival mean." The LIFETEST program focuses on statistical analysis of the occurrence and timing of events. LIFETEST is a univariate analysis that produces life tables and graphs of survival curves. By analyzing the survival curves of two or more groups, LIFETEST assesses data for statistical significance, and facilitates development of a survival distribution function. The survival distribution function is useful in understanding when a specific event occurs (mortality) and whether that event can be reproduced and considered significant. In this study LIFETEST was used to characterize pooled survival data collected in each challenge for each variety and to calculate a standard error for each treatment.

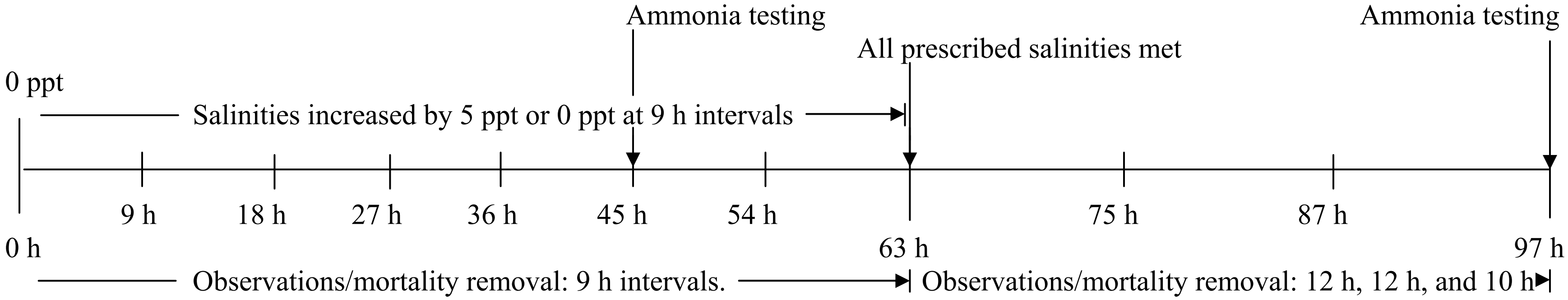

Figure 2-1. Description of procedures implemented during salinity challenges. Tilapia were acclimated to transition salinities for 8 hours. Water changes increasing salinity by 5 ppt were performed every 9 hours until prescribed salinity was achieved. At 45 h and 97 h ammonia was recorded in control containers and tilapia fed in all containers. All tilapia reached target salinity by 63 h, after which observations were made at 75 h, 87 h, and 97 h.

Results

Preliminary Stocking Density Study

Only the lowest density (0.50 fish/L) resulted in ≤ 10 ppm ammonia (Table 2-4). Nitrite was ≤ 0.15 mg/L in all varieties and stocking densities. Total hardness remained unchanged at 147 mg/L.

Table 2-4. Relationship between stocking densities and water quality for juvenile (mean 4 g) Mississippi commercial tilapia (MCT), Florida red tilapia (FRT), Nile tilapia (NT), and blue tilapia (BT) held in 20 L of freshwater for 96 hours.

	Stocking densities (fish/L)											
	MCT			FRT			NT			BT		
	0.50	0.75	1.25	0.50	0.75	1.25	0.50	0.75	1.25	0.50	0.75	1.25
Ammonia (mg/l)	7	14	8	8	10	8	7	7	10	5	6	10
Nitrite (mg/l)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2

Blue Tilapia

On 16 September (Trial 1), 4 October (Trial 2), and 6 November (Trial 3), 2001 separate groups of 120 blue tilapia juvenile (mean 3.72 ± 0.42 g) were subjected to four salinity treatments. Temperatures ranged between 26 C and 33 C during trials and ammonia ranged from 6 to 10 mg/L. Blue tilapia challenged at 0 ppt and 10 ppt exhibited 100% survival throughout trials. Although survival was high for blue tilapia held at 20 ppt (≥ 96%), those at 35 ppt salinity exhibited significant mortality (Fig. 2-2). Reduced survival at 35 ppt was consistent in all three trials with standard deviation less than ± 3% among all trials.

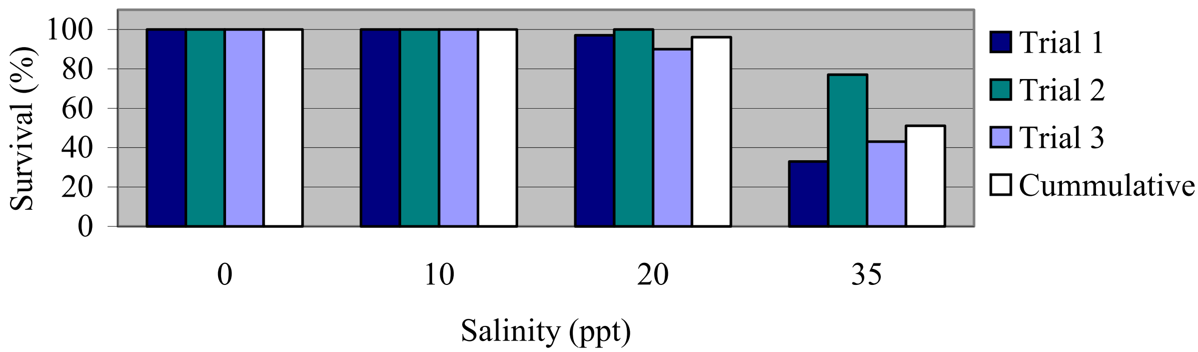

Figure 2-2. Survival of juvenile blue tilapia exposed to four salinity regimes (0, 10, 20, 35 ppt) during a 97-h period.

Florida Red Tilapia

On 24 September (Trial 1), 6 (Trial 2) and 16 November (Trial 3), 2001 separate groups of 120 juvenile (4.45 ± 0.77 g) Florida red tilapia were subjected to four salinity treatments. Temperatures ranged from 23-30 C, and ammonia ranged from 12 to 16 mg/L. Florida red tilapia challenged at 0 and 10 ppt exhibited a survival of ≥ 97%. At 20 ppt survival ranged from 100% to 70%. Survival at 35 ppt salinity varied greatly among trials exhibiting a standard deviation of ± 34% (Fig. 2-3).

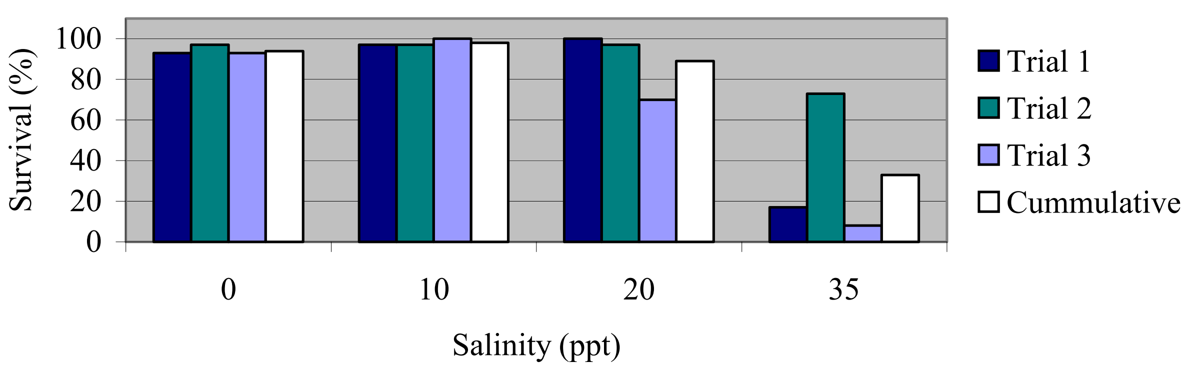

Figure 2-3. Survival of juvenile Florida red tilapia exposed to four salinity regimes (0, 10, 20, 35 ppt) during a 97-h period.

Mississippi Commercial Tilapia

On 16 September (Trial 1), 18 October (Trial 2), and 16 November, 2001 (Trial 3) separate groups of 120 juvenile (3.46 $\pm$ 0.81 g) Mississippi commercial tilapia (MCT) were subjected to four salinity treatments. Temperatures ranged from 26-31 C, and ammonia ranged 9 to 14 mg/L. Mississippi commercial tilapia subjected to 0 and 10 ppt exhibited 100% survival. At 20 ppt MCS expressed a high degree of variation in survival among trials (SD $\pm$ 49%). Variation in survival among replicates within trials were minimal except in Trial 2 where a standard deviation of $\pm$ 53% was observed. Mean survival for MCT at 20 ppt salinity was 54%. All MCT in the 35 ppt treatment died prior to reaching final salinity (Fig. 2-4).

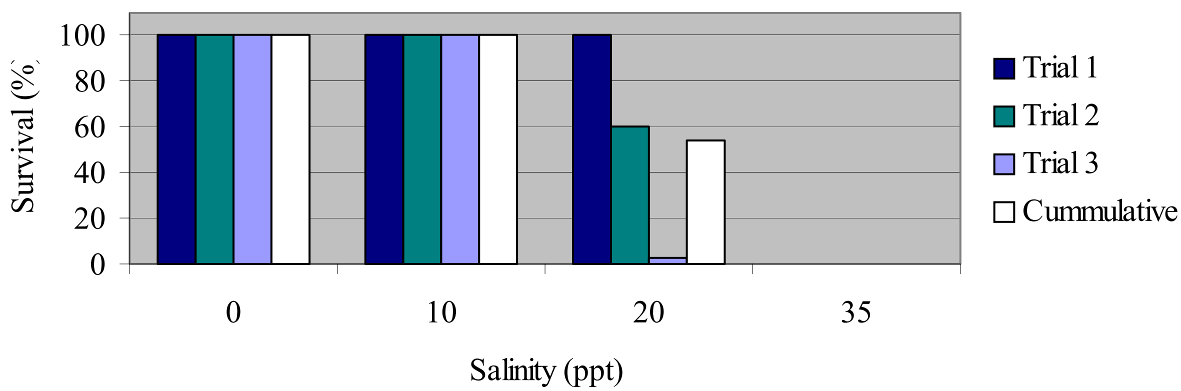

Figure 2-4. Survival of juvenile Mississippi commercial tilapia exposed to four salinity regimes (0, 10, 20, 35 ppt) during a 97-h period.

Nile Tilapia

On 24 September (Trial 1), 4 (Trial 2) and 18 October (Trial 3) separate groups of 120 juvenile (mean 3.0 $\pm$ 0.23 g) Nile tilapia were subjected to four saltwater treatments. Temperatures ranged from 23-33 C, and ammonia ranged from 13 to 16 mg/L. Survival at 0 and 10 ppt salinity regimes was 100%. Nile tilapia exposed to 20 ppt had a mean survival of 81% $\pm$ 12% (Fig. 2-5), and all Nile tilapia exposed to 35 ppt died.

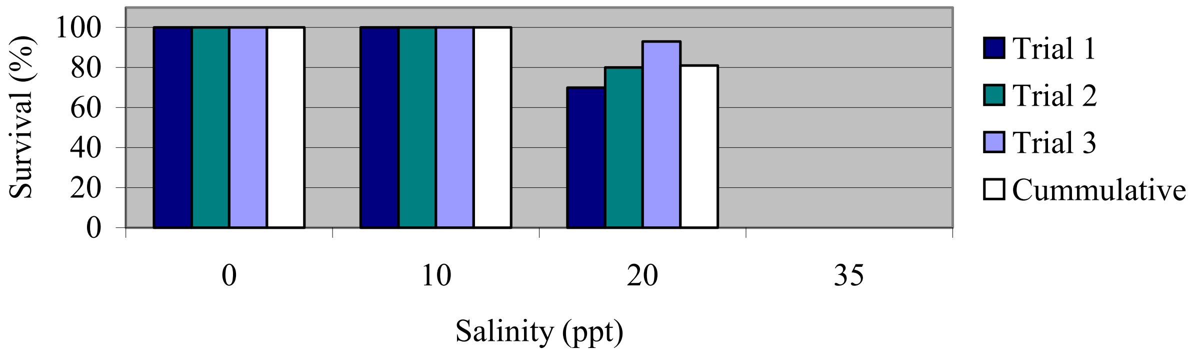

Figure 2-5. Survival of juvenile Nile tilapia exposed to four salinity regimes (0, 10, 20, 35 ppt) during a 97-h period.

Statistical Analysis

A two-way ANOVA for salinity tolerance was performed on the three replicates included in each challenge. There were no significant differences in survival among replicate containers within any variety (Table 2-5). Significant differences were found among treatment salinities for all varieties ($P < 0.01$). No significant interaction was noted between replicates and salinities (Table 2-5).

Table 2-5. Probability (*P*) values expressing no significant differences in survival among replicate containers and *P* values expressing significance ($P < 0.01$) in the interaction between replicates and salinities.

	P values expressing significance	
	Survival among replicate containers	Interaction between replicates and salinities
Oreochromis aureus	0.36	0.51
Mississippi commercial	0.86	0.89
Florida red	0.79	0.96
O. niloticus	0.95	1

A pair-wise comparison test was performed to evaluate survival differences of container replicates and challenge salinities. *Oreochromis aureus* showed no significant difference in

survival among salinities 0 ppt, 10 ppt, and 20 ppt. There was a highly significant difference in survival among replicates at 35 ppt salinity (containers 1 and 2, $P \leq 0.0001$; container 3 P= 0.0002). Florida red, Mississippi commercial, and Nile tilapia exhibited no significant differences at salinities of 0 ppt, 10 ppt, 20 ppt. Significant differences were found in Florida red ($P \leq 0.0002$), Mississippi commercial ($P \leq 0.0069$), and Nile ($P \leq 0.0001$) tilapias at 35 ppt salinity.

Proc LIFETEST predicted 100% survival among blue tilapia exposed to 0 and 10 ppt, high survival ($\geq$ 80%) at 20 ppt, and reduced survival ($\leq$ 50%) at 35 ppt (Fig. 2-6). Florida red

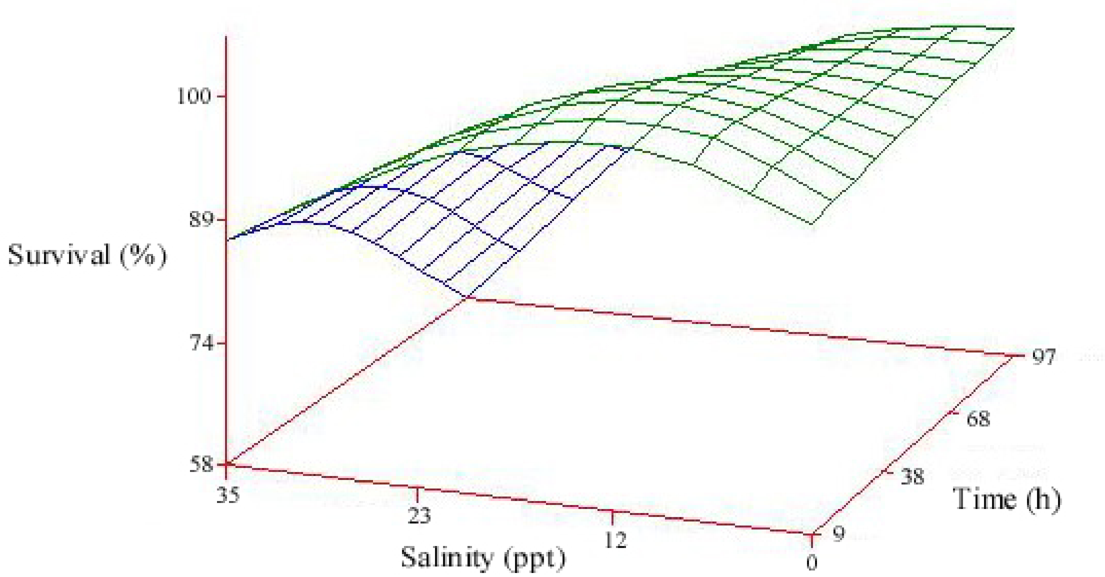

Figure 2-6. Results of proc LIFETEST performed on blue tilapia exposed to four salinity regimes (0, 10, 20, 35 ppt) for 97 hours.

tilapia had high survival ($\geq$ 80%) through 0, 10, and 20 ppt salinities with a reduction in survival (34 $\pm$ 5%) in the 35 ppt salinity regimes (Fig. 2-7). Mississippi commercial tilapia expressed complete survival at 0 and 10 ppt, moderate survival ($\leq$ 60%) at 20 ppt, and 100%

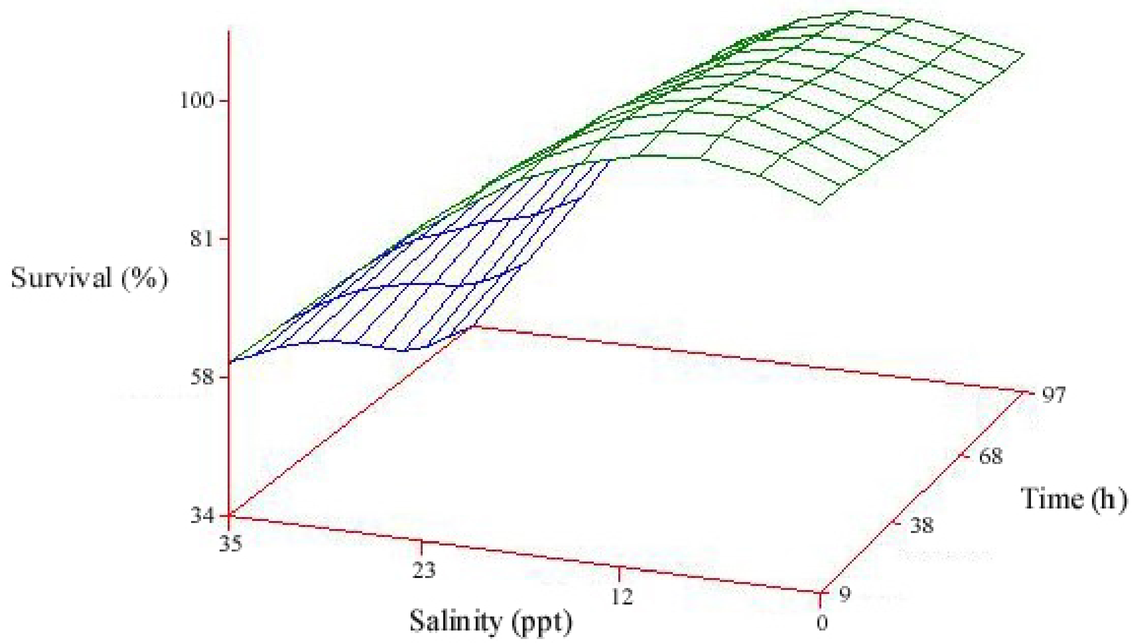

Figure 2-7. Results of proc LIFETEST performed on Florida red tilapia exposed to four salinity regimes (0, 10, 20, 35 ppt) for 97 hours.

mortality at 35 ppt (Fig. 2-8). Results of Nile tilapia closely corresponded more closely with

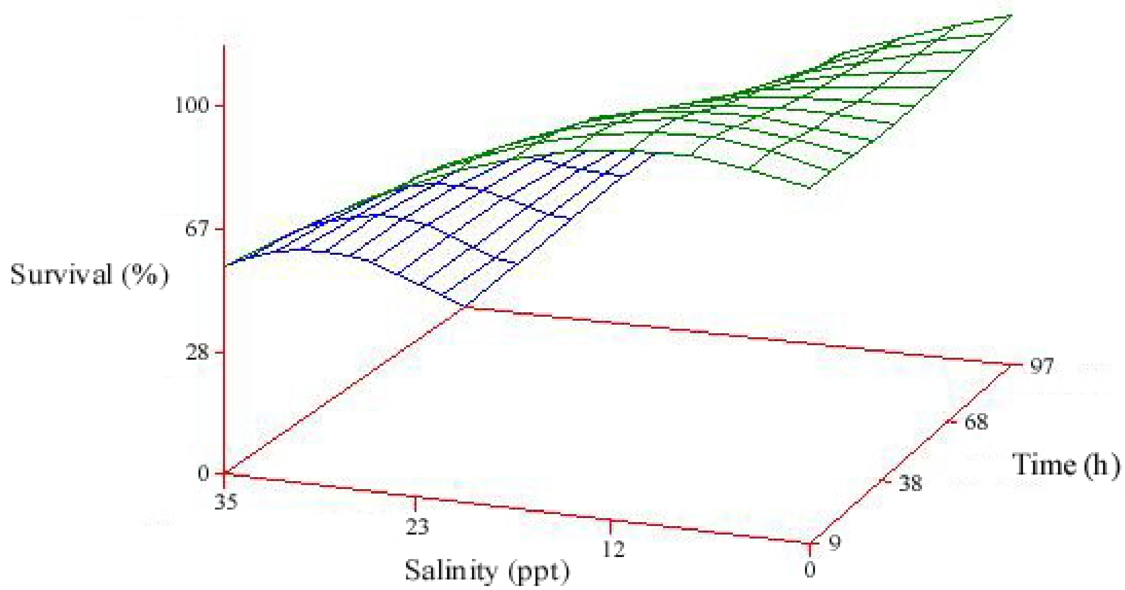

Figure 2-8. Results of proc LIFETEST performed on Mississippi commercial tilapia exposed to four salinity regimes (0, 10, 20, 35 ppt) for 97 hours.

those of Mississippi commercial tilapia, with complete survival at 0 and 10 ppt, high survival (≥ 83% among trials) at 20 ppt, and 100% mortality at 35 ppt (Fig. 2-9).

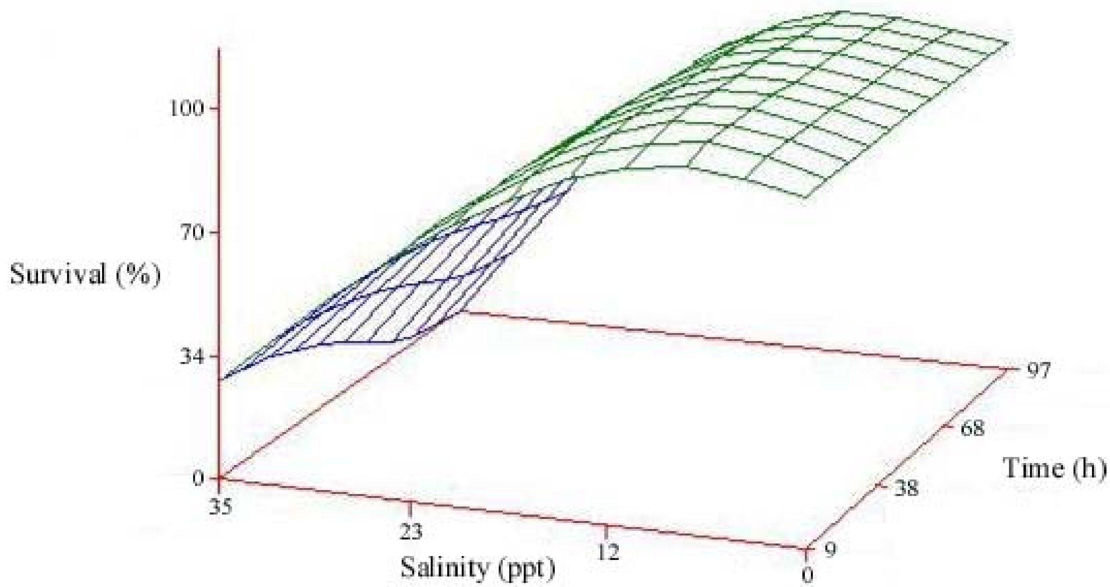

Figure 2-9. Results of proc LIFETEST performed on Nile tilapia exposed to four salinity regimes (0, 10, 20, 35 ppt) for 97 hours.

Discussion

Tilapia are widely cultured throughout the world, and many tilapia species have been evaluated for culture purposes (Wohlfarth and Hulata, 1981). Hybridization of tilapia is practiced worldwide (Lahav and Ra'anan, 1997; Wohlfarth and Hulata, 1981), allowing for beneficial traits such as salt tolerance to be incorporated into new tilapia varieties. Several recent research initiatives in tilapia aquaculture involve genetic improvement of these stocks (Stickney, 2000; Hallerman, 2002). As hybrid-based varieties are developed there is a need to characterize their environmental tolerances. Concerns about environmental tolerances in hybrids are twofold: in terms of commercial performance and out of growing concerns over possible detrimental effects aquaculture may have on native species and habitats. This study addressed these issues by evaluating salinity tolerance of two commercially produced hybrid-based varieties.

Salt tolerance for the purpose of this paper may be defined as the measurement of a tilapia's ability to survive elevated salt concentrations. Tolerance of moderate salinity (20 ppt) in Nile tilapia and high salt tolerance (> 35 ppt) in blue tilapia have been documented (Wohlfarth and Hulata, 1981) and were determined to be suitable for comparisons of salinity tolerance with the two hybrid-based varieties. Test salinities used in the study reflected those commonly found in estuaries in coastal Louisiana (NOAA, 1998), since tilapia accidentally released into the environment may attempt to over winter in coastal waters where temperatures are more favorable to survival. Temperatures during the experiment were not identified as an influencing factor. There was minimal fluctuation (23-33 C) during the experiment with temperatures varying < 5 C within a trial.

Results suggest several notable characteristics among varieties. Blue tilapia expressed the greatest tolerance for elevated salinity levels, with 96% survival at 20 ppt and 30 ppt salinities. As blue tilapia were exposed to 35 ppt salinity, survival decreased abruptly to 49%. Blue tilapia have been recognized to have high salt tolerance, in excess of 35 ppt in prior studies (Wohlfarth and Hulata, 1981) with survival at as high as 53 ppt after acclimation (Chervinski and Yashouv, 1971). Poor survival of blue tilapia juveniles within this study (mean survival of 54% over 34 h after acclimation) at 35 ppt was unexpected and no direct explanation could be found. Clearly, a prolonged acclimation period (5 ppt/8 h interval until 35 ppt at 63 h) may have attributed to mortality, but previous studies have indicated while direct transfer to seawater can result in almost complete mortality, graduated acclimation is sufficient to acclimate salt tolerant tilapia successfully (Villegas, 1990b; Philippart and Ruwet, 1982; Lotan, 1960).

Results for Florida red tilapia in this study indicate high survival ($\geq$ 89%) in salinities up to 20 ppt. Survival in the 35 ppt salinity regime varied among trials. Survival among replicates

within each of the three trials was uniform with < ± 2% difference in average survival. Previous studies suggest that FRT is very salt tolerant, withstanding salinities ≤ 37 ppt (Watanabe, 1989) and having a lower feed conversion ratio at (≥ 18 ppt) salinity versus freshwater (Watanabe et al., 1990). Unpublished data also suggested that survival among freshwater acclimated Florida red tilapia is much lower upon transfer to saline waters (Watanabe et al., 1989a). With high variation in survival among trials at 35 ppt, further investigation is warranted on why the Florida red tilapia in this study had such disparities in survival at 35 ppt.

Results of Mississippi commercial tilapia trials indicated this variety could survive in salinities up to 10 ppt for at least 97 hours. The majority of Mississippi commercial tilapia in the 35 ppt treatment died prior to reaching that salinity, with only 9% surviving to 35 ppt and the rest dying immediately upon transfer to 35 ppt salinity. No published research to date has investigated environmental tolerances of Mississippi commercial tilapia or closely related varieties and so no comparisons could be drawn from other work.

Juvenile Nile tilapia have been documented as surviving in salinities up to 25 ppt with acclimation (Al Asgah, 1984). Within this study, Nile tilapia expressed high survival (>81%) through 20 ppt salinity. All Nile tilapia died prior to reaching 35 ppt, with a significant amount (56%) dying at 25 ppt after 45 h of acclimation. Villegas (1990b) found while Nile tilapia could survive direct transfer to 15 ppt well (87%), direct transfers of Nile tilapia to >15 ppt resulted in 50% mortality at 20 ppt and complete mortality at 32 ppt. With stepwise acclimation of 5 ppt per 48 h till 20 ppt and 1 ppt thereafter, juvenile *O. niloticus* were found to tolerate salinities up to 28 ppt (Al Asgah, 1984).

Results of this study suggest that blue tilapia juveniles can withstand rapid acclimation to seawater and that Florida red tilapia can be acclimated rapidly to seawater with some mortality.

Nile and Mississippi commercial tilapias withstood rapid acclimation up to 20 ppt salinity. A more gradual acclimation of tilapia might increase survival, but may not properly model an accidental release of tilapia into Louisiana coastal waters. Studies have also indicated that early exposure to salinity while in the egg or larval stage increases salinity tolerance significantly (Al Asgah, 1984; Watanabe et al., 1985b; Watanabe et. al., 1989a). Beyond initial survival in brackish and seawater salinities debate continues as to whether tilapia could survive external factors (predators, current, temperatures, disease) they might encounter. Most notable of these factors is temperature. There is little information of the interactive effects of temperature and salinity tolerance in tilapia (Watanabe et al., 1993) and further examination is warranted.

References

Allen, K.O. 1971. Effects of salinity on growth and survival of channel catfish, *Ictalurus punctatus* (Rafinesque), eggs through yearlings. Ph.D. dissertation, Louisiana State University and Agricultural and Mechanical College, Baton Rouge, Louisiana.

Al Asgah, N.A. 1984. The effect of salinity on eggs and larvae of *Oreochromis niloticus* (Linnaeus). Arab Gulf Journal of Scientific Research 2:673-681.

Avella, M. and Doudet, T. 1993. Physiological adaptation of *Oreochromis niloticus* and *O. aureus* to salinity. International Center for Living Aquatic Resources Management Conference Proceedings 41:575.

Chervinski, J. and Yashouv, A. 1971. Preliminary experiments on the growth of *Tilapia aurea* Steindachner (Pisces, Cichlidae) in seawater ponds. Bamidgeh 23:125-129.

Clark, A.E., Watanabe, W.O., Olla, B.L. and Wicklund, R.I. 1990. Growth, feed conversion and protein utilization of Florida red tilapia fed isocaloric diets with different protein levels in seawater pools. Aquaculture 88:75-85.

Ernst, D.H.; Ellingson, L.J., Olla, B.L., Wicklund, R.I., Watanabe, W.O. and Grover, J.J. 1989. Production of Florida red tilapia in seawater pools: nursery rearing with chicken manure and growout with prepared feed. Aquaculture 80:247-260.

Fryer, G. and Iles, T.D. 1972. The Cichlid Fishes of the Great Lakes of Africa- Their Biology and Evolution. Oliver and Boyd, Edinburgh, Scotland.

Hallerman, E.M. 2002. Genetic improvement of tilapia at commercial RAS. Global Aquaculture Advocate 5:16-17.

Hickling, C.F. 1960. The malacca *Tilapia* hybrids. Journal of Genetics 57:1-10.

Hulata, G., Wohlfarth, G.W. and Halevy, A. 1986. Mass selection for growth rate in the Nile tilapia (*Oreochromis niloticus*). Aquaculture 57:177-184.

Lahav, E. and Ra'anan, Z. 1997. Salinity tolerance of genetically produced tilapia (Oreochromis) hybrids. Bamidgeh 49:160-165.

Lotan, R. 1960. Adaptability of *Tilapia aurea* to various saline conditions. Bamidgeh 12:96-100.

Lutz, C.G. 2000. Personal communication on Mississippi Commercial tilapia Ancestry, LSU Agricultural Center, Baton Rouge, Louisiana.

Mair, G.C. 2000. Genetics in tilapia culture. pages 136-148. in S. Subasinghe and T. Singh (eds.) Tilapia 2000: Tilapia: production, marketing, and technological developments: Proceedings of the Tilapia 2001 International Technical and Trade Conference on Tilapia. Kuala Lumpur, Malaysia.

National Oceanographic and Atmospheric Administration, 1998. Seasonal Salinity Isohaline Distribution Maps. http://biogeo.nos.noaa.gov/gom-efh/lma.htm.

Perez, J.E. and Maclean, N. 1976. The haemoglobins of the fish *Sarotherodon mossambicus* (Peters): functional significance and ontogenetic changes. Journal of Fisheries Biology 9:447-455.

Perry, W.G. and Avault, J.W. 1972. Comparison of striped mullet and tilapia for added production in caged catfish studies. The Progressive Fish Culturist 34:229-232.

Phillippart, J-Cl. and Ruwet, J-Cl. 1982. Ecology and distribution of tilapias. in R. Pullin and R. Lowe-McConnell, eds. The biology and culture of tilapias. International Center for Living Aquatic Resource Management, Manila, Philippines. pages 15-59.

Potts, W.T.W., Toster, M.A., Rudy, P.P. and Hewells, G.P. 1967. Sodium and water balance in the cichlid teleost *Tilapia mossambica*. Journal of Experimental Biology 47:461-470.

Rakocy, J.E., Hargreaves, J.A. and Bailey, D.S. 1993. Comparison of tilapia species for cage culture in the Virgin Islands. UVI Research 5:13-17.

Stickney, R.R. 2000. Status of research on tilapia. Pages. 23-26 in B.A. Costa-Pierce and J.E. Rakocy, editors. Tilapia Aquaculture in the Americas, Vol. 2. The World Aquaculture Society, Baton Rouge, Louisiana.

United States Congress, Office of Technology Assessment. 1993. Harmful Non-Indigenous Species in the United States, OTA-F-565. Washington, District of Columbia.

Villegas, C.T. 1990a. Growth and survival of *Oreochromis niloticus*, *O. mossambicus*, and their F_1 hybrids at various salinities. Proceedings of the Second Asian Fisheries Society. Manila, Philippines. 507-510.

Villegas, C.T. 1990b. Evaluation of salinity tolerance of *Oreochromis mossambicus*, *O. niloticus*, and their F1 hybrids. Aquaculture 85:281-282, 287.

Watanabe, W.O., Kuo, C.M. and Huang, M.C. 1985a. The ontogeny of salinity tolerance in the tilapias *Oreochromis aureus*, *O. niloticus*, and an *O. mossambicus* x *O. niloticus* hybrid, spawned and reared in freshwater. Aquaculture 47:353-367.

Watanabe, W.O., Kuo, C.M. and Huang, M.C. 1985b. Salinity tolerance of Nile tilapia fry (*Oreochromis niloticus*), spawned and hatched at various salinities. Aquaculture 48:159-176.

Watanabe, W.O., Ellingson, L.J., Wicklund, R.I. and Olla, B.L. 1988. The effects of salinity on growth, food consumption and conversion in juvenile, monosex male Florida red tilapia. International Center for Living Aquatic Resource Management Conference Proceedings 15. 515-523.

Watanabe, W.O., French, K.E., Ernst, D.H., Olla, B.L. and Wicklund, R.I. 1989a. Salinity during early development influences growth and survival of Florida red tilapia in brackish and seawater. Journal of the World Aquaculture Society 20:134-142.

Watanabe, W.O., Burnett, K.M., Olla, B.L. and Wicklund, R.I. 1989b. The effects of salinity on reproductive performance of Florida red tilapia. Journal of the World Aquaculture Society 20:223-229.

Watanabe, W.O., Ellington, L.J., Olla, B.L., Ernst, D.H. and Wicklund, R.I. 1990. Salinity tolerance and seawater survival vary ontogenetically in Florida red tilapia. Aquaculture 87:311-321.

Watanabe, W.O., Ernst, D.H., Chasar, M.P., Wicklund, R.I. and Olla, B.L. 1993. The effects of temperature and salinity on growth and feed utilization of juvenile, sex-reversed male Florida red tilapia cultured in a recirculating system. Aquaculture 112:309-320.

Watanabe, W.O., Olla, B.L., Wicklund, R.I. and Head, W.D. 1997. Saltwater culture of the Florida red tilapia and other saline-tolerant tilapias: a review. Pages 54-141 in B.A. Costa-Pierce and J.E. Rakocy, eds. Tilapia Aquaculture in the Americas, Volume 1. The World Aquaculture Society, Baton Rouge, Louisiana.

Wohlfarth, G.W and Hulata, G.I. 1981. Intraspecific Hybridization. Applied Genetics of Tilapias. International Center for Living Aquatic Resources Management Studies and Reviews. 2-18.

CHAPTER 3- A REVIEW OF THE POTENTIAL FOR FOUR COMMERCIAL TILAPIA VARIETIES TO ADAPT TO SALINITIES FOUND IN THE COASTAL WATERSHEDS OF LOUISIANA

Coastal habitats in Louisiana serve as a natural nursery for many native species found in the Gulf of Mexico and provide the state with an abundance of unique natural resources. Coastal habitats in Louisiana encompass approximately 2.8 million hectares of watersheds and marshes along the Gulf of Mexico, divided by the Mississippi river and its delta (Figure 3-1) (Chabreck, 1972). These coastal lands and water bottoms support important commercial and recreational

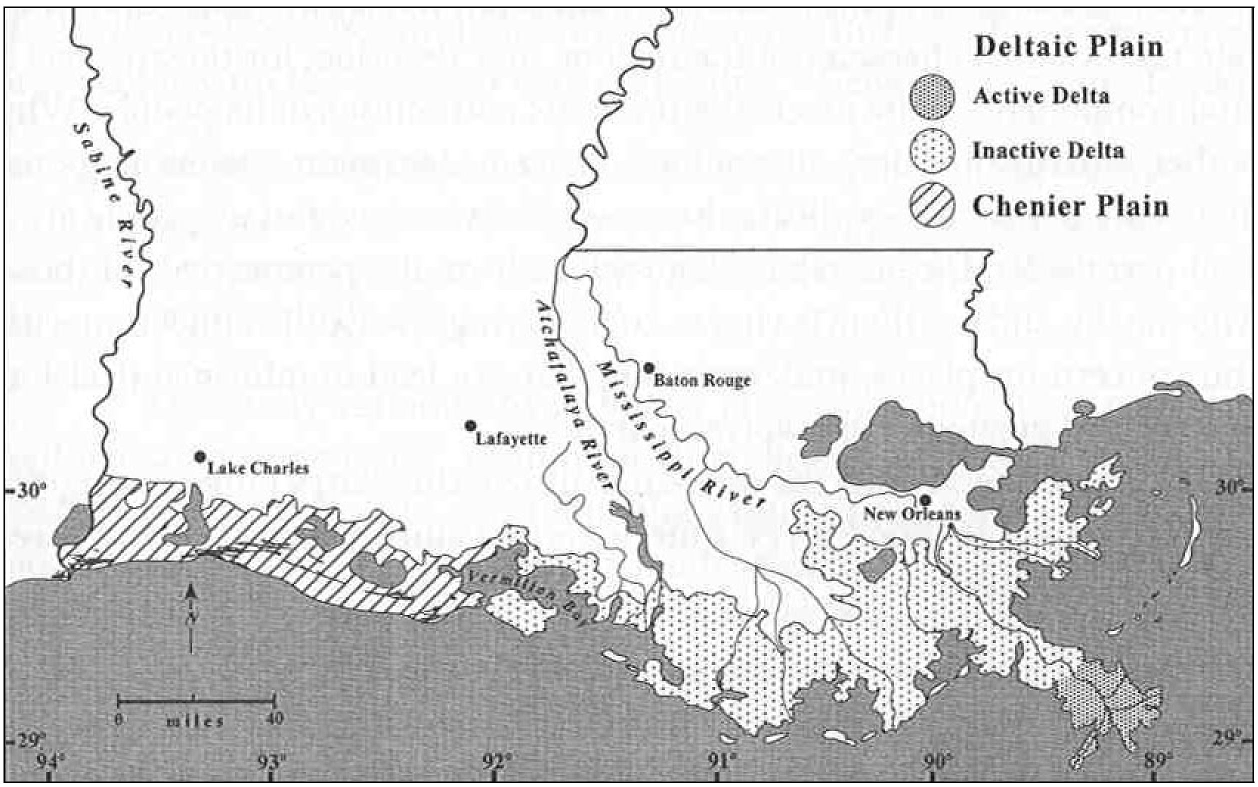

Figure 3-1. Map of Coastal Louisiana identifying the Deltaic and Chenier Plains (Chabreck, 1972).

fisheries in Louisiana that directly contribute more than three billion dollars to the state economy annually (Committee on the Future of Coastal Louisiana, 2002). Louisiana leads all other Gulf of Mexico states in fisheries production, contributing to 66% of landings and 39% of dollar value (Chesney et al., 1999). Additionally, approximately 70% of migratory fowl using the

Mississippi and Central flyways overwinter in coastal Louisiana (Louisiana Coastal Wetlands Conservation and Restoration Task Force and the Wetlands Conservation and Restoration Authority, 1998), making these wetlands important economically and ecologically not only to the state, but the region as well.

Louisiana has a history of seafood production and its proximity to established markets and plentiful water resources have allowed for a burgeoning aquaculture industry that was valued at approximately 203 million dollars in 2001 (Louisiana Cooperative Extension Service, 2001). While aquaculture is ancient in its practice, many sectors of the industry are in their infancy with extensive research dedicated to the development of stocks and production techniques. One offshoot of this research is genetic improvement of aquaculture species. In tilapia, hybridization has been successful with new varieties being developed for production (Wohlfarth and Hulata, 1981). Some varieties have been rushed into production before investigations into their environmental tolerances are complete (Wohlfarth and Hulata, 1981).

Accidental introduction and establishment of an invasive exotic species such as tilapia could result in detrimental changes to native ecosystems that may include habitat and recruitment loss of native flora and fauna (Courtenay and Robbins, 1973). However, it has long been assumed that most of the southeastern United States (U.S.) could not support feral populations of accidentally released tilapia due in large part to their inherent need for temperatures $\geq$ 10 C (Hargreaves, 2000). Tilapia are tropical species that are not well suited for cold temperatures that occur periodically in the southeast (Wohlfarth and Hulata, 1981).

Nonetheless, wild tilapia populations have been observed in Texas, Mississippi, Alabama, Georgia, and Florida where climate and water resources allow. Many of these environments closely resemble those of Louisiana. In Mississippi, tilapia populations have been

observed in coastal canals following accidental releases by farmers evaluating tilapia (Lutz, 2001). Tilapia have been intentionally stocked into lakes, reservoirs, and rivers of Texas, Alabama and Florida as prey for game fish or as sportfish and have persisted where temperature allows (Hargreaves, 2000).

Although it is widely accepted that tilapia, a tropical fish, would be unable to survive sub-tropical temperatures (< 20 C) experienced in most of the southeastern U.S. during winter, there could be exceptions if released tilapia located "thermal refuges", where waters are less affected by winter temperatures. Prior studies have shown that tilapia will actively seek out warmer temperatures when necessary (McBay, 1961) and that tilapia kept at elevated salinity may have broader tolerances to low temperatures than previously thought (Watanabe et al., 1989).

One such thermal refuge may possibly be the Gulf coast of the U.S. where water temperatures are generally warmer than inland waters. The brackish estuaries found along the Gulf of Mexico could provide wintering grounds for tilapia able to survive ambient salinity. The coastal estuaries of Louisiana could be particularly well suited as a thermal refuge, where in addition to favorable temperatures, salinities drop throughout winter (5-10 ppt) due to winds, currents, and the outflow of the Mississippi river (National Oceanic and Atmospheric Administration, 1998). With temperature being the sole condition limiting survival of tilapia year round in the southeastern U.S. (Hargreaves, 2000), the ability for tilapia to over winter in thermal refuges should be addressed and potential thermal refuges identified. The purpose of this review is to identify factors that could influence the ability of four tilapia varieties: Nile (*Oreochromis niloticus*), blue (*O.* aureus), Florida red (*O. hornorum* x *O. mossambicus*), and

Mississippi commercial (ancestry undetermined) tilapias to become established in south Louisiana.

Geographic Considerations

The coastal wetlands of Louisiana developed during the Pleistocene Age as the Mississippi river and its delta changed course and meandered across its flood plain, depositing layers of sedimentation that formed marshes and marsh prairie lands (Chabreck, 1972). The coast of Louisiana can be divided into two separate and distinctive geographic regions: the Deltaic Plain and the Chenier Plain (Fig. 3-1). The Chenier Plain is comprised of all wetlands west of Vermillion Bay. The Deltaic Plain is comprised of all coastal wetlands east of Vermillion Bay. It is younger and is still geologically active along the mouth of the Mississippi river. The Deltaic Plain is comprised of many types of aquatic habitat, including rivers, streams, bays, lakes, ponds, and numerous canals. Because the Deltaic Plain includes so many different habitats, the drainage of water is complex and allows for several stratifications in salinity.

Drainage is an important determinant of the offshore salinity of these coastal areas, although not the only one. Seasonal changes dramatically affect salinity along the coast as well. Each season has characteristic salinity stratification due to currents, tides, prevailing wind directions, precipitation, and the Mississippi and other river discharges (Chabreck, 1972).

The semi-tropical climate of Louisiana and year-round accessibility to groundwater provide tilapia culturists with advantages not found in much of the United States. Tilapia are a particularly invasive fish due to their reproduction at very young ages and spawn up to every 30 days (Avault, 1996). Tilapia are very adaptable and may be found in many differing habitats (e.g. rivers, lakes, estuaries) and are able to survive on many types of vegetation (Rakocy et al.,

1993). These characteristics have allowed them to crowd out native species, interfering with recruitment by competing for nesting sites (Butnz and Manooch, 1969; Noble et al., 1976) and by preying on native fry (Lobel, 1980). With few studies performed on tilapia and their effects on native species and habitats it has been difficult to assess the threat tilapia may pose to native environments. Due in part to this lack of information on tilapia the state of Louisiana has been conservative in its approach to allowing tilapia aquaculture in Louisiana (see Regulation of Tilapia in Louisiana).

In Florida released tilapia have become so prevalent the state has established the first capture fishery for tilapia in the United States (Costa-Pierce and Riedel, 2000). Tilapia are currently found in at least eighteen counties in Florida and have become established in many of the environments (e.g. lakes, rivers, canals) they have had access to (Courtenay et al., 1984), becoming the most common exotic fish encountered in Florida (Costa-Pierce and Riedel, 2000).

While it has been demonstrated that some salt tolerant tilapia (e.g. *O. mossambicus, O. aureus*) could survive exposure to seawater and possibly become established in marine environments (Lobel, 1980) there is little evidence to suggest they would (Hargreaves, 2000). Few studies have evaluated the risk tilapia introductions pose to native habitats, and while tilapia have been implicated in vegetation loss and a reduction in native fish populations (Courtenay and Robins, 1973; Schoenherr, 1988) little concrete evidence exists (Hargreaves, 2000). Butnz and Manooch (1969) speculated that tilapia in Florida lakes were competing with bream (*Lepomis macrochirus, L. microlophulus*) for nesting habitats and juveniles competed for food. Competition for nesting sites was confirmed by Noble et al. (1976) where by three ponds were stocked with largemouth bass (*Micropterus salmoides*) and varying densities of blue tilapia. Recruitment of largemouth bass was highest in the pond with no tilapia present and lowest (no

recruitment) where tilapia densities were highest. Those tilapia that have become established in salt water habitats in such diverse locations as Fanning Atoll of the Line Islands of the central Pacific Ocean and the Salton Sea in California have shown a preference for estuaries and brackish inshore waters that contain vegetative littoral habitats with low water current (Lobel, 1980; Costa-Pierce and Riedel, 2000; Hargreaves, 2000), habitats similar to coastal Louisiana.

The extensive, naturally occurring waterways and navigational canals found in coastal Louisiana could allow tilapia numerous avenues to every part of costal Louisiana. *Oreochromis mossambicus* released in canals in Arizona eventually spread via canals into southern California, and are now established in the Salton Sea (Costa –Pierce and Doyle, 1997). Instances in which introduced tilapia have altered aquatic biota have been observed and recorded. Crutchfield (1995) found when *Tillapia zilli* and *Oreochromis aureus* were accidentally released into a power plant cooling reservoir that *T. zilli* eliminated all aquatic macrophyte growth from the reservoir within two years and adversely affected native populations of golden shiner (*Notemigonus crysoleucas*), eastern mosquito fish (*Gambusia holbrooki*), and green sunfish (*Lepomis cyanellus*) that required macrophytes as shelter. The study also stated *T. zilli* was so prolific it became the forth most abundant fish in the 1,760-ha reservoir only 3 years after the release of approximately 100 individuals. Schoenherr (1988) implicated tilapia introductions in the decline of the desert pupfish (*Cyprinodon macularius*) along the Colorado river and resident populations of *Tilapia zilli* have been identified by Horn (1988) and Knaggs (1976) near power plant effluents in Huntington Beach and in Upper Newport Bay in California. Courtenay (1991) states that wherever tilapia have been released for vegetation control they have crowded out native fishes and altered the native biota. However, findings implicating tilapia in native habitat

destruction are rejected by some scientists as being based on anecdotal evidence, with a lack of empirical data supporting it (Courtenay, 1997).

More intensive studies on tilapia introduction and disruptive influences on native habitat are lacking. If tilapia were introduced into the coastal watersheds of Louisiana and were able to become established this would alter the ecology. Destruction of vegetation by tilapia could not only alter important habitats for many aquatic species, but could also increase erosion and coastal land loss.

Regulation of Tilapia in Louisiana

The possession, transport, production, and sale of tilapia in Louisiana is tightly controlled. No person may possess tilapia in Louisiana without a permit, which calls for a $25,000 performance bond. The permit is granted only after investigation and inspection of the premises proposed to hold the tilapia. Additional permits are needed to transport or sell live animals and the production and sale of live tilapia must be reported to the state annually.

Regulation of tilapia in Louisiana (Appendix C) is stricter than most surrounding states with Mississippi, Alabama, and Arkansas having no restriction on tilapia. Texas and Florida have enacted some regulation, but much of it addresses importation of new tilapia stocks (Courtenay, 1997).

There are few federal regulations designed to curtail the spread of non-indigenous species. Statutes that allow the federal government to restrict non-indigenous species include: the 1948 amendment to the Lacey Act of 1900 (18 United States Code 42); the Fish and Wildlife Act of 1956 (16 United States Code 742a-742j); the 1972 Migratory Bird Convention with Japan (24 United States Treaty 3329); and the Endangered Species Act of 1973 (16 United States Code 1531-1543). Of these regulations the Lacey Act is the most encompassing, allowing for

prohibition of the import, possession, and transport of species deemed dangerous to humans and the natural resources of the United States (Courtenay, 1997). However, the Lacey Act amendment and other statutes have been underutilized and reactionary. The Secretary of the Interior has authority through these regulations to prohibit non-indigenous species, but only air-breathing catfish (family *Clariidae*) and some salmonid fishes have been prohibited to date (Courtenay, 1997). Some have suggested that instead of reacting to species that have become problematic it would be far better for the United States Department of the Interior to institute mandatory comprehensive testing prior to introduction. Due to weak federal regulations, non-indigenous species regulation has been left up to state governments.

With no encompassing federal regulation of tilapia over state borders, each state acts in its own interests with little or no coordination among states. Regulation of tilapia on a state-by-state basis could allow for introduction of tilapia into Louisiana watersheds through migration from adjacent states. *Tilapia zilli* released in Arizona irrigation canals for aquatic weed control migrated through these canals into California where it became established in the Salton Sea (Costa–Pierce and Doyle, 1997).

Salinity and Temperature and the Role they Play in Tilapia Survival

The coast of Louisiana may be divided into five "bio-salinity zones" (NOAA, 1998) that fluctuate seasonally (Table 3-1). These zones were developed from data published by the

Table 3-1. Five distinct salinity zones found in coastal Louisiana.

Bio-Salinity Zones	Salinities encountered
Zone I	0.0 - 0.5 ppt
Zone II	0.5 - 5.0 ppt
Zone III	5.0 - 15.0 ppt
Zone IV	15.0 - 25.0 ppt
Zone V	> 25.0 ppt

National Oceanic and Atmospheric Association (NOAA). They have been plotted on seasonal salinity isohaline distribution maps of coastal Louisiana (Figure 3-2; NOAA, 1998). Based on available salinity tolerance data (Table 3-2) it appears likely that if *O. niloticus*, *O. aureus*, or Florida red tilapia (*O. mossambicus* x *O. hornorum*) were to be introduced to prevailing coastal

Table 3-2. Percent survival recorded from Chapter 2 in which juvenile (mean 4 g) tilapia of four varieties were investigated for salinity tolerance by exposure to four regimes (0, 10, 20, 35 ppt) over a 97-h period.

Variety	Salinity (ppt)			
	0	10	20	35
Blue (*Oreochromis aureus*)	100	100	96	54
Florida red (*O. hornorum x O. mossambicus*)	94	98	89	33
Mississippi commercial tilapia	100	100	5	0
Nile (*O. niloticus*)	100	100	81	0

salinities they could survive given suitable temperatures in up to four of the five winter bio-salinity zones recognized by NOAA. The results indicate that juvenile Mississippi commercial tilapia (MCT), while not surviving well beyond 10 ppt challenge salinity, would not immediately die due to exposure to low salinity ($\leq$ 10 ppt) waters encountered in inshore and coastal habitats, such as Lake Pontchartrain and Lake Borne. This would allow MCT to survive in the lower three recognized winter bio-salinity zones.

The idea that tilapia would be prone to lethal temperatures and therefore not establish themselves in Louisiana (Hargreaves, 2000) will depend on temperatures of coastal waters in light of the current information which indicates that as inland temperatures decline through the

winter months so do coastal salinities (Fig. 3-2). The complex river systems found in Louisiana could allow tilapia, a fish recognized for its ability to migrate to favorable conditions (e.g. temperature [Hargreaves, 2000; Costa –Pierce and Riedel, 2000] and habitat [Courtenay, 1991]), to enter coastal sites that could act as "thermal refuges" in response to unfavorable temperatures

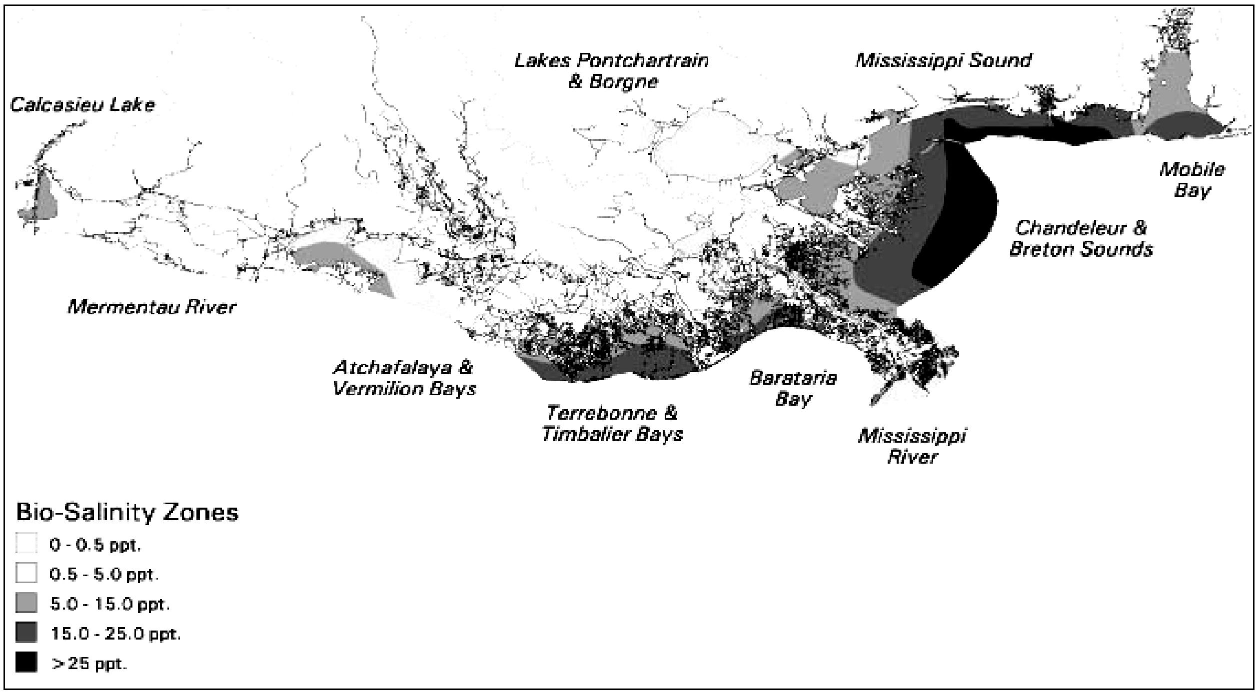

Figure 3-2. Coastal salinity map indicates five separate salinity strata develop during winter in Louisiana (NOAA, 1998).

(≤ 10 C). The concept of tilapia using thermal refuges has been validated. In southern California tilapia have been observed in coastal waters along Huntington Beach (Costa –Pierce and Doyle, 1997) and upper Newport Bay (Knaggs, 1976) around power plant effluent. In several instances tilapia have been released into power plant cooling reservoirs and established themselves in regions where surrounding water temperatures were unsuitable (< 10 C) (Kirk, 1972; Crutchfield, 1995). In Hargreaves (2000) review of tilapia aquaculture in the southeast

United States he stated that the ability for tilapia to survive year round in the United States was largely a function of minimum temperature.

Many euryhaline tilapia can reproduce at elevated salinity levels. The mossambique tilapia (*O. mossambicus*) is regarded as one of the most salt tolerant with reproduction at 45 ppt (Popper and Lichatowich, 1975). The blue tilapia *O. aureus* survives salinities as high as 52 ppt and reproduce at 20 ppt (Perry and Avault, 1972). Initial studies of the Florida red tilapia have concluded it can reproduce in salinities up to 36 ppt, but reproduces optimally at 18 ppt (Watanabe et al., 1993). Nile tilapia (*O. niloticus*) possess some tolerance for salinity, with survival and even reproduction up to 20-25 ppt (Kirk, 1972; Al Asgah, 1984). No data are currently available for the ability of Mississippi commercial tilapia (MCT) to reproduce at elevated salinity.

The potential in particular for FRT to not only survive, but reproduce in brackish water along with the establishment of a reproductive salinity tolerance for MCT must also be addressed. The ability for these tilapias to reproduce at salinity would not automatically predict success in introduction to new habitats; several factors could prevent their establishment (e.g. pathogens and native predators). Data suggest that several species and varieties of tilapia could withstand and even reproduce at salinities commonly found in coastal Louisiana and could pose some risk of introduction to the coast if temperatures allowed their survival.

Of the two hybrid-based varieties in this review, the Florida red tilapia appears to hold the greatest potential for survival in coastal habitats based on the findings in this study, and from studies that document the affinity of FRT for saline waters (Watanabe et al., 1989; Watanabe et al., 1990, Watanabe et al., 1993). Results from this study and others (Watanabe et al., 1989; Watanabe et al., 1990, Watanabe et al., 1993) indicate FRT could survive salinities found at bio-

salinity zones from 0-25 ppt with a reduction in survival at salinities $\geq$ 35 ppt. Previous papers indicate that FRT can survive full-strength seawater (Ernst et al., 1989), and can be most successfully spawned at 18 ppt (Watanabe et al., 1989). Florida red tilapia physiologically function more efficiently in brackish and saline waters, with lower feed conversion ratios and faster weight gain than FRT raised in freshwater (Head et al., 1994). Concerns have been raised over possible release of FRT into marine ecosystems (Watanabe et al., 1989). If FRT could survive and reproduce in coastal marshes under favorable temperatures, they would pose an introduction risk to coastal Louisiana.

Juveniles of a second hybrid-based variety, the MCT, exhibited survival in salinities of 10 ppt, which may be encountered in coastal areas of the state during winter. High variation in survival within individual challenge salinities of MCT salinity trials (Chapter 2) warrant further investigation into the salinity tolerance range of MCT.

Juvenile Nile tilapia exhibited high survival ($\geq$ 81%) at challenge salinities up to 20 ppt (Table 3-2) in recent trials (Chapter 2). Early studies in Hawaii found Nile tilapia at 20 ppt were able to grow and reproduce (Brock and Takata, 1955). These findings were confirmed by Al Asgah (1984) reporting reproduction in salinities up to 20 ppt and survival at 20-25 ppt (Watanabe et al., 1985), with successful direct transfer from freshwater to salinities up to 15 ppt (Villegas, 1990). Recent trials (Chapter 2) and previous work indicate Nile tilapia could withstand salinities up to 20 ppt, salinities commonly found in Louisiana coastal estuaries (NOAA, 1998). Based solely on salinity data, the Nile tilapia poses a possible introduction risk to coastal Louisiana wherever favorable water temperatures prevail.

The blue tilapia, *O. aureus*, also exhibited typically high salt tolerance with survival up to 35 ppt in a recent study (Chapter 2). Previous studies indicate its survival in salinities up to 53

ppt and reproduction in salinities as high as 19 ppt (Chervinski and Yashouv, 1971), similar to the reproduction range of FRT (Watanabe et al., 1989). Blue tilapia are also widely cited as among the most cold tolerant tilapia (Wohlfarth and Hulata, 1981), with evidence of increased survival at 5 ppt versus 0 ppt at cold temperatures (Chervinski, 1966), and this species might make use of coastal habitats as thermal refuges during winter months.

The role salinity plays in temperature tolerance is not fully understood. Traditionally, elevated salinity has been considered a stessor that would not provide any benefit to tilapia (Allanaon et al, 1971; Tilney and Hocutt, 1987), but studies conducted on Florida red tilapia (Watanabe et al., 1989) have suggested there may be benefit from exposure to salinity during cold episodes. Gradual acclimation to low temperatures may also increase low temperature tolerance. Further, Watanabe et al. (1989) suggests tolerance to lowered temperatures is more a function of acclimation time rather than mean lethal temperature. Results of the study indicate that the four tilapia varieties evaluated in Chapter 2 could survive Louisiana's coastal salinities if favorable temperatures or thermal refuges were available. As temperatures drop through the winter, coastal salinities parallel this trend with lowest salinities being recorded when tilapia would most need thermal refuges.

Increased coordination and cooperation among state governments, tilapia producers, and aquaculture researchers would best protect Louisiana's coastal habitats from the possible threat of tilapia introduction (Fig. 3-3). State governments must work closely to develop regulations that could prevent tilapia introductions across state borders. Researchers should continue to work with tilapia producers in developing improved stocks and further examine environmental tolerances of common varieties. These initiatives will lead to more efficient production of tilapia, increasing revenue for producers. Additionally, it will provide better understanding of

tilapia biology and allow for a more comprehensive risk assessment of the potential threat tilapia pose to the native environments.

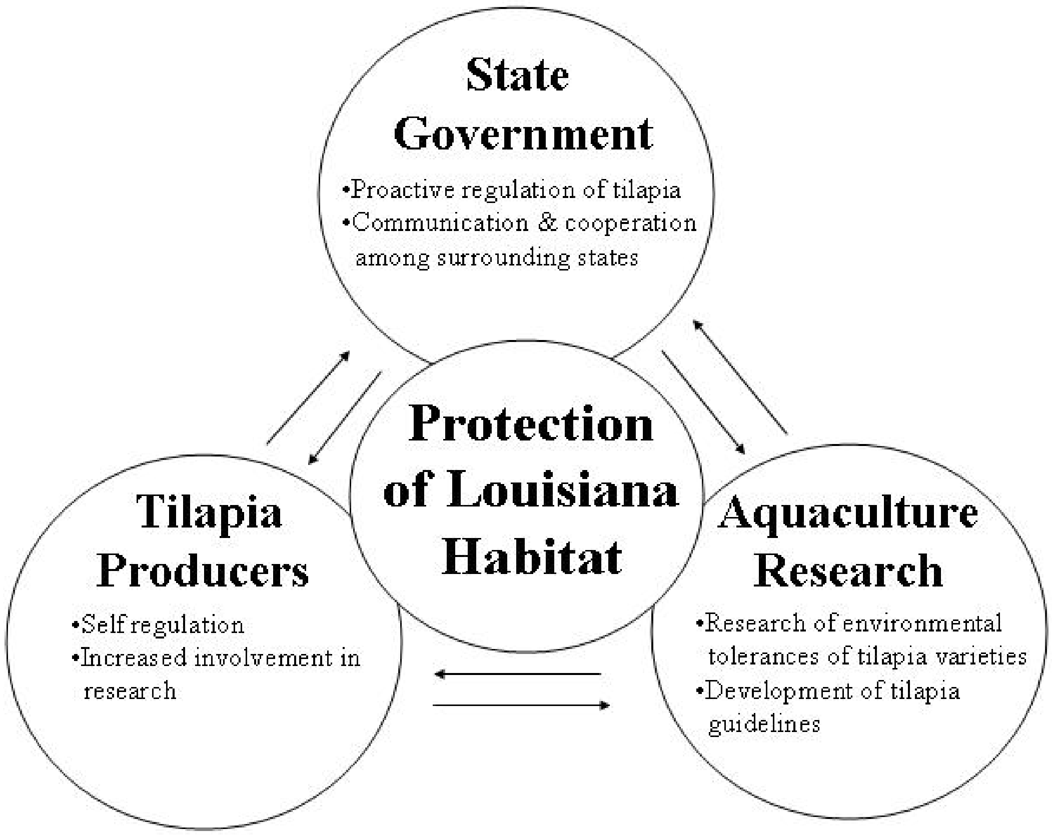

Figure 3-3. Entities involved in successful tilapia regulation and production in Louisiana.

References

Al Asgah, N.A. 1984. The effect of salinity on eggs and larvae of *Oreochromis niloticus* (Linnaeus). Arab Gulf Journal of Scientific Research 2:673-681.

Allanson, B.R., Bok, A. and van Wyk, N.I. 1971. The influence of exposure to low temperature on *Tilapia mossambica* Peters (Cichlidae). II. changes in serum osmolarity, sodium and chlorides ion concentrations. Journal of Fish Biology 3:181-185.

Avault, J.W. 1996. Fundamentals of Aquaculture. AVA Publishing Company, Baton Rouge, Louisiana.

Brock, E. and Takata, M. 1955. Contribution to the problems of boat capture and mortality together with experiments in the use of *Tilapia* as live bait. Industrial Research Advisory Council Hawaii, Final Report 49:39-40.

Butnz, J. and Manooch, III. C.S. 1969. *Tilapia aurea* (Steindacher), a rapidly spreading exotic in south central Florida. Proceedings of the South-eastern association of Game and Fish Commissioners 22:495-501.

Chabreck, H. Robert. 1972. Vegetation, Water and Soil Characteristics of the Louisiana Coastal Region. LSU Agricultural Experiment Station Bulletin 664:5-17.

Chervinski, J. 1966. Growth of *Tilapia aurea* in brackish water ponds. Bamigeh 18:81-83.

Chervinski, J. and Yashouv, A. 1971. Preliminary experiments on the growth of *Tilapia aurea* Steindachner (Pisces, Cichlidae) in seawater ponds. Bamidgeh 23:125-129.

Chesney, E.J., Baltz, D.M. and Thomas, R.G. 1999. Louisiana estuarine and coastal fisheries and habitats: perspectives from a fish's eye view. Ecological Applications 10:350-366.

Committee on the Future of Coastal Louisiana. 2002. Saving coastal Louisiana: recommendations for implementing an expanded coastal restoration program. Governor's Office of Coastal Activities, February. Baton Rouge, Louisiana. page 5.

Costa-Pierce, B.A. and Doyle, R.W. 1997. Genetic identification and status of tilapia regional strains in southern California. pages 1-17. <u>in</u> B.A. Coasta-Pierce and J.E. Rockocy eds. Tilapia Aquaculture in the Americas, Volume one. World Aquaculture Society. Baton Rouge, Louisiana.

Costa-Pierce B.A. and Riedel, R. 2000. Fisheries ecology of the tilapias in subtropical lakes in the United States. pages 1-20. <u>in</u> B.A. Coasta-Pierce and J.E. Rockocy eds. Tilapia Aquaculture in the Americas, Volume two. World Aquaculture Society. Baton Rouge, Louisiana.

Courtenay, jr. W.R. and Robbins, C.R. 1973. Exotic aquatic organisms in Florida with emphasis on fishes: a review and recommendations. Transactions of the American Fisheries Society 102:1-13.

Courtenay, jr. W.R., Hensley, D.A., Taylor, J.N. and McCann, J.A. 1984. Distribution of exotic fishes in the continental United States. pages 41-77. <u>in</u> W.R. Courtenay, jr. and J.R. Stauffer, jr., eds. Distribution, Biology, and Management of Exotic Fishes. John Hopkins University Press, Baltimore, Maryland.

Courtenay, jr. W.R. 1991. Biological pollution through fish introductions. pages 35-61 <u>in</u> McKnight, B.N. Biological Pollution: The Control and Impact of Invasive Exotic Species. Indiana Academy of Science, Indianapolis, Indiana.

Courtenay, jr. W.R. 1997. Tilapias as non-indigenous species in the Americas: environmental, regulatory and legal issues. pages 18-33. <u>in</u> B.A. Coasta-Pierce and J.E. Rockocy eds. Tilapia Aquaculture in the Americas, Volume one. World Aquaculture Society. Baton Rouge, Louisiana.

Crutchfield, jr. J.U. 1995. Establishment and expansion of redbelly tilapia and blue tilapia in a power plant cooling reservoir. pages 452-461. <u>in</u> Schramm, H.L., Piper, R.G. Uses and Effects of Cultured Fishes in Aquatic Ecosystems. American Fisheries Society, Bethesda, Maryland.

Ernst, D.H., Ellingson, L.J., Olla, B.L., Wicklund, R.I., Watanabe, W.O. and Grover, J.J. 1989. Production of Florida red tilapia in seawater pools: nursery rearing with chicken manure and growout with prepared feed. Aquaculture 80:247-260.

Hargreaves, J.A., 2000. Tilapia culture in the southeast United States. pages 60-81. <u>in</u> Costa-Pierce and Rakocy, J.E. Tilapia Aquaculture of the Americas, volume 2. The World Aquaculture Society, Baton Rouge, Louisiana.

Head, W.D. and Zerbi A. 1994. Preliminary observations on the marketability of salt-water-cultured Florida red tilapia in Puerto Rico. Journal of the World Aquaculture Society 25:432-441.

Horn, M. 1988. The fish community of the upper Newport Bay ecological reserve. pages 80-92. <u>in</u> The Natural and Social Sciences of Orange County. Natural History Foundation of Orange County, Newport Beach, California.

Kirk, R.G. 1972. A review of recent developments in Tilapia culture, with special reference to fish farming in the heated effluents of power stations. Aquaculture 1:45-60.

Knaggs, E. 1976. Status of the genus *Tilapia* in California's estuarine and marine waters. Unpublished Report, Operations Research Branch, California Department of Fish and Game, November 1976.

Lobel, P.S. 1980. Invasion by the mozambique tilapia (*Sarotherodon mossambicus*; Pices; Cichlidae) of a pacific atoll marine ecosystem. Micronesica 16:349-355.

Louisiana Coastal Wetlands Conservation and Restoration Task Force and the Wetlands Conservation and Restoration Authority. 1998. Coast 2050: Toward a Sustainable Coastal Louisiana. Louisiana Department of Natural Resources, Baton Rouge, Louisiana. page 57.

Louisiana Cooperative Extension Service. 2001. Louisiana Summary, Agriculture and Natural Resources 2001, Publication 2382. Louisiana Cooperative Extension Service. Baton Rouge, Louisiana. Pages 5-24.

Lutz, C.G. 2001. Personal communication on Mississippi commercial tilapia ancestry. Louisiana State University Agricultural Center, Baton Rouge, Louisiana.

McBay, L.G. 1961. The biology of *Tilapia nilotica* Linneaus. Proceedings of the Annual Conference of the Southeastern Association of game and Fish Commissioners 15:208-218.

National Oceanographic and Atmospheric Administration. 1998. Seasonal Salinity Isohaline Distribution Maps. http://biogeo.nos.noaa.gov/gom-efh/lma.htm.

Noble, R.L., Germany, R.D. and Hall, C.R. 1976. Interactions of blue tilapia and largemouth bass in a power plant cooling reservoir. Proceedings of the Southeastern Association of Game and Fish Commissioners 29:247-251.

Perry, W.G. and Avault, J.W. 1972. Comparison of stripped mullet and tilapia for added production in caged catfish studies. Progressive Fish Culturist 34:229-232.

Popper, D. and Lichatowich, T. 1975. Preliminary success in predator control of *Tilapia mossambica*. Aquaculture 5:213-214.

Schoenherr, A. 1988. A review of the life history of the dessert pupfish, *Cyprinodon macularius*. Bulletin of the Southern California Academy of Sciences 87:104-134.

Tilney, R.L. and Hocutt, C.H., 1987. Changes in gill epithelia of *Oreochromis mossambicus* subjected to cold shock. Environmental Biology of Fishes 19:35-44.

U.S. Congress, Office of Technology Assessment. 1993. Harmful Non-Indigenous Species in the United States, OTA-F-565, Washington, District of Columbia. page 263.

Villegas, C.T. 1990. Evaluation of salinity tolerance of *Oreochromis mossambicus*, *O. niloticus*, and their F1 hybrids. Aquaculture 85:281-282.

Watanabe, W.O., Kuo, C.M. and Huang, M.C. 1985a. The ontogeny of salinity tolerance in the tilapias *Oreochromis aureus*, *O. niloticus*, and an *O. mossambicus* x *O. niloticus* hybrid, spawned and reared in freshwater. Aquaculture 47:353-367.

Watanabe, W.O., French, K.E., Ernst, D.H., Olla, B.L. and Wicklund, R.I. 1989. Salinity during early development influences growth and survival of Florida red tilapia in brackish and seawater. Journal of the World Aquaculture Society 20:134-142.

Watanabe, W.O., Clark, J.H., Dunham, J.B., Wicklund, R.I. and Olla, B.L. 1990. Production of fingerling Florida red tilapia (*Tilapia hornorum* x *T. mossambica*) in floating marine cages. The Progressive Fish Culturist 52:158-161.

Watanabe, W.O., Ernst, D.H., Chasar, M.P., Wicklund, R.I. and Olla, B.L. 1993. The effects of temperature and salinity on growth and feed utilization of juvenile, sex-reversed male Florida red tilapia cultured in a recirculating system. Aquaculture 112:309-320.

Wohlfarth, G.W and Hulata, G.I. 1981. Intraspecific Hybridization. Applied Genetics of Tilapias. International Center for Living Aquatic Resources Management Studies and Reviews. Pages 2-18.

CHAPTER 4- GENERAL CONCLUSIONS

Regulations were enacted in Louisiana in 1992 to allow for tilapia culture and simultaneously reduce the possibility of escape of these fish into native habitats. Capture and recreational fisheries have been a major source of revenue for the state, contributing three billion dollars annually to the economy of the state (Committee on the Future of Coastal Louisiana, 2002). These fisheries depend on healthy nursery habitats located within the coastal marshes of the state. The potential for introduced tilapia to become established in these habitats has been debated for a number of years (U.S. Congress, Office of Technology Assessment, 1993; Arnoldi, 1998). It has been reported that tilapia would not survive low temperatures (< 10 C) occasionally encountered in most of southeastern United States (Hargreaves, 2000). However, little information has been compiled regarding the ability of coastal saline waters to serve as thermal refuges during cold episodes.

This study was designed to address some of these concerns by generating data to more objectively evaluate salinity tolerances of commercially available tilapia varieties. The four challenge salinities (0 ppt, 10 ppt, 20 ppt, 35 ppt) used in the study are commonly encountered in coastal Louisiana waters. Juvenile tilapia that weighed approximately 4 grams were used in this study for several reasons: the frequency with which this size of fish may be moved during sale or transport to production facilities, the fact that their size could allow such tilapia to escape unnoticed, and because of the potential concentrations of numbers that might accidentally be released in one incident. It was equally important to evaluate tilapia with sufficient body mass to initially withstand immediate stressors such as handling and increased salinity that may occur during an accidental release.

The two hybrid-based varieties used in this study, Florida red and Mississippi commercial tilapia, were included for several reasons: their present commercial use, the prospect that they may become more important to the U.S. tilapia industry, and because of the inadequate amount of knowledge collected to date on their environmental tolerances (e.g. salinity and temperature). Two well-established species, *O. niloticus* and *O. aureus*, were used for benchmark comparisons because of their broad differences in salinity tolerance. It was anticipated that the salinity tolerance of the two hybrid tilapia varieties would fall between those of these two species and that direct comparisons among all varieties would aid in the determination of how tolerant each was to salinity. After all tolerances were evaluated, they were compared to common coastal salinities.

Reasoning for studying salinity tolerances of these four tilapia varieties and their potential for survival in common coastal salinities include the possibility that tilapia released into Louisiana could survive and become established in spite of temperate conditions that occasionally occur during winter. Although, tilapia are tropical species that are not well adapted for the semi-tropical climate of Louisiana, it has been suggested that introduced tilapia could survive cold temperatures by migrating to coastal habitats where they would encounter warmer waters due to the proximity of the Gulf of Mexico. If tilapia could survive the brackish conditions and winter temperatures found in coastal marshes of Louisiana they could become environmental pests and possibly damage marshes and the state's capture fisheries through vegetation loss and interfering with native fish recruitment. Problems with introduced tilapia expanding their ranges, eliminating vegetation, and disrupting native species have been encountered in southern Florida, southeast Georgia, and the Salton Sea in California (Courtenay, 1993; U.S. Congress Office of Technology Assessment, 1993; Arnoldi, 1998).

Tilapia characteristically are prolific and very adaptive to many different environments. It is these traits that have allowed tilapia to spread from just a few introduction points into eighteen counties in Florida (Courtenay et al., 1984). Tilapia released into Arizona irrigation canals for weed control eventually spread across the border of Arizona into California where they have become established in the Salton sea (Costa-Pierce and Riedel, 2000) and parts of Colorado river (Schoenherr, 1988).

Results of the study indicate that the blue tilapia, Nile tilapia, and Florida red tilapia (FRT) could survive salinities found throughout the year along coastal Louisiana. Data collected for the Mississippi commercial tilapia (MCT) suggest that salinity tolerance along coastal Louisiana would be poor, with only 5% survival at 20 ppt (Chapter 2). Mississippi commercial tilapia could be restricted to more inland waters that remain at 5-15 ppt salinity. Due to high variation in survival among replicates at 20 ppt salinity (Chapter 2) and no other data available on their salinity tolerance it is impossible to predict the exact salinity tolerance of the Mississippi commercial tilapia. All containers of each selected salinity were compared for independence with no significant differences found among containers; mortalities observed were not associated with any particular container. As a result, the possibility exists that at least some Mississippi commercial tilapia could exhibit salinity tolerance similar to those of blue, Nile, and Florida red tilapia.

While this study evaluated juvenile tilapia survival at four salinities, it did not account for other factors that introduced tilapias might encounter in natural habitats. Temperature, predation, and disease will all negatively impact the survival of tilapias released in Louisiana. An unrelated factor that could influence survival is coloration. Bright coloration is important for tilapia marketability, as Florida red tilapia are similar in coloration to highly desired snapper

species (family *Lutjanidae*), but could make some varieties targets for predation, particularly by birds (Myer, 2001). Florida red tilapia are red to red-calico and Mississippi commercial tilapia can range from grey to pearly white. Whether these coloration patterns would persist in released populations is uncertain. Reproduction of *O. aureus*, *O. niloticus* (Wohlfarth and Hulata, 1981), and FRT (Watanabe, 1989) have been reported at or above most commonly occurring Louisiana coastal salinities. The long-term survival and reproductive performance of Mississippi commercial tilapia at elevated salinities has not been studied.

Investigation of the temperature tolerances of the varieties used in this study and the combined effects of temperature and salinity on mortality would provide insight into the potential for tilapia to inhabit the coastal waters of Louisiana. Some research suggests that Florida red tilapia exhibit increased cold tolerance at higher salinity (Watanabe et al., 1993). A more complete description of the environmental tolerances of Florida red tilapia and Mississippi commercial tilapia is also warranted based on their use in commercial aquaculture throughout the southeastern United States.

References

Arnoldi, D.C. 1998. Tilapia research needs. Project Statement. Department of Wildlife and Fisheries. Baton Rouge, Louisiana.

Committee on the Future of Coastal Louisiana. 2002. Saving coastal Louisiana: recommendations for implementing an expanded coastal restoration program. Governor's Office of Coastal Activities, February. Baton Rouge, Louisiana. page 5.

Costa-Pierce B.A. and Riedel, R. 2000. Fisheries ecology of the tilapias in subtropical lakes in the United States. pages 1-20. in B.A. Coasta-Pierce and J.E. Rockocy eds. Tilapia Aquaculture in the Americas, Volume two. World Aquaculture Society. Baton Rouge, Louisiana.

Courtenay, jr. W.R., Hensley, D.A., Taylor, J.N. and McCann, J.A. 1984. Distribution of exotic fishes in the continental United States. pages 41-77. in W.R. Courtenay, jr. and J.R. Stauffer, jr., eds. Distribution, Biology, and Management of Exotic Fishes. John Hopkins University Press, Baltimore, Maryland.

Courtenay, jr. W.R. 1993. Biological pollution through fish introductions. Pages 35-61 in McKnight, B.N. Biological Pollution: The Control and Impact of Invasive Exotic Species. Indianapolis, Indiana.

Hargreaves, J.A. 2000. Tilapia culture in the southeast United States. pages 60-81. in Costa-Pierce and Rakocy, J.E. Tilapia Aquaculture of the Americas, volume 2. The World Aquaculture Society, Baton Rouge, Louisiana.

Meyer, D.E. 2001. Small scale Tilapia culture in rural Central America. Global Aquaculture Advocate 4:47-48.

Schoenherr, A. 1988. A review of the life history of the dessert pupfish, *Cyprinodon macularius*. Bulletin of the Southern California Academy of Sciences 87:104-134.

United States Congress, Office of Technology Assessment. 1993. Harmful Non-Indigenous Species in the United States, OTA-F-565. Washington, District of Columbia.

Villegas, C.T. 1990. Evaluation of salinity tolerance of *Oreochromis mossambicus*, *O. niloticus*, and their F1 hybrids. Aquaculture 85:281-282.

Watanabe, W.O., French, K.E., Ernst, D.H., Olla, B.L. and Wicklund, R.I. 1989. Salinity during early development influences growth and survival of Florida red tilapia in brackish and seawater. Journal of the World Aquaculture Society 20: 134-142.

Watanabe, W.O., Ernst, D.H., Chasar, M.P., Wicklund, R.I. and Olla, B.L. 1993. The effects of temperature and salinity on growth and feed utilization of juvenile, sex-reversed male Florida red tilapia cultured in a recirculating system. Aquaculture 112:309-320.

Wohlfarth, G.W and Hulata, G.I. 1981. Intraspecific Hybridization. Applied Genetics of Tilapias. International Center for Living Aquatic Resources Management Studies and Reviews. pages 2-18.

APPENDIX A- OUTLINE OF PROCEDURES USED DURING SALINITY CHALLENGES

I. Collection of tilapia from outside tanks
   A. Water in outside tanks drained
      1. Standpipes are removed and a net is placed over drain to prevent tilapia from escaping
      2. If drains are clogged then a large siphon pipe and a bucket are used to drain tanks
   B. Tilapia are netted as the water drains
   C. Tilapia are placed into 96-liter coolers with the water from their tank and aerated via an airstone
   D. After all tilapia are removed from tank they are carried to the laboratory

II. Introduction to laboratory systems: One system per variety
   A. A cooler is placed next to a 4-tank closed recirculating system and aeration is again provided with airstone, note: temperatures in field and in laboratory were similar during collection
   B. Tilapia of each variety are separately netted and equally distributed among the four tanks

III. Preparation for challenge
   A. Tilapia are maintained in recirculating systems for $\geq$ 48 hours
   B. Tilapia are fed to satiation once a day including the day of the trial

IV. Introduction to containers for challenge
   A. Water supply to each holding tank is turned off
   B. Holding water for each system is measured for salinity and is recorded
   C. Water is lowered using a siphon to allow fish to be caught easily, reducing stress
   D. Tilapia are netted, and a sample weighed
   E. Tilapia are stocked from left to right in a serpentine fashion, 10 fish per container with every fourth container stocked until completion

V. Acclimatization to challenge salinity
   A. Tilapia are held at 0.0 ppt salinity for 8 hours
   B. After the initial eight hour holding period
      1. The water of each experimental container is replaced every 8 hours with water 5 ppt higher salinity, with one additional hour for transfer. This continues till challenge salinity is met
      2. Temperature is recorded from the holding system closest to containers every eight hours
      3. Every container is checked for mortalities every eight hours
         a. Mortalities are observed and recorded
         b. A picture is taken of each container’s mortalities
         c. Dead tilapia are disposed of
      4. At 40 hours into the acclimatization process
         a. Water in the control container is tested for ammonia and recorded
         b. All container’s tilapia are fed 1 gram of catfish diet and observed for feeding

VI. Tilapia are observed for at least 34 hours at their challenge salinity
   A. Temperature is recorded from holding system closest to containers every eight hours
   B. Every container is checked for mortalities every twelve hours
         a. Mortalities are observed and recorded
         b. A picture is taken of each container’s mortalities

c. Dead tilapia are disposed of through incineration

VII. Tilapia are collected from challenge and stocked into the recirculating holding systems

A. A final count is made of all living tilapia

B. The control container's water is tested for ammonia and recorded

C. Each container's tilapia are fed 1 gram of catfish diet and observed for feeding

D. Tilapia are netted and released into a holding system

E. Containers are rinsed

VIII. Challenge ended

APPENDIX B- DATA OF: TRIAL, VARIETY, SALINITY, AND SURVIVAL USED IN CHAPTER 2

September 16, 2001 Percent Survival

Variety	0 ppt	5 ppt	10 ppt	15 ppt	20 ppt	25 ppt	30 ppt	35 ppt
Blue	100							
Blue	100							
Blue	100							
Blue	100	100	100					
Blue	100	100	100					
Blue	100	100	100					
Blue	100	100	100	100	100			
Blue	100	100	100	100	90			
Blue	100	100	100	100	100			
Blue	100	100	100	100	90	90	40	20
Blue	100	100	100	100	100	100	100	70
Blue	100	100	100	100	100	100	50	10
Mississippi Commercial	100							
Mississippi Commercial	100							
Mississippi Commercial	100							
Mississippi Commercial	100	100	100					
Mississippi Commercial	100	100	100					
Mississippi Commercial	100	100	100					
Mississippi Commercial	100	100	100	100	100			
Mississippi Commercial	100	100	100	100	100			
Mississippi Commercial	100	100	100	100	100			
Mississippi Commercial	100	100	100	100	100	70	0	0
Mississippi Commercial	100	100	100	100	100	60	10	0
Mississippi Commercial	100	100	100	100	100	60	20	0

September 24, 2001 Percent Survival

Variety	0 ppt	5 ppt	10 ppt	15 ppt	20 ppt	25 ppt	30 ppt	35 ppt
Florida Red	100							
Florida Red	90							
Florida Red	90							
Florida Red	100	100	100					
Florida Red	100	100	90					
Florida Red	100	100	100					
Florida Red	100	100	100	100	100			
Florida Red	100	100	100	100	100			
Florida Red	100	100	100	100	100			
Florida Red	100	100	100	100	100	50	0	0
Florida Red	100	100	100	100	80	80	30	30
Florida Red	100	100	100	100	100	60	20	20
Nile	100							
Nile	100							
Nile	100							
Nile	100	100	100					
Nile	100	100	100					
Nile	100	100	100					
Nile	100	100	100	100	80			
Nile	100	100	100	100	60			
Nile	100	100	100	100	70			
Nile	100	100	100	100	90	0	0	0
Nile	100	100	100	100	100	0	0	0
Nile	100	100	100	100	90	0	0	0

October 4, 2001 Percent Survival

Variety	0 ppt	5 ppt	10 ppt	15 ppt	20 ppt	25 ppt	30 ppt	35 ppt
Nile	100							
Nile	100							
Nile	100							
Nile	100	100	100					
Nile	100	100	100					
Nile	100	100	100					
Nile	100	100	100	100	80			
Nile	100	100	100	100	90			
Nile	100	100	100	100	70			
Nile	100	100	100	100	100	20	10	0
Nile	100	100	100	100	100	40	0	0
Nile	100	100	100	100	100	30	0	0
Blue	100							
Blue	100							
Blue	100							
Blue	100	100	100					
Blue	100	100	100					
Blue	100	100	100					
Blue	100	100	100	100	100			
Blue	100	100	100	100	100			
Blue	100	100	100	100	100			
Blue	100	100	100	100	100	100	100	70
Blue	100	100	100	100	100	100	100	70
Blue	100	100	100	100	90	100	100	90

October 14, 2001 Percent Survival

Species or Variety	0 ppt	5 ppt	10 ppt	15 ppt	20 ppt	25 ppt	30 ppt	35 ppt
Nile	100							
Nile	100							
Nile	100							
Nile	100	100	100					
Nile	100	100	100					
Nile	100	100	100					
Nile	100	100	100	100	90			
Nile	100	100	100	100	90			
Nile	100	100	100	100	100			
Nile	100	100	100	100	0	0	0	0
Nile	100	100	100	100	0	0	0	0
Nile	100	100	100	100	10	0	0	0
Mississippi Commercial	100							
Mississippi Commercial	100							
Mississippi Commercial	100							
Mississippi Commercial	100	100	100					
Mississippi Commercial	100	100	100					
Mississippi Commercial	100	100	100					
Mississippi Commercial	100	100	100	100	100			
Mississippi Commercial	100	100	100	100	80			
Mississippi Commercial	100	100	100	100	0			
Mississippi Commercial	100	100	100	100	100	30	20	0
Mississippi Commercial	100	100	100	100	100	50	0	0
Mississippi Commercial	100	100	100	100	90	20	10	0

November 6, 2001 Percent Survival

Species or Variety	0 ppt	5 ppt	10 ppt	15 ppt	20 ppt	25 ppt	30 ppt	35 ppt
Florida Red	90							
Florida Red	100							
Florida Red	100							
Florida Red	100	100	100					
Florida Red	100	100	90					
Florida Red	100	100	100					
Florida Red	100	100	100	100	100			
Florida Red	100	100	100	100	100			
Florida Red	100	100	100	100	90			
Florida Red	100	100	100	100	100	70	50	50
Florida Red	100	100	100	100	100	80	80	80
Florida Red	100	100	100	100	90	90	90	90
Blue	100							
Blue	100							
Blue	100							
Blue	100	100	100					
Blue	100	100	100					
Blue	100	100	100					
Blue	100	100	100	100	80			
Blue	100	100	100	100	100			
Blue	100	100	100	100	90			
Blue	100	100	100	100	100	100	80	30
Blue	100	100	100	100	100	90	70	70
Blue	100	100	100	100	100	80	40	30

November 16, 2001 Percent Survival

Species or Variety	0 ppt	5 ppt	10 ppt	15 ppt	20 ppt	25 ppt	30 ppt	35 ppt
Florida Red	100							
Florida Red	80							
Florida Red	100							
Florida Red	100	100	100					
Florida Red	100	100	100					
Florida Red	100	100	100					
Florida Red	100	100	100	100	80			
Florida Red	100	100	100	100	60			
Florida Red	100	100	100	100	70			
Florida Red	100	100	100	100	70	50	30	20*
Florida Red	100	100	100	100	60	30	0	0
Florida Red	100	100	100	100	30	10	10	10
Mississippi Commercial	100							
Mississippi Commercial	100							
Mississippi Commercial	100							
Mississippi Commercial	100	100	100					
Mississippi Commercial	100	100	100					
Mississippi Commercial	100	100	100					
Mississippi Commercial	100	100	100	100	0			
Mississippi Commercial	100	100	100	100	0			
Mississippi Commercial	100	100	100	100	10			
Mississippi Commercial	100	100	100	70	20	0	0	0
Mississippi Commercial	100	100	100	40	0	0	0	0
Mississippi Commercial	100	100	100	30	0	0	0	0

- Denotes one fish removed from study for dissection

APPENDIX C- REGULATION OF TILAPIA SPECIES IN LOUISIANA

Louisiana Revised Statute, Title 56, Section 319. Exotic fish; importation, sale, and possession of certain exotic species prohibited; permit required; penalty.

A. No person, firm, or corporation shall at any possess, sell, or cause to be transported into this state by any other person, firm, or corporation, without first obtaining the written permission of the secretary of the department of Wildlife and Fisheries, any of the following species of fish: carnero catfish (*Clarias batrachus*), all of the family clariidae, freshwater electric eel (*Electrophorus* sp.), carp [except those taken in state waters, provided such fish shall be dead when in a person's possession, common carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*)], rudd (*Scardinius erythrophthalmus*), and all species of tilapia.

B. All permits granted under the provisions hereof shall be on an animal basis except for permits issued for triploid grass carp possession and transportation for aquatic plant control. All requests for such permission shall indicate the source, number, and destination of the species named therein.

C. The provisions contained herein shall be enforced by the commission, and its decision in the granting or denial of the permission referred to herein shall be final.

D. No person shall have in possession or sell in this state a piranha or Rio Grande Tetra; except that, piranha may be possession and displayed at the Aquarium of the Americas, Audubon Institute, New Orleans, as authorized by a special permit issued by the department, under conditions the department deems necessary to prevent their introduction into waters of the state. Neither the permit nor the conditions and requirements thereof, shall be required to be adopted pursuant to the provisions of the Administrative Procedure Act.

E. Violation of any of the provisions of this section constitutes a class four violation.

Louisiana Revised Statute, Title 77, Section 903. Tilapia.

A. Rules and Regulations on Importation, Culture, Possession, Disposal and Sale of Tilapia in Louisiana. The following terms shall have the following meanings in this section.

Culture – all activities associated with the propagation and nurturing of tilapia.

Culture Permittee – the individual or organization that possesses a valid Louisiana tilapia permit.

Culture System – shall be an approved indoor system designed such that all water containing, or that at any time might contain, tilapia (adult fish, juvenile fish, fry, or fish eggs) is filtered, screened and/or sterilized in such a manner as the department deems adequate to prevent any possibility of escape from the system.

Department – the Louisiana Department of Wildlife and Fisheries or an authorized employee of the Department.

Disposal – the business of processing, selling, or purposely removing tilapia from the culture system.

Live Holding Permittee – the individual or organization that possesses a valid Louisiana tilapia live holding permit.

Live Holding System – an approved indoor holding or display system designed such that all water containing, or at any time might contain, tilapia (adult fish, juvenile fish, fry or fish eggs) is filtered, screened and/or sterilized prior to release in such a manner as the department deems adequate to prevent any possibility of escape.

Process – the act of chill killing whole tilapia in an ice slurry for a period of not less than 60 minutes, or removal of tilapia intestines followed by immersion in an ice slurry for a period of not less than two minutes or removal and proper disposal of tilapia heads in such manner as the department deems necessary to prevent any possibility of accidental release of fry or fertilized eggs.

Secretary – secretary of the department of Wildlife and Fisheries.

Tilapia – eggs, fish, or body parts belonging to the genera Tilapia, Sarotherdon, or Oreochromis and their hybrids.

Tilapia culture permit – official document pertaining to culture that identifies the terms of, and allows for the importation, exportation, transport, culture, possession, disposal, transfer and sale of tilapia in Louisiana as approved by the secretary or his designee.

Tilapia live holding permit – official document pertaining to live holding for retail sale that identifies the terms of, and allows for the possession and sale of tilapia in Louisiana as approved by the secretary of his designee.

B. Tilapia Permit Request Procedures

1. Individuals or organizations wishing to import, export, transport, culture possess, dispose, transfer or sell live tilapia in Louisiana must first request a tilapia culture or live holding permit from the secretary or his designee of the Department of Wildlife and Fisheries. The following procedures will be necessary:

a. Applications for permits can be obtained by contacting the Administrator, Inland Fisheries Division, Department of Wildlife and Fisheries, P. O. Box 98000, Baton Rouge, LA 70898-9000.

b. The completed applications should be returned to the same address whereby Inland Fisheries Division personnel or a department approved contractor, at the applicant's expense, will then make an on-site inspection of the property and culture or live holding system.

c. After the on-site inspection has been completed, department personnel will make a final determination as to whether the applicant is in full compliance with all rules for a tilapia culture or live holding permit. Department personnel will then recommend to the secretary or his designee if the applicant's request should be approved or disapproved.

d. The Secretary or his Designee will notify the applicant, in writing, as to whether or not the permit has been granted and if not, why. In the event of disapproval, applicants may reapply after correcting specified deficiencies noted in the secretary's or his Designee's letter of denial.

C. Rules on Transport of Live Tilapia

1. The department shall be notified in writing at least 24 hours prior to shipments of live tilapia form one Louisiana culture permit holder to another Louisiana culture permit holder or live holders within the state or shipments out-of-state on a form provided by the department. Notification shall include Louisiana tilapia culture permit number, route, date and time(s) of transport, destination, owner of transport vehicle, total number of each species, and a copy or reference to electrophoretic certification of shipped stock by species. Anyone possessing live tilapia within the State must have a tilapia culture or live holding permit. Live tilapia showing signs of diseases shall not be transported into or within the State of Louisiana.

2. For each occurrence of tilapia being imported into Louisiana from out of state to a permitted resident culturer or live holder, the permittee must obtain, in writing, approval from the department. Procedures and necessary information for obtaining approval are:

a. Requests shall be made to: Administrator, Inland Fisheries Division, Louisiana, P. O. Box 98000, Baton Rouge, Louisiana 70898-9000.

b. Requests shall include:

i. Louisiana tilapia permit number, or a copy of the permit;
ii. Route of transport;
iii. Date of transport;
iv. Time(s) of transport;
v. Destination;
vi. Owner of transport vehicle;
vii. Electrophoretic certification made within the past thirty days identifying shipped stock(s) to species.
viii. Total number of species;
ix. Identification of seller and buyer.

3. A bill of lading must accompany the live tilapia during import, export, transport, transfer or sale and shall include:

a. Copy of the permittee's written approval as described in Louisiana Administrative Code 76:VII.903C.2. above.

b. Date and approximate time of shipment;

c. Route of shipment;

d. Source of tilapia (culture facility);

e. Name, address and phone number of seller;

f. Name, address and phone number of buyer;

g. Identification and certification as to species;

h. Total number of each species;

i. Destination;

j. Letter from source stating that tilapia are not showing signs of diseases;

k. Display the word "TILAPIA" prominently on at least two sides of the vehicle or hauling tank with letters that are no less than six inches high.

D. Rules for Security of Tilapia Culture or Live Holding Facility

1. Applicant must demonstrate to the satisfaction of Department officials that adequate security measures are in place at the culture facility that will guard against vandalism and theft of tilapia.

2. Any changes or modification of a permitted security system must first have the approval of Department officials.

3. The Department will have just cause to revoke a tilapia permit for lapses in security if: 1) the permittee is found to be in noncompliance with Paragraphs 1 and 2 above; 2) the permittee is determined to be derelict in maintaining the security measures that were approved for the permit; 3) failure to take appropriate measures when vandalism, theft, or accidental release of fish occurs.

4. It shall be the responsibility of the permittee to immediately notify the secretary or his designee of any tilapia that leave the facility for any reason other than those specifically identified and allowed for under their current permit, including but not limited to accidental releases, theft, etc.

5. It shall be the responsibility of the permittee to have at least one individual who is familiar with the culture system readily available for emergencies, inspections, etc.

E. Rules of Tilapia Culture and Live Holding Site

1. A legal description of the tilapia culture facility site that shows ownership must be submitted along with the permit request.

2. The applicant must agree to allow department officials or a department approved contractor, at the applicant's expense, to conduct unannounced random inspections of the transport vehicle, property, culture system or live holding system, and fish. Department officials may request other officials to accompany them performing these inspections.

Additionally, those individuals performing these inspections may remove or take fish samples for analysis and/or inspection.

3. All aspects of the tilapia culture facility must be at least one foot above the 100-year flood elevation. Additionally, the Department may require a surface hydrological assessment of the proposed site at permittee's expense.

4. The department will require a live holding contingency plan for disposal of live tilapia in the event of impending flooding or other natural disasters.

F. Rules for the Tilapia Culture and Live Holding System.

1. Applicant must provide a detailed narrative description, including scale drawings, of the tilapia culture or live holding system.

2. The tilapia culture or live holding system shall be an approved indoor system designed such that tilapia eggs, larvae, juveniles or adults cannot escape.

3. All water utilized in the culture or live holding of tilapia shall be accounted for and shall be filtered, screened, and/or sterilized prior to leaving the culture or live holding system and the permittee's property in such a manner as the department deems adequate to prevent any possibility of escape from the system.

4. All aspects of the tilapia culture or live holding system and processing shall be completely enclosed so that predation from birds, mammals, amphibians, and reptiles is precluded.

5. A means to dispose of tilapia through chlorination, desiccation, or other appropriate methods, in the event of an emergency must be included as a component of any department-approved live-holding system.

6. One or more persons responsible for the operation of the live holding system must demonstrate to the department's satisfaction a basic knowledge and understanding of the culture, biology, and potential local ecological impacts of tilapia.

G. Rules for the Processing of Tilapia

1. All tilapia and tilapia parts other than live tilapia specifically permitted by the department must be properly processed and killed prior to leaving the tilapia culture or live holding facility.

2. All tilapia, other than live tilapia specifically permitted by the department, being brought into the state from without the state must be dead.

3. Records shall be kept of all tilapia processed at a culture or live holding facility and shall include the following information:

a. Source of fish;
b. Processed pounds;
c. Date processed;

4. Permits are not transferable from person to person, or property to property.

5. Live tilapia, may be sold within the state only to a holder of a valid tilapia culture or live holding permit. A tilapia culture permit shall be required for the possession or transport of tilapia eggs, fry or juveniles.

6. No person may release live tilapia, fish or eggs, into the waters of Louisiana (whether public or private) without the written approval of the Secretary.

7. Permittee must agree to collect and provide an adequate number of tilapia to the Department or a Department approved contractor upon request for identification and analysis, at the permittee's expense.

8. Only those persons or organizations with valid tilapia permits may propagate, culture or possess the following species and/or hybrids produced from their crosses.

Tilapia *aurea*	*Tilapia nilotica*
Tilapia mossambica	*Tilapia hornorum*

9. Tilapia culturers shall be required to submit an annual report to the secretary or his designee on a form provided by the department.

10. The Department may employ whatever means it deems necessary to prevent the release or escapement of tilapia or their eggs into the environment. The permittee shall agree to reimburse the Department for all costs including, but not limited to, man hours and materials utilized during corrective actions.

11. The department shall be overseer of all escape incidents and may implement or require to be implement or require to be implemented whatever measures deemed necessary to contain, kill or recapture fish. The permittee shall agree to reimburse Wildlife and Fisheries for all department costs including, but not limited to, man hours and materials utilized during these corrective actions. In order to assure the secretary that the permittee will fulfill their financial obligations, the tilapia culturer shall, at the option of the department, post a $25,000 performance bond, or present a letter of credit from a financial institution stating that the $25,000 is available to the department on a certificate of deposit. Tilapia live holder permittees will be required to post a $10,000 performance bond, or present a letter of credit from a financial institution stating that the $10,000 is available to the department on a certificate of deposit.

12. If a permittee terminates tilapia production or live holding, the permittee shall notify the secretary or his designee immediately and dispose of the tilapia according to methods approved by the department.

13. In addition to all other legal remedies, including provisions of Revised Statute, Title 56, section 319.E., failure to comply with any of the provisions herein shall be just cause to

immediately suspend and/or revoke the permittee's permit. All tilapia shall be destroyed at permittee's expense under the department's supervision within 30 days of permit revocation.

14. Any permittee allegedly in violation of the above rules has a right to make a written response of the alleged violation(s) within five days.

VITA

Robert Nugon was born in New Orleans on June 24, 1972. He received his high school diploma from Isadore Newman School in 1991. Robert attended Millsaps College in Jackson, Mississippi, where he received his Bachelor of Science in biology with a minor in studio art in May of 1997. He was a biology laboratory assistant throughout his undergraduate career and helped in preparation and instruction of cell biology, zoology, botany, genetics, and ecology laboratories. In 1993 he spent a summer as a cotton plant analyst in the Mississippi Delta. He was given the title of Biology Animal Manger at Millsaps College in 1995 and served an internship at the University of Mississippi Medical Center in psychological research that same year. He also interned in herpetological reproduction under Dr. Richard Highfill at Millsaps in 1997. After graduation he was hired by the Louisiana University Marine Consortium (1997) and the University of Delaware (1998) as a scientific oceanographic instrument technician and worked offshore in the Gulf of Mexico and off the coast of New England. He entered the master's program in the School of Renewable Natural Resources, Louisiana State University and Agricultural and Mechanical College, Baton Rouge, in 1997, and is currently a candidate for the degree of Master of Science in spring of 2003.