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EVALUATION OF GROWTH, PRODUCTION, AND COLD TOLERANCE OF FOUR VARIETIES OF TILAPIA

A Thesis

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ABSTRACT

As global tilapia production grows, it is important to characterize varieties available for production and generate data on environmental tolerances. This study generated data on tolerances, adaptabilities and production characteristics of four tilapia varieties. Goals of this study were to: (1) compare growth of four tilapia varieties in two types of recirculating systems, (2) evaluate juvenile growth of seven tilapia varieties in fresh and brackishwater mesocosms, and (3) estimate cold tolerance of juveniles of four tilapia varieties in fresh and brackishwater.

Nile tilapia (*Oreochromis niloticus*), blue tilapia (*Oreochromis aureus*), Mississippi commercial strain tilapia (MCS), and Florida red tilapia (FRT) were grown in a clear-water recirculating system for eight months. Blue tilapia yields were greater ($P \leq 0.05$) than those of Nile tilapia and FRT. Yields of Nile tilapia, MCS, and FRT were not significantly different.

Seven tilapia varieties: Nile, blue, FRT, MCS, blue x FRT, Mozambique x Nile, and Nile x blue were grown in fresh and brackishwater mesocosms. Nile tilapia was stocked in three freshwater pools. Every other variety was stocked into six pools, in three of which salinity was increased to 23 ppt over 14 days. In freshwater, yield of MCS was greater ($P \leq 0.05$) than that of all other varieties.

Four tilapia varieties: Nile, blue, FRT and MCS were subjected to three temperature reduction regimes: rapid (-0.5 C/ 5 h), moderate (-1 C/ 24 h) and gradual (-1 C/ 48 h) and to the moderate reduction regime at three salinities (0, 5 and 10 ppt). No significant differences were observed in cold tolerance among varieties within temperature reduction regimes. Salt did not improve the cold tolerance of blue, Nile and MCS but slightly improved that of FRT.

Performance of four tilapia varieties: Nile, blue, MCS and FRT in four culture systems was ranked to develop index values. Cold tolerance of four tilapia varieties was described from an environmental standpoint, with no significant differences found between varieties. The influence of the temperature reduction regimes on cold tolerance was significant.

CHAPTER 1
INTRODUCTION

The earliest known representation of a fish culture pond, a bas-relief from an Egyptian tomb dating from before 3000 BC, shows a pair of small fish that can be identified as *Oreochromis niloticus*, a species still abundant in the Nile Valley (Hickling 1963). The modern history of tilapia culture however is believed to have begun in 1939. In that year, five fish of the species *Oreochromis mossambicus*, originally from southern Africa, were discovered in a lagoon in Java (Hickling 1963). From 1942 on, wartime conditions made the fry of the local milkfish scarce and in a short time tilapia replaced the milkfish as the predominant pond cultured fish of Java (Hickling 1963).

The estimated contribution of aquaculture to global supplies of fish, crustaceans and mollusks increased from 3.9% in 1970 to 27.3% in 2000 (FAO 2002). In the last 30 years capture fisheries and land animal production systems have had 1.4% and 2.8% annual increases respectively, while aquaculture has grown at 9.2% annually (FAO 2002). In 2000, total aquaculture production, including aquatic plants, reached 46 million metric tons (MT), worth $56.5 million; Chinese production represented 71% of the total weight and 50% of the total value (FAO 2002).

Tilapia belong to the family Cichlidae which is widely distributed in Africa, the Middle East, South and Central America, southern India and Sri Lanka. It contains approximately 1,300 species, of which approximately 150 can be called tilapia (www.fishbase.org). Tilapia are perciform fish that originated in Africa and the Jordan Valley (www.fishbase.org). They have adapted to diverse habitats: permanent and temporary rivers, rivers with rapids, large equatorial lakes, tropical and subtropical rivers, open and closed estuaries, lagoons, swampy lakes, deep lakes and coastal brackish lakes (Trewavas 1983).

Tilapia was originally considered for aquaculture as a means of producing cheap protein and also because of the readiness of the species to breed in almost any type of water body, being omnivorous and hardy enough for fish farming (Pillay 1990). Today, tilapia has become one of the most common farm raised fish in the world, second only to carps in terms of total production (Watanabe et al. 2002). Tilapia has been produced since the 1950s in the USA, when they were introduced into Alabama. Imports into the USA represent approximately 2.8% of worldwide production.

The most commercially important tilapia genera are: *Oreochromis*, *Tilapia* and *Sarotherodon*. The genus *Oreochromis* is the largest, with approximately 79 species, followed by *Tilapia* with approximately 41 species and the genus *Sarotherodon* with approximately 10 species. *Oreochromis* is typical of the rivers and lakes of East and Central Africa and the Jordan valley. *Tilapia* distribution coincides with that of *Sarotherodon* and in addition with *Oreochromis* in the Zambezi basin and southwards.

Nile tilapia (*Oreochromis niloticus*), Mozambique tilapia (*Oreochromis mossambicus*) and blue tilapia (*Oreochromis aureus*) are the most commercially important species found in the genus *Oreochromis*. Red tilapia were first isolated in Taiwan by crossing a red *O. mossambicus* with a *O. niloticus*. They are currently produced in the USA, Philippines, Greece, Israel, Jamaica, India and other tilapia producing countries (Wohlfarth et al. 1990). Their price in local markets is often considerably higher than that of wild-type colored fish. The Florida red tilapia (FRT) used in this study was developed from the cross of *O. urolepis hornorum* with *O.*

mossambicus. Other red synthetic strains used today are the Taiwan red tilapia, also the Jamaican Red tilapia with *O. niloticus, O. mossambicus and O. hornorum* ancestry. The Mississippi commercial strain was derived from the "Rocky Mountain White®" and has *O. aureus* and *O. mossambicus* ancestry (Lutz, personal communication 2000).

Varietal Characteristics

Nile tilapia is a benthopelagic fish adapted to freshwater and low salinity brackish water conditions. It is naturally distributed in Africa and coastal rivers of Israel and is capable of tolerating temperatures from 8 to 42 C (Trewavas 1983). Nile tilapia was first introduced into the USA from the Ivory Coast via Brazil in 1974; the Ghana and the Egypt strains were introduced in 1982 into Alabama (Hargreaves 2000). The strain of Nile tilapia that was used in all the experiments for this thesis originated in northern Egypt as the Auburn-Egypt strain. Nile tilapia has been reported to tolerate low temperatures between 11 and 13 C (Chervinski 1976). Li et al. (2002) demonstrated variation in cold tolerance within and between three Nile tilapia strains. The Egyptian strain is believed to have better cold tolerance than that of the Ivory Coast strain when exposed to decreasing ambient temperature (Tave et al. 1990).

Produced worldwide and marketed both fresh and frozen, Nile tilapia is the most widely farmed tilapiine species in the world, representing approximately 83% of total tilapia production (Hempel 2002). 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 is also widely practiced (Villegas 1990).

The blue tilapia is a benthopelagic fish adapted to freshwater and brackishwater conditions and is also capable of tolerating sea water, although it does not reproduce at 36 ppt and higher (McGeachin et al. 1987). Salinity tolerance for blue tilapia has been reported as high as 54 ppt (Lotan 1960). Chervinski and Yashouv (1971) showed this species to reproduce in salinities up to 19 ppt, grow in salinities of 36 ppt, and die at 53 ppt. The species is naturally distributed in Africa and Eurasia and is capable of tolerating temperatures from 8 to 30 C (Trewavas 1983) although lethal temperatures of 7 C have been reported (Perry and Avault 1972). Blue tilapia can be differentiated from Nile tilapia by vermillion edges in the dorsal fin and pink to red color in the caudal fin (Eccles 1992). It was first introduced into the USA from Israel in 1957, for aquaculture purposes in Alabama. The strain used in this thesis originated in Lake Manzala, Egypt.

The Mozambique tilapia is a benthopelagic fish adapted to freshwater and brackishwater conditions, also tolerating salinities well above normal sea water. Naturally distributed in southern Africa and found in blind estuaries, coastal lakes and warm weedy pools, *O. mossambicus* can tolerate temperatures from 8 to 42 C. Mozambique tilapia has been reported to survive and reproduce in salinities as high as 49 ppt (Popper and Lichatovich 1975). It was first introduced into the southeastern USA from Africa in 1951 by Homer Swingle while earlier introductions were made into Latin America (FAO 1997).

O. urolepis hornorum is benthopelagic, adapted to freshwater and brackish water. Naturally distributed in East Central Africa, it can tolerate temperatures from 22 to 26 C. *O. urolepis hornorum* has been observed surviving salinities as high as 35 ppt in seawater ponds (Fryer and Iles 1972; Philippart and Ruwet 1982). This species is an original parental line of the FRT, which is derived from a cross of *O. mossambicus* with *O. urolepis hornorum* developed in the late 1970s by Natural Systems in Florida (Rakocy et al. 1993). MCS was developed in Colorado as the "Rocky Mountain White" ® tilapia. Blue and Mozambique tilapia are included in its ancestry and is characterized by its white coloration (Lutz, personal communication 2002).

Worldwide Tilapia Production

For the year 2002, estimated global tilapia production was 1,374,239 MT (Fitzsimmons 2003a). Major producers were China (629,182 MT/yr), Mexico (102,000 MT/yr), Thailand (100,000 MT/yr), the Philippines (92,284 MT/yr), Taiwan province (85,000 MT/yr), Brazil (65,000 MT/yr) and Indonesia (50,000 MT/yr) (Fitzsimmons 2003a). Estimated global tilapia sales for the year 2000 were valued at $1,706,538,200 (FAO 2001). Cultured tilapia increased from 29% of total tilapia production in1980 to 68% in 2000. Of the total, Nile tilapia currently represents approximately 83% (Hempel 2002). In the Americas, major producers are Mexico, Brazil, Cuba, Ecuador, Colombia, Costa Rica, the USA, Honduras and Jamaica.

Tilapia Production and Imports in the USA

Tilapia production in the USA has been increasing over the last decade, and total production has surpassed 9,000 MT (www.noaa.gov). Tilapia is also imported into the USA from throughout the world. These imports represent about 2.8% of world production (Harvey 2003). Tilapia is imported as frozen whole fish, fresh fillets and frozen fillets. Whole frozen tilapia represented 61% of all imports in 2002, a 5% increase from 2001. The USA has now become the largest market for frozen round and fresh or frozen fillets (Hempel 2002). China and Taiwan supplied 99% of all frozen whole tilapia to the USA. Fresh fillets composed 21% of all 2002 imports, produced primarily in Honduras, Costa Rica and Ecuador. Frozen fillets composed 18% of all 2002 imports, originating primarily in China, Taiwan and Indonesia (Harvey 2003). Projected tilapia imports into the USA for 2003 are estimated at over $224,000,000. Over the last ten years an estimated $705,000,000 worth of tilapia products have been imported into the USA (Fitzsimmons 2003b).

Table 1.1 Total tilapia imports into the USA, in thousands.

Categories	2000	2001	2002
	Mass (kg) / Value ($)	Mass (kg) / Value ($)	Mass (kg) / Value ($)
Whole, frozen	27,782 / 33,701	38,720 / 38,052	40,748 / 44,031
Fillet, fresh	7,502 / 44,455	10,236 / 60,839	14,187 / 81,694
Fillet, frozen	5,186 / 23,222	7,372 / 28,905	12,255 / 48,490
Total	**40,469 / 101,378**	**56,338 / 127,797**	**67,191 / 174,215**

(Source: USDA 2002)

Tilapia in Louisiana

When compared to neighboring states, 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 in Louisiana and a permit is needed for their possession (Louisiana Revised Statutes 56:319). Facilities that produce tilapia in Louisiana must be fully enclosed and at least 30 cm above the 100-year flood plain (Louisiana Administrative Code 76:VII.903). These protections are in response to the previous establishment of tilapia released into natural habitats in Florida, Georgia, and California (USA Congress, Office of Technology Assessment 1993). Mississippi, Alabama, and Arkansas have no restrictions on tilapia (Courtenay 1997). While Texas and Florida have enacted some regulation, much of it addresses importation of tilapia stocks from out of state (Courtenay 1997).

A number of federal regulations have been designed to limit the introduction and spread of exotic and non-indigenous species within the USA. Statutes that allow the federal government to restrict non-indigenous species include: the 1948 amendment to the Lacey Act of 1900 (18 USA Code 42); the Fish and Wildlife Act of 1956 (16 USA Code 742a-742j); the 1972 Migratory Bird Convention with Japan (24 USA Treaty 3329); and the Endangered Species Act of 1973 (16 USA Code 1531-1543). The Lacey Act is the most encompassing, controlling the import, possession, and transport of species deemed dangerous to humans and the natural resources of the USA (Courtenay 1997).

Tilapia culture is rapidly growing in the USA, but production has not grown as fast as in other tilapia-producing countries in the Americas. The southern USA possesses a range of environments where tilapia can be produced seasonally, but with temperature restrictions placed on tilapia growth during the winter months. Throughout the world, arid areas not suitable for agriculture are being evaluated for tilapia production in brackishwater. Tilapia production is also practiced in recirculating systems where fresh product can be supplied to large ethnic communities in urban areas.

Culture systems such as raceways, ponds and recirculating tank systems have all been used successfully. In the USA and elsewhere, there are important considerations that tilapia producers need to take into account. Some of the characteristics of tilapia that are important components of the production performance are presented in this study. These include temperature tolerance and adaptability to the production system that will be used.

It is important to have adequate information on available tilapia varieties and the characteristics of each to avoid losses of time and money.

Cold tolerance is an important consideration in temperate countries that do not possess year round climates suitable for tilapia production in ponds. Although the major tilapia species have similar geographic origins, differences in cold tolerance exist among and within species. In general, the further the geographic origin from the equator, the more cold tolerance is displayed by tilapia species (Li et al. 2002). The blue tilapia is believed to have better cold tolerance than Nile and Mozambique tilapia (Chervinski and Lahav 1976; Shafland and Pestrak 1982). In commercial stocks, selection and hybridization can be exploited to improve the cold tolerance of in tilapia (Maruyama 1978).

Recirculating systems are used for tilapia production where water availability is limited or represents a high cost. In countries with temperate climates, where ambient winter temperatures can be lethal to tilapia, over-wintering and/or growout can also be carried out in recirculating systems if adequate water quality parameters can be maintained. Israel is one of the countries that lead in water re-use technology due to limited freshwater availability. Recirculation systems have also been used in the southeastern USA for over-wintering. Legislation in Louisiana prohibits the culture of tilapia in outdoor ponds to minimize the potential for adverse ecological impacts, and recirculation systems have been employed as an economically competitive alternative for production of tilapia for live markets.

Production of tilapia in brackish and salt water has been evaluated in experimental conditions in the past but little data is available from commercial facilities. Brackishwater can be used for tilapia production in places where freshwater is limiting and there is an abundance of brackish water. Sea water can also be employed for tilapia culture where fresh water is limiting. The general approach to commercial saltwater tilapia culture has been to produce seed and juveniles in freshwater, followed by growout in brackish or seawater (Watanabe 1985). Apart from several studies of pure species many years ago little recent information is available to evaluate which species, strains and hybrids possess natural characteristics that will allow them to perform well in brackish or seawater production systems.

Some characteristics of tilapia, including fast growth, make it a very invasive fish that can establish populations outside of its natural range under the proper conditions (Pillay 1990). Assessments must continue over the impacts, favorable or adverse, that can come from the establishment of tilapia outside their natural range. Cold tolerance in fresh and brackishwater habitats is a key consideration in evaluating potential establishment of introduced tilapia stocks.

Tilapia are very adaptable fish that can be produced in many culture systems, of which the most important and popular are reviewed herein. Research presented in this thesis involves two types of production systems, greenwater mesocosms (0 and 23 ppt salinity) and recirculating aquaculture systems.

Pond Culture

Pond culture is the most widely used method for tilapia production. Its success is due in part to the fact that fish may use natural food sources found in ponds (Rakocy and McGinty 1989). In temperate countries, pond culture is seasonally dependent on water temperature. Two approaches to pond production are utilized: extensive and intensive.

Extensive pond culture depends on organic and inorganic fertilizers to increase natural productivity. In intensive pond culture, high protein commercial feeds are used with aeration or water exchange.

Small shallow ponds between 1 and 10 hectares are generally recommended for tilapia production. One of the negative aspects of pond culture is that reproduction in mixed sex cultures is uncontrolled. Harvesting is also labor intensive. To control overpopulation, predators such as *Hemichromis fasciatus, Lates niloticus, Clarias lazera* and *Micropterus salmoides* have been used (2-10% of the stock) (Pillay 1990).

Polyculture can be used to take better advantage of natural foods available and to produce secondary crops, or to control tilapia recruitment (Rakocy and McGinty 1989). Tilapia has been produced seasonally in polyculture in the southeast USA with channel catfish, Chinese carp and freshwater prawns with productivity exceeding 4,000 kg/ha (Hargreaves 2000). Polyculture is an important tilapia production system in many countries, such as in Indonesia where tilapia has been grown together with shrimp in brackish water (Guerrero 2001). There is a renewed interest in shrimp producing countries such as Honduras and Ecuador to produce tilapia alongside shrimp during rainy season production cycles when water salinities are depressed. Most commercial tilapia varieties do not perform well at higher salinities found in many shrimp farming areas

Cage Culture

Cage culture is commonly used in bodies of water that cannot be drained, such as lakes, estuaries or coastal marsh areas. Advantages of cage culture include low investment costs, ease of management, ease and low cost of harvesting, opportunities for close observation of feeding and health. Its disadvantages include vulnerability to poachers and structural damage, less tolerance of fish to poor water quality, more dependence on commercial diets and the increased risk of disease outbreaks (McGinty and Rakocy 1989).

Cage culture is very popular because mixed sex tilapia can be produced without the risk of reproduction when mesh is large enough to let eggs fall through. From a water quality standpoint, cage culture is similar in some ways to open pond aquaculture. Although the fish are confined, metabolic wastes leave the cages and are broken down throughout the body of water (Boyd 1990). Cage culture lends itself to the production of tilapia in polyculture with shrimp during crop cycles when salinity is low enough to enable tilapia production. Cages are an alternative for shrimp producers to avoid nest building by tilapia where shrimp can become stranded when the ponds are emptied.

Marine cage culture is an alternative in arid and tropical coastal regions (McGeachin et al. 1987; Watanabe et al. 1990b, 1990c). Watanabe et al. (1990a) specifies that maximum yields for tilapia in marine cages have not been reached, but yields as high as 52 kg/m^3 have been attained using the Florida red tilapia. Salt water cage culture has also been attempted in Colombia with an overall survival of 65% and yield of 31 kg/m^3 in 5 months (Popma and Rodriguez 2000).

Tank Culture

Tank culture is important in areas where water supply is restricted or insufficient, also with unsuitable soil and terrain. Adequate water quality is indispensable for tank culture; this can be achieved with aeration and water exchange or filtration and re-use (Rakocy 1989). In flow through systems water is discarded after use, recirculation systems involve more water conservation through filtration and recycling (Rakocy 1989).

There are two common types of recirculating systems in tilapia production, usually defined by the color of the water: greenwater and clear-water (Hargreaves 2000). Greenwater systems allow for phytoplankton to proliferate, allowing tilapia to filter feed on the bloom. This natural productivity supplements the commercial feed that is normally given in pelleted form, and also allows for partial assimilation of tilapia wastes by the phytoplankton. In Louisiana, greenwater recirculating systems normally use net pens suspended within rectangular tanks to facilitate concurrent batch stocking and harvesting, allowing isolation of specific size or family groups in a system (Abernathy and Lutz 1998). In clear-water systems, phytoplankton growth is restricted and nutrition is provided solely by the feed. Recirculating systems normally include biological and mechanical filtration to reduce the effects of toxic waste produced by the fish and fowling of water caused by uneaten feed.

In tank production, feeding and harvesting require less time, labor and treatment of diseases is relatively easy, although the fish have limited access to natural food (Rakocy 1989). Costs also increase based on feed, facilities and equipment. Recirculating systems generally recycle 90 to 99% of culture water daily, and biofilters are required for conversion of toxic nitrogenous waste products to non-toxic forms (Hallerman 2000). Recirculating systems are biologically intense; fish are usually reared intensively (59 kg/m^3 or greater) for recirculation to be cost effective (Masser et al. 1999).

Recirculating pond systems possess moderate (2-15 kg/m^3) to high (>100 kg/m^3) fish density and moderate (0.3-5/d) to high (50-100/d) volumetric exchange (Hargreaves 2000). Two examples of recirculating pond systems are the combined intensive-extensive (CIE) system practiced in Israel that is characterized by 2-3 days of hydraulic retention time and low population density, and the partitioned aquaculture system (PAS) system, which is composed of raceways, a sump for fecal matter retention and an algae pond where tilapia are used to maintain a young, active phytoplankton population (Hargreaves 2000). The feasibility of producing four tilapia varieties in recirculating systems was addressed in this study.

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CHAPTER 2
GROWTH OF FOUR TILAPIA VARIETIES IN RECIRCULATING SYSTEMS

Recirculating systems are used for tilapia production in countries with temperate climates in North America and Europe or where availability of freshwater is limiting. Tilapia are highly suited for production in recirculating systems because of their high tolerance to crowding, ready acceptance of a wide range of foods, efficient feed conversion, rapid growth, and ease of production (Hallerman 2000).

Compared to outdoor culture systems used in the tropics, the cost of producing fillets in recirculating culture systems can be 2.2 times higher (Lutz 2000). Direct and depreciation costs are substantially higher than those of less intensive production systems (Lutz 2000). Recirculating systems are suited for tilapia production in large cities with live fish markets, as they allow for year-round production and can be near those markets, reducing shipping costs (Kirkup 2000). Approximately 70% of domestic tilapia production is sold live and recirculating aquaculture accounts for 70% of the total United States tilapia production (Fitzsimmons 2003a).

There are several types of recirculating systems that can be used successfully with tilapia: microbial based systems, greenwater raceways in greenhouses, intensive tank systems, and controlled environment systems (Fitzsimmons 2003b). Microbial-based systems take advantage of the ability of tilapia to tolerate water quality conditions that would be lethal to many other fish. These systems employ substantial aeration to maintain solids in suspension, allowing for nitrification. Greenwater recirculating systems allow phytoplankton to grow in the water, from which tilapia obtain some nutrition; floating bead filters and sediment traps are used for nitrification and solids removal. Controlled environment systems are the most intensive approach used in the

United States and Europe, with densities of up to 100 kg/m^3 normally achieved. This type of system normally integrates many designs, depending on the producer.

Variety[1] selection for a culture system is crucial for the success of any commercial tilapia venture. Nile tilapia is the most commonly produced fish in the world, and it adapts well to most culture conditions with good growth and yields. Nile tilapia is considered the best tilapia species for tropical freshwater culture and can be considered as a standard to which other varieties of tilapia can be compared. Blue tilapia, not as commercially important as Nile tilapia, is a versatile fish that is often used for hybridization with Nile tilapia (Fitzsimmons 2003a). Blue tilapia is considered very cold tolerant (Mair 2001). Florida red tilapia (FRT) is an important variety in places with low freshwater availability, such as the Caribbean region, where they can be produced in brackishwater. Mississippi commercial strain tilapia (MCS) was developed in North America. It is a very prolific variety with good potential for commercial production, showing great adaptability to most culture conditions.

New strains have been created from accidental crossing of pure species and also have developed within a species when there is physical isolation (Rakocy et al. 1993). For Nile tilapia, this is illustrated with the Ghana, Ivory and Egypt strains which vary among each other in growth rates (Rakocy 1993).

Many comparisons among tilapia varieties have been reported although none have been in clear-water recirculating systems. Nile tilapia grows better than blue tilapia in concrete tanks (Siddiqui et al. 1995). Yields of Nile tilapia and FRT were greater than that of blue tilapia in cage culture, although survival of blue tilapia was greater (Rakocy et al. 1993). In this study, we compared the performance of four tilapia varieties.

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[1] The term variety will be used to describe species, strains within species, and synthetic strains

Materials and Methods

The varieties used were a line derived from the Auburn-Egypt strain of Nile tilapia, an Egyptian (Lake Manzala) strain of blue tilapia, a synthetic strain of red tilapia developed in the 1970's from "Florida Red" *O. mossambicus x O. urolepis hornorum* hybrids (FRT), and a commercially-available silver tilapia synthetic strain with ancestry including *O. aureus, O. mossambicus* and *O. niloticus* referred to as Mississippi commercial strain tilapia (MCS). All fish were obtained from stocks maintained at the LSU Agricultural Center Aquaculture Research Station, Baton Rouge, LA.

Clear-water Recirculating Systems

Four recirculating systems at the Aquaculture Research Station were used in the study. Each system included four 299-L (0.3-m^3) fiberglass circular tanks, a reservoir sump, a 0.5-kW (0.75-hp) pump and a biological/mechanical filter. The total volume of each system, including sump and filter, was 1,600 L. Systems were back-flushed once weekly, resulting in a 9.5% water exchange (151L/filter). Prior to stocking, water quality (pH, nitrite, and ammonia) was measured to ensure that environmental conditions were suitable.

One hundred juvenile fish (0.7 g to 1.2 g) of each variety were randomly selected and weighed in groups of five to the nearest 0.1 g, to minimize handling stress. After weighing, 25 fish of each variety were stocked into separate tanks in each system. Each system contained one variety per tank, and all varieties were present in each system. Plastic orchard netting was secured over each tank to prevent fish from jumping out.

Initial daily feeding rate was 2% of individual tank biomass. Fish were fed once daily with a floating pelleted feed (28% protein and 4% fat, Cargill, Nutrena Feed

Division, Minneapolis, Minnesota). Fecal matter and uneaten feed were removed continuously through upflow stand pipe sleeves.

Fish were weighed monthly to the nearest 0.1 g using a digital scale, pooling five fish at a time to obtain a combined weight. Survival was calculated after weight sampling and feeding rates were subsequently adjusted. As growth accelerated after the first 3 months, the daily feeding rate was adjusted to 6% of the biomass per tank. At 5 months, the daily feeding rate was reduced to 4% in response to declining consumption and excessive loss of uneaten feed through the drains in some tanks. Total daily ration was also divided into two portions until the end of the study. The length of the study was 242 d.

Statistical Analysis

The experimental design was a randomized block, with systems as blocks and varieties as treatments. Statistical analysis was performed using the Statistical Analysis Software (SAS) system version 8.1 for Windows ™ (SAS Institute Inc., 1999-2000, Cary, North Carolina). The general linear model procedure (GLM) with least squares means was employed for analysis of individual weight, in addition to Duncan's multiple range and Scheffe's tests for yield, survival, and feed conversion. Treatment means were declared significantly different at $P \leq 0.05$. The GLM procedure was used to perform an analysis of variance and covariance and determine significant differences between varieties and effects of system and survival on weight and yield.

Greenwater Recirculating Systems

Five grow-out trials (~350 d each) were conducted in a commercial greenwater recirculating tilapia facility (Til-Tech Aquafarms) located in Robert, Louisiana, USA. In each trial, contemporaneous fry of each variety were segregated in recirculating greenwater nursery systems to produce fingerlings for stocking. Fry were fed to satiation with a 36% protein diet four times daily and raised under similar conditions within the same recirculating systems. Once fingerlings were approximately 10 g (range: 5 to 16 g), 250 fingerlings of each variety were stocked into separate net pens (12-mm mesh, 2 m x 2 m x 1 m depth) suspended within 76-m^3 recirculating production tanks. Production tanks were outfitted with 0.6-m^3, floating bead, bio-mechanical filters, recirculating pumps providing approximately 20 system turnovers per day, and aeration systems consisting of floating vertical pump sprayers (Kasco Industries) and compressed air delivered via airstones.

Fish were fed a 32%-protein, floating pellet (Burris Feed and Mill, Franklinton, LA) twice daily. Daily feed amount for all varieties was based on satiation feeding level for Nile tilapia to allow for feeding efficiency comparisons. Growth and survival were monitored throughout the production cycle in each trial. Sampling was conducted at irregular time intervals due to the need to avoid conflicts with commercial operations at the facility. On each sampling date, all fish from each variety were counted and weighed individually. Between 10 and 100 g, fish were weighed to the nearest 0.1 g; at weights above 100 g fish were weighed to the nearest 1 g.

Statistical Analysis

Statistical analysis was performed using the SAS system version 8.1 for Windows ™ (SAS Institute Inc., 1999-2000, Cary, North Carolina). Significance was set at $P \leq 0.05$. The GLM procedure was used to perform an analysis of variance and determine significant differences between varieties, and trial effects on individual fish weight, specific daily growth, feed conversion, survival and yields from net pens.

Results

Clear-water Recirculating Systems

Recirculating system (block) did not affect average size at harvest, yield, or survival ($P \geq 0.05$). Average yield (mean ±SD) ranged from 2.9 ±3.0 kg/m^3 (FRT) to 13.5 ±4.0 kg/m^3 (blue tilapia) (Table 2.1). No significant difference ($P = 0.07$) was observed between blue tilapia yield and MCS yield. Blue tilapia yield was significantly greater ($P \leq 0.05$) than that of Nile tilapia and FRT. Yields of Nile tilapia, MCS and FRT were not different.

Mean weights by variety ranged from 83 g (FRT) to 179 g (blue tilapia). No significant difference ($P = 0.23$) was observed between the mean weight of blue tilapia (179 ±26 g) and MCS (146 ±17 g) (Table 2.1). The mean weight of blue tilapia was different from that of Nile tilapia (88 ±14 g) and FRT (83 ±26 g). The mean weight of MCS was not significantly different from that of Nile tilapia or FRT ($P \geq 0.05$) (Table 2.1). Specific daily growth (SDG) ranged from 0.8 ± 0.5% (FRT) to 1.3 ± 0.2% (blue tilapia) (Table 2.1). Specific daily growth for varieties in this study did not differ significantly ($P \geq 0.05$).

Differences among varieties in feed conversion ratio (FCR) were not significant ($P > 0.98$). Average FCRs (mean±SD), ranged from 3.3 ±0.3 for blue tilapia, to 10 ±6 for FRT (Table 2.1). Within replicate tanks, feed conversion ratios ranged from 2.8 (in both blue and Nile tilapia) to 16 (FRT). Survival (mean±SD) was 83 ±13% for blue tilapia, 73 ±15% for MCS, 70 ±12% for Nile tilapia, and 28 ±17% for FRT.

Table 2.1 Varietal yields, mean weights, specific daily growth (SDG), feed conversion ratio (FCR) and survival of tilapia in clear-water recirculating systems.

Variety	n	Yield kg/m³	Mean Weight g	SDG %	FCR	Survival %
Blue	4	13.5 ±3.9a	179 ±26a	1.3 ±0.2a	3.3 ±0.3a	83 ±13a
MCS	4	9.5 ±2.2ab	146 ±17ac	1.2 ±0.2a	3.8 ±0.6ab	73 ±15a
Nile	4	5.6 ±1.6b	88 ±14b	1.2 ±0.2a	3.5 ±0.7ab	70 ±12a
FRT	4	2.9 ±3.2b	83 ±67bc	0.8 ±0.5a	10.0 ±6.0b	28 ±17b

Values are means ±SD
Means within columns with same letter are not significantly different ($P>0.05$)
MCS-Mississippi commercial strain tilapia; FRT- Florida red tilapia

Greenwater Recirculating Systems

Mean weights of Nile tilapia (453 ±8 g) and blue tilapia (433 ±6 g) did not differ significantly. Mean weights of Nile tilapia were significantly higher than those of MCS (407 ±10 g) and FRT (404 ±29 g). No significant difference was observed between final mean weights of blue tilapia, MCS and FRT. Specific daily growth was not significantly different in all varieties, with an overall average of 2.8 ±0.3 %(Table 2.2).

Average yields in greenwater trials ranged from 22 kg/m³ (FRT) to 45 kg/m³ (Nile tilapia) (Table 2.2). No significant difference ($P \geq 0.05$) was observed between Nile tilapia and blue tilapia yield. There were differences between Nile tilapia yield and those of MCS and FRT. Feed conversion ratio for Nile tilapia was not significantly different from blue tilapia, but was significantly lower than that of MCS or FRT. There was no significant difference in FCR between blue tilapia and MCS, but there was a significant difference in FCR between blue tilapia and FRT. Average FCRs (mean±SD), ranged from 1.6 ±0.1 for Nile tilapia to 3.4 ±0.5 for FRT (Table 2.2). Survival (mean ±SD) averaged 61 ±3 %. There was no significant difference in survival between Nile tilapia and blue tilapia or between survival in MCS and FRT, but survival rates for Nile tilapia and blue tilapia were significantly higher than those of MCS and FRT.

Table 2.2 Varietal yields, mean weights, specific daily growth (SDG), feed conversion ratio (FCR) and survival of tilapia in greenwater recirculating systems.

Variety	n	Yield kg/m³	Mean Weight g	SDG %	FCR	Survival %
Nile	5	22 ±2a	453 ±8a	2.8 ±0.1a	1.6 ±0.1a	79 ±2a
Blue	5	20 ±1a	433 ±6ab	2.8 ±0.1a	1.8 ±0.0a	73 ±1a
MCS	5	12 ±1b	407 ±10b	2.6 ±0.2a	2.6 ±0.2ab	48 ±3b
FRT	5	11 ±3b	405 ±14b	2.8 ±0.1a	3.4 ±0.5b	43 ±4b

Values are means ±SD
Means within columns with same letter are not significantly different ($P>0.05$)
MCS- Mississippi commercial strain tilapia; FRT- Florida red tilapia

Discussion

Clear-water Recirculating Systems

Although the amount of water exchanged was relatively low, clear-water conditions were maintained throughout the study. The configuration of the systems permitted easy sampling or harvesting by lowering the water level and capturing the fish with a handheld dip net. Modifying feeding rates as growth rates changed minimized uneaten feed losses via the drains.

In a comparison involving floating cages, Nile tilapia had higher yield and mean weight than blue tilapia and FRT (Rakocy et al. 1993). Papoutsoglou and Tziha (1996) obtained final body weights from blue tilapia ranging from 125 g to 314 g in recirculating systems. Although the final weights (reached in 243 days) of the blue tilapia and MCS in this study were within those reported by Papoutsoglou and Tziha, their weights were achieved in 200 days. Rosati et al. (1997) reported individual fish weights in Nile tilapia ranging from 600 g to 700 g reached in 239 days when starting with juveniles. Cole et al. (1997) also reported high yields in all-male Nile tilapia, produced in semi-recirculating tank systems in 168 days, with mean final weights of 514 g. Similar results were reported by Rakocy et al. (1997), with 487.2 g fish produced in 168 days.

A reason in some cases for low yields observed was low survival. Low growth observed in this study may have arisen because there was no grading during growout. Lack of grading may have increased variation in size and made feed conversion less efficient. Smaller sizes produced in commercial recirculating systems can be prevented by adjusting size classes (grading) within systems to allow fish to grow larger (Lutz 2000). Grading fingerlings reduces the tendency of larger fish to out-compete small fish for feed (Losordo 1997). A more complex production strategy, concurrent batch stocking and harvesting, focuses on stocking individual groups into the same system at intervals, culturing them until the average has reached market size and harvesting the entire individual group (Summerfelt et al. 1993). Due to the nature of the experimental units used in this trial, neither grading nor concurrent stocking and harvesting was possible.

Greenwater Recirculating System

Greenwater recirculating systems are cost-competitive when compared to other tilapia growout systems (Abernathy and Lutz 1998). Martin et al. (2000), employing greenwater recirculating systems, reported after 24 weeks, a yield of 13.4 kg/m^3, FCR of 1.4. Rakocy et al. (1992) did observe lower yields and higher FCR with a greenwater recirculating system than in systems where algal growth was inhibited, probably caused by the uptake of metabolic breakdown products of the phytoplankton.

Clear-water vs Greenwater Recirculating Systems

Although no comparison can be made between the two systems because of differences in growth conditions in the two studies, stocking densities were considerably lower in clear-water systems than in greenwater systems. Blue tilapia growth was best in clear-water, while FRT was least. In greenwater, Nile tilapia growth was best, while FRT growth was least. In both production systems survival of FRT was least of all varieties, thereby reducing yield. The FCR was also lower for all varieties in greenwater, which may illustrate the added nutritional effects of phytoplankton grazing. Growth of all varieties was lower in clear-water than in greenwater.

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CHAPTER 3
GROWTH OF SEVEN VARIETIES OF TILAPIA IN FRESH AND BRACKISHWATER MESOCOSMS

Tilapia culture is limited primarily to freshwater and low salinity brackishwater, but some varieties[2] can be cultured in higher salinity brackishwater and marine systems (Watanabe et al. 1985a). Tilapia tolerate various ranges of salinity; some, like the Mozambique tilapia (*Oreochromis mossambicus*) and its hybrids, tolerate full strength seawater (Watanabe et al. 1985b). The production of tilapia in brackishwater is important in coastal areas, regions with limited supplies of freshwater, and in countries where tilapia producers can make use of infrastructure developed for shrimp production.

Differences in salinity tolerance within tilapia species are typically related to strain, age, and body size (Villegas 1990). Nile tilapia (*O. niloticus*) is the least salt tolerant commercial species with lethal salinities of 12.5 ppt reported in experimental conditions (Watanabe 1985b; Avella and Doudet 1993), although it has been cultured at salinities of 15 ppt (Popma and Masser 1999). Significant mortality of Nile tilapia occurs at 25 ppt although larger fish are less affected by salinity than smaller fish (Villegas 1990). Another major commercial species, the blue tilapia (*O. aureus*) has been grown at a salinity of 20 ppt (Popma and Masser 1999).

Tilapia are presumed to have an ancestry that includes marine fishes, which gives them some advantage in adaptation to saline conditions (Kirk 1972). Salinity tolerance in tilapia can be improved to some extent with acclimation (Avella et al. 1992). Fingerlings

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[2] The term variety will be used to describe species, strains within species, and synthetic strains.

of Florida red tilapia (FRT) reared in brackishwater grew faster than freshwater progeny at salinities less than 18 ppt (Watanabe et al. 1989).

In the Americas, commercial tilapia production generally involves four phases. The first phase is spawning and sex reversal followed by a nursery phase. After the nursery phase is advanced fingerling production, which corresponds to Phase II in hybrid striped bass production, followed by the final growout phase (Cohen and Interiano 1999). Brackishwater culture, where practiced, usually begins in either the nursery phase or with advanced fingerling production.

Because of the salinity tolerance of some tilapia, there is some risk that they may become established in areas where they do not occur naturally but are used for aquaculture. The purpose of this study was to evaluate the performance of commercially available varieties of tilapia in freshwater and brackishwater during the advanced fingerling production phase.

Materials and Methods

The study included seven varieties of tilapia: the Auburn-Egypt strain of Nile tilapia, an Egyptian (Lake Manzala) strain of blue tilapia, a synthetic strain of red tilapia developed in the 1970's from "Florida Red" *O. mossambicus x O. urolepis hornorum* hybrids (FRT), a commercially-available silver tilapia synthetic strain with ancestry including *O. aureus, O. mossambicus* and *O. niloticus* (MCS), and three hybrids (blue tilapia x Nile tilapia hybrid (BxN), YY Nile tilapia x Mozambique tilapia hybrid (MxN), and blue tilapia x Florida red tilapia hybrid (BxFRT)). Mean weight of fish at stocking was 9.5 ± 8 g. Forty 2.2-m^3 fiberglass pools were used, each containing approximately 20 cm of soil to simulate biochemical processes found in culture ponds. Water was not exchanged throughout the study, but losses by evaporation were replaced by filtered pond water.

Due to dilution by rainfall, salt was added as needed to brackishwater pools to maintain salinity at 23 ppt. Constant aeration was provided by diffused air. Orchard netting was secured over the pools to prevent losses by predation or escapement. After pools were filled with filtered water from a nearby pond, and before stocking, water quality parameters (pH, nitrite, ammonia and dissolved oxygen) were measured.

Each variety (except Nile tilapia) was stocked at 14 fish per pool into six separate pools, Salt was added to three pools for each variety (except Nile tilapia) to gradually raise the salinity to 23 ppt. One week after stocking, 3.2 kg (3 ppt) of salt (NaCl) was added to pools intended for brackishwater every two days to raise the salinity to 21 ppt. Once 21 ppt was reached in all pools, salinity was measured with a salinity probe in all the marked pools in order to determine the amount of salt needed to reach the target of 23 ppt. Nile tilapia was evaluated in only three freshwater pools due to previous studies, including one using the same strain (Nugon 2003) in which high mortalities were observed above 10 ppt (Watanabe 1985b; Avella and Doudet 1993).

Feeding was done by hand with a commercial, floating, 28%-protein pelleted feed. Initially, each pool was fed 1.1 g once daily (0.24 kg/ha or 0.15% bodyweight/day). After 7 days, all fish were fed to satiation.

Sampling

Forty days after stocking, at least 5 fish in each pool were captured and weighed individually to the nearest 0.1 g. Based on biomass estimates from the first sampling, feeding was adjusted to 4.3 g/day (0.92 kg/ha/day or 0.3% bodyweight/day) for all pools to expedite feeding and simplify FCR comparisons among varieties and treatments. Fish were harvested after 107 days by completely draining the pools. Fish were individually weighed to the nearest 0.1 g.

Statistical Analysis

A randomized design was employed for this study. Results were analyzed using the Statistical Analysis Software system version 6.12 for Windows ™ (SAS Institute Inc., 1996, Cary, North Carolina). The general linear model (GLM) procedure and the least squares means method were used in addition to the Duncan multiple range test and Scheffe's test. The significance level was set at $P \leq 0.05$. The GLM procedure was used to determine effects of salinity and variety on weight and survival. Duncan and Scheffe's tests were performed to compare weight means between the species and varieties used. The treatment combination of Nile tilapia and brackishwater culture conditions were treated as missing values to allow for statistical comparisons (Littell et al. 1991)

Results

Freshwater

In freshwater, MCS had a yield of 0.40 ±0.10 kg/m^2 and mean weight of 151 ±51 g (Table 3.1), FRT had a final yield of 0.35 ±0.05 kg/m^2 with a mean weight of 121 ±11 g, similar to that of blue tilapia (*P*=0.45) which had a final yield of 0.33 ±0.06 kg/m^2 and mean weight of 127 ±16 g. Nile tilapia had a yield of 0.31 ±0.03 kg/m^2 and a mean weight of 111 ±7 g, with no significant difference between FRT and blue tilapia.

Of the hybrids used, B x N had a yield of 0.29 ±0.01 kg/m^2 with a mean weight of 110 ±1 g; B x FRT had a yield of 0.26 ±0.07 kg/m^2 with a mean weight of 117 ±28 g and M x N had a yield of 0.21 ±0.03 kg/m² with a mean weight of 82 ±10 g. Feed conversion ratios (FCR) ranged from 3.3 (MCS) to 8.4 (MxN hybrid).

Specific daily growth (SDG) ranged from 3.6 ±0.2% (Nile tilapia) to 5.6 ±0.4% (blue tilapia). No significant differences ($P \geq 0.05$) were found for SDGs in freshwater. Nile tilapia and FRT had 100 ±0 % survival, B x N had 98 %, MCS had 95 ±8 %, and blue tilapia had 93 ±0 % survival, M x N had 81 ±4 % and B x FRT had 79 ±0 % survival (Table 3.1).

Table 3.1 Varietal yield, mean weight, specific daily growth (SDG), feed conversion ratio (FCR) and survival of tilapia in freshwater mesocosms

Variety	Yield kg/m²	Mean Weight g	SDG %	FCR	Survival %
MCS	0.40 ±0.10a	151 ±51a	4.6 ±0.3a	3.3 ±0.8a	95 ±8b
FRT	0.35 ±0.05a	121 ±11a	3.9 ±0.2a	4.4 ±0.4ac	100 ±0a
Blue	0.33 ±0.06ab	127 ±16a	5.6 ±0.4a	3.7 ±0.6a	93 ±0b
Nile	0.31 ±0.03a	111 ±7a	3.6 ±0.2a	5.4 ±0.4b	100 ±0a
BxN	0.29 ±0.01a	110 ±1a	3.9 ±0.0a	4.9 ±0.2bc	98 ±4ab
BxFRT	0.26 ±0.07ab	117 ±28ab	3.7 ±0.2a	6.1 ±1.9bcd	79 ±0c
MxN	0.21 ±0.03b	82 ±10b	3.9 ±0.3a	8.4 ±1.2d	81 ±4c

Brackishwater

In brackishwater, all yields, mean weights and SDGs were lower than in freshwater. In brackishwater, blue tilapia had a yield of 0.07 ±0.13 kg/m² and a mean weight of 72 ±124 g, which were significantly higher ($P \leq 0.05$) than the yields and mean weights of the rest of the varieties and hybrids used. The FRT had a yield of 0.25 ±0.02 kg/m² and a mean weight of 93 ±9 g, and the MCS had a final weight of 0.01 ±0.02 kg/m² with a mean weight of 23 ±41 g (Table 3.2).

Of the hybrids used, B x FRT had a final weight of 0.10 ±0.14 kg/m² and a mean weight of 85 ±73 g, MxN had a final weight of 0.04 ±0.02 kg/m² with a mean weight of

77 ±20 g and BxN suffered complete mortality (Table 3.2). Feed conversion ratios (FCR) ranged from 6 (FRT) to >10. Specific daily growth (SDG) ranged from 0% (BxN) to 3.8 ±0.1% (FRT). No significant differences were observed among SDGs in brackishwater. Survivals ranged from 100% for FRT to 2.4% for MCS; for the hybrids survival ranged from 29 ±38% for BxFRT to 0% for BxN (Table 3.2).

Table 3.2 Varietal yield, mean weight, specific daily growth (SDG), feed conversion ratio (FCR) and survival of tilapia in brackishwater (23 ppt) mesocosms

Variety	Yield kg/m²	Mean Weight g	SDG %	FCR	Survival %
MCS	0.01 ±0.02b	23 ±41bc	1.0 ±2.0b	> 10b	2 ±4b
FRT	0.25 ±0.02a	93 ±9a	3.8 ±0.1a	6a	100 ±0a
Blue	0.07 ±0.13ab	72 ±12ab	1.7 ±3.0ab	> 10b	12 ±21b
BxN	0.00 ±0.00b	-	-	-	0.0 ±0b
BxFRT	0.10 ±0.14ab	85 ±73ab	1.9 ±1.8ab	> 10b	29 ±38b
MxN	0.04 ±0.02b	77 ±20ab	2.2 ±0.6b	> 10b	19 ±11b

Discussion

Freshwater vs Brackishwater

The productivity of all the varieties of tilapia in freshwater was higher. The final biomass produced in brackishwater was low primarily as a result of lower survival than in freshwater. The general approach to saltwater tilapia culture has been to produce seed and juveniles in freshwater, followed by growout in brackishwater (Watanabe 1985a). Selection for salinity could be beneficial for lines to be used in brackishwater production, especially if they could be exposed early to salinity to promote good survival. Fry from salinity-selected broodstock were found to survive at higher salinities than fry that were not selected for salinity (Watanabe 1985a). Although the brackishwater results were not comparable to freshwater results in terms of weight and survival.

Freshwater Mesocosms

Some varieties had lower weights due to the onset of sexual maturation and reproduction. Fry were observed in FRT, MCS and BxN pools approximately 60 days after stocking. No fry were observed when the fish were sampled 40 days after stocking. Lower individual weight in pools can be partially attributed to slower growth resulting from reproduction and subsequent competition for space, food and oxygen.

Brackishwater Mesocosms

Varieties with Mozambique tilapia ancestry had better weight gain in brackishwater. FRT final weights were significantly greater than ($P \leq 0.05$) those of the MCS and BxFRT. The BxN, which had complete mortality, was not appropriate for brackishwater culture at 23 ppt suggesting that hybrids with Nile tilapia ancestry may express that species' reduced performance in salinities above 10-15 ppt although MLS-96 values for a *O. mossambicus* x *O. niloticus* hybrid were between 23 and 23.2 ppt (Villegas 1990).

Another reason for low weights and survival in brackishwater can be explained by Chervinski's (1961) and Villegas' (1990) observations that tilapia fingerlings are less salt tolerant than larger fish. These results imply that advanced fingerling production for many varieties may need to be carried out in freshwater prior to growout in brackish ponds. Brackishwater culture would involve access to both fresh and brackishwater. If access to brackishwater is not possible, handling and transportation costs would make brackishwater culture less profitable than freshwater culture.

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CHAPTER 4
COLD TOLERANCE OF FOUR TILAPIA VARIETIES

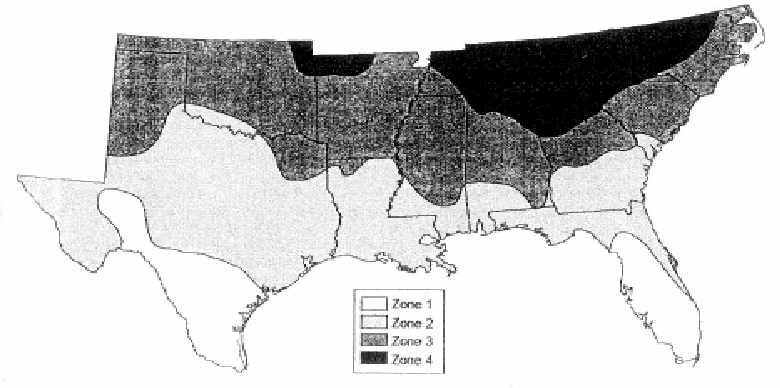
The potential for introduced tilapia to survive and become established makes them an environmental concern in areas beyond their natural range where winter temperatures may not reach lethal levels. Cold tolerance is also of great importance in tilapia production in many regions, dictating the length of the outdoor growing season and costs involved in the operation of indoor holding or production facilities during winter months. The temperature at which 100% mortality occurs for most tilapia species has been reported as 10-11 C for several days (Popma and Masser 1999), but little data is available to evaluate mortality patterns at intermediate temperatures.

Tilapia are generally characterized by a temperature preference in the range of 12-42 C (Avella et al. 1992), and optimal water temperature for tilapia growth is about 29-31 C (Popma and Masser 1999). Apart from genetics, variation in cold tolerance can be attributed to a number of factors, including test conditions. Rate of temperature decrease or “thermal schedule” (Zale and Gregory 1989; Starling et al. 1995) and duration of exposure at low temperatures (Hargreaves 2000) appear to affect tilapia survival significantly.

Results of previous cold tolerance studies with tilapia indicate a fairly wide variation in lethal temperatures for any particular species (Villegas 1990; Chervinski and Lahav 1976). Blue tilapia is considered the most cold tolerant of the major tilapia species (Rakocy et al. 1993). In areas outside their natural range, the major limiting factor determining the possibility for establishment of tilapia populations is the normal minimum temperature during the coldest period of the year (Hargreaves 2000) (Figure

4.1). Based on normal winter temperatures, it has been generally accepted that tilapia survival in the southeastern USA would be limited to coastal habitats along the Gulf and Atlantic coasts from Texas to South Carolina, and in more inland waters only in southern regions of Florida and Texas (Hargreaves 2000).

Determining the cold tolerance and lethal temperatures is important from a production standpoint when fish begin to die, especially terms of defining acceptable or unacceptable losses. Lethal temperatures are also important from an environmental standpoint to assess the risk of establishment of tilapia populations and in the development of culture regulations. The objective of this study was to evaluate the influence of temperature reduction regimes and salinity (0, 5 and 10 ppt) on cold tolerance of juveniles of four varieties of tilapia.



Tilapia Culture Suitability Zone	Estimated Growing Season (d)	Regional Geographic Extent
1	150-210	central and southern FL, south TX
2	120-150	central and southeast TX, coastal to north LA, coastal MS and AL, FL panhandle, southern GA, southern SC
3	90-120	north TX, north LA, AR, central and north MS, central AL and GA, eastern SC, eastern NC
4	60-90	north AR, AL and GA, TN, western SC, central NC

Figure 4.1 Culture suitability zones for tilapia in the southeast USA (from Hargreaves 2000).

Materials and Methods

The varieties[3] used in this study were the Auburn-Egypt strain of Nile tilapia, an Egyptian (Lake Manzala) strain of blue tilapia, a synthetic strain of red tilapia developed in the 1970's from "Florida Red" *O. mossambicus x O. urolepis hornorum* hybrids (FRT), a commercially-available silver tilapia synthetic strain with ancestry including *O. aureus, O. mossambicus* and *O. niloticus* (MCS). To determine variability and differences in the cold tolerance of these fish, three temperature reduction regimes were used and defined as rapid, moderate, and gradual. The rapid reduction regime consisted of an acclimation period at 20 C for 36 h followed by a 0.5 C reduction every 5 h. This temperature reduction regime was repeated once with two replicates per variety. The moderate reduction regime consisted of an acclimation period at 22 C for 36 h followed by a 1 C reduction every 24 h. This temperature reduction regime was repeated three times (six replicates per variety). The gradual temperature reduction consisted of an acclimation period at 22 C for 36 h followed by a 1 C reduction every 48 h. This temperature reduction regime was repeated three times (six replicates per variety). To determine the effect of salinity on cold tolerance of these varieties, the moderate reduction regime was repeated with salinities of 5 and 10 ppt. Salinity was measured with a refractometer prior to stocking fingerlings.

Temperature reduction was accomplished using two in-line titanium chillers connected to two 280-L circular fiberglass tanks. Twenty fingerlings (2 to 5 g) of each variety were segregated into two 3.8-L plastic containers with perforated bottoms. Mouths of the jars were secured with fiberglass screen to allow mortality to be monitored. In all trials, containers were checked before each temperature reduction and

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[3] The term variety will be used to describe species, strains within species and synthetic strains.

mortalities removed. Mortality was defined as loss of equilibrium, failure to respond to handling, and cessation of opercular movement. Prior to the trials, water quality parameters (ammonia, nitrite, and pH) in the system were measured.

Statistical Analysis

Results were analyzed using the Statistical Analysis Software system version 6.12 for Windows ™ (SAS Institute Inc., 1996, Cary, North Carolina). The PROC LIFETEST method was used to generate survivorship curves for each replicate within each trial. Survivorship curves were used to extract temperatures at which survival was 90% (LT_{90}), 50% (LT_{50}), 10% (LT_{10}), and 0% (LT_0). Significance level was set at $P \leq 0.05$. Trials and replicates within trials that were not different at $P \leq 0.05$ were pooled. The general linear models procedure (PROC GLM) method was used to evaluate differences in lethal temperatures among varieties and temperature reduction regimes.

Results

Temperature Reduction Regimes

Differences among varieties were very small and generally not significant when temperature was reduced rapidly (Table 4.1). The LT_{90} for blue tilapia occurred at 8.2 C, for Nile tilapia, MCS and FRT this value was 8.8 C. The LT_{50} values were 6.3 C for MCS, 6.8 C for blue and Nile tilapia, and 8.0 C for FRT. The LT_{10} values were 5.5 C for MCS, 6.5 C for blue tilapia and FRT and 7.2 C for Nile tilapia. The LT_0 was 5.3 C for MCS, 5.5 C for blue tilapia and FRT and 5.6 C for Nile tilapia.

In the moderate reduction regime, lethal temperatures were uniformly higher, and differences between varieties became more apparent (Table 4.2). LT_{90}s were observed from 19 ±0 (FRT) to 14 ±2 (MCS), while LT_{50} values were 10 ±1C for blue tilapia, 12 ±1

C for Nile tilapia and FRT, and 9 ±1 for MCS. LT_{10}s ranged from 10 ±1 (FRT) to 7.3 ±0.6 C (blue tilapia) and LT_0s ranged from 9.5 ±1.0 (FRT) to 7.2 ±0.6 (blue tilapia).

In the gradual reduction regime, lethal temperatures were similar to those observed with moderate reduction. Again, differences between varieties became more apparent (Table 4.3). LT_{90}s ranged from 17.5 ±4.2 C (Nile tilapia) to 14.0 ±0.0 C (FRT). The LT_{50} values were 13 ±0 C for Nile tilapia, 12.0 ±0.7 C for FRT, 11.6 ±0.0 C for blue tilapia and 11 ±0 C MCS. LT_{10}s ranged from 10.6 ±2.6 C in Nile tilapia to 10.1 ±2.1 C in MCS, and LT_0s ranged from 11.0 ±0.0 C (FRT) to 7.5 ±0.0 C (blue tilapia)

Table 4.1 Mean (±SD) lethal temperature at which survival is equal to 90% (LT_{90}), 50% (LT_{50}), 10% (LT_{10}) and 0% (LT_0) where tilapia were exposed to a rapid (-0.5 C/5 h) reduction regime.

Variety	n	LT_{90}	LT_{50}	LT_{10}	LT_0
Blue tilapia	2	8.2 ±0.2a	6.8 ±0.1b	6.5 ±0.5b	5.5 ±0.1a
Nile tilapia	2	8.8 ±0.4ab	6.8 ±0.4ab	7.2 ±0.3bc	5.6 ±0.3a
MCS	2	8.8 ±0.1b	6.3 ±0.2a	5.5 ±0.2a	5.3 ±0.1a
FRT	2	8.8 ±0.2b	8.0 ±0.2c	6.5 ±0.3b	5.5 ±0.2a

Numbers with same letter within a column are not significantly different.

Table 4.2 Mean (±SD) lethal temperature at which survival is equal to 90% (LT_{90}), 50% (LT_{50}), 10% (LT_{10}) and 0% (LT_0) where tilapia were exposed to a moderate (-1 C/24 h) reduction regime.

Variety	N	LT_{90}	LT_{50}	LT_{10}	LT_0
Blue tilapia	6	15.2 ±3.2a	9.5 ±1.0a	7.3 ±0.6a	7.2 ±0.6a
Nile tilapia	6	19.4 ±0.0b	12.2 ±1.5b	8.6 ±1.0ab	7.8 ±1.5ab
MCS	6	14.5 ±2.7a	9.2 ±0.6a	7.6 ±1.0a	7.5 ±1.0ab
FRT	6	19.4 ±0.0b	11.8 ±0.6b	10.2 ±0.6b	9.5 ±1.0b

Numbers with same letter within a column are not significantly different.

Table 4.3 Mean (±SD) lethal temperature at which survival is equal to 90% (LT_{90}), 50% (LT_{50}), 10% (LT_{10}) and 0% (LT_0) where tilapia were exposed to a gradual (-1 C/48 h) reduction regime.

Variety	N	LT_{90}	LT_{50}	LT_{10}	LT_0
Blue tilapia	6	14.1 ±0.8a	11.6 ±0.0a	10.5 ±0.0a	7.5 ±0.0a
Nile tilapia	6	17.5 ±4.2a	12.6 ±0.0b	10.6 ±2.6a	10.0 ±2.1b
MCS	6	15.6 ±5.6a	11.1 ±2.1ab	10.1 ±2.1a	9.5 ±1.4b
FRT	6	14.0 ±0.0a	12.0 ±0.7ab	11.1 ±0.6a	11.0 ±0.0b

Numbers with same letter within a column are not significantly different.

Although differences between varieties were generally small, differences between regimes reflected the effect of thermal schedules on the cold tolerance of tilapia (Table 4.5). For LT_{50} and LT_{10} values, thermal schedules produced significant differences in cold tolerance, while the moderate and gradual reduction regimes did not differ significantly in terms of LT_{90} and LT_0. Differences between the cold tolerance indicators in the moderate (-1 C/24 h) and gradual (-1 C/48 h) reduction regimes were low when compared to the rapid reduction regime.

Table 4.4 Comparison of mean (±SD) lethal temperatures at which survival is equal to 90% (LT_{90}), 50% (LT_{50}), 10% (LT_{10}) and 0% (LT_0) when four tilapia varieties were exposed to three temperature reduction regimes.

Regime	LT_{90}	LT_{50}	LT_{10}	LT_0
- 0.5 C/ 5h	8.7 ±0.3 a	6.9 ±0.7 a	6.0 ±0.7 a	5.5 ±0.1 a
- 1 C/ 24 h	17.1 ±1.5 b	10.7 ±0.9 b	8.4 ±0.8 b	8.0 ±1.0 b
- 1 C/ 48 h	15.3 ±2.8 b	11.8 ±0.8 c	10.6 ±1.3 c	9.4 ±1.0 b

Numbers with same letter within a column are not significantly different.

Varieties

Blue tilapia

Approximately 90 % of the blue tilapia in the rapid reduction regime died between 7 C and 6.5 C, following survival above 80 % at temperatures above 7.5 C

(Figure 4.2). In the rapid reduction regime, the LT_{90} for blue tilapia was 10 C lower than in the moderate and gradual reduction regimes and complete mortality (LT_0) followed at 5.5 C.

In the moderate reduction regime, the LT_{90} of blue tilapia was higher than in the rapid and gradual reduction regimes. Survival was observed to decline slowly as the temperature decreased (Figure 4.2). Approximately 60 % of the population died between 17 C and 9 C, a decline that did not occur at the same temperatures in the rapid reduction regime.

In the gradual reduction regime, the LT_{90} occurred 6 C higher than in the rapid reduction regime and 4 C lower than in the moderate reduction regime (Figure 4.2). As in the moderate reduction regime, a notable decline in survival was observed between 15 C and 11 C, at which point approximately 60 % of the population died. The LT_0 was reached 1 C higher than in the moderate reduction regime and 2.5 C higher than in the rapid reduction regime (Figure 4.2). Cold tolerance of blue tilapia was affected by the rate of temperature reduction, as evidenced by temperatures at which mortalities began to occur. In the rapid reduction regime, mortalities began at much lower temperatures than in the moderate (+11 C) and gradual reduction regimes (+7 C). Temperature reduction regime also affected rate at which survival decreased, from very steep declines in the rapid reduction regime to more continual declines in survival in the moderate and gradual reduction regimes. The final lethal temperature (LT_0) reached by blue tilapia in all the trials was also affected by the rate at which temperature was reduced. The more rapid the temperature reduction, the lower the final lethal temperature.

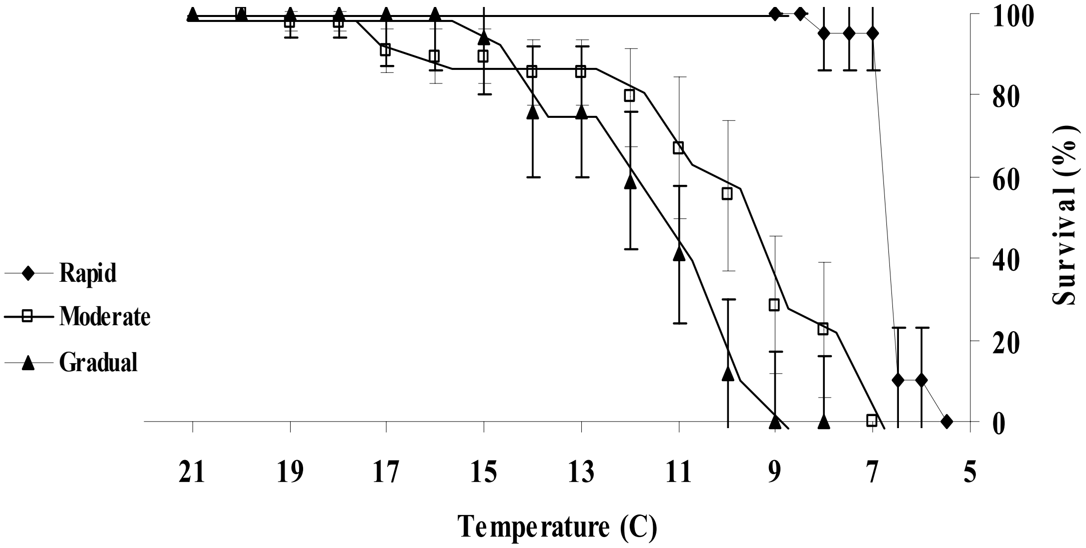

Figure 4.2 Survival of blue tilapia (*O. aureus*) juveniles exposed to three temperature reduction regimes.

In the moderate reduction regime with 5 ppt, LT_{90} occurred 1 C higher than in freshwater (Figure 4.3). Survival declined slowly until 11 C, following the mortality curve observed in freshwater. The LT_0 in 5 ppt was reached 0.5 C above freshwater. In the moderate reduction regime with 10 ppt, survival was above 80 % at 12 C (Figure 4.3). The LT_0 reached at 10 ppt was 2 C below freshwater. At 11 C survival reached 70 ±11%, until 10 C was reached. After initial mortalities, fish in both the 5 ppt and 10 ppt salinity had higher survivals than those in 0 ppt. The LT_0 was lowered by salinity and both 5 ppt and 10 ppt salinities had the same effect in reducing final lethal temperature. However, survivals were higher above 8 C in 10 ppt than in 5 ppt. The effect of salinity on blue tilapia cold tolerance was significant ($P \leq 0.05$) compared to temperatures reached in freshwater.

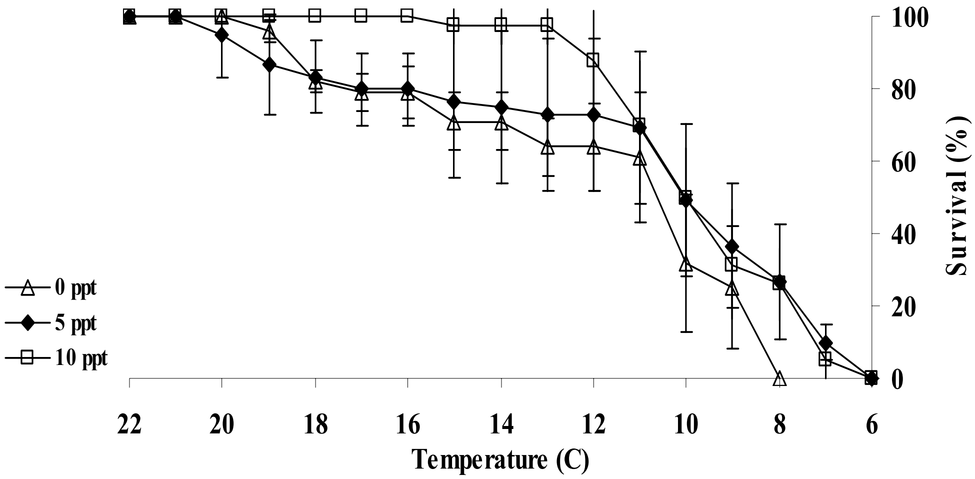

Figure 4.3 Survival of blue tilapia (*O. aureus*) juveniles exposed to a temperature reduction of -1 C/24 h at 0, 5 and 10 ppt.

Nile Tilapia

In the rapid reduction regime, survival dropped abruptly after reaching 9 C (Figure 4.4). In the moderate and gradual reduction regime, mortalities began at 19 C and 21 C, respectively. In the rapid reduction regime, mortalities began 10.5 C lower than in the moderate reduction regime and 12.5 C lower than in the gradual reduction regime (Figure 4.4). In the rapid reduction regime, more than 60 % of the population died between 9 C and 7.5 C. In the moderate reduction regime, LT_{90} began 1 C lower than in the gradual reduction regime. As in the blue tilapia in the moderate reduction regime, survival declined slowly as temperature was reduced, although lower survivals were observed than in the blue tilapia at equal temperatures (Figure 4.4). The LT_0 in the moderate reduction regime was observed at the same temperature as in the gradual reduction regime and 1.5 C higher than in the rapid reduction regime (Figure 4.4). In the gradual reduction regime, mortalities began at 20 C (Figure 4.4). At 12 C, survival dropped to 42 ±17% (Figure 4.4). The LT_0 occurred 1 C higher in the gradual reduction regime than in the rapid reduction regime (Figure 4.4).

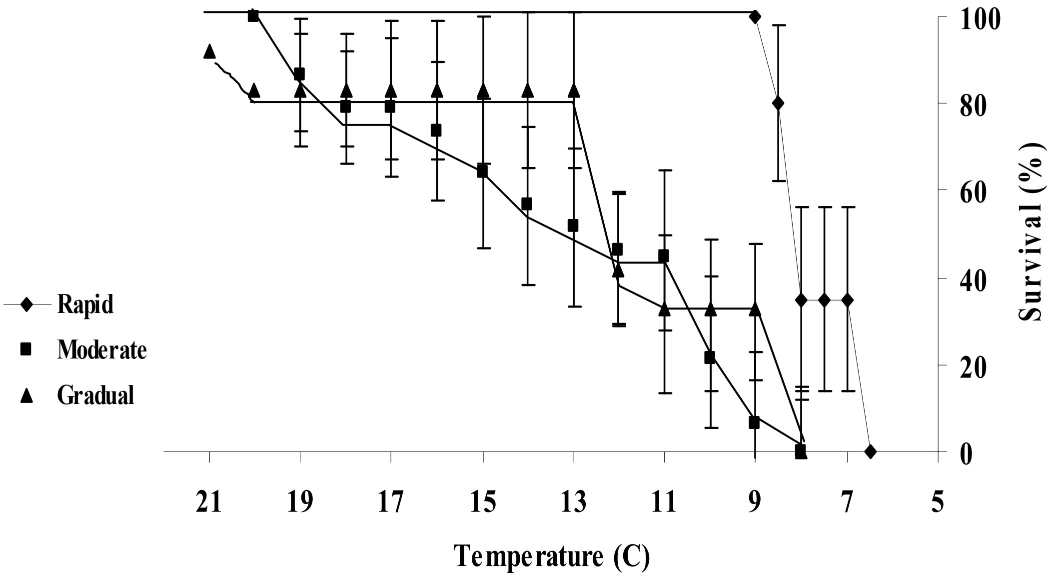

Figure 4.4 Survival of Nile tilapia (*O. niloticus*) juveniles exposed to three temperature reduction regimes.

In the moderate reduction regime at 5 ppt, mortalities began 1 C higher than in freshwater (Figure 4.5). Approximately 50 % of the population died from 19 C to 10 C (Figure 4.5). The LT_0 was observed at the same temperature as in freshwater. In the moderate reduction regime with 10 ppt, survival decreased slowly, approximately 70 % from 20 C to 9 C (Figure 4.5). Mortalities in 10 ppt began 1 C lower than in freshwater. The LT_0 was observed at the same temperature as in freshwater and 5 ppt.

MCS

In the rapid, moderate and gradual reduction regimes, mortalities began at 9 C, 17 C and 11 C, respectively. In the rapid reduction regime, mortalities began 8.5 C lower than in the moderate reduction regime and 3.5 C than in the gradual reduction regimes (Figure 4.6).

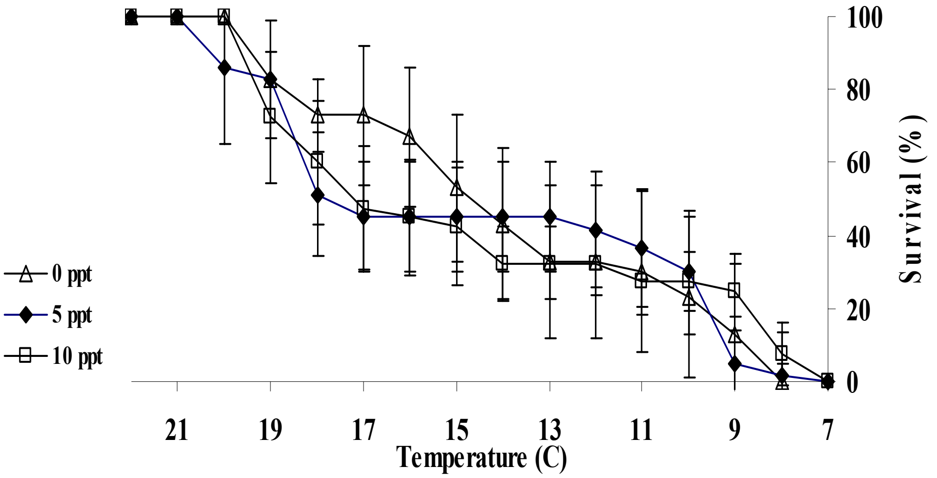

Figure 4.5 Survival of Nile tilapia (*O. niloticus*) juveniles exposed to a temperature reduction of -1 C/24 h at 0, 5 and 10 ppt.

Approximately 50 % of the population in the rapid reduction regime died between 8.5 C and 6.5 C. survival (Figure 4.6). The LT_0 in the rapid reduction regime occurred 2.5 C lower than in the moderate reduction regime and 1.5 C lower than in the gradual reduction regime.

In the moderate reduction regime, mortalities began 5 C higher than in the gradual reduction regime (Figure 4.6). Approximately 50 % of the population died between 19 C and 9 C. The LT_0 was reached 1 C higher than the gradual reduction regime. In the gradual reduction regime, survival was higher than in the moderate reduction regime at temperatures above 10 C (Figure 4.6). Approximately 60 % of the population died between 12 C and 8 C. In the moderate reduction regime at 5 ppt, mortalities began 1 C higher than in freshwater.

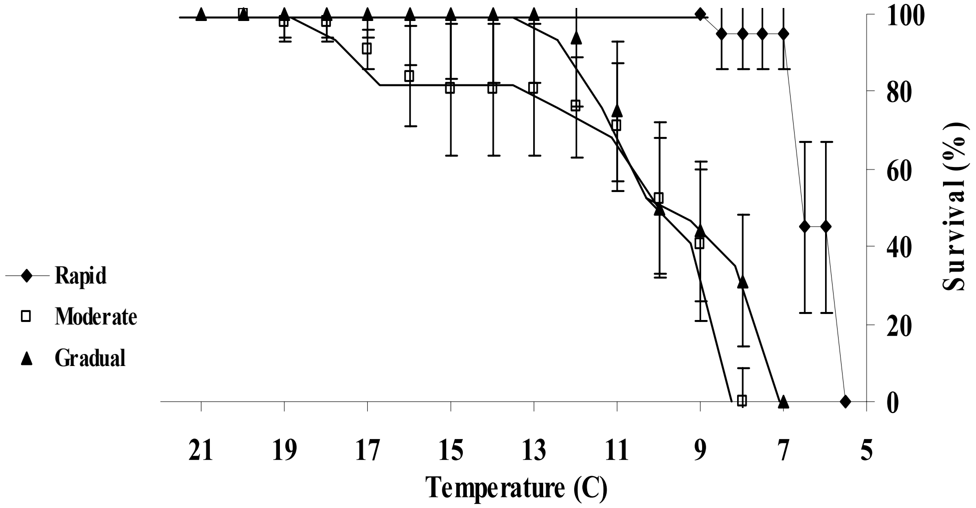

Figure 4.6 Survival of MCS juveniles exposed to three temperature reduction regimes.

Mortalities at 10 ppt started at the same temperature as in freshwater. Approximately 60 % of the population died between 19 C and 10 C (Figure 4.7). The LT_0 in 5 ppt occurred 1 C higher than in freshwater and the LT_0 in 10 ppt occurred 1.3 C than in freshwater (Figure 4.7). In the moderate reduction regime with 10 ppt, mortalities began at the same temperature of the freshwater trial. Approximately 70 % of the population died between 19 C and 8 C (Figure 4.7). The LT_0 was reached at the same temperature as in the freshwater trial and 1 C lower than in 5 ppt.

The rate of temperature reduction influenced cold tolerance in MCS. In the gradual reduction regime, survival between 20 C and 12 C respectively, was considerably higher than that of the moderate reduction regime. Below 11 C, survival in both regimes decreased more rapidly until final lethal temperatures were reached. As in the rest of the varieties, survival in the rapid reduction regime was better than in the moderate and gradual reduction regimes, allowing fish to reach lower LT_0.

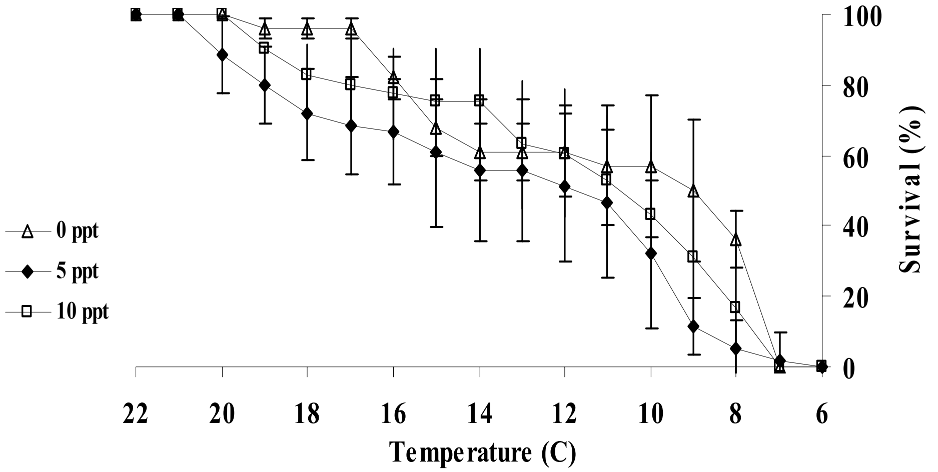

Figure 4.7 Survival of MCS juveniles exposed to a temperature reduction of -1 C/24 h at 0, 5 and 10 ppt.

FRT

In the rapid, moderate and gradual reduction regimes, mortalities began at 8.5 C, 19 C and 17 C, respectively (Figure 4.8). Approximately 80 % of the population died between 9 C and 8.5 C in the rapid reduction regime, with mortalities continuing below 7 C. Below 8 C, survival was constant until the LT_0 was reached at 6.5 C. The LT_0 in the rapid reduction regime occurred 3.5 C lower than in the moderate and gradual reduction regime. In the moderate reduction regime, mortalities began 2 C higher than in the gradual reduction regime (Figure 4.8). Approximately 55 % of the population died off between 19 C and 11 C. The LT_0 was observed at the same temperature as in the gradual reduction regime. In the gradual reduction regime, approximately 80 % of the population died off between 19 C and 10 C. Mortalities were not as constant as those observed for other varieties (Figure 4.8).

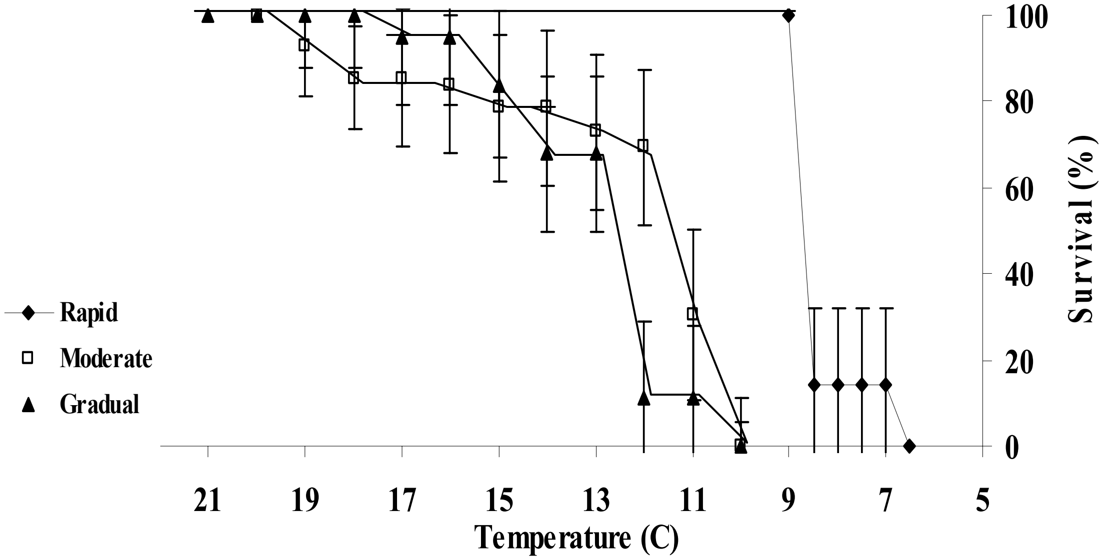

Figure 4.8 Survival of FRT juveniles exposed to three temperature reduction regimes.

In the moderate reduction regime with 5 ppt, mortalities began at the same temperature as in freshwater. Mortalities in 10 ppt began 4 C lower than in freshwater. In 5 ppt mortalities began 3 C higher than in 10 ppt. Survival did not decline to under 80 % until the temperature had reached 10 C, 9 C lower than in freshwater. The LT_0 occurred 4 C lower than in freshwater (Figure 4.9). In the moderate reduction regime with 10 ppt, following the acclimation period no mortalities were observed above 16 C. Between 16 C and 10 C 50 % of the population died (Figure 4.9). The LT_0 was observed 2 C lower than that of freshwater and 2 C higher than that of the moderate reduction with 5 ppt (Figure 4.9). In all temperature reduction regimes, LT_0 were higher for FRT than other varieties. In the moderate and gradual reduction regimes, between 20 C and 13 C, survival declined slowly. Below 13 C, survival was observed to decline more rapidly.

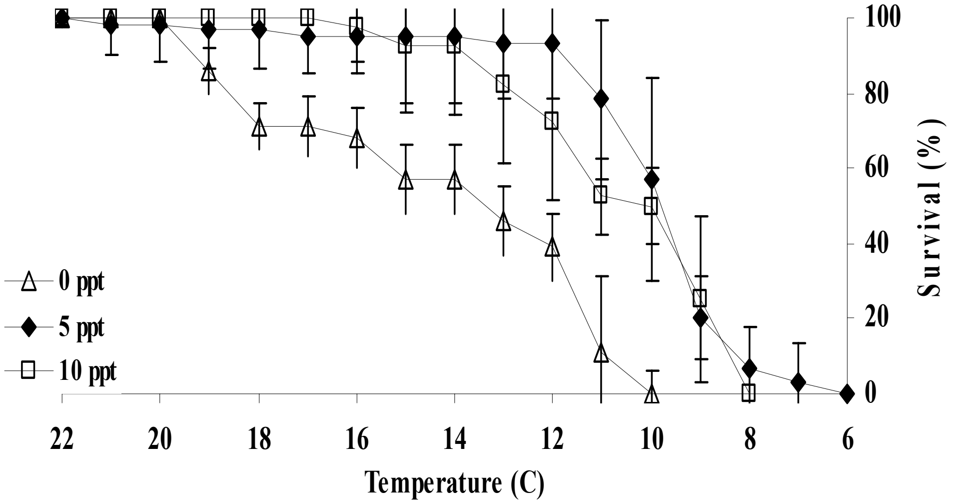

Figure 4.9 Survival of FRT juveniles exposed to a temperature reduction of -1 C/24 h at 0, 5 and 10 ppt.

Salinity Effect on Cold Tolerance

The mean LT_{90} in 5 ppt was higher than in 0 ppt and 10 ppt. The LT_{90} values for blue tilapia in 10 ppt and FRT in 5 and 10 ppt were the only LT_{90}s reduced by salinity (Table 4.5). Mean LT_{50} values for all varieties in 5 and 10 ppt were higher than in 0 ppt, but no significant difference ($P \leq 0.05$) was observed among the three. No significant difference was observed between the mean LT_{10} at 0 ppt and 5 ppt, but there was a significant difference between the mean LT_{10} at 0 ppt and 10 ppt.

The mean LT_0 reached in the 5 and 10 ppt trials were 0.9 C higher and 1.7 C lower than in 0 ppt, respectively. Between 20 C and 10 C, survival above 80% was observed in 5 ppt and 10 ppt, while in 0 ppt it was considerably lower, and the final lethal temperature was reached at 10 C. In 5 ppt and 10 ppt, survival decreased rapidly below 10 C. Mean LT_0s were reached at 9.7 ±1.9 C (10 ppt) and 8.9 ±2.7 C (5 ppt) (Table 4.4).

No significant difference was observed among varieties but there was a trend of decreased cold tolerance with salinity (Table 4.5). This trend can be observed with FRT (salinity tolerant) with LT_{50} lower in 5 and 10 ppt than in freshwater, while LT_{50}s of Nile tilapia were higher in 5 and 10 ppt than in freshwater.

Table 4.5 Mean (±SD) lethal temperature at which survival is equal to 90% (LT_{90}), 50% (LT_{50}), 10% (LT_{10}) and 0% (LT_0) where tilapia were exposed to a moderate (-1 C/24 h) reduction regime at 0, 5 and 10 ppt.

Salinity	Variety	n	LT_{90}	LT_{50}	LT_{10}	LT_0
	Blue tilapia	6	15.2 ±3.2	9.5 ±1.0	7.3 ±0.6	7.2 ±0.6
0 ppt	Nile tilapia	6	19.4 ±0.0	12.2 ±1.5	8.6 ±1.0	7.8 ±1.5
	MCS	6	14.5 ±2.7	9.2 ±0.6	7.6 ±1.0	7.5 ±1.0
	FRT	6	19.4 ±0.0	11.8 ±0.6	10.2 ±0.6	9.5 ±1.0
	Overall Mean	24	**17.1 ±1.5 a**	**10.7 ±0.9 a**	**8.4 ±0.8 a**	**8.0 ±1.0 a**
	Blue tilapia	6	18.4 ±2.5	9.6 ±1.0	7.9 ±1.5	7.8 ±1.5
5 ppt	Nile tilapia	6	20.1 ±0.6	13.5 ±4.4	12.5 ±5.1	11.2 ±5.5
	MCS	6	18.3 ±2.8	13.5 ±5.1	9.5 ±1.0	7.8 ±1.5
	FRT	6	14.5 ±4.5	10.0 ±1.5	9.1 ±2.2	8.8 ±2.1
	Overall Mean	24	**17.8 ±2.6 a**	**11.7 ±3.0 a**	**9.8 ±2.5 ab**	**8.9 ±2.7 a**
	Blue tilapia	6	12.5 ±0.8	10.5 ±1.7	9.5 ±1.7	7.8 ±1.2
10 ppt	Nile tilapia	6	19.5 ±0.9	16.0 ±5.7	13.8 ±4.6	12.2 ±4.0
	MCS	6	18.3 ±2.4	11.6 ±2.6	9.5 ±1.7	8.8 ±1.2
	FRT	6	15.5 ±0.0	11.5 ±1.7	10.5 ±1.7	9.8 ±1.2
	Overall Mean	24	**16.4 ±1.0 a**	**12.4 ±2.9 a**	**10.8 ±2.4 b**	**9.7 ±1.9 a**

Numbers with same letter within a column are not significantly different.

Discussion

In the rapid reduction regime, as temperatures were reduced from 20 C survivals did not typically decline until below 9.5 C. This effect was also described by Starling et al. (1995), where rapid temperature reductions actually allow fish to reach lower temperatures because the total time of exposure was reduced. In all temperature reduction trials, blue tilapia and MCS had similar LT_0s, while Nile tilapia and FRT had similar somewhat higher LT_0s. Notable declines in survival for blue tilapia, MCS and FRT generally occurred between 7 C and 6.5 C, while Nile tilapia had notable declines in survival between 8.5 C and 8 C.

In the moderate and gradual reduction regimes, survival began to decline at higher temperatures than in the rapid reduction regime. This may be attributed to a longer acclimation and exposure time to each temperature, while in the rapid reduction regime fish were exposed to decreasing temperatures for shorter periods of time.

Mortalities for blue tilapia and MCS began at the same temperature (19 C) in the moderate reduction regime. As in the rapid reduction regime, Nile tilapia and FRT also exhibited similar survival as temperatures declined. Blue tilapia, Nile tilapia and MCS generally had significant declines in survival between 11 C and 9 C; FRT had a significant decline in survival between 12 C and 10 C.

In the gradual reduction regime, final lethal temperatures were similar to those observed in the moderate reduction regime. Extending the exposure time of the fish to cold beyond what occurred in the moderate reduction regime apparently did not reduce their cold tolerance. In this trial, MCS had the lowest final lethal temperature (7 C), while blue tilapia and Nile tilapia both had the same final lethal temperature (8 C).

The effect of salinity on cold tolerance was not apparent in all varieties. Salinity did not improve survivals at intermediate temperatures (temperatures between acclimation temperature and final lethal temperature). In 5 ppt, cold tolerance of Nile tilapia was apparently reduced by salinity, and even more so in 10 ppt.

Florida red tilapia exhibited the most enhanced cold tolerance in brackishwater, reaching an LT_{50} lower than that reached in freshwater. Salinity (10 ppt) slightly improved the LT_{90} for blue tilapia and FRT compared to freshwater. Similar results were obtained in cold tolerance studies where blue tilapia survival improved with salinity (5 ppt) when compared to freshwater survival (Chervinski and Lahav 1976).

Cold tolerance of tilapia varieties is important from production and environmental standpoints. Choosing a variety with better cold tolerance may reduce costs for a producer by extending the growing season and reducing mortalities resulting from sudden temperature decrease. Temperature at which mortality begins and the LT_{90} values are the most important considerations in terms of cold tolerance in commercial tilapia production, inasmuch as regular losses greater than 10% to cold temperatures would probably not be economically sustainable.

From an environmental standpoint, defining the cold tolerance of tilapia varieties should help to formulate or revise tilapia regulations. Additional work is needed to establish mortality patterns in degree-days, with these and other tilapia varieties exposed to declining temperatures to simulate ambient conditions in various locations.

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CHAPTER 5
GENERAL CONCLUSIONS

Accidental introduction of a non-native species such as tilapia into the southeast USA would be expected to have negative impacts to local ecosystems. These might include habitat damage and losses in indigenous populations through competition for space and food, predation or disease introduction (Courtenay and Robbins 1973). Tilapia are produced in many types of production systems and different varieties may perform differently under different conditions. This thesis presents some aspects on cold tolerance and adaptability of tilapia to several culture systems.

Performance Comparisons

Comparisons were made between varieties in each production system used in the thesis. These comparisons were based on important production traits, as well as environmental tolerances. An index was developed to include growth and survival as indicators of production, and FCR as an indication of production efficiency. For index comparisons between varieties, growth and survival were weighed 25% each and FCR was weighed 50%.

For clear-water and greenwater recirculating systems, performance in growth, survival and FCR were ranked separately from 1 (best) to 4 (worst) for each variety. Index score represented the product of the weighting and rank for each trait. In freshwater mesocosms, performance scores were ranked from 1 (best) to 7 (worst) due to the additional use of three hybrids. In brackishwater mesocosms, performance scores were ranked from 1 (best) to 6 (worst) since Nile tilapia was not suitable for this production environment and therefore not included. Index scores in fresh and brackishwater mesocosms were calculated as in the recirculating systems. Cold tolerance of varieties was compared using LT_{50} values.

Blue Tilapia

Blue tilapia had the highest index score of all varieties evaluated in clear-water recirculating systems (Table 5.1) and the second highest in greenwater recirculating systems (Table 5.2). In freshwater mesocosms, it also had the second highest score (Table 5.3) while in brackishwater it had an intermediate performance (Table 5.4). In cold tolerance trials, based on LT_{50}, it was the second most cold tolerant in freshwater and the most cold tolerant in brackishwater.

Mississippi Commercial Strain Tilapia (MCS)

Index scores for MCS were intermediate in clear-water (Table 5.1) and greenwater recirculating systems (Table 5.2). MCS had the best overall performance in freshwater mesocosms (Table 5.3) and third best in brackishwater mesocosms (Table 5.4). Based on LT_{50}, it was the most cold tolerant variety in freshwater and the second most cold tolerant in brackishwater.

Nile Tilapia

Nile tilapia had the highest index score in greenwater recirculating systems (Table 5.2) but third highest in clear-water recirculating systems (Table 5.1). Its performance was relatively poor in freshwater mesocosms (Table 5.4). Based on LT_{50}, its cold tolerance was relatively poor in freshwater and in brackishwater.

Florida Red Tilapia (FRT)

The performance of FRT was the best in brackishwater (23 ppt) mesocosms (Table 5.4) and second best in freshwater mesocosms (Table 5.3). In clear-water and greenwater recirculating systems its performance was relatively poor (Tables 5.1 and 5.2). Based on LT_{50}, its cold tolerance was relatively poor in freshwater, with a slight improvement in brackishwater (both 5 and 10 ppt).

Table 5.1 Relative performance of four tilapia varieties in clear-water recirculating systems.

Variety	Growth (25%)	Survival (25%)	FCR (50%)	Index Score
Blue tilapia	1	1	1	1.0
MCS	2	2	2	2.0
Nile tilapia	3	3	3	3.0
FRT	4	4	4	4.0

Table 5.2 Relative performance of four tilapia varieties in greenwater recirculating systems.

Variety	Growth (25%)	Survival (25%)	FCR (50%)	Index Score
Blue tilapia	2	2	2	2.0
MCS	3	3	3	3.0
Nile tilapia	1	1	1	1.0
FRT	4	4	4	4.0

Hybrids

Of the hybrids used in fresh and brackishwater mesocosms, all performed relatively poorly, when compared to the four main varieties evaluated in the thesis. Most of the hybrids were worse than their parent lines, except for B x N which had a better index score in freshwater mesocosms than Nile tilapia.

Table 5.3 Relative performance of seven tilapia varieties in outdoor freshwater mesocosms.

Variety	Growth (25%)	Survival (25%)	FCR (50%)	Index Score
Blue tilapia	2	5	2	2.8
MCS	1	4	1	1.8
Nile tilapia	6	1	5	4.3
FRT	3	1	3	2.5
B x N	5	3	4	4.0
B x FRT	4	7	6	5.8
M x N	7	6	7	6.8

Table 5.4 Relative performance of six tilapia varieties in outdoor brackishwater (23 ppt) mesocosms

Variety	Growth (25%)	Survival (25%)	FCR (50%)	Index Score
Blue tilapia	4	4	3	2.5
MCS	5	5	2	3.5
FRT	1	1	1	1.0
B x N	6	6	6	6.0
B x FRT	2	2	5	4.5
M x N	3	3	4	3.5

Appraisal of Cold Tolerance of Tilapia Varieties

Differences in cold tolerance between varieties were slight, with no significant difference ($P \leq 0.05$) between any two varieties. Temperature reduction regimes had a noticeable effect on the cold tolerance of all varieties. Fish in the rapid (-0.5 C/5 h) reduction regime reached lower temperatures than in the moderate (-1 C/24 h) and gradual (-1 C/48 h) reduction regimes.

The assumptions that salinity may improve cold tolerance by facilitating osmoregulation (Hargreaves 2000) and that isosmotic salinity (12 ppt) reduces thermal stress (Zale and Gregory 1989) were not confirmed. Mean LT_{90}s were 17.1 C (0 ppt), 17.8 C (5 ppt) and 16.5 C (10 ppt). Mean LT_{50}s were 10.7 C (0 ppt), 11.7 C (5 ppt) and

12.4 C (10 ppt). Mean LT_{10}s were 8.4 C (0 ppt), 9.8 C (5 ppt) and 10.8 C (10 ppt). Mean LT_0s were 8 C (0 ppt), 8.9 C (5 ppt) and 9.7 C (10 ppt). Based on LT_{50} and LT_0 values, as salinity increased from 0 ppt to 10 ppt, osmotic stress may actually have occurred in addition to thermal stress. Lethal temperatures were higher at 5 ppt than at 0 ppt for blue tilapia, Nile tilapia and MCS. FRT was the only variety in which the LT_{50} and LT_0 were reduced by salinity in 5 ppt and the only variety in which the LT_{50} was reduced in 10 ppt. Nugon (2003) reported survivals above 97% for all varieties in 10 ppt at temperature between 26 C and 30 C. Although a gradual decline in survival, at intermediate temperatures, was observed at 10 ppt for all varieties, salinity stress apparently added to the thermal stress caused most varieties to die at higher temperatures than in freshwater.

In Louisiana, annual water temperatures near the coast range from 5.9 C to 30 C (www.nodc.noaa.gov) and annual salinities range from 0.2 ppt to 18.7 ppt. During colder months, salinities drop between 5 and 10 ppt (www.lumcon.lsu.edu). This indicates that introduced populations might be restricted by salinity and temperature in the coastal areas of Louisiana, but the possibility of establishment must not be discounted. Establishment might be possible where warmer low salinity gulf waters are present. In southeastern USA, there have been reports of established populations in Florida, North Carolina and Texas (Hale et al. 1995; Hargreaves 2000). Blue tilapia LT_0s were 7.2 ±0.6 C (0 ppt), 7.8 ±1.5 C (5 ppt), and 7.8 ±1.2 C (10 ppt). Nile tilapia LT_0s were 7.8 ±1.5 C (0 ppt), 11.2 ±5.5 C (5 ppt), and 12.2 ±4 C (10 ppt). MCS LT_0s were 7.5 ±1 C (0 ppt), 7.8 ±1.5 C (5 ppt), and 8.8 ±1.2 C (10 ppt). FRT LT_0s were 9.5 ±1 C (0 ppt), 8.8 ±2.1 C (5 ppt), and 9.8 ±1.2 C (10 ppt).

From a production standpoint, lower LT_{90} mean less mortalities at the onset of the colder months, less dependence on indoor facilities and an extension of the growout period outdoors. Culturing varieties with lower LT_{90} will also reduce costs associated with the use of indoor facilities and make available additional fish to increase production biomass and returns on investment.

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APPENDIX A- POOLED WEIGHTS OF 4 TILAPIA VARIETIES IN RECIRCULATING AQUACULTURE SYSTEMS

Recirculating Aquaculture System Growout
12/14/01

System 1	Blue	Nile	MCS	FRT
1	17.70	35.70	29.70	40.30
2	27.50	36.20	47.20	36.90
3	12.80	35.20	31.60	33.80
4	15.10	29.00	27.70	29.10
5	13.40	39.80	31.90	18.20
Total (g)	86.50	175.90	168.10	158.30
STDEV	6.01	3.90	7.78	8.58
Weight/fish (g)	3.46	7.04	6.72	6.33

System 2	Blue	Nile	MCS	FRT
1	14.90	30.20	36.00	29.70
2	13.10	24.50	28.30	26.60
3	15.20	19.40	34.30	26.40
4	14.50	68.90	29.30	26.40
5	8.80	38.20	33.20	23.80
Total (g)	66.50	181.20	161.10	132.90
STDEV	2.64	19.55	3.30	2.09
Weight/fish (g)	2.66	7.25	6.44	5.32

System 3	Blue	Nile	MCS	FRT
1	14.80	29.20	19.10	16.90
2	14.20	35.40	45.80	31.90
3	23.90	29.80	21.70	17.40
4	13.10	33.20	43.80	30.10
5	12.10	23.20	14.50	16.30
Total (g)	78.10	150.80	144.90	112.60
STDEV	4.74	4.64	14.69	37.43
Weight/fish (g)	3.12	6.03	5.80	4.50

System 4	Blue	Nile	MCS	FRT
1	23.80	33.30	25.90	26.00
2	32.60	19.90	27.40	24.80
3	16.80	20.20	24.60	28.60
4	18.60	22.40	22.10	19.40
5	14.90	16.40	16.10	10.60
Total (g)	106.70	112.20	116.10	109.40
STDEV	7.11	6.44	4.43	7.14
Weight/fish (g)	4.27	4.49	4.64	4.38

Recirculating Aquaculture System Growout
1/14/02

System 1	Blue	Nile	MCS	FRT
1	21.16	68.39	75.42	41.03
2	53.93	92.67	95.84	96.54
3	48.90	93.95	70.73	43.76
4	49.48	80.67	102.16	10.52
5	26.46	94.00	42.20	0.00
Total (g)	199.93	429.68	386.35	191.85
STDEV	15.01	11.29	23.67	37.64
Weight/fish (g)	8.00	17.19	16.10	11.29

System 2	Blue	Nile	MCS	FRT
1	35.22	34.65	68.85	57.12
2	32.39	69.46	57.88	42.60
3	30.56	52.49	35.14	45.46
4	0.00	79.68	51.41	23.12
5	0.00	62.84	36.24	0.00
Total (g)	98.17	299.12	249.52	168.30
STDEV	18.00	17.20	14.40	22.44
Weight/fish (g)	6.14	13.01	9.98	8.42

System 3	Blue	Nile	MCS	FRT
1	30.06	82.37	46.23	74.46
2	42.52	93.48	46.81	42.91
3	27.92	46.58	58.40	41.74
4	31.91	79.21	99.17	0.00
5	10.15	52.86	28.82	0.00
Total (g)	142.56	354.50	279.43	159.11
STDEV	11.70	20.17	26.40	31.88
Weight/fish (g)	6.48	14.18	12.15	10.61

System 4	Blue	Nile	MCS	FRT
1	29.90	62.80	46.73	77.74
2	45.55	67.71	53.04	63.93
3	76.00	38.89	70.54	21.06
4	47.15	55.63	65.17	22.90
5	0.00	28.59	22.17	0.00
Total (g)	198.60	253.62	257.65	185.63
STDEV	27.75	16.50	18.94	32.43
Weight/fish (g)	9.46	11.03	11.20	8.84

Recirculating Aquaculture System Growout
03/29/02

System 1	**Blue**	**Nile**	**MCS**	**FRT**
1	461.15	176.12	356.84	148.9
2	460.91	217.27	416.15	184.73
3	400.56	174.50	415.12	46.90
4	312.82	176.85	208.81	0.00
5	357.86	94.07	158.63	0.00
Total (g)	1993.30	838.81	1555.55	380.53
STDEV	64.84	44.94	120.06	85.93
Weight/fish (g)	83.05	34.95	67.63	31.71

System 2	**Blue**	**Nile**	**MCS**	**FRT**
1	104.44	282.57	204.15	115.89
2	135.15	261.09	192.67	145.36
3	61.78	289.00	207.52	14.46
4	0.00	128.73	304.07	0.00
5	0.00	961.39	0.00	0.00
Total (g)	301.37	1922.78	908.41	275.71
STDEV	60.88	329.00	111.00	69.94
Weight/fish (g)	21.53	76.91	37.85	21.21

System 3	**Blue**	**Nile**	**MCS**	**FRT**
1	433.54	178.60	230.09	213.77
2	324.70	146.72	305.46	289.75
3	304.53	136.56	389.62	96.42
4	327.19	140.52	256.93	0.00
5	150.09	66.58	175.36	0.00
Total (g)	1540.05	668.98	1357.46	599.94
STDEV	101.68	41.06	81.05	129.39
Weight/fish (g)	66.96	30.41	59.02	42.85

System 4	**Blue**	**Nile**	**MCS**	**FRT**
1	469.10	191.18	260.12	237.86
2	334.74	211.32	282.05	209.64
3	258.46	199.85	244.93	157.24
4	352.30	124.20	231.55	133.16
5	0.00	0.00	107.15	0.00
Total (g)	1414.60	726.55	1125.80	737.90
STDEV	175.22	88.03	68.59	92.31
Weight/fish (g)	70.73	36.33	51.17	40.99

Recirculating Aquaculture System Growout
05/17/02

System 1	Blue	Nile	MCS	FRT
1	211.54	141.262	372.94	155.96
2	300.72	146.668	432.48	127.72
3	196.51	127.456	624.50	0
4	379.27	105.678	646.50	0
5	342.20	119.594	0	0
Total (g)	1430.24	640.66	2076.42	283.68
STDEV	80.04	16.53	136.88	19.97
Weight/fish (g)	57.21	25.63	230.71	13.51

System 2	Blue	Nile	MCS	FRT
1	250.00	393.54	315.16	129.91
2	200.50	496.68	203.54	149.03
3	120.30	425.57	899.40	0
4	0.00	337.20	0.00	0
5	0.00	0	0	0
Total (g) weight	570.80	1652.99	1418.10	278.94
STDEV	114.03	66.54	373.72	13.52
Weight/fish (g)	40.77	91.83	88.63	30.99

System 3	Blue	Nile	MCS	FRT
1	206.42	243.56	556.60	200.67
2	560.30	328.62	543.16	490.66
3	457.73	224.59	381.34	106.49
4	573.04	353.90	396.43	0
5	667.65	0	250.30	0
Total (g) weight	2465.14	1150.67	2127.83	797.82
STDEV	176.64	139.81	127.01	200.23
Weight/fish (g)	107.18	52.30	96.72	61.37

System 4	Blue	Nile	MCS	FRT
1	326.25	275.06	312.91	341.36
2	473.90	216.13	415.06	365.17
3	687.71	246.38	383.04	352.60
4	447.51	262.60	531.45	0
5	0	0	0	0
Total (g) weight	1935.37	1000.17	1642.46	1059.13
STDEV	150.35	25.48	91.15	11.91
Weight/fish (g)	101.86	50.01	74.66	66.20

Recirculating Aquaculture System Growout
6/18/02

System 1	Blue	Nile	M. C.	FRT
1	720	360	630	135
2	855	225	652.5	90
3	742.5	315	450	0
4	540	292.5	337.5	0
5	855	247.5	0	0
Total (g)	3712.5	1440	2070	225
STDEV	129.25	53.72	110.99	31.82
Weight/fish (g)	148.50	60.00	108.95	28.13

System 2	Blue	Nile	MCS	FRT
1	427.5	292.5	787.5	135
2	427.5	247.5	495	135
3	697.5	90	405	0
4	202.5	0	0	0
5	0	0	0	0
Total (g)	1755	630	1687.5	270
STDEV	155.88	106.33	199.98	77.94
Weight/fish (g)	103.24	45.00	120.54	30.00

System 3	Blue	Nile	MCS	FRT
1	585	315	495	450
2	787.5	315	472.5	270
3	855	247.5	607.5	112.5
4	427.5	292.5	517.5	0
5	292.5	90	247.5	0
Total (g)	2947.5	1260	2340	832.5
STDEV	985.66	420.13	773.51	322.20
Weight/fish (g)	128.15	57.27	106.36	69.38

System 4	Blue	Nile	MCS	FRT
1	652.5	292.5	495	697.5
2	630	315	427.5	540
3	630	337.5	495	0
4	405	225	315	0
5	0	0	0	0
Total (g)	2317.5	1170	1732.5	1237.5
STDEV	116.73	48.61	84.94	362.98
Weight/fish (g)	121.97	61.58	91.18	112.50

Recirculating Aquaculture System Growout
7/16/02

System 1	Blue	Nile	MCS	FRT
1	726.4	363.2	544.8	163.44
2	976.1	431.3	544.8	0
3	635.6	295.1	735.48	0
4	1135	249.7	522.1	0
5	771.8	204.3	0	0
Total (g)	4244.9	1543.6	2347.18	163.44
STDEV	202.78	90.23	99.70	81.72
Weight/fish (g)	169.80	67.11	123.54	32.69

System 2	Blue	Nile	MCS	FRT
1	749.1	454	817.2	158.9
2	499.4	136.2	726.4	0
3	726.4	295.1	476.7	0
4	0	0	0	0
5	0	0	0	0
Total (g)	1974.9	885.3	2020.3	158.9
STDEV	373.55	196.85	389.36	71.06
Weight/fish (g)	116.17	59.02	144.31	31.78

System 3	Blue	Nile	MCS	FRT
1	885.3	317.8	508.48	476.7
2	885.3	317.8	635.6	431.3
3	590.2	431.3	735.48	0
4	749.1	440.38	635.6	0
5	408.6	0	0	0
Total (g)	3518.5	1507.28	2515.16	908
STDEV	204.93	178.58	292.51	249.18
Weight/fish (g)	152.98	75.36	125.76	90.80

System 4	Blue	Nile	MCS	FRT
1	408.6	771.8	499.4	590.2
2	317.8	908	544.8	544.8
3	431.3	544.8	544.8	249.7
4	249.7	499.4	454	0
5	0	0	0	0
Total (g)	1407.4	2724	2043	1384.7
STDEV	173.32	347.24	231.50	284.61
Weight/fish (g)	74.07	143.37	102.15	115.39

Recirculating Aquaculture System Growout
8/16/02

System 1	Blue	Nile	MCS	FRT
1	917.08	635.6	635.6	22.7
2	1180.4	499.4	794.5	0
3	885.3	295.1	635.6	0
4	885.3	385.9	590.2	0
5	817.2	0	0	0
Total (g)	4685.28	1816	2655.9	22.7
STDEV	140.835	239.697	306.912	10.152
Weight/fish (g)	195.22	95.58	147.55	11.35

System 2	**Blue**	**Nile**	**MCS**	**FRT**
1	817.2	454	703.7	249.7
2	408.6	340.5	590.2	0
3	930.7	204.3	635.6	0
4	295.1	0	0	0
5	0	0	0	0
Total (g)	2451.6	998.8	1929.5	249.7
STDEV	382.75	202.65	354.59	111.67
Weight/fish (g)	144.21	66.59	148.42	49.94

System 3	**Blue**	**Nile**	**MCS**	**FRT**
1	1044.2	544.8	771.8	590.2
2	1180.4	476.7	771.8	317.8
3	908	499.4	635.6	0
4	908	499.4	1271.2	0
5	590.2	0	0	0
Total (g)	4630.8	2020.3	3450.4	908
STDEV	219.15	227.23	455.59	266.66
Weight/fish (g)	201.34	96.20	164.30	100.89

System 4	**Blue**	**Nile**	**MCS**	**FRT**
1	1225.8	454	703.7	998.8
2	862.6	499.4	476.7	703.7
3	658.3	431.3	454	317.8
4	590.2	0	930.7	0
5	0	0	0	0
Total (g)	3336.9	1384.7	2565.1	2020.3
STDEV	447.60	254.00	345.98	440.87
Weight/fish (g)	175.63	92.31	122.15	168.36

APPENDIX B- FINAL INDIVIDUAL WEIGHTS FOR 4 TILAPIA VARIETIES IN GREENWATER RECIRCULATING SYSTEMS

Trial 1

Day	MCS	Blue	Nile	FRT
1	0.02	0.02	0.02	0.31
37	2.17	1.55	1.67	5.83
44	3.57	2.18	2.15	6.57
47	6.38	6.35	5.46	15.75
117	29.13 ±27.7	26.15 ±21.4	26.25 ±21.4	60.3 ±41.8
124	44.67 ±15.5	48.67 ±22.5	52.12 ±25.9	80.3 ±55.7
301	357 ±152.4	322.5 ±120.9	301.1 ±103.6	250 ±103.3
370	413 ±88.3	379 ±139.1	393 ±83.3	337 ±145.2
405	443 ±102.3	409 ±140.7	425.0 ±83.7	345 ±116.6

Trial 2

Day	MCS	Blue	Nile	FRT
1	0.02	0.02	0.02	0.02
47	5.73	6.1	5.8	3.82
71	9.3	9.1	9.4	14.7
136	66.5 ±37.3	38.8 ±30.4	30.5 ±19.3	66.4 ±49.3
158	94.3 ±23.7	78.3 ±41.8	74.1 ±39.8	114.8 ±72.8
204	217 ±44.5	219 ±83.0	230 ±87.6	249 ±102.8
254	315 ±94.8	330 ±121.4	337 ±101.4	367 ±168.1
290	346 ±111.8	386 ±131.6	399 ±105.3	387 ±183.4
333	389 ±126.0	440 ±115.3	453 ±107.4	414 ±211.5

Trial 3

Day	MCS	Blue	Nile	FRT
1	0.02	0.02	0.02	0.02
34	1.67	1.11	1.43	1.92
59	7.39	6.42	7.10	7.98
88	11.4 ±9.0	9.33 ±7.8	11.2 ±10.1	14.7 ±11.3
121	35.9 ±19.1	36.7 ±17.6	38.1 ±23.2	43.6 ±29.6
197	185 ±61.0	181 ±72.4	179 ±73.4	185 ±92.5
230	234 ±81.9	244 ±82.9	266 ±90.4	271 ±111.1
285	311 ±93.3	344 ±120.7	351 ±112.3	357 ±160.6
310	368 ±111.5	382 ±114.6	388 ±89.2	381 ±160.6
356	406 ±117.7	439 ±118.5	463 ±97.2	418 ±163.9

Trial 4

Day	**MCS**	**Blue**	**Nile**	**FRT**
1	0.02	0.02	0.02	0.02
91	10.7 ±8.3	8.91 ±6.6	12.1 ±9.3	11.9 ±9.8
122	33.1 ±16.9	34.4 ±21.3	39.9 ±27.5	34.6 ±24.9
191	166 ±54.8	174 ±78.3	168 ±53.8	159 ±109.7
234	211 ±73.9	239 ±86.0	254 ±78.7	244 ±161
288	301 ±84.3	337 ±107.8	343 ±99.5	339 ±206.8
348	391 ±93.8	436 ±139.5	455 ±109.2	423 ±228.4

Trial 5

Day	**MCS**	**Blue**	**Nile**	**FRT**
1	0.02	0.02	0.02	0.02
91	11.1 ±9.2	10.4 ±8.0	11.9 ±9.9	10.7 ±7.6
131	34.4 ±21.7	40.7 ±18.3	43.7 ±27.1	38.8 ±27.9
191	172 ±68.8	199 ±77.8	166 ±73.0	168 ±95.8
239	218 ±74.1	259 ±103.6	269 ±80.7	236 ±115.6
299	293 ±105.8	351 ±122.8	356 ±85.4	342 ±164.2
330	361 ±111.9	398 ±135.3	411 ±102.7	389 ±175.0
361	406 ±109.6	444 ±142.1	471 ±103.6	419 ±171.8

APPENDIX C- INDIVIDUAL WEIGHTS OF FISH HARVESTED FROM FRESH AND BRACKISHWATER MESOCOSMS

Freshwater Nile tilapia

Pool	A3	D5	B7
	100.40	84.94	89.32
	123.07	109.22	140.00
	113.03	153.12	100.30
	109.30	115.14	179.61
	111.81	70.86	108.34
	83.76	109.57	150.90
	125.89	95.10	124.17
	111.58	111.06	104.92
	109.15	145.55	132.42
	112.10	82.25	155.12
	141.40	123.30	91.82
	110.50	91.37	79.36
	60.95	124.39	122.19
	80.50	73.68	85.88
Total Wgt (g)	**1493.44**	**1489.55**	**1664.35**
Mean Wgt (g)	**106.67**	**106.40**	**118.88**
St. Deviation	**20.25**	**25.07**	**29.88**
Survival %	**100.00**	**100.00**	**100.00**

Freshwater Blue tilapia

Pool	A6	B1	C4
	166.50	96.50	119.70
	114.40	137.50	79.80
	103.70	101.30	108.10
	105.30	104.40	132.90
	166.10	118.30	94.50
	130.00	123.60	119.50
	143.50	101.20	155.70
	108.80	122.00	137.10
	197.00	83.90	149.90
	210.70	89.80	99.90
	137.70	144.20	135.20
	185.50	129.60	120.00
	113.60	117.30	136.90

	0.00	0.00	0.00
Total Wgt (g)	**1882.80**	**1469.60**	**1589.20**
Mean Wgt (g)	**144.83**	**113.05**	**122.25**
St. Deviation	**21.82**	**23.07**	**28.95**
Survival %	**93.00**	**93.00**	**93.00**

	Freshwater MCS		
Pool	**A7**	**B5**	**D6**
	86.10	248.30	134.50
	93.60	175.20	156.60
	94.10	143.40	232.40
	130.40	252.10	88.70
	125.80	290.70	140.50
	139.40	145.30	115.40
	102.70	145.70	210.40
	95.20	248.40	108.00
	77.30	255.50	68.30
	139.80	240.00	107.60
	101.40	130.60	131.90
	98.80	238.10	89.10
	168.00	0.00	94.80
	146.90	0.00	117.00
Total Wgt (g)	**1599.50**	**2513.30**	**1795.20**
Mean Wgt (g)	**114.25**	**209.44**	**128.23**
St. Deviation	**27.07**	**56.61**	**46.03**
Survival %	**100.00**	**86.00**	**100.00**

	Freshwater FRT		
Pool	**A4**	**A8**	**C1**
	129.75	141.50	159.30
	123.90	112.90	162.80
	65.48	125.70	144.40
	104.24	184.50	150.20
	138.32	169.50	199.90
	115.84	121.10	107.30
	99.60	165.60	91.10
	158.80	113.80	159.20
	134.60	109.80	127.20
	90.90	89.10	82.90
	38.90	118.40	121.40

	105.90	91.20	107.00
	97.80	84.90	53.10
	115.90	135.00	75.20
Total Wgt (g)	**1519.93**	**1763.00**	**1741.00**
Mean Wgt (g)	**108.57**	**125.93**	**128.14**
St. Deviation	**30.54**	**30.55**	**39.81**
Survival %	**100.00**	**100.00**	**100.00**

Freshwater B x F

Pool	**A9**	**B2**	**C10**
	63.20	148.30	91.20
	108.70	101.60	171.40
	65.20	170.50	89.80
	63.60	149.70	118.90
	64.80	157.60	133.90
	112.00	117.90	104.10
	67.60	110.00	122.30
	115.50	125.70	168.30
	114.60	110.10	146.30
	56.80	165.10	136.60
	98.70	112.20	170.30
	0.00	0.00	0.00
	0.00	0.00	0.00
	0.00	0.00	0.00
Total Wgt (g)	**930.70**	**1468.70**	**1453.10**
Mean Wgt (g)	**84.61**	**133.52**	**132.10**
St. Deviation	**21.82**	**23.07**	**28.95**
Survival %	**79.00**	**79.00**	**79.00**

Freshwater B x N

Pool	**C6**	**C8**	**D10**
	126.50	127.40	125.80
	116.60	118.90	108.10
	114.60	91.10	106.10
	103.40	118.60	121.60
	110.10	36.40	33.30
	118.10	71.80	136.70
	142.00	144.40	141.00
	69.40	106.90	89.90
	107.90	122.00	107.40
	91.40	129.40	123.70

	103.30	103.40	126.30
	160.00	81.00	108.70
	93.70	128.30	115.10
	62.30	157.40	0.00
Total Wgt (g)	**1519.30**	**1537.00**	**1443.70**
Mean Wgt (g)	**108.52**	**109.79**	**111.05**
St. Deviation	**25.61**	**31.46**	**27.14**
Survival %	**100.00**	**100.00**	**93.00**

Freshwater M x N

Pool	**B6**	**B9**	**C9**
	87.40	75.20	48.60
	96.80	103.50	58.50
	97.60	51.10	79.70
	97.40	89.80	87.00
	77.50	56.10	112.90
	75.60	87.90	77.10
	76.70	72.90	70.60
	116.10	122.50	56.90
	89.60	76.40	74.50
	122.70	68.30	75.00
	82.60	133.90	281.00
	277.00	0.00	0.00
	0.00	0.00	0.00
	0.00	0.00	0.00
Total Wgt (g)	**1297.00**	**937.60**	**1021.80**
Mean Wgt (g)	**92.73**	**80.37**	**74.08**
St. Deviation	**15.59**	**21.46**	**18.00**
Survival %	**86.00**	**79.00**	**79.00**

Brackishwater Blue tilapia

Pool	**B3**	**C2**	**D8**
	173.30	0.00	0.00
	184.70	0.00	0.00
	241.10	0.00	0.00
	243.90	0.00	0.00
	231.70	0.00	0.00
Total Wgt (g)	**1074.70**	**0.00**	**0.00**
Mean Wgt (g)	**214.94**	**0.00**	**0.00**
St. Deviation	**33.36**	**0.00**	**0.00**
Survival %	**35.71**	**0.00**	**0.00**

Brackishwater MCS

Pool	A5	C3	D7
	0.00	210.30	0.00
Total Wgt (g)	**0.00**	**210.30**	**0.00**
Mean Wgt (g)	**0.00**	**70.10**	**0.00**
St. Deviation	**0.00**	**0.00**	**0.00**
Survival %	**0.00**	**7.14**	**0.00**

Brackishwater FRT

Pool	A2	C7	D1
	68.70	104.90	102.00
	69.30	68.70	86.10
	88.10	77.10	84.00
	98.00	115.70	94.90
	93.60	46.00	78.70
	80.80	74.40	71.60
	70.50	68.80	94.30
	84.00	99.70	111.60
	87.60	88.90	98.90
	75.70	67.90	86.70
	100.30	94.40	89.60
	100.80	103.40	64.50
	114.30	64.10	82.90
	89.90	361.50	102.20
Total Wgt (g)	**1221.60**	**1435.50**	**1248.00**
Mean Wgt (g)	**87.26**	**102.54**	**87.89**
St. Deviation	**13.54**	**20.16**	**13.17**
Survival %	**100.00**	**100.00**	**100.00**

Brackishwater B x F

Pool	B4	D2	D3
	0.00	116.40	158.00
	0.00	136.20	108.20
	0.00	0.00	80.60
	0.00	0.00	119.20
	0.00	0.00	156.80
	0.00	0.00	114.80

	0.00	0.00	103.70
	0.00	0.00	137.50
	0.00	0.00	178.90
	0.00	0.00	120.90
Total Wgt (g)	**0.00**	**252.60**	**1278.60**
Mean Wgt (g)	**0.00**	**126.30**	**127.86**
St. Deviation	**0.00**	**14.00**	**29.71**
Survival %	**0.00**	**14.29**	**71.43**

Brackishwater B x N

Pool	**A1**	**B10**	**D4**
	0.00	0.00	0.00
Total Wgt (g)	**0.00**	**0.00**	**0.00**
Mean Wgt (g)	**0.00**	**0.00**	**0.00**
St. Deviation	**0.00**	**0.00**	**0.00**
Survival %	**0.00**	**0.00**	**0.00**

Brackishwater M x N

Pool	**A10**	**B9**	**C5**
	67.50	83.50	86.30
	0.00	110.50	47.60
	0.00	105.70	59.20
	0.00	19.80	0.00
Total Wgt (g)	**67.50**	**319.50**	**193.10**
Mean Wgt (g)	**67.50**	**99.9**	**64.37**
St. Deviation	**0.00**	**41.74**	**19.86**
Survival %	**7.14**	**28.6**	**21.42**

APPENDIX D- SURVIVAL OF 4 VARIETIES OF TILAPIA EXPOSED TO TEMPERATURE REDUCTION REGIMES

Rapid Temperature Reduction Regime (0.5 C/ 5 hrs)

Temperature	Nile	Blue	MCS	FRT
9.0	20	20	20	20
8.5	16	20	19	8
8.0	7	19	19	8
7.5	7	19	19	8
7.0	7	19	19	8
6.5	0	2	9	0
6.0	0	2	0	0
5.5	0	0	0	0
5.0	0	0	0	0

Moderate Temperature Reduction (1 C/ 24 hrs)

Temperature	Nile	Blue	MCS	FRT
20	30	30	30	30
19	25	29	29	26
18	25	28	29	26
17	22	25	29	21
16	22	24	25	21
15	20	24	20	20
14	16	21	18	17
13	13	21	18	17
12	10	19	18	14
11	10	19	17	12
10	9	18	17	3
9	7	10	15	0
8	4	8	11	0
7	0	0	0	0

Gradual Temperature Reduction (1C/48 Hours)

Temperature	Nile	Blue	MCS	FRT
21	18	20	20	20
20	17	20	20	20
19	17	20	20	20
18	17	20	20	20
17	17	20	20	19
16	17	20	20	19
15	17	19	20	17
14	17	15	20	14
13	17	15	20	14
12	8	12	19	2

11	7	8	15	2
10	7	2	10	0
9	7	0	9	0
8	0	0	6	0
7	0	0	0	0
6	0	0	0	0

Moderate Reduction Regime - 5 ppt

Temperature	Nile	Blue	MCS	FRT
20	16	18	22	22
19	16	18	22	22
18	2	18	22	22
17	0	18	22	22
16	0	18	22	22
15	0	16	18	22
14	0	16	18	22
13	0	16	18	22
12	0	16	16	22
11	0	16	14	22
10	0	6	8	20
9	0	0	0	0

Moderate Reduction Regime- 5 ppt

Temperature	Nile	Blue	MCS	FRT
22	20	20	20	20
21	20	20	20	19
20	17	17	13	19
19	17	15	9	18
18	15	15	6	18
17	14	15	6	17
16	14	15	6	17
15	14	15	6	17
14	14	14	3	17
13	14	14	3	17
12	12	14	3	17
11	9	12	3	16
10	8	11	2	16
9	2	11	2	12
8	1	8	2	4
7	0	6	1	2
6	0	0	0	0

Moderate Reduction Regime- 5 ppt

Temperature	Nile	Blue	MCS	FRT
20	20	20	20	20
19	18	20	20	20
18	17	20	20	20
17	17	20	20	20
16	16	20	20	20
15	15	20	20	20
14	14	20	20	20
13	14	20	20	20
12	12	19	19	20
11	12	14	17	10
10	4	10	11	0
9	0	5	9	0
8	0	4	0	0
7	0	0	0	0

Moderate Reduction Regime- 10 ppt

Temperature	Nile	Blue	MCS	FRT
19	20	20	20	20
18	19	19	20	20
17	15	19	19	20
16	15	19	19	20
15	14	18	18	20
14	13	18	18	20
13	13	18	16	20
12	13	18	16	20
11	11	17	16	20
10	11	17	16	20
9	10	11	13	10
8	3	5	7	0
7	0	1	0	0
6	0	0	0	0

Moderate reduction regime- 10 ppt

Temperature	Nile	Blue	MCS	FRT
20	20	20	20	20
19	9	17	18	16
18	8	16	17	16
17	5	13	16	13
16	4	12	16	13
15	4	10	16	13
14	4	8	16	12

13	3	7	16	12
12	3	5	14	12
11	3	4	14	12
10	3	4	12	7
9	3	4	12	6
8	1	4	10	0
7	0	0	0	0

Moderate reduction regime- 10 ppt

Temperature	Nile	Blue	MCS	FRT
20	20	20	20	20
19	9	20	16	20
18	5	20	14	20
17	4	20	14	20
16	3	20	13	19
15	3	20	13	17
14	0	20	13	17
13		20	10	13
12		16	9	9
11		10	6	1
10		2	2	0
9		1	0	
8		0		

APPENDIX E. STANDARD OPERATING PROCEDURES FOR THE STUDIES IN THIS THESIS

SOP-1. Determination of Cold Tolerance of Juveniles of Seven Varieties of Tilapia in 0 ppt Water Salinity

Materials Needed:

2 Titanium in-Line Chillers
2 280-L Circular fiberglass tanks
0.5 hp Submersible Pump
Fiberglass screen
Insulating Tape
Sump
Jars

Procedures:

1. Insulate two 280-L tanks and all inlet and outlet water pipes from the chillers with insulating tape.
2. Connect in-line chillers to pump and connect pipes with valves that will supply chilled water to the tanks. Fill up tanks and sump with water and start circulating water through the system.
3. Drill 0.32 cm holes on bottom of plastic jars to allow water circulation inside the jars and cut fiberglass screen to cover the jars mouths to prevent fish from escaping into the tank.
4. Turn in-line chillers on, manually setting the acclimation temperature on the chillers dial at which experiment will start.
5. Obtain 20 juvenile tilapia of each variety that will be used in each experiment and separate 10 fish per jar (one variety per jar), placing jars containing the same variety in different tanks. Cover jars with fiberglass screen after fish are placed inside.

6. Start acclimation period for each temperature reduction regime to allow fish to adapt to the starting temperature of each trial. Mortalities in this period are replaced so that all jars contained the same number of fish.

7. Reduce temperature at the predetermined regime reduction rate and record mortalities between each reduction phase until complete mortality is reached.

SOP-2. Determination of Cold Tolerance of Juveniles of Seven Varieties of Tilapia in 5 ppt and 10 ppt

Materials Needed:

2 Titanium in-Line Chillers	2 280-L circular fiberglass tanks
Insulated Tanks	0.5 hp Submersible Pump
Coarse granular salt	Fiberglass screen
Jars	

Procedures:

1. Add 4.3 kg of salt to the previously insulated tanks to reach 5 ppt salinity.

2. Obtain 20 fish of each variety and place 10 fish per jar (one variety per jar). Cover jars with fiberglass screen and place jars into tanks, placing jars containing the same variety in different tanks.

3. Start acclimation period at 20°C for 36 hours to allow fish to adapt to starting temperature.

4. Reduce the temperature using the protocols for the moderate reduction regime used in the cold tolerance determination study using the in-line chillers to control temperature and monitor mortality before reducing temperature.

5. After the lethal temperature for all fish has been reached, increase the temperature to start a new trial.

6. To the water with 5 ppt of salinity, add 4.3 kg of salt to increase salinity to 10 ppt.

7. Obtain 20 fish of each variety and place 10 fish per jar (one variety per jar). Cover jars with fiberglass screen and place jars into the tanks repeating the steps followed in the previous trial.

8. Start acclimation period at 20° C for 36 hours to allow fish to adapt to starting temperature.

9. Reduce temperature repeating the steps of the previous trial.

SOP-3. Growth of Four Tilapia Varieties in Clearwater Recirculating Systems

Materials Needed:

4 Recirculating Systems
Digital Scale
28 % protein commercial Feed
Notebook
Graduated cups
Dip nets

Procedures:

1. 100 fish of each species were used in the study: blue, Nile, Florida red and Mississippi commercial tilapia were chosen, weighed and separated into four systems (random blocks). Each recirculating system is composed of four tanks, each holding one of the species mentioned above.

2. Adjust feeding to 2% total biomass/tank/day to start the experiment.

3. First weigh-in is made one month after stocking; all fish are removed from the tanks by lowering the water and then weigh groups of five and an average and standard deviation is obtained to record growth and adjust feeding.
4. The second weigh-in is made three months after stocking to record growth and adjust feeding, still use 2% total biomass/tank/day.
5. The third weigh-in is made five months after stocking, growth is recorded and feeding is increased to 6% total biomass/tank/day due to the increased appetite of the fish. Fish are weighed individually due to their size.
6. The fourth weigh-in is made six months after stocking, growth is recorded and feeding is adjusted.
7. The fifth weigh-in is made seven months after stocking to record growth, feeding is adjusted to 4% total biomass/tank/day due to reduced intake and loss of feed through drainage.
8. The sixth weigh-in is made eight months after stocking to record growth and adjust feeding.
9. The fish from each tank are weighed, to determine weight gain, feed conversion ratios and specific daily growths for all varieties are also calculated.

SOP-4. Growth of Seven Varieties of Tilapia in Fresh and Brackish water Mesocosms.

Materials Needed:

Salt
40 2134-L tanks
Seine
28% protein Pelleted feed
Hand Nets
Digital Scale

Procedures:

1. The fiberglass tanks used are inspected for incidence of weeds and classified according to degree of weed coverage of the tank floor to determine which pools are to be used as brackish water pools.
2. Fill tanks with pond water and stock with 14 fish. Each variety is assigned 6 pools of which 3 will be fresh water and the remaining will be brackish water. Nile tilapia are only assigned 3 freshwater pools.
3. Salt is added to the brackish water tanks 14 days post stocking (14.9 kg/pool) to raise the salinity to 7 ppt. Salt is added every other day increasing salinity by 7 ppt until 21 ppt is reached.
4. Subsequent salt additions are made to reach the goal of 23 ppt.
5. Feeding is set to 1.10 g/day for all the tanks; it is increased to 2.2 g/day 20 days after stocking. Feeding is increased to 4.26 g/day 40 days after stocking.
6. Sampling is made 40 days after stocking by seining the tanks. The goal is to obtain five fish from each tank to record weight. The seine is passed at the most three times if five fish are not caught with the first two passes.
7. Salinity is checked with a portable salinity meter before adding more salt. After the 23 ppt is reached, salinity is measured biweekly.
8. Tanks are drained; all fish are weighed and sexed at 107 days after stocking.

VITA

Patricio E. Paz was born in Santo Domingo, Dominican Republic, on February 21, 1976. He received his high school diploma from Escuela Internacional Sampedrana in Honduras in 1994. He later attended the Escuela Agricola Panamericana (EAP) in Honduras where he received his title as Agronomo in December 1997, soon after marrying Maria Francisca Corrales, and later obtained the Ingeniero Agronomo (Bachelor of Science) title, specializing in wild shrimp seed by-catch in December 12, 1998. In December 1998 he began working with beef cattle production in the south of Honduras. While working with beef cattle he also had the opportunity to work in shrimp production. In 1999, at the same cattle farm, he became involved in sugar cane production, working with this crop for 2 years, after which he decided to apply to LSU. In fall 2001 he joined the School of Renewable Natural Resources (then School of Forestry, Wildlife and Fisheries) to obtain his master’s degree. He is currently a candidate for the degree of Master of Science in Fisheries (aquaculture) in spring 2004.