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GENETIC EFFECTS INFLUENCING SALINITY AND COLD TOLERANCE IN TILAPIA

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Renewable Natural Resources

by
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To my family

for their support

encouragement

patience

and unconditional love

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Genetic effects influencing salinity tolerance (ST) and cold tolerance (CT) were evaluated in two full diallel mating designs using six tilapia varieties: *Oreochromis aureus* (BL), *O. mossambicus* (MO), *O. niloticus* (NI), *O. niloticus* crossbreds (RE), Mississippi commercial strain (MC) and Florida red tilapia (FL). Statistical analyses provided estimates of direct heterosis (h_i), cross heterosis (h_{ij}), maternal effects (m_i), line effects (l_i), reciprocal and specific reciprocal effects (r_{ij} and r_{**ij}), and general and specific combining abilities (GCA and S_{ij}).

Analysis of genetic effects for ST indicated that FL exhibited significant GCA ($P < 0.01$). BL, FL, and MO exhibited highly significant l_i ($P < 0.01$). Highly significant m_i ($P < 0.01$) was apparent for FL and RE. Highly significant S_{ij} and h_{ij}% ($P < 0.01$) were exhibited in two and eight variety combinations (VCs), respectively. Highly significant r_{ij} ($P < 0.01$) was observed in BL-MO, and MC-RE. In addition, highly significant r_{**ij} ($P < 0.05$) was noted in BL-MO and FL-RE.

Analysis of genetic effects for CT indicated that BL, MC and RE exhibited significant GCA's ($P < 0.05$). Highly significant l_i and m_i ($P < 0.01$) were apparent in BL and RE, respectively. Significantly negative S_{ij} ($P < 0.05$) was exhibited only in BLxMC, while negative and significant h_{ij} % ($P < 0.05$) was apparent in BLxMC, FLxMO and FLxRE. Highly significant r_{ij} ($P < 0.01$) was apparent in FL-MC and MC-RE, while negative and significant r_{**ij} ($P < 0.05$) was exhibited only in FL-MC. No significant direct heterosis (h_i) was apparent in ST or CT.

Improvement in ST in could be accomplished by developing a breeding program combining selection, hybridization and backcrossing in MO, BL and FL, while improvement of CT may be accomplished by selection and hybridization in BL. The potential environmental and commercial implications of developing salinity-tolerant and cold-tolerant tilapia varieties and crosses are discussed.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Tilapia is a common name given to various cichlid species native to Africa and the Middle East. Tilapia are taxonomically divided into three genera according to their brooding behavior: Tilapia (substrate spawners), Sarotherodon (biparental mouth brooders), and Oreochromis (maternal mouth brooders). Approximately 70 species of tilapia have been described, but only nine are cultured commercially (Gupta and Acosta 2004a). Several tilapia species and their hybrids have been introduced for aquaculture throughout tropical and subtropical regions of the world. The Nile tilapia Oreochromis niloticus is the main species cultured worldwide, followed by hybrids and hybrid- based synthetic lines.

A number of biological characteristics make tilapia excellent candidates for culture: acceptance of formulated feeds, efficient food conversion ratios (Jauncey 2000), tolerance of handling (Little 2000), tolerance of high stocking densities (Popma and Masser 1999), tolerance of marginal water quality (Fitzsimmons 2000), year-around spawning (Beardmore 2001) and market demand (Harvey 2005).

Throughout the past century, tilapia were introduced worldwide for various purposes, most often as a source of protein for human nutrition, but also for stock enhancing, live bait for fishing, and as a biological control for aquatic weeds (FAO 2004). Initially cultivation on aquaculture was carried out in small ponds. Today, tilapia are cultured in 85 countries in ponds of all sizes, raceways, net-pens and floating cages (Gupta and Acosta 2004a, FAO 2004). They are also grown in indoor recirculating aquaculture systems in several temperate regions (Hargreaves 2000). This variety of culture and production systems illustrates the high level of environmental adaptability of these fishes.

Tilapia production accounts for approximately 4 % of the cultured fish and shellfish production in the world (FAO 2004). Among aquacultured species, production of tilapia is second only to carp Cyprinus carpio (Young and Muir 2000), accounting for 1.5 million MT in 2002, an increase of 15% over the previous 2 years (FAO 2004). The Nile tilapia Oreochromis niloticus is reported to account for 75 to 83% of global tilapia production (Dey and Eknath 1997; Shelton 2002).

Globally, tilapia production occurs primarily in Asia (70%) and Latin America (19%). China is the major tilapia producer in the world with 42% of total production (USDA-FAS 2006). A large portion of the tilapia produced in China is based on hybrids of O. niloticus and blue tilapia O. aureus (Mair 2002). Besides China, other major tilapia producing countries in Asia are Taiwan, the Philippines, and Thailand. Most farmers in those countries produce tilapia in intensive and semi-intensive systems in polyculture with native fish species or integrated with livestock (Little 2000; Gupta and Acosta 2004a).

Mexico is the largest tilapia producer in the Americas with 128,696 MT reported in 2003 (Anuario estadistico de Pesca 2003) with only 5% of the total from aquaculture industries. Production is carried out in ponds, net pens, cages, rice fields, raceways, and in polyculture ponds with shrimp. In the Americas, other main tilapia producers are Ecuador, Costa Rica and Honduras. In Honduras and Costa Rica, intensive tilapia production began in the early 1990's and at present is carried out in flow-through ponds or large cages with the objective of supplying fresh fillets to the North American market (Teichert-Coddington and Green 1997, Fitzsimmons 2000). In Ecuador, tilapia were initially grown in polyculture in shrimp ponds. After the Taura syndrome virus almost destroyed the Ecuadorian shrimp industry, many shrimp farmers switched to producing tilapia as the infrastructure for aquaculture was already in place (Fitzsimmons 2000).

Tilapia imports in the USA are mainly in the form of frozen and fresh fillets. Seventy five percent of frozen fillet imports originate in China; the remaining 25% are from other Asian nations. Since the year 2000, frozen fillet imports into the USA have increased 900 % (Harvey 2005). Fresh fillet imports are dominated by Ecuador, Costa Rica, and Honduras (Harvey 2005). Up to 2000, Costa Rica dominated Latin-American tilapia imports into the USA (Fitzsimmons 2000), however, due to disease problems production and exports have decreased. Currently, more than 50% of USA fresh fillet imports originate in Ecuador (Harvey 2005).

Geographic Distribution

Globally, most tilapia culture occurs in tropical and subtropical regions at ambient temperatures of between 20 and 30 C (Wohlfarth and Hulata 1983). Due to natural temperature tolerances geographic distribution is largely dependent on latitude and elevation. The most northerly natural occurrence of tilapia is in Lake Huleh, Israel (33° 04'N) (Philippart and Ruwet 1982) where native O. aureus, Tilapia zilli and introduced O. niloticus are found at winter water temperatures as low as 8 C. The southernmost natural occurrence of tilapia is Port Elizabeth, South Africa (33° 42'S) where T. sparrmanii can be found at winter water temperatures of 7 C (Chimits 1957).

In continental Africa and Madagascar, tilapia can be found at 2,000 m above sea level. Oreochromis niloticus, T. zilli and Sarotherodon leucostictus have been considered among the most cold-tolerant tilapia because they can survive at elevations of between 1,500 and 2,000 m (Trewavas 1982). Small-scale production of introduced O. niloticus has been conducted in Burundi and Rwanda at elevations of 1.300 and 2,300 m, respectively (Veverica et al. 1999). Commercial pond production of Mozambique tilapia, O. mossambicus, has been reported in Ecuador at elevations of 2,400 m. (FAO 1977). The reported cold tolerances of some of the major cultured species and hybrids are presented in Table1.1.

When tilapia are exposed to sub-optimum temperatures (lower than 20 C), respiration, food consumption, growth and reproduction are reduced (Ross 2000; Baras et al. 2001). Most tilapia display cold stress symptoms when exposed to temperatures below 15 C. Such symptoms include cessation of feeding, rapid movement, disorientation, and darkening of the skin (Al Amoudi et al. 1996). Mortality typically begins between 10 and 15 C, limiting the potential for outdoor pond culture in many areas of the world. With the exception of T. zilli, and T. sparrmanii, most tilapia cannot tolerate temperatures below 10 C for extended periods of time (Watanabe et al. 1985).

Ionic balance is disturbed when tilapia are stressed due to extreme temperatures. Low temperatures impede osmoregulation by decreasing Na^+ serum and chloride concentrations (Allanson et al. 1971). After extended exposure to low temperatures, tilapia enter a comatose state due to osmoregulatory failure.

Apart from temperature and elevation, tilapia distribution in many coastal regions is limited by salinity tolerance. Improvement of salinity tolerance in tilapia could provide revenue in zones where over-fishing of wild stocks or low prices for farmed shrimp production have affected local economies. Reported salinity tolerance of some of the major tilapia species and hybrids cultured in many regions of the world is presented in Table 1.2**.**

There are concerns that tilapia tolerant to high-salinity or low temperature might escape from farms and become established in coastal areas where they previously were not present. Introduction of tilapia in Asian and Latin American countries has been associated with predation on native species, disease transmission, and eutrophication (Gutierres and Reaser 2005). Tilapia can aggressively compete with other species for nesting sites and food sources (Muoneke 1988). In the USA, tilapia are an considered exotic species in certain states and their culture is regulated (Courtenay 1997, Hargreaves 2000).

Table 1.1. Reported tolerated temperature range and lethal tolerances of cultured tilapia (various sources).

Species / Variety	Temperature range (C)	Lower lethal temperature (C)	Source
O. niloticus	8 – 42	9 – 13 7.4	Chervinski and Lahav 1976; Trewavas 1982; Khater and Smitherman 1988;
O. aureus	8 - 30	3 - 13	McBay 1961; Trewavas 1982; Zale and Gregory 1989; Starling 1995.
O. mossambicus	8 – 42	8 – 13.2	Popper and Lichatovich 1975; Behrends et al. 1990.
Mississippi commercial strain (MCS)	8 – 42	7.5	Paz 2004
Florida red tilapia (FRT)	8 – 42	9.5	Paz 2004

Table 1.2. Reported salinity tolerances of cultured tilapia (various sources).

Species / Variety	Growth (ppt)	Reproduction (ppt)	Death (ppt)	Source
O. niloticus	0 - 15	10 - 20	25 - 30	Yashouv 1960; Chervinski and Lahav 1976; Al Asgah 1984; Khater and Smitherman 1988; Villegas 1990a; Avella et al. 1993, Kamal and Mair 2005
O. aureus	5 - 19	10 - 19	53	Chervinsky and Yashouv 1971; Perry and Avault 1972; Avella et al. 1993.
O. mossambicus	36	49	68	Popper and Lichatowich 1975; Lothan 1960;
Mississippi commercial strain (MCS)	N/A	N/A	30 - 35	Nugon 2003
Florida red tilapia (FRT).	36	18 - 36	35 – 40*	Watanabe et al. 1997; Nugon 2003

* estimated value based on 30% survival at 35 ppt
N/A data not available

Despite regulatory precautions, populations of O. aureus have been reported in reservoirs receiving heated effluents in Texas (Muoneke 1988), in tidal creeks in Georgia (Hales and MESA 1991) and in lakes in Florida (Costa-Pierce and Riedel 2000). Similarly, O. niloticus have been collected in Pascagoula Bay (10 – 15 ppt) and other coastal areas of Mississippi (Peterson et al. 2004, 2005). In Puerto Rico, O. mossambicus was introduced in the late 1950s to control algae in sugar cane irrigation canals. Since its introduction, this species has colonized many lowland areas on the island (Austin 1971). Populations of introduced O. mossambicus have been reported to support commercial fisheries in the Salton Sea (45 ppt), California (Costa-Pierce and Riedel 2000). Prior research suggests that O. niloticus, O. aureus and the Florida red tilapia could overwinter and potentially establish breeding populations if they were to migrate downstream to low salinity coastal waters in Louisiana (Nugon 2003, Paz 2004).

Exposure to elevated levels of salinity results in osmoregulatory failure and subsequent death in tilapia (Ross 2000) although tolerance levels vary widely among species. In tilapia, as in many other freshwater fish, exposure to salinity triggers prolactin synthesis to prevent Na^+ diffusion and decrease membrane permeability (Avella et al. 1990). Among cultured tilapias, Oreochromis mossambicus were reported to exhibit the highest salinity tolerance (Table 1.2). In euryhaline fish such as O. mossambicus pituitary prolactin levels are lower in saltwater than in freshwater (Nicoll et al. 1981). Prolactin synthesis was reduced by 50 % when O. mossambicus were exposed to 10mM Ca^{2+} (Wendelaar Bonga et al. 1985). Cortisol synthesis was triggered when euryhaline O. mossambicus were exposed to salt water conditions, resulting in proliferation of chloride cells (CC) (Foskett et al. 1981). Exposing O. niloticus and O. aureus to increasing salinities (10, 20, 30 ppt) resulted in an increase in CC on gill filaments proportional to the increase in salinity, a characteristic found in seawater teleosts (Avella et al. 1993). Freshwater-acclimated O. mossambicus and O. niloticus displayed normal-size mature CC, while

saltwater acclimated fish exhibited CC twice as large in O. niloticus and three times larger in O. mossambicus (Cioni et al. 1991).

Rate of acclimation to salt water influences salinity tolerance in tilapias. Abrupt transfer of O. mossambicus from 0 to 35 ppt resulted in complete mortality within six hours (Wang et al. 2001). Mortality was attributed to an increase in blood osmolality, suggesting that the increase in blood Na^+, and Na-K-ATPase was not enough to compensate for ionic exchange requirements at high salinities.

Phenotypic Variation

Quantitative phenotypes exhibit continuous variation, therefore their analysis allows the partitioning of phenotypic variance into independent components. Phenotypic variance (V_P) reflects the sum of genetic variance (V_G), environmental variance (V_E), and the genetic-environmental interaction variance (V_{G-E}).

$$V_P = V_G + V_E + V_{G-E}$$

From a commercial standpoint V_G and V_E may be equally important. A breeding program is designed to exploit and maximize available genetic variance through the use of its main components: additive variance (V_A), dominance variance (V_D), and epistatic variance (V_I).

$$V_G = V_A + V_D + V_I$$

Additive variance is attributed to additive effects which are related to the heritability of various traits and consequently are the basis for selection (Tave 1993). When additive effects influence a continuous trait, choosing those organisms presenting the best performing traits as broodstock should result in the overall improvement of the offspring (Lutz 2001). Dominance variance (V_D) describes the variance associated with dominance genetic effects which are expressed based on combinations of specific alleles, or combinations of individuals, strains or species that carry those alleles. Dominance effects are the basis of hybridization and

crossbreeding and are expressed as heterosis, commonly described as hybrid vigor, increase in the performance of hybrids or crossbred individuals over that of the parental lines (Hallauer and Miranda 1988).

Other genetic effects, such as maternal effects (m_i) and line effects (l_i) include both additive and dominance effects. Maternal effects are the influences that maternal genotype, phenotype and environment have on the offspring (Falconer and McKay 1996). Maternal effects are important in early development and reproductive traits such as egg quality and size, and may be equally important in mouth-brooding species such as tilapias (Lutz 2001). In a study comparing egg production and size in one, two, and three-year old O. niloticus females, three-year-old females produced larger eggs than did one-year-old females, influencing fitness of embryos and larvae (Siraj et al. 1983). In addition, fecundity and egg weight were negatively correlated, but maternal effects on age or size would not be detectable beyond 20 days after hatching. Maternal effects may also have a genetic component determined by the evolution of maternal genotype under variable environmental conditions (Wade 1998), therefore, it has been suggested that maternal effects may have evolved as adaptations to environmental variations (Heath et al. 1999).

Line effects (l_i) influence the performance (e.g. growth, salinity tolerance, cold tolerance, disease resistance) of species, crossbreds or varieties. Certain strains or varieties are assumed to be more tolerant to high levels of salinity or low ambient temperatures than others, and the determination of line effects can be used to statistically separate those lines based on their performance. Evaluation of line effects denotes the combined influence of V_A and V_D on their phenotype and this influence can't be partitioned.

Breeding Programs

Breeding programs require data collection and data analysis to determine if the phenotypic goal can be achieved through selection or crossbreeding and hybridization (Tave 1993). Breeding programs in various tilapia species and hybrids have been conducted to improve growth (Bentsen et al. 1998) viability, sex ratios (Hulata et al. 1986; Teichert-Coddington and Smitherman 1988), and salinity tolerance (Bentsen et al. 1998; Likongwe 2002; Tayamen et al. 2002). Perhaps, the most successful breeding program in tilapia is the Genetic Improvement of Farmed Tilapia (GIFT), based on selection in O. niloticus combining strains from Ghana, Egypt, Kenya, Senegal, Israel, Singapore, Thailand and Taiwan (Eknath et al. 1993). Growth improvement per generation was close to 17% across five generations (Bentsen et al. 1998). Similarly, the GIFT methodology was used to test and improve salinity tolerance in interspecific hybrids of O. spilurus, O. aureus, O. mossambicus and three genetically improved strains of O. niloticus (GIFT, YY male, and FAC –Philippine strain) in ten environments with salinities ranging from 0 to 42 ppt (Tayamen et al. 2002). The authors evaluated 27 crosses and selected 14 based on their performance in terms of growth and survival to develop a synthetic variety, a population produced crossing genotypes which are known to improve one or more traits.

Diallel Mating Design

A diallel cross is an experimental breeding design used to test all possible combinations of distinct varieties or inbred lines. Diallel crosses are used for estimation of genetic effects and evaluating quantitative traits of economic or biological importance. In a number of aquatic species diallel crosses have been used to evaluate traits such as growth, yield, weight, maturation, disease resistance, temperature tolerance and salinity tolerance (Tave 1990ab; Wolters 1995; Bentsen et al. 1998; Marengoni et al. 1998; Muñoz-Cordova 2000; Quinton et al. 2004; Maluwa and Gjerde 2006).

The number of all potential crosses (parentals, F1 and reciprocals) within a diallel is n^2, where "n" is the number of varieties. The number of F1 crosses is estimated by the formula: n* (n-1)/2. For example, a diallel cross of six varieties would produce the following number of F1 crosses: 6 (6-1)/2 = 15. If F1 and reciprocal crosses are needed, such as the case of evaluating of reciprocal effects, the equation becomes n *(n-1), producing 30 crosses. Depending on the number of parental lines tested, the diallel analysis can be considered to test fixed (Model I) or random (Model II) effects. Model I tests the entire population while Model II requires a population sample. A sample of less than 10 parents can still be considered a fixed effect (Hallauer and Miranda 1988).

The estimation of heterotic components using diallel crosses was devised by Griffing (1956) resulting in four analytical methods, all of which use F1 progeny in their calculations. Method 1 uses data from F1s, reciprocals and parents. Method 2 includes only F1 progeny and parents. Method 3 uses F1 and reciprocal progeny (required for evaluation of maternal effects). Method 4 uses only F1 progeny.

Many statistical approaches to diallel analyses have been proposed (Hallauer and Miranda 1983), however, Griffing's method remains the most used because parents may be clones, pure lines, inbred lines, or distinct species. Diallel crosses are sometimes useful in prediction of the performance of line combinations not included in the original diallel (Gardner and Eberhart 1966). A full diallel cross (Method I) allows estimation of general combining ability (GCA), specific combining ability (SCA), reciprocal effects and specific reciprocal effects.

When a line is crossed with other lines, that line's GCA is expressed as a deviation of its offspring from the mean of all crosses (Falconer and Mackay 1996). The GCA contains dominance and additive genetic effects. When a cross is produced, the combination of the

parental GCAs results in the expected combining ability of the cross. However, if the expected value is different than the actual value, then the deviation is the SCA or specific heterosis (S_{ij}). The SCA measures the performance of the hybrid over or below that expected based on the performance of the parental lines (Falconer and Mackay1996).

Reciprocal effects are defined as the difference in the performance of F1 and reciprocal crosses**.** Reciprocal effects can be separated into general reciprocal effects (maternal) and specific reciprocal effects (derived from the interaction between progeny genotype and maternal effect) which in some cases are described as cytoplasmatic effects (Eisen et al. 1983, Hallauer and Miranda 1988, Tave et al. 1990a).

An 8 x 8 diallel cross in tilapia was conducted in the development of the GIFT program in the Philippines (Eknath et al. 1993, Bentsen et al. 1998). The cross generated 64 different groups (crosses) which were reared for 10 to 20 weeks, tagged then harvested 90 days later. Heterosis for growth was exhibited in 22 crosses; however, only seven were significantly superior to the performance of their parents. Based on the results, it was determined that an adequate breeding program would have to be based on additive genetic effects. Similar approaches using diallel mating designs were conducted using four pure species (O. spilurus, O. aureus, O. mossambicus, O. niloticus (Tayamen et al. 2002). Parents and hybrids were tested in nine different sites with different levels of salinity (0 to 42 ppt). Survival varied according to each cross and the interaction with test environments. Highest cross heterosis across environments was observed in O.aureus x O. spilurus (improving ST by 22 ppt and O. mossambicus x O. niloticus (improving ST by 25 ppt).

Despite the fact that diallel analyses have potential to elucidate genetic influences over commercially important traits, they have not been widely used to the need for extensive breeding facilities and due the complexities in their computation and genetic interpretation. Also, there

are few ways to analyze a full diallel design and statistical packaged programs are not available. Most diallel-based software programs have been written for plant breeding studies with distinct approaches to data analysis that may not be practical or applicable in commercial aquaculture.

The goal of the research described in this thesis was to evaluate genetic influences over salinity and cold tolerances in tilapia. To accomplish that, a diallel design was carried out using six tilapia varieties (species, strains within species, and synthetic lines of commercial importance). Varieties were chosen for inclusion in diallel crosses on the basis of their availability, commercial attributes, and expected environmental tolerances. Oreochromis aureus was chosen due to its cold and salinity tolerance; Florida red tilapia (FL) and O. mossambicus (MO) were chosen due to their salinity tolerance. Mississippi commercial strain (MC) is known to be a hardy, cold-tolerant line. Nile tilapia O. niloticus (NI) was chosen due to its worldwide importance in commercial aquaculture, and the Nile tilapia red phenotype (RE) used in this analysis was chosen for its red coloration and crossbred genotype (as an F1 cross between Stirling Red x Auburn-Egypt lines). Offspring were produced, subjected to tolerance trials, and survival patterns recorded and analyzed. Results were used to estimate the following parameters: 1) mean salinity tolerance (MST) and mean temperature tolerance (MTT) of purebred varieties and interspecific hybrids and crossbreds; 2) best linear unbiased predictors for the estimation of parental contributions towards ST and CT in all crosses; 3) cumulative survivals of parental varieties, F1 and reciprocal hybrids; and 4) genetic effects influencing ST and CT.

This study of cold tolerance and salinity tolerance in tilapia species and crosses is particularly important in the Southern United States where tilapia could be used as a rotational aquaculture crop along with catfish or other fish species, however, their availability is highly regulated due to the potential for displacement of native fish species. This study will generate information on which species or varieties are suitable for culture under current environmental

conditions (salinity and temperature) in the region. Research presented in this thesis has produced an abstract and presentation at the Seventh International Symposium of Tilapia in Aquaculture (ISTA 7) held in Veracruz, Mexico. In addition, two other abstracts were submitted for presentation at the 2007 Annual Meeting of the World Aquaculture Society to be held in San Antonio, TX. This thesis was written following guidelines for paper presentation for the Journal of the World Aquaculture Society. It is anticipated that Chapters 2 and 3 will be submitted for publication in peer-reviewed journals.

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CHAPTER 2
SALINITY TOLERANCE

There is increasing commercial interest in tilapia species or hybrids that can tolerate salinity and still exhibit acceptable growth. This is particularly true in zones with abundant brackish and saltwater resources, as well as in some arid regions where freshwater resources are limited (Watanabe et al. 1997; Pruginin et al. 1988). Currently, commercially important salinity-tolerant tilapia varieties are hybrid-based, as is the case for most red tilapia (Watanabe et al. 1993, 1997), such as Taiwanese red tilapia and Florida red tilapia (FL). The Taiwanese red tilapia is derived from an O. mossambicus x O. niloticus hybrid (Liao and Chang 1983), and FL is derived from the hybrid of O. urolepis hornorum and O. mossambicus (Watanabe et al. 1997).

Most tilapia have limited salinity tolerance. Effects of increasing salinity in tilapia can be observed as sub-lethal end points wherein normal fish biology and behavior are disrupted. Sub-lethal end points observed in tilapia species and hybrids include low reproductive performance, increased incidence of disease, cessation of feeding, disease development, sluggishness with rapid pectoral and opercular movements, and erratic swimming behavior. High salinity appears to delay gonadal development in O. aureus and O. niloticus (Chervinski and Yashouv 1971; Fineman-Kalio 1988). Egg and fry production per body weight in FL decreased with salinities above 18 ppt (Watanabe et al. 1989a).

Lethal end points have been described as observable thresholds to determine mortality in toxicity studies. These thresholds may occur in the following order in tilapia exposed to increasing salinity: sinking, cessation of opercular movement and failure to respond to gentle touch (Watanabe 1985a; McGeachin et al. 1987; Perschbacher and McGeachin 1988).

Early studies with tilapia described salinity tolerance (ST) as the highest salinity withstood before death (Lothian 1960; Chervinski and Yashouv 1971). Throughout this thesis,

different levels of tolerance are referred to. Mean salinity tolerance (MST) is defined as the salinity at which 50% mortality was reached, it is also described as LT_{50} in certain studies. Incipient salinity tolerance (IM) also described as cumulative survival (CS_{100}) is defined as the level at which a variety initially exhibits mortality associated with increasing salinity. Similarly, commercially incipient mortality level is defined as the salinity at which a variety reaches 15% mortality (CS_{85}). Lastly, lethal salinity is defined as the salinity at which a variety suffers complete mortality (CS_0).

Factors Affecting Salinity Tolerance in Tilapia

Various factors affect ST in tilapia, such as natural history (Trewavas 1982) species and strain, size, age (Watanabe et al. 1985a, Villegas 1990), salinity exposure time, rate of salinity increase (Watanabe et al. 1984, Suresh and Lin, 1992, Lemaire et al. 2004, Paz 2004), temperature (Linkongwe et al. 1996) and genetic effects (Lutz 2006). In addition, interaction between some of these factors may determine ST under different culture conditions.

There has been emphasis on developing hybrids and hybrid-based tilapia varieties with improved ST and growth (Liao and Chang 1983; Watanabe et al. 1997). Most of the available red hybrids (with certain degrees of O. mossambicus inheritance) tolerate salinities above 36 ppt, but exhibit limited growth. Although the FL can tolerate salinities above 36 ppt (Watanabe et al 1989, 2006), growth studies have shown that this variety grows at a slower rate than Nile tilapia (Paz 2004). As in most red tilapia hybrids, limited growth of the FL has been attributed to presence of O. mossambicus genes in their original cross (Watanabe et al. 1989).

Age or size at seawater transfer may influence ST and growth in tilapia (Watanabe et al. 1985a). Although O. niloticus x O. aureus hybrids exhibited increasing ST with age, this pattern was not exhibited in either parental species (Watanabe et al. 1985a). Hybrids derived from O. niloticus x O. aureus developed ST much earlier than in parental species suggesting heterosis for

salinity tolerance (Watanabe et al. 1985a). In O. mossambicus a second hemoglobin type has been reported to develop 45 - 50 days after hatching. This hemoglobin exhibits higher affinity for oxygen at higher osmotic pressure and temperature (Perez and Mclean 1976).

Villegas (1990) exposed O. niloticus, O. mossambicus, and their F1 hybrids to 0, 10, 15, 25, and 32 ppt, at 15, 30, 45, 60 and 75 days after hatching. Results suggested that fish size had a greater effect on ST than fish age. Similarly, Watanabe (1985a) reported differences in ST when comparing stunted and non-stunted (fish grown to regular size) O. aureus and O. niloticus. Stunted fish were not as salinity tolerant as non-stunted fish.

Perschbacher and McGeachin (1988) evaluated ST of Florida red tilapia fry, sex-reversed juveniles and adults at 2 ppt intervals from 19 ppt (for fry and juveniles) and 25 ppt (for adults) to 37 ppt. At all salinities, adults exhibited significantly higher survival than did fry and juveniles. All fish adapted to direct transfer from groundwater (1.5 – 2 ppt) to approximately 18 ppt. Similarly, Watanabe et al. (1990a) reported that newly hatched FL fry were not as salinity tolerant as older juveniles. Nugon (2002) reported that size of fingerling O. niloticus (2.8 g ± 1.8), O. aureus (4.0 g ± 2.4), Florida red tilapia (4.9 g ± 1.6), and Mississippi commercial strain (4.35 ± 1.08) did not significantly affect tolerance to various salinity levels (0, 10, 20, 35 ppt). Similarly, no correlation between fish size (> 1g to <5g) and ST was found in O. mossambicus, O. niloticus or their hybrids when exposed to a salinity increase of 6 ppt/d until reaching 100 % mortality (Mateo et al. 2004).

The combined effect of temperature (24, 28 and 31 C) and salinity on growth was studied in O. niloticus (Linkongwe et al. 1996). Fish were acclimated to target temperatures at a rate of 1 C/ 24 h. and to experimental salinities (0, 8, 12, 16 ppt) at 2 ppt for 24 h. At all temperatures an increase in salinity tended to inhibit growth and increased mortality. Similarly, Al Amoudi et al. (1996) reported that survival of O. mossambicus abruptly transferred from freshwater to salt

water (26 ppt) was not affected by temperature suggesting that fish were less tolerant to cold stress in saltwater than in freshwater. On the contrary, no significant benefit of salinity on CT was found in the Florida red tilapia, O. niloticus, O. aureus, and the Mississippi commercial strain (Paz 2004).

Duration of exposure is another factor affecting ST in tilapia. Gradual transfers appear to allow fish to adapt to increasing salinity (Watanabe et al. 1984) while rapid changes can result in physiological stress, osmoregulatory failure and death. Complete mortality of O. mossambicus within 6 hours of abrupt transfer from 0 to 35 ppt was attributed to insufficient time to conduct osmoregulation (Wang et al. 2001).

Salinity tolerance of some of the main commercial tilapia varieties was presented in Table 1.2. Lethal salinity of O. niloticus was reported as between 25 and 30 ppt (Villegas 1990; Avella et al. 1993). Similarly, lethal salinity of the Mississippi commercial strain (MC) ranged from 20 to 30 ppt (Nugon 2002), while the Florida red tilapia could stand a maximum of 35 to 40 ppt (Watanabe et al. 1989). In contrast, O. aureus was able to tolerate as high as 53 ppt (Avella et al. 1993), while O. mossambicus was reported as the most salinity-tolerant species at 68 ppt (Popper and Lichatowich 1975; Lothan 1960). Similarly, ST of of O. niloticus, O.aureus, the Mississippi commercial strain (MC) and Florida red tilapia (FL) was determined increasing salinity at 5 ppt for 8 h from 0 - 35 ppt (Nugon 2002). Results agreed with previous studies for O. niloticus, O. aureus and the Florida red tilapia (Chervinsky and Yashouv 1971; Perry and Avault 1972, Al Asgah, 1984, Watanabe 1997). Cumulative survival at 35 ppt was 49% in O. aureus and 34% in the Florida red tilapia. The lowest ST was exhibited by the MC strain with this variety not being able to rapidly acclimating beyond 20 ppt.

The goal of the research described in this chapter was to evaluate genetic effects influencing tilapia ST using a diallel mating design including six parental varieties with differing

ST. Specific objectives were to: (1) determine ST in all varieties and their crosses, and (2) estimate genetic effects influencing ST. To accomplish these objectives, the following hypotheses were tested:

1) Mean salinity tolerance (MST = μ) is equal among all varieties and their crosses.

H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6$

H_1: $\mu_1 \neq \mu_2 \neq \mu_3 \neq \mu_4 \neq \mu_5 \neq \mu_6$

2) Crosses between parental varieties exhibit no heterosis

H_0: $V_{1x}\, V_2 = V_{1x}\, V_3 = V_{1x}\, V_3 \ldots V_{5x}\, V_6$

H_1: $V_{1x}\, V_2 = V_{1x}\, V_3 = V_{1x}\, V_3 \ldots V_{5x}\, V_6$

3) Reciprocal crosses present no differences in ST

H_0: $r_{ij} = r_{ji}$

H_1: $r_{ij} \neq r_{ji}$

4) Maternal effects (m_i) are no different among dams.

H_0: $m_i = m_j$

H_1: $m_i \neq m_j$

Materials and Methods

Parental Varieties

Five purebred and one crossbred tilapia varieties maintained at the Aquaculture Research Station (ARS), Louisiana State University Agricultural Center (LSU Agcenter), were used in this research. Origins of these varieties were as follows:

Oreochromis aureus (BL) was obtained from Hofstra University after being transported from Lake Manzala Egypt to Ivory Coast, and subsequently to the U.S.

Florida red tilapia (FL) was obtained from the University of the Virgin Islands. The FL variety was initially developed from an O. urolepis hornorum female x O. mossambicus male

hybrid (Watanabe et al. 1997). Resulting F1 fish and subsequent generations were selectively bred in Florida, then Jamaica, and finally the Virgin Islands.

Mississippi Commercial strain (MC) tilapia is descended in large part from a variety originally developed in Colorado, USA known as the Rocky Mountain White Tilapia®. Electrophoretic analysis suggests that this variety was based on O. aureus and O. mossambicus, but MC may include an undetermined degree of O. niloticus ancestry as well as a result of hybridizing and back-crossing (Lutz 2006).

O. mossambicus (MO) used in these experiments were descendents of two South African stocks referred to as Kasintula and Ndumu strains. These stains were raised in captivity at Stellenbosch University in South Africa, transferred to the University of Wales, Swansea, UK and samples were forwarded to Til-Tech Aquafarm in Robert, LA. Offspring from these strains were pooled and offspring were allowed to mate randomly for three generations to form the line of O. mossambicus used in this research.

O. niloticus (NI) used in this experiment were descendants of the Auburn-Egypt (A-E) line. The A-E line was derived from a group of fish originating in northern Egypt and brought to Auburn University in 1982. The A-E line in these trials had the same origins as those used by Khater and Smitherman (1988) and Tave et al. (1990ab), but has been maintained separately since 1988. This line was obtained from Star Aquaculture in Belle Rose, Louisiana.

Red O. niloticus (RE) used in these trials were F1 A-E female x Stirling red Nile (STR) male crossbreds. The original STR line was developed from a population of O. niloticus collected in Lake Manzala**,** Egypt in 1979. The STR line was developed as a subpopulation of the original O. niloticus - University of Stirling strain (McAndrew et al. 1988).

Mating Design

All experiments were conducted at the ARS. Over the summers of 2004 and 2005, fish from the parental varieties described above were used to produce two diallel crosses, one each year. Five trials were conducted to estimate ST and genetic effects influencing this trait.

Broodstock were stocked in outdoor fiberglass pools, with 2-3 females per male (SOP # 1). Eight to 12 fish were stocked per pool, depending on individual weights. Each pool (0.5 m height x 2.45 m diameter) contained approximately 2.3 m^3 of water and 0.35 m^3 of soil, and all pools were provided with 8.5 lpm of air through airstones.

Broodstock were fed 28% protein floating feed to satiation daily. Fish were allowed to spawn naturally and females were allowed to incubate eggs within the pools. Fingerlings were fed the same feed as their parents. At approximately 45 d tanks were partially drained and fingerlings were collected and moved to an indoor recirculating holding system (SOP #2). The holding system consisted of a 525-L sump, and a floating bead filter, and 36 individual 25-L tanks, one for each cross in the diallel design.

Salinity Tolerance Trials

A separate recirculating system containing four 280-L circular fiberglass tanks and a sump (1.35 m x 0.60 m x 0.45 m) was used for ST trials (SOP # 3). Prior to the beginning of each trial, water quality was adjusted to: pH 7-8, alkalinity 220 ± 50 mg/l as $CaCO_3$, total hardness 280 ± 50 mg/l as $CaCO_3$, chloride 300- 400 mg/l. Samples of 10 to 20 fish (average size 1.5 - 6.5 g) from each cross in the diallel design were placed in separate 3.8-L food-grade polyethylene jars (Consolidated Plastics Co. Inc., Ohio) holding approximately 2-L of tank water. Each jar was marked with the corresponding parental line information. If less than 10 fish were available from a cross, all fish were used. The mouth of each jar was fitted with soft

knotless nylon netting (16 mm^2) secured by two rubber bands. Jars were submerged in three of the four fiberglass tanks.

Fish immersed in jars were acclimated to 0.2 ppt salinity for 24 h before the beginning of the trial and any initial mortality was recorded. Mortality within the first 24 h was assumed to be a result of handling and stress. Jars were checked for dead fish every 24 ± 1 h (noon) thereafter. To minimize handling stress throughout the trials, dead fish were removed from jars with a small net every 24 h. Each day after mortality was recorded, salinity was raised by an increment of 7 ppt by adding 9.35 Kg of NaCl (Diamond Crystal® Solar Salt, Cargill™ Minneapolis, MN) in the system sump (1.25 m x 0.50 m x 0.45 m). Target salinities were obtained within 60 - 120 min of adding salt into the system. Salinity concentrations were measured with a temperature-compensated hand-held refractometer (Aquatic Ecosystems Inc. Florida, Model SR5). Trials continued until 100% was obtained in all fish.

Statistical Analysis

Data were analyzed with Statistical Analysis Software 9.1 (SAS) (SAS Institute, North Carolina). A total of five trials were conducted over the 2 years of research. A single trial was conducted in 2004 and four trials were conducted in 2005. In the absence of significant year effects, data from the two diallels (2004 and 2005) were pooled and each trial was considered a replicate. Fish died at different times over a specified interval (e.g. 7 ppt for 24 h), therefore data were analyzed as interval sensitive.

The mating of a specific group of Dams x Sires was defined as a cross. Analysis of variance (ANOVA) was conducted on the data with the model salt = cross (model y = x) where the variable cross (number of dead fish per cross) was dependent on the independent variable salt (level of salinity 0 – 84 ppt). Mean salinity tolerance (MST) was defined as the salinity at which a tilapia cross reached 50% mortality. MSTs were estimated using Tukey's analysis for all pair-

wise mean comparisons and were separated into letter groups using a SAS macro (%PDMIX800, Saxton 1998) which transforms pair wise statistical differences into letter groups.

Best linear unbiased predictors (BLUP) were developed in PROC MIXED (SAS) to partition sire and dam contributions to ST in all varieties. BLUPs were used to estimate values for two missing cells in the diallel crosses (SOP #5) resulting from apparent incompatibility of breeding stocks.

Cumulative survival of all crosses exposed to salinity challenges was calculated using PROC LIFETEST (SAS) and plotted in MS-Excel (Microsoft Corp. Redmond, WA). Based on CS data, PROC MIXED was used to develop lethal salinity (LS) estimates at various survival levels.

Least squares estimates of genetic effects (line, maternal, reciprocal and specific reciprocal, GCA and SCA) were conducted using a statistical model developed by Eisen et al. (1983) and adapted for this analysis by Dr. Arnold Saxton (Dept. Animal Science, University of Tennessee, Knoxville). The following equations were used in the estimation of genetic parameters.

Individual heterosis was calculated using the equation: (Equation 1)

$$H = \frac{\text{Mean survival of all reciprocal F1 hybrids} - \text{Mean of survival all parental varieties}}{\text{Mean of all parental varieties}} * 100$$

Partitioning of cross heterosis (h_{ij}) into its components (Gardner and Everhart 1966) was as follows:

$$h_{ij} = \bar{h} + h_i + h_j + S_{ij} \quad \text{(Equation 2)}$$

Where h_{ij} is the heterosis exhibited by a particular cross, $\bar{h}$ is the average heterosis of all parental varieties, hi and hj are direct heterosis of varieties *i* and *j*, and S_{ij} is the specific heterosis more commonly described as SCA) that occurs when variety *i* is mated to variety j.

Line effects $l_i = \bar{y}_{ij} - \bar{y}_a - m_i$ (Equation 3)

Where $\bar{y}_{ij}$ is the mean performance of the offspring of sire variety *i* mated to dam variety *j*, $\bar{y}_a$ is the mean of the parental varieties, and m_i is the maternal effect.

General combining ability (GCA) was estimated as follows:

$$GCA = (1/2)\, l_i + h_i \quad \text{(Equation 4)}$$

Where l_i is the direct line effect and h_i is the direct heterosis of line *i*.

Net crossing effect was calculated as:

$$\bar{y}^*_{ij} = (\bar{y}^*_i + \bar{y}^*_j) / 2 \quad \text{(Equation 5)}$$

Where $\bar{y}^*_i$ is the net variety effect of dams in variety *i*, and $\bar{y}^*_j$ is the net variety effect of sires in variety *j*. Net crossing effect is also known as net breeding value (Venkowsky 1970).

Results

The first diallel cross (summer 2004) resulted in 27 of 36 possible crosses, whereas the second diallel (summer 2005) produced 34 of the 36. Two crosses FL x NI and MO x NI were not produced over the two years. A total of 2,003 fish were tested for ST over 2 years (327 in 2004 and 1875 in 2005). Among parental varieties MST ranged from 25.03 (NI) to 48.66 (FL) (Table 2.1). FL and MO were significantly more salinity tolerant ($\underline{P} < 0.05$) than BL or NI, MC and RE.

Among crosses, MST ranged from 25.2 ppt (NI x RE) to 52.5 ppt (FL x NI) (Table 2.2). No crosses involving NI dams exhibited MST greater than 32.9 ppt regardless of the sire line.

Similarly, no crosses involving MC dams with sires other than MO exhibited MST greater than 36 ppt. Crosses involving RE dams had a wide range of MST depending on sire variety. For example, RE x MC exhibited MST of 30.8 ppt, while RE x BL, RE x MO and RE x FL had significantly ($\underline{P} < 0.05$) higher MST (38.2, 42.8 and 45.7 ppt, respectively) than RE x MC. While the parental mean of MST was 35.2 ppt, MSTs above 36 ppt were exhibited in 21 of the 34 crosses. Five crosses exhibited MST above 50 ppt, all of which included FL or MO dams. Analysis of variance indicated highly significant ($\underline{P} < 0.0001$) dam and sire effects for ST (Table 2.2). In addition, the interaction was also highly significant.

Tukey-Kramer adjustment allowed the partitioning of maternal and paternal effects for ST from all crosses (Tables 2.3 and 2.4). Non-estimable contributions of some parental varieties were due to the two missing crosses required to evaluate all potential variety combinations. Lowest MST was exhibited by offspring MC dams (31.45 ppt) while the highest was exhibited by those of FL dams (49.34 ppt). Offspring of RE sires exhibited the lowest MST (32.3 ppt) while those of BL exhibited the highest (40.99 ppt).

Cumulative Survival

Cumulative survival curves among parental varieties (Figure 2.1) indicated MO was the most ST (with individuals surviving to 84 ppt), followed by FL (77 ppt), and BL (63 ppt). MC and NI each tolerated as much as 49 ppt while RE was the least ST variety (35 ppt). Three distinct survival patterns were observed including MO and FL together, BL, and MC, NI and RE together. All three groupings were highly significantly different ($\underline{P} < 0.0001$) from each other.

Cumulative survival of BL and FL varieties and their crosses is shown in Figure 2.2. BL exhibited highly significantly ($\underline{P} < 0.0001$) lower maximum ST (70 ppt) than FL (63 ppt). In the BL variety, salinity affected survival starting at 28 ppt with CS declining to 64% at 35 ppt. In the case of purebred FL, exposure to 35 ppt only reduced survival by 10%.

Table 2.1. Mean salinity tolerance (MST) of tilapia F1 offspring resulting from 2004 and 2005 diallel crosses (Tukey-Kramer). Purebred parental varieties are in italics.

Dam x	Sire	N	MST	SE	Mean days until death	Letter Group
FL	NI	12	53	3.14	8	ABCD
FL	MO	64	52	1.36	8	A
MO	BL	71	51	1.29	8	AB
FL	MC	43	51	1.66	8	AB
MO	FL	74	51	1.27	8	AB
FL	BL	56	49	1.45	7	ABC
FL	*FL*	*61*	*49*	*1.39*	*7*	*ABC*
MO	MC	45	48	1.62	7	ABCD
MO	*MO*	*77*	*46*	*1.24*	*7*	*ABCD*
RE	FL	83	46	1.19	7	ABCD
BL	FL	59	45	1.42	7	ABCDEF
MC	MO	71	45	1.29	7	BCDE
BL	NI	35	43	1.84	7	BCDEFG
RE	MO	77	43	1.24	7	CDEFG
MO	NI	77	43	1.24	7	CDEFG
FL	RE	55	43	1.47	7	CDEFG
MO	RE	69	41	1.31	6	DEFG
BL	MC	77	39	1.24	6	EFGH
RE	BL	72	38	1.28	6	EFGHI
BL	*BL*	*83*	*38*	*1.19*	*6*	*FGHI*
MC	BL	79	37	1.22	6	GHIJ
BL	RE	53	33	1.50	5	HIJK
NI	BL	82	33	1.20	5	HIJK
RE	MC	60	31	1.41	5	JKL
RE	NI	36	30	1.81	5	IJKL
MC	FL	83	28	1.19	4	KL
MC	NI	76	27	1.25	4	KL
BL	MO	40	27	1.72	4	KL
MC	*MC*	*62*	*27*	*1.38*	*4*	*KL*
RE	*RE*	*72*	*26*	*1.28*	*4*	*KL*
NI	MC	68	26	1.32	4	KL
MC	RE	88	25	1.16	4	L
NI	RE	70	25	1.30	4	L
NI	*NI*	*73*	*25*	*1.27*	*4*	*L*

Varieties and crosses with the same letters were not significantly different ($\underline{P} > 0.05$).

Table 2.2. Analysis of variance of dam and sire contributions to ST in tilapia varieties.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
Dam	5	2169	165.39	<. 0001
Sire	5	2169	40.92	<. 0001
Dam*Sire	23	2169	13.08	<. 0001

Table 2.3. Estimated maternal effects for ST (Mean ± SE).

Dams	Estimate (ppt)	SE	Letter group
FL	49.34	0.76	A
MO	46.71	0.55	B
BL	37.65	0.61	C
RE	35.70	0.57	C
MC	31.45	0.51	D
NI	N/E	N/E	-

Groups with the same letters were not significantly different (P > 0.05).
N/E = non estimable due to missing crosses in the diallel.

Table 2.4. Estimated paternal effects for ST (Mean ± SE).

Sire	Estimate (ppt)	SE	Letter group
BL	40.99	0.52	A
MC	36.91	0.59	B
NI	36.86	0.77	B
RE	32.30	0.55	C
FL	N/E	N/E	-
MO	N/E	N/E	-

Groups with the same letters were not significantly different (P > 0.05).
N/E = non estimable due to missing crosses in the diallel.

The FL x BL cross was more tolerant than the reciprocal BL x FL. FL x BL was also significantly more tolerant ($\underline{P}$ <0.0001) than BL. The hybrid exhibited approximately 10% heterosis for survival at salinities between 49 and 63 ppt. Maternal effects and heterosis were apparent as at least one of the crosses exhibited higher CS than parental varieties at intermediate salinities.

Cumulative survival of BL and MC varieties and their crosses (Figure 2.3) indicated no significant differences in ST up to 28 ppt. Minimum lowest lethal salinity was exhibited by purebred MC (49 ppt) while highest lethal salinity was exhibited by BL (63 ppt). Purebred MC were significantly less tolerant ($\underline{P} < 0.001$) than the other three groups.

Cumulative survival of BL and MO varieties and their crosses (Figure 2.4) indicated that purebred BL and MO were significantly different ($\underline{P} < 0.001$) from each other, tolerating up to 63 ppt and 84 ppt, respectively. Cumulative survival of MO x BL was higher than all other crosses at intermediate salinities (35 – 63 ppt). Cumulative survival patterns suggested MO x BL exhibited positive heterosis, while it's reciprocal displayed negative heterosis. The cross BL x MO survival was significantly lower ($\underline{P} < 0.01$) than the other three groups at all salinities above 21 ppt. Salinity tolerance of the parental varieties was intermediate to that of their crosses.

Cumulative survival of BL and NI varieties and their crosses is presented in Figure 2.5. Cumulative survival of purebred BL was significantly higher ($\underline{P} < 0.0001$) than that of NI at salinities above 21 ppt. The BL x NI was superior ($\underline{P} < 0.0001$) to its reciprocal and to purebred NI, indicating heterosis for ST. Performance of NI x BL was intermediate to that of parental varieties. Cumulative survival in NI purebreds was highly significantly lower ($\underline{P} < 0.0001$) at salinities between 21 and 49 ppt. Genetic effects such as maternal, line, and reciprocal genetic effects were suggested by differences between CS patterns.

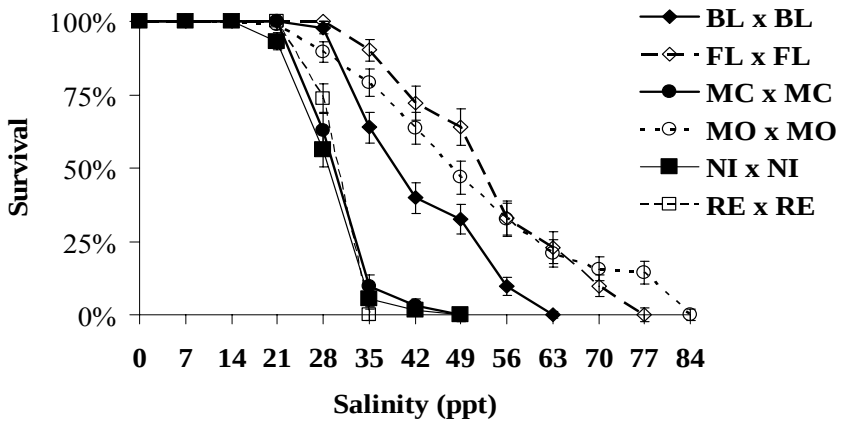

Figure 2.1 Cumulative survival of six tilapia varieties at various salinities (Mean ± SE).

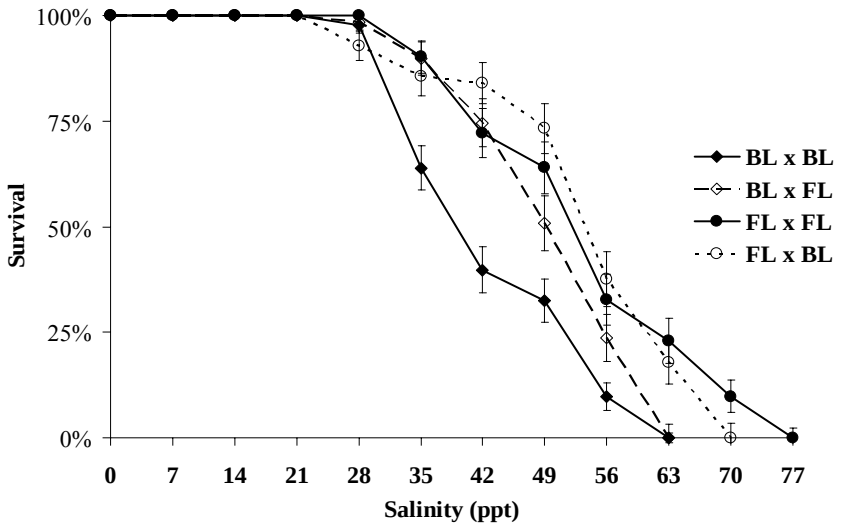

Figure 2.2. Cumulative survival of BL and FL varieties and their crosses (Mean ± SE).

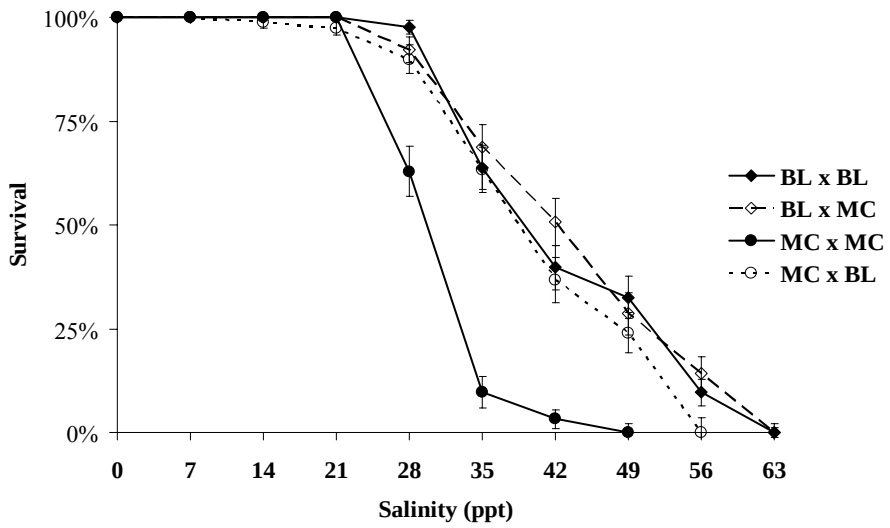

Figure 2.3. Cumulative survival of BL and MC varieties and their crosses (Mean ± SE).

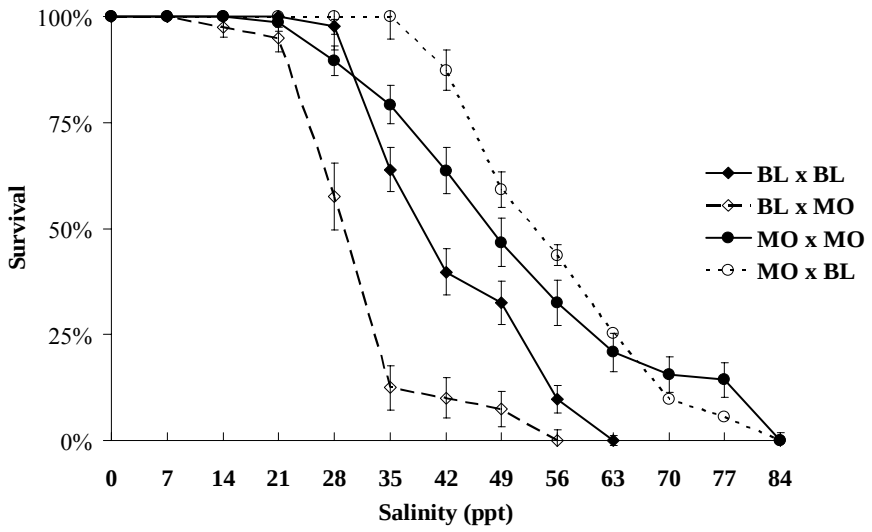

Figure 2.4. Cumulative survival of BL and MO varieties and their crosses (Mean ± SE).

Cumulative survival of purebreds BL and RE and those of their crosses (Figure 2.6) indicated that survival was not affected at salinities up to 21 ppt. Cumulative survival of the RE variety declined steeply above 21 ppt, reaching 74% at 28 ppt, and 3% at 35 ppt, while CS of BL was still 64% at 35 ppt. Cumulative survival of RE x BL was higher than all other groups between 42 ppt and 49 ppt. Maximun salinity tolerance was exhibited by purebred BL and BL x RE, each tolerating 63 ppt. In addition, the BL x RE cross was significantly more tolerant ($\underline{P} < 0.01$) than was RE.

Cumulative survival curves of purebreds FL and MC (Figure 2.7) were significantly different ($\underline{P} < 0.0001$) from each other at salinities above 14 ppt. Crosses exhibited similar ($\underline{P} > 0.05$) survival curves as their maternal varieties, and were significantly different ($\underline{P} < 0.05$) from each other.

Purebred FL and MO and their crosses (Figure 2.8) exhibited similar survival patterns with increasing salinity. The purebred FL exhibited lethal salinity of 77 ppt, while MO tolerated 84 ppt. No statistical differences ($\underline{P} < 0.05$) were found among varieties and crosses.

Cumulative survival of FL, NI and the FL x NI cross is presented in Figure 2.9. The missing reciprocal cross (NI x FL) was not available for analysis. Cumulative survival of purebred FL was significantly more tolerant ($\underline{P} < 0.001$) than purebred NI. The cross FL x NI exhibited the highest MST (52.5 ppt) among all 34 groups tested in this study. However, its MST was not significantly different ($\underline{P} > 0.05$) from the purebred FL.

Cumulative survival of FL and RE varieties and their crosses (Figure 2.10) indicated purebred FL was the most tolerant (lethal salinity of 77 ppt), while RE was the least tolerant (35 lethal salinity of ppt). No significant differences ($\underline{P} > 0.05$) were apparent among purebred FL and the reciprocal crosses, but purebred RE was significantly less tolerant ($\underline{P} < 0.01$) to other groups. Heterosis and maternal influence were suggested in the CS curves.

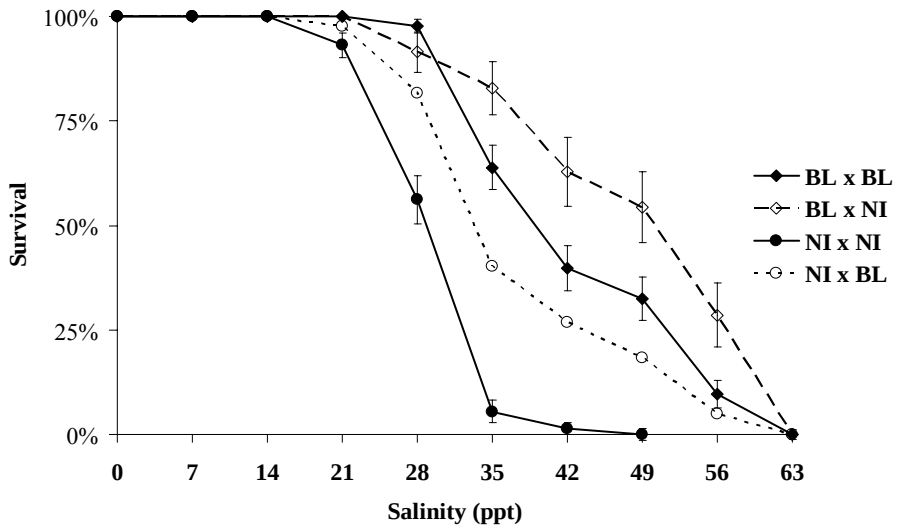

Figure 2.5. Cumulative survival of BL and NI varieties and their crosses (Mean ± SE).

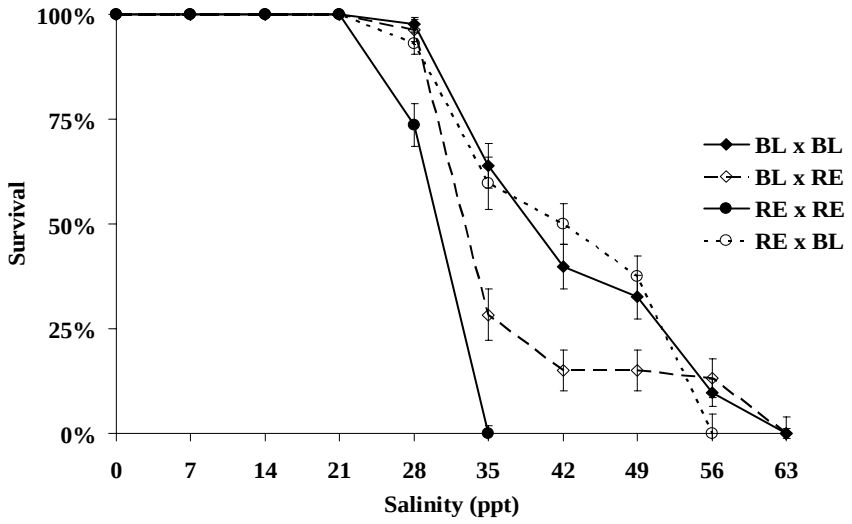

Figure 2.6. Cumulative survival of BL and RE varieties and their crosses (Mean ± SE).

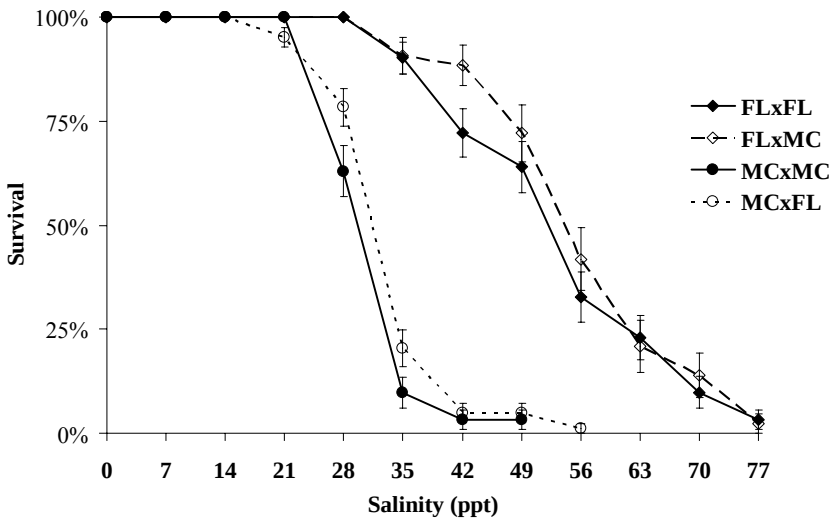

Figure 2.7 Cumulative survival of FL and MC varieties and their crosses (Mean ± SE).

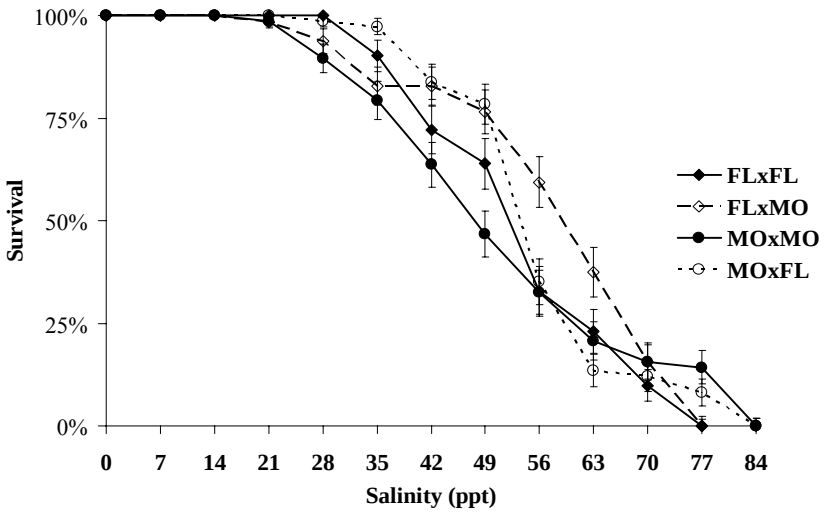

Figure 2.8 Cumulative survival of FL and MO varieties and their crosses (Mean ± SE).

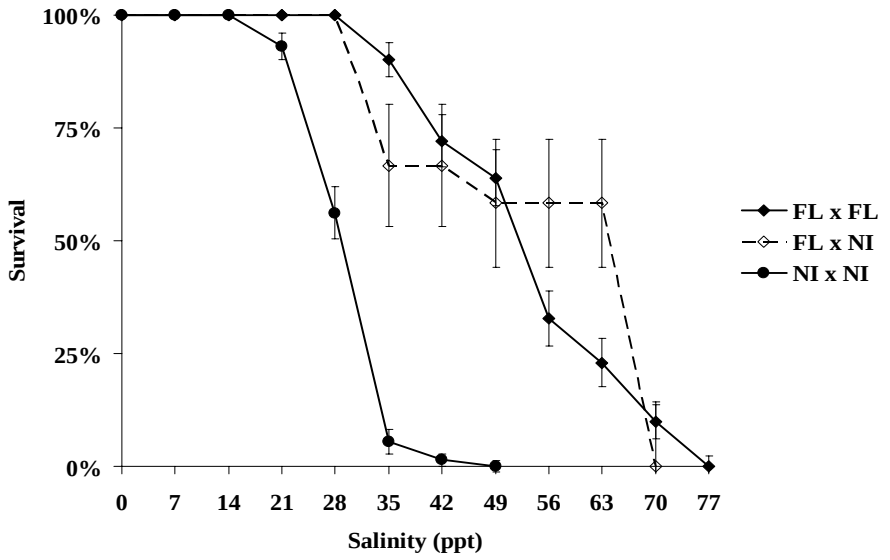

Figure 2.9. Cumulative survival of FL and NI varieties and their crosses (Mean ± SE).

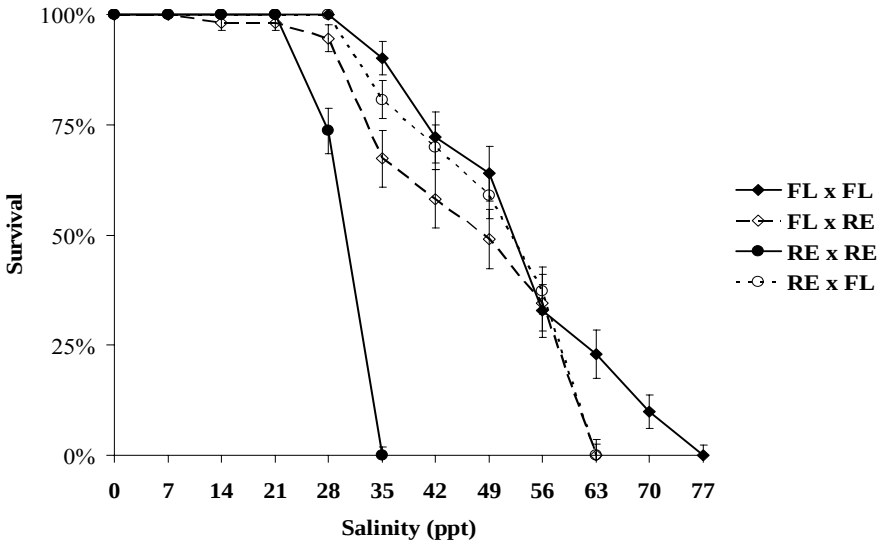

Figure 2.10. Cumulative survival of FL and RE varieties and their crosses (Mean ± SE).

Cumulative survival patterns of MC and MO varieties (Figure 2.11) indicated that the purebred MO was more tolerant ($P < 0.01$) than the purebred MC at salinities > 21 ppt. Crosses exhibited superior ST than MO between 35ppt and 56 ppt, however MO exhibited the highest overall ST among all groups.

MC and NI varieties and their crosses (Figure 2.12) exhibited no significant differences in CS with increasing salinity. Both purebreds reached 100% mortality at 49 ppt, while NI x MC died at 56 and MC x NI died at 63 ppt. Cumulative survival of all crosses reached 10% by 35 ppt. All four CS curves followed a similar distribution.

Cumulative survival of MC and RE varieties and their crosses (Figure 2.13) were not significantly different ($P > 0.05$) although RE x MC exhibited higher tolerance than its reciprocal cross or the parental varieties. MC x RE was the least salinity tolerant of the four groups tested.

Groups MO and MO x NI were significantly more salinity tolerant ($P < 0.01$) than NI (Figure 2.14). Cumulative survival of NI fell below 10% above salinities of 35 ppt, while CS of the other two groups still exceeded 75% at that salinity. Maximum lethal salinity was exhibited by MO (84 ppt) followed by MO x NI (70 ppt).

Cumulative survival of MO and RE varieties and their crosses (Figure 2.15) exhibited highly significant differences ($P< 0.01$). Cumulative survival of MO followed a gradual decrease from 96% at 28 ppt to 1% at 84 ppt. In contrast, CS of RE drastically declined from 75% at 28 ppt to 1% at 35 ppt and was significantly ($P < 0.01$) lower than the other three groups.

Cumulative survival of NI and RE varieties and their crossbreds (Figure 2.16) indicated RE x NI was highly significantly more tolerant ($P < 0.0001$) than the other three groups. All groups exhibited similar ST patterns, with crossbred RE x NI exhibiting heterosis.

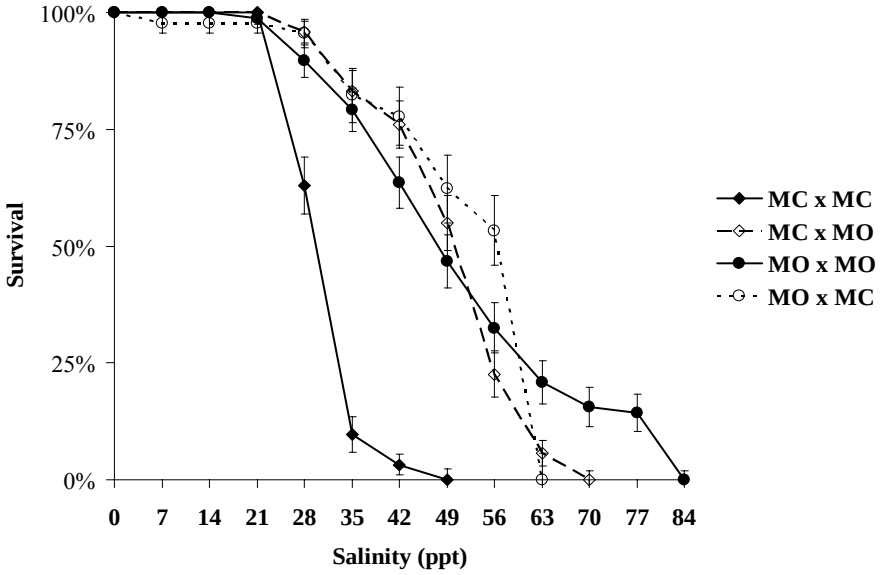

Figure 2.11. Cumulative survival of MC and MO varieties and their crosses (Mean ± SE).

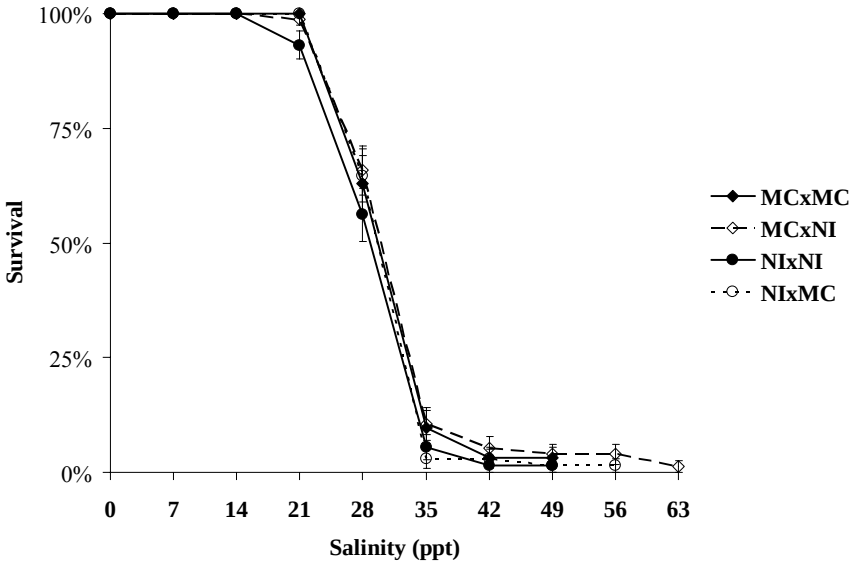

Figure 2.12. Cumulative survival of MC and NI varieties and their crosses (Mean ± SE).

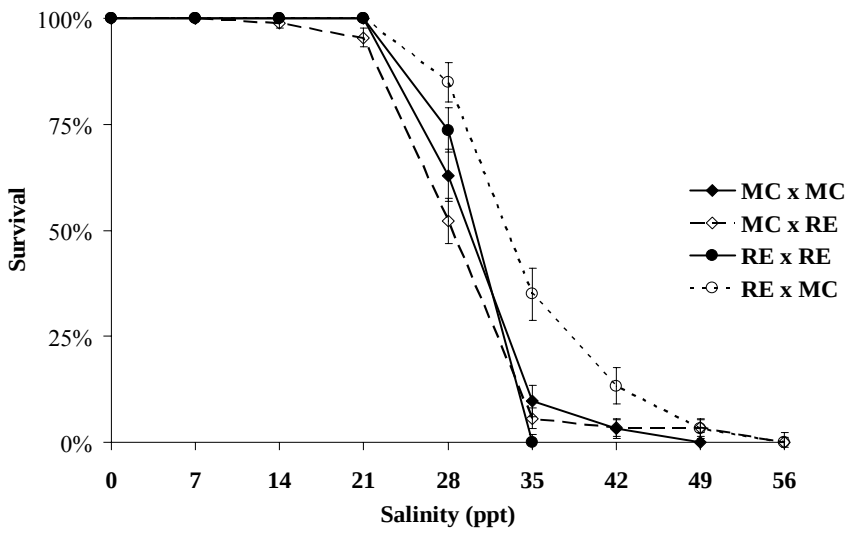

Figure 2.13. Cumulative survival of MC and RE varieties and their crosses (Mean ± SE).

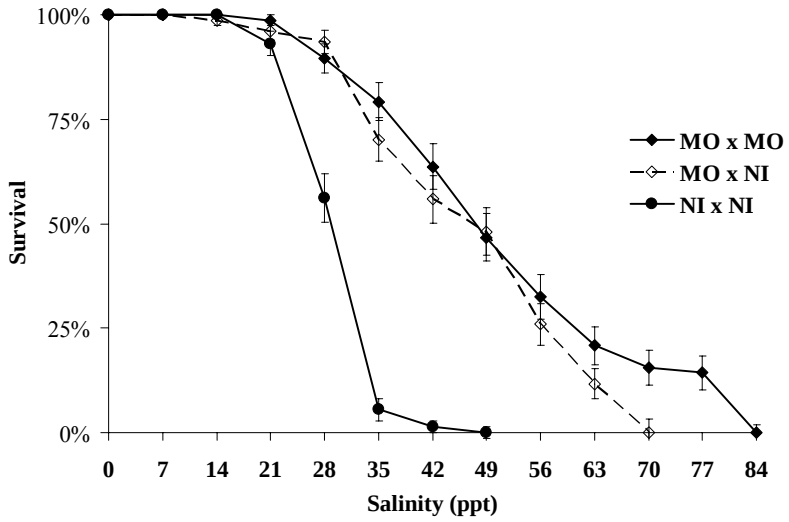

Figure 2.14. Cumulative survival of MO and NI and of one of their crosses (Mean ± SE).

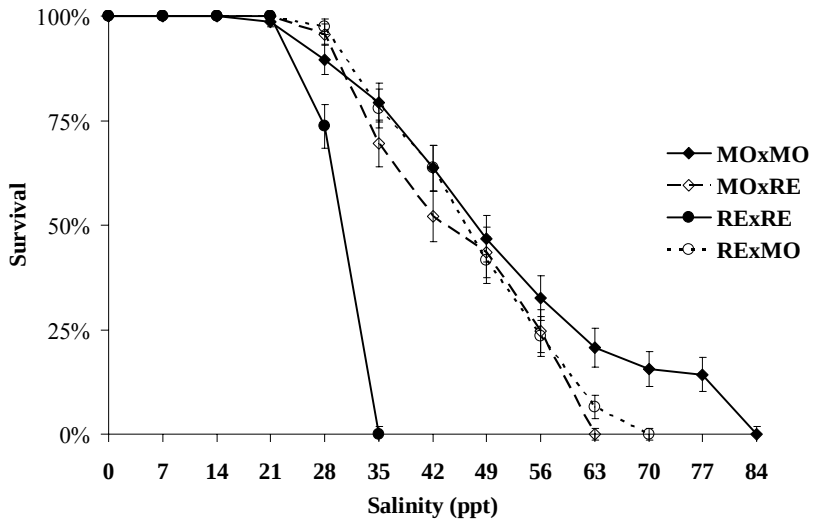

Figure 2.15. Cumulative survival of MO and RE varieties and their crosses (Mean ± SE)

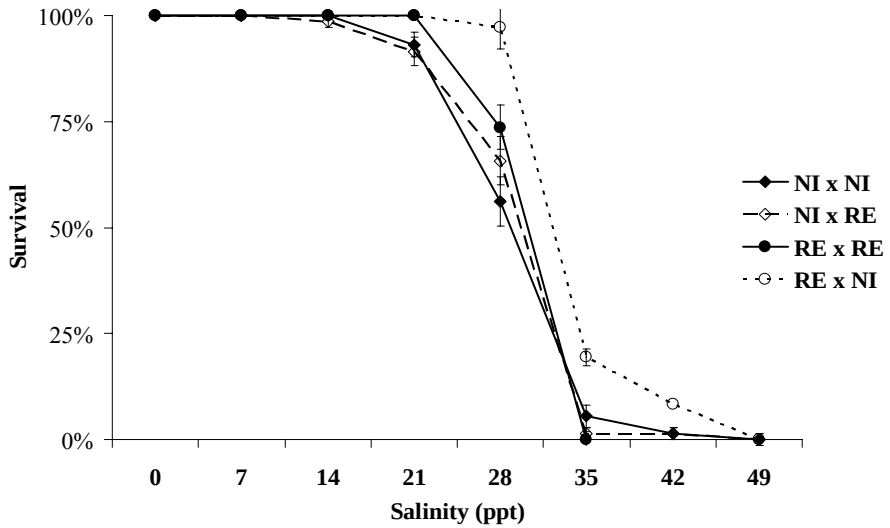

Figure 2.16. Cumulative survival of RE and NI varieties and their crosses (Mean ± SE).

Cumulative Survival Thresholds of Economic and Environmental Importance

Cumulative survival thresholds of economic and environmental importance (Table 2.5.) indicated that CS_{100} (0% mortality) ranged from 14 ppt to 28 ppt. Incipient mortality (first mortalities in the group) (IM) were recorded in NI at 14 ppt, while FL tolerated twice the salinity level before mortalities were observed. Cumulative survival (CS_{85}) (15% mortality) followed a trend similar to Figure 2.1 in which parental varieties could be separated into three different groups: FL, followed by MO and BL grouped together, and MC, RE and NI grouped together. Total mortality (CS_0) denoted that variety RE was the least tolerant followed by NI and MC. The other three varieties exhibited CS_0 of 63 ppt and above.

Incipient mortality of 7 ppt was observed in crosses containing one or both parents of varieties RE, MC and NI. In addition, 50% of all crosses exhibited incipient mortality at 21 ppt. Four of the five remaining crosses tolerated salinities of at least 28 ppt, and were produced by either FL sires or dams. Maximum value for CS_{100} was exhibited by MO x BL at 35 ppt.

Incipient mortality (IM) for all crosses produced by BL dams was 21 ppt. Similarly, IM for crosses produced by FL dams was between 7 to 28 ppt. Among crosses of by MC dams, IM was between 7 to 21 ppt. Similarly, among crosses produced by MO dams, IM was between 7 to 35 ppt. Crosses produced by NI dams exhibited IM from 7 ppt to 21 ppt. Crosses produced by RE dams exhibited IM between 21 to 28 ppt.

Crosses produced by BL sires exhibited IM between 7 ppt and 35 ppt. Crosses produced by FL sires to other dams produced offspring exhibiting IM from 14 and 28 ppt. Similarly, crosses produced by MO sires to other dams exhibited IM between 14 and 28 ppt. MC sires crossed to other dams produced offspring with IM between 21 and 28 ppt. NI sires crossed to dams of all varieties produced offspring with IM between 7 ppt and 28 ppt. The most tolerant

Table 2.5. Cumulative survival (%) thresholds of economic and environmental importance (n=2203).

Cross	**Cumulative Survival (%)**		
	100	**85**	**0**
MO x NI	*7*	28	70
FL x RE	*7*	28	63
MC x BL	*7*	28	56
MC x RE	*7*	*21*	56
NI x RE	*7*	*21*	49
MO x MO	14	28	*84*
FL x MO	14	28	77
MC x NI	14	*21*	63
NI x BL	14	*21*	63
MC x FL	14	*21*	56
NI x NI	14	*21*	49
MO x FL	21	35	*84*
FL x BL	21	35	70
BL x FL	21	35	63
MC x MO	21	28	70
RE x MO	21	28	70
BL x BL	21	28	63
BL x MC	21	28	63
BL x RE	21	28	63
MO x MC	21	28	63
MO x RE	21	28	63
BL x NI	21	28	56
RE x BL	21	28	56
RE x NI	21	28	49
RE x MC	21	24.5	56
BL x MO	21	*21*	56
NI x MC	21	*21*	56
MC x MC	21	*21*	49
RE x RE	21	*21*	*35*
FL x MC	28	*42*	77
FL x FL	28	35	77
FL x NI	28	28	70
RE x FL	28	28	63
MO x BL	*35*	*42*	*84*

Maximun and minimum salinity tolerance within each column is represented in italics.

crosses and their CS_0 were: MO x BL and MO x FL (84 ppt), FL x MC and FL x MO (77 ppt), and FL x BL, FL x NI, MC x MO, and MO x NI (70 ppt). All other crosses exhibited complete mortality between 49 and 63 ppt.

Best Linear Unbiased Predictors (BLUPs)

Best linear unbiased predictors estimates (Table 2.6) indicated that offspring of BL, FL, and MO dams and sires were more tolerant ($P < 0.0001$) than those of NI, MC and RE dams and sires. Analysis of interaction between dams and sires indicated that crossing BL dams to BL, FL, MO, and RE sires produced offspring significantly more salinity tolerant ($P < 0.0001$) than those produced by BL dams and MC or NI sires. Crosses produced by FL dams with FL, BL or MO sires produced offspring with the highest ST. Crossing of MC dams with FL sires produced the least tolerant offspring. On the contrary, MO dams with MO or FL sires produced the most salinity tolerant offspring. Crossing of RE dams with any sire did not improve ST in any cross (Table 2.7) nor was there a significant improvement in ST when either NI dams or sires were used to produce crosses.

Estimated values of two missing crosses were calculated using BLUPs. These estimates required information for varieties NI, FL, and MO, therefore the BLUP for NI (Table 2.6) was replaced with a BLUP for RE (Table 2.7). The substitution of BLUP NI by BLUP RE was necessary as estimates for a particular cross could not be calculated from the same table were the estimate originated. For example, no estimates of NI crosses could be obtained when NI was used as the intercept value in a table. Estimated MSTs were 44.56 ppt (NI x FL) and 41.67 ppt (NI x MO). Values were appended to the original data set to carry out the diallel analysis.

Table 2.6. Best linear unbiased predictors for ST using the NI variety as an intercept.

Effect	Dam	Sire	Estimate		SE
Intercept	--	--	25.03	***	1.27
Dam	BL	--	18.37	***	2.24
Dam	FL	--	27.47	***	3.39
Dam	MC	--	2.24		1.78
Dam	MO	--	17.61	***	1.78
Dam	NI	--	0.00	.	.
Dam	RE	--	5.31	***	2.22
Sire	--	BL	7.92	***	1.75
Sire	--	FL	15.38	***	2.17
Sire	--	MC	1.12		1.83
Sire	--	MO	12.48	***	2.20
Sire	--	NI	0.00	.	.
Sire	--	RE	0.17		1.82
Dam*Sire	BL	BL	-13.20	***	2.81
Dam*Sire	BL	FL	-13.69	***	3.18
Dam*Sire	BL	MC	-5.43	**	2.88
Dam*Sire	BL	MO	-29.11	***	3.34
Dam*Sire	BL	NI	0.00	.	.
Dam*Sire	BL	RE	-10.16	***	2.99
Dam*Sire	FL	BL	-11.55	***	3.88
Dam*Sire	FL	FL	-19.22	***	4.07
Dam*Sire	FL	MC	-2.50		4.00
Dam*Sire	FL	MO	-12.59	***	4.07
Dam*Sire	FL	NI	0.00	.	.
Dam*Sire	FL	RE	-10.16	***	3.92
Dam*Sire	MC	BL	1.32		2.48
Dam*Sire	MC	FL	-14.30	***	2.78
Dam*Sire	MC	MC	-1.85		2.61
Dam*Sire	MC	MO	5.11	*	2.84
Dam*Sire	MC	NI	0.00	.	.
Dam*Sire	MC	RE	-2.22		2.49
Dam*Sire	MO	BL	0.71		2.51
Dam*Sire	MO	FL	-6.93	***	2.80
Dam*Sire	MO	MC	4.00		2.75
Dam*Sire	MO	MO	-8.67	***	2.81
Dam*Sire	MO	NI	0.00	.	.
Dam*Sire	MO	RE	-1.72		2.56
Dam*Sire	RE	BL	-0.05		2.83
Dam*Sire	RE	FL	0.00		.
Dam*Sire	RE	MC	-0.65		2.94
Dam*Sire	RE	MO	0.00		.
Dam*Sire	RE	NI	0.00	.	.
Dam*Sire	RE	RE	-4.16	.	2.87
Dam*Sire	NI	BL	0.00	.	.
Dam*Sire	NI	MC	0.00	.	.
Dam*Sire	NI	RE	0.00	.	.
Dam*Sire	NI	NI	0.00	.	.

* $\underline{P} < 0.10$, ** $\underline{P} < 0.05$, *** $\underline{P} < 0.01$; Dots (.) are part of the BLUP calculation related to the intercept value.

Table 2.7. Best linear unbiased predictors for ST using the RE variety as an intercept.

Effect	Dam	Sire	Estimate		SE
Intercept			26.35	***	1.28
Dam	BL	--	7.07	***	1.97
Dam	FL	--	16.16	***	1.95
Dam	MC	--	-1.13		1.73
Dam	MO	--	14.74	***	1.83
Dam	NI	--	-1.15		1.83
Dam	RE	--	0.00	.	.
Sire	--	BL	11.86	***	1.81
Sire	--	FL	19.36	***	1.75
Sire	--	MC	4.45	**	1.90
Sire	--	MO	16.47	***	1.78
Sire	--	NI	3.99	*	2.22
Sire	--	RE	0.00	.	.
Dam*Sire	BL	BL	-7.16	***	2.64
Dam*Sire	BL	FL	-7.69	***	2.71
Dam*Sire	BL	MC	1.22		2.72
Dam*Sire	BL	MO	-23.11	***	2.90
Dam*Sire	BL	NI	6.00	*	3.25
Dam*Sire	BL	RE	0.00	.	.
Dam*Sire	FL	BL	-5.50	**	2.75
Dam*Sire	FL	FL	-13.22	***	2.68
Dam*Sire	FL	MC	4.15		2.92
Dam*Sire	FL	MO	-6.59	***	2.68
Dam*Sire	FL	NI	6.00		4.12
Dam*Sire	FL	RE	0.00	.	.
Dam*Sire	MC	BL	-0.57		2.48
Dam*Sire	MC	FL	-16.24	***	2.42
Dam*Sire	MC	MC	-3.14		2.62
Dam*Sire	MC	MO	3.17		2.49
Dam*Sire	MC	NI	-1.94		2.80
Dam*Sire	MC	RE	0.00	.	.
Dam*Sire	MO	BL	-1.68		2.58
Dam*Sire	MO	FL	-9.37	***	2.53
Dam*Sire	MO	MC	2.22		2.82
Dam*Sire	MO	MO	-11.10	***	2.54
Dam*Sire	MO	NI	-2.44		2.86
Dam*Sire	MO	RE	0.00	.	.
Dam*Sire	NI	BL	-4.11		2.54
Dam*Sire	NI	MC	-3.51		2.66
Dam*Sire	NI	NI	-4.16		2.87
Dam*Sire	NI	RE	0.00	.	.
Dam*Sire	RE	BL	0.00	.	.
Dam*Sire	RE	FL	0.00	.	.
Dam*Sire	RE	MC	0.00	.	.
Dam*Sire	RE	MO	0.00	.	.
Dam*Sire	RE	NI	0.00	.	.
Dam*Sire	RE	RE	0.00	.	.

* $\underline{P} < 0.10$, ** $\underline{P} < 0.05$, *** $\underline{P} < 0.01$; Dots (.) are part of the BLUP calculation related to the intercept value.

Diallel Analysis

Mean salinity tolerance of parental varieties and crosses (Table 2.8) showed that highest MST in offspring was obtained using varieties FL, MO and BL. Similarly, the breeding values ($\bar{y}*_{ij}$) denoted that these varieties would be the most useful for ST.

Table 2.8 Mean salinity tolerance (MST) of parental varieties and crosses produced in two diallel crosses (n = 2005, includes data from two missing crosses). Shaded cells denote parental crosses.

Sire	Dam							
	BL	FL	MC	MO	NI	RE	$\bar{y}_i$	$\bar{y}*_i$
BL	38.1	48.9	36.5	51.3	33.0	38.2	41.0	41.6
FL	45.1	48.7	28.3	51.1	44.6	45.7	43.9	43.0
MC	39.1	51.1	26.5	47.8	26.2	30.8	36.9	39.0
MO	26.8	52.4	44.9	46.5	41.7	42.8	42.5	41.7
NI	43.4	52.5	27.3	42.6	25.0	30.3	36.9	39.2
RE	33.4	42.5	25.2	41.1	25.2	26.4	32.3	33.5
$\bar{y}_j$	37.7	49.3	31.5	46.7	32.6	35.7		
$\bar{y}*_j$	37.6	49.5	32.4	46.8	34.1	37.6		

Purebred mean = 35.19 $\bar{y}_i$ = mean of sire line i; $\bar{y}*_i$: Net variety effect of sires i

Overall mean = 38.91 $\bar{y}_j$ = mean of dam line j; $\bar{y}*_j$: Net variety effect of dams j

Genetic Effects

Net variety effect of sires ($\bar{y}*_i$) exhibited a ranking of ST: FL>MO>RE>BL>NI>MC. The differences between net variety estimates of sires of varieties RE and BL was 0.02. Variety

ranking according to variety effects of dams ($\bar{y}$ *$_j$) was somewhat different:

FL>MO>BL>NI>MC>RE. In addition, $\bar{y}$ *$_j$ denoted that FL and MO dams produced offspring with ST > 45 ppt. Net crossing effect (Equation 5) followed the same pattern as the variety effects of dams: FL>MO>BL>NI>MC>RE.

Estimates of line effects (l_i) and maternal effects (m_i) are presented in Table 2.9. Offspring of purebred BL, FL and MO were significantly more tolerant ($\underline{P} < 0.01$) than those of MC, NI and RE. In addition, purebred RE offspring were significantly ($\underline{P} < 0.01$) less salinity tolerant than all other purebred offspring. The RE variety was 12.3 ± 1.4 ppt less salinity tolerant than the average purebred mean.

Maternal effects estimates (m_i) indicated that FL and RE dams significantly ($\underline{P} < 0.01$) improved ST of their offspring. On the contrary, MC and BL dams had a highly significant ($\underline{P} < 0.01$) negative effect on ST of their offspring, decreasing ST between 3.3 and 5.5 ppt from the purebred mean.

Table 2.9. Least square estimates of direct line genetic effect (l_i) and maternal effects (m_i) in parental varieties (Mean ± SE)

Variety	l_i		m_i	
BL	6.27 ± 1.34	***	-3.34 ± 0.75	***
FL	8.03 ± 2.36	***	5.44 ± 2.00	***
MC	-3.20 ± 1.43	**	-5.46 ± 0.71	***
MO	7.05 ± 2.25	***	4.22 ± 1.94	**
NI	-5.89 ± 2.94	**	-4.27 ± 2.70	
RE	-12.25 ± 1.38	***	3.41 ± 0.73	***

* $\underline{P} < 0.10$, ** $\underline{P} < 0.05$, *** $\underline{P} < 0.01$

Estimates of general combining ability (GCA) and direct heterosis (h_i) are presented in Table 2.10. GCA estimates indicated that FL would provide most salinity tolerant variety combinations ($\underline{P} < 0.01$) for ST. In addition, varieties BL and MO would also improve ST ($\underline{P} < 0.10$) of their offspring. In contrast, the RE variety would significantly ($\underline{P} < 0.01$) decrease ST when used in crosses. Similarly, MC and NI would also have a significant ($\underline{P} < 0.05$) negative effect in ST.

Mean heterosis ($\bar{h}$) for ST was significant ($\underline{P} < 0.01$), and indicated that crosses were on average 4.46 ppt more tolerant than their parental varieties. No significant direct heterosis was exhibited among varieties; however, varieties with positive estimates would contribute towards increasing ST of offspring in the crosses because cross heterosis (equation 2) is dependent on individual direct heterosis values, $\bar{h}$ and specific heterosis values.

Table 2.10. Least square estimates of GCA and direct heterosis in parental varieties (Mean ± SE).

Variety	GCA		h_i	
BL	1.55 ± 0.85	*	-1.58 ± 0.97	
FL	5.49 ± 1.93	***	1.47 ± 1.29	
MC	-2.2 ± 0.89	**	-0.6 ± 1	
MO	3.62 ± 1.95	*	0.1 ± 1.25	
NI	-1.6 ± 0.78	**	1.35 ± 1.49	
RE	-6.86 ± 0.86	***	-0.73 ± 0.98	
$\bar{h}$	--	--	4.46 ± 0.78	***

* $\underline{P} < 0.10$, ** $\underline{P} < 0.05$, *** $\underline{P} < 0.01$

In Tables 2.12 and 2.12 crosses were combined as variety combinations (F1 and reciprocal crosses together). For example, the variety combination (VC) of MO-BL would include the F1 MO x BL (Dam x Sire) and its reciprocal cross (BL x MO). Statistical comparisons were done only within each VC and not among VCs.

The estimation of cross heterosis (h_{ij}), percent cross heterosis (h_{ij} %), and specific heterosis (S_{ij}), is presented in Table 2.11. Eight of the fifteen VCs exhibited highly significant ($\underline{P} < 0.01$) h_{ij} estimates ranging from -8% to +32%. Lowest significant and negative h_{ij} ($\underline{P} < 0.05$) was exhibited by BL-MO, while the highest significant and positive h_{ij} ($\underline{P} < 0.05$) was expressed in FL-NI.

Specific heterosis (S_{ij}) was positive and highly significant ($\underline{P} < 0.01$) for BL-MC and MC-MO, and was positive but less significant ($\underline{P} < 0.10$) for BL-NI. These values would reflect improvement in ST independent of h_i. On the contrary, negative and significant S_{ij} ($\underline{P} < 0.05$) exhibited by NI-RE decreased ST in their hybrids. Similarly, negative and highly significant S_{ij} ($\underline{P} < 0.01$) was exhibited by BL-MO, FL-MC, and MC-NI.

Differences among reciprocal crosses (r_{ij}) and specific reciprocal effects (r**ij) are presented in Table 2.12. Significant positive r_{ij} ($\underline{P} < 0.05$) was exhibited by BL-RE, and NI-RE. In addition, highly significant positive r_{ij} ($\underline{P} < 0.01$) was apparent by BL-MO and MC-RE. Highly significant negative reciprocal effects ($\underline{P} < 0.01$) were exhibited in BL-NI and FL-MC.

Positive and significant r_{**ij} ($\underline{P} < 0.05$) was found in FL-RE. In addition, a positive and highly significant difference ($\underline{P} < 0.01$) was exhibited in BL-MO. Negative and significant r_{**ij} ($\underline{P} < 0.05$) was found in MC-RE and highly significant negative r_{**ij} ($\underline{P} < 0.01$) was exhibited in BL-NI, FL-MC, and MC-MO.

Table 2.11. Least square estimates of cross heterosis (h_{ij}), and specific heterosis (S_{ij}) effects influencing ST in tilapia crosses

Variety combinations[1]	h_{ij} ± SE		h_{ij} %	S_{ij} ± SE	
BL-FL	3.59 ± 1.37	***	8	-0.76 ± 1.26	
BL-MC	5.47 ± 1.26	***	17	3.19 ± 1.06	***
BL-MO	-3.27 ± 1.38	**	-8	-6.24 ± 1.27	***
BL-NI	6.60 ± 1.4	***	21	2.37 ± 1.42	*
BL-RE	3.58 ± 1.32	***	11	1.43 ± 1.09	
FL-MC	2.13 ± 1.42		6	-3.20 ± 1.26	**
FL-MO	4.18 ± 1.32	***	9	-1.85 ± 1.38	
FL-NI	11.69 ± 5.74	**	32	4.41 ± 3.53	
FL-RE	6.61 ± 1.34	***	18	1.41 ± 1.24	
MC-MO	9.82 ± 1.39	***	27	5.86 ± 1.26	***
MC-NI	0.93 ± 1.31		4	-4.29 ± 1.37	***
MC-RE	1.57 ± 1.31		6	-1.56 ± 1.07	
MO-NI	6.41 ± 5.55		18	0.51 ± 3.43	
MO-RE	5.55 ± 1.27	***	15	1.73 ± 1.22	
NI-RE	2.08 ± 1.44		8	-3.00 ± 1.42	**

* $\underline{P} < 0.10$, ** $\underline{P} < 0.05$, *** $\underline{P} < 0.01$ [1] includes reciprocal crosses

Table 2.12. Least square estimates of reciprocal (r_{ij}) and specific reciprocal effects (r_{**ij}) influencing ST in tilapia hybrids (Mean ± SE).

Variety combinations[1]	r_{ij} ± SE.			r_{**ij} ± SE.			
BL-FL	1.90	±	1.02 *	-2.49	±	1.24	*
BL-MC	-1.29	±	0.87	-0.23	±	0.75	
BL-MO	12.25	±	1.08 ***	8.47	±	1.24	***
BL-NI	-5.22	±	1.1 ***	-4.76	±	1.56	***
BL-RE	2.40	±	0.99 **	-0.98	±	0.81	
FL-MC	-11.39	±	1.02 ***	-5.94	±	1.24	***
FL-MO	-0.65	±	0.93	-0.05	±	1.51	
FL-NI	-3.97	±	5.66	0.88	±	3.91	
FL-RE	1.60	±	0.95 *	2.62	±	1.22	**
MC-MO	1.45	±	1.04	-3.39	±	1.22	***
MC-NI	-0.56	±	0.91	-1.15	±	1.5	
MC-RE	2.79	±	0.91 ***	-1.64	±	0.76	**
MO-NI	-0.48	±	5.48	3.76	±	3.8	
MO-RE	0.87	±	0.9	1.27	±	1.18	
NI-RE	2.57	±	1.12 **	-1.27	±	1.56	

* $\underline{P} < 0.10$, ** $\underline{P} < 0.05$, *** $\underline{P} < 0.01$ [1] includes reciprocal crosses

Discussion

The existing literature on salinity tolerance in tilapia usually provides comparisons among two or three crosses. In the study presented in this thesis, 36 crosses were compared. In addition, the fact the parental varieties tested included pure species, crossbreds and established commercial lines provided information up to now not available. The rate of salinity increase used in this study (7 ppt / day) was slightly higher than other rates (6 ppt / d). Salinity intervals of at least 6 ppt / day have proven to sufficient to determine differences between parental varieties and crosses (Lemaire 2001, Mateo et al. 2004).

Differences Among Parental Varieties

Comparisons among MST of parental varieties indicated that BL, FL and MO had superior ST than did MC, RE and NI (Figure 2.1). Similar differences in ST were also apparent in the estimation of net variety effects, denoting that BL, MO and FL would contribute the most ST when used to develop a synthetic variety (Venkowsky 1970). In addition, FL would provide a better alternative than MO as a culture species in brackish and saline water. These findings agreed with previous reports on the ST of FL (Watanabe et al. 2000 and 2006). In the case of MO, maximum ST was similar to previous reports (Popper and Lichatowich 1975; Lothan 1960, Trewavas 1982). The FL variety in this study tolerated up to 77 ppt, which was also similar to previous reports (Watanabe et al. 1985a, 1985b, 1988, 1990). The FL variety was developed from an O. urolepis hornorum female x O. mossambicus male cross (Watanabe et al. 1997). Both of these species can tolerate high salinities. Salinity tolerance of the BL variety (63 ppt) was superior to previous reports for this species (Chervinsky and Yashouv 1971; Perry and Avault 1972; Nugon 2002). Phenotypic differences between NI and RE in this study were not associated with differences in ST in between these varieties. Among purebred varieties, NI exhibited the lowest MST (25.03 ppt). This result agreed with previously reported MST values

(Avella et al. 1993), but it was notably lower than the MST of 56.8 ppt reported for Nile tilapia (originating in the Philippines) by Mateo et al. (2004). Information on ST of MC is limited. In our trials, MC exhibited greater ST than previously reported (Nugon 2002, Paz 2003) although it represented the same population evaluated in prior research. Higher ST obtained in our trials might be attributable to differences in experimental design or testing conditions. Purebred MC exhibited MST and CS_0 values similar to those of NI and RE (Figures 2.12 and 2.13). This variety was not developed to be cultured at high salinities, and it should be considered that MC contains an unknown degree of O. niloticus, O. aureus and O. mossambicus ancestry (Lutz 2006), and inheritance from the later two species should provide some degree of ST. However, it is possible that introgression with O. niloticus has decreased overall ST in this variety. Comparison in ST between MC and MO-NI denoted that crosses MO x NI and NI x MO (estimated MST from BLUP) would have exhibited greater tolerance than purebred MC. Similarly, NI-BL would have also exhibited greater tolerance than MC. Such improvement in MSTs in MO-NI and NI-BL over purebred MC suggests that MC may have reached a plateau in ST. In addition, that VCs originally found in MC most likely produce fish more tolerant that the current MC population denoting potential heterosis.

The higher ST of O. aureus over O. niloticus (Avella et al. 1993) was corroborated in this study. The poor ST of O. niloticus has been previous reported (Villegas 1990, Tayamen et al. 2002). Strains of different geographical origin and selected strains of NI have shown differences in ST in when tested under different environments.

Differences Among Crosses

The positive influence of FL and MO dams (maternal effects) on ST of offspring was a novel finding. The fact that all crosses produced by FL dams (regardless of sire variety) were able to tolerate salinities above 42 ppt provides a point of consideration for the development of

salinity tolerant crosses for commercial operations. The cross FL x NI exhibited the highest MST among all crosses (52.5 ppt). However, maximum salinity tolerance was exhibited in purebred MO, and the crosses MO x FL and MO x BL all tolerating 84 ppt, while purebred FL and crosses FL x MC and FL x MO all tolerated 77 ppt.

Two crosses, NI x MO and NI x FL did not produce offspring over consecutive years. Unsuccessful production of O. niloticus x O. mossambicus has been reported previously (Mateo et al. 2004, Kamal and Mair 2005). The fact that both missing crosses in this study (NI x MO and NI x FL) were not produced in two consecutive years may be the result of incompatible mating behavior. However, NI x MO offspring have been obtained in extensive and intensive ponds, with 96.7% heterosis for growth (Rosario et al. 2004). This cross was also produced using an O. niloticus strain developed in the Philippines (FAC strain) from populations originating in Israel, Singapore and Taiwan (Tayamen et al. 2002). Villegas (1990) suggested the removal of the male pre-maxillae to reduce aggressive behavior and therefore improve spawning in NI x MO. Tayamen et al. (2002) removed the MO male pre-maxillae, and their NI x MO cross exhibited an average of 24.88 % heterosis for growth across 10 salinity levels ranging from 0 to 42 ppt.

In this study, MO x NI exhibited an MST of 42.6 ppt, not different ($P > 0.05$) from that exhibited by purebred MO (46.5 ppt). In addition, MO x NI exhibited maximum ST of 70 ppt. Similarly, Taiwanese red tilapia (developed from the same cross, MO x NI) has been reported to obtain faster growth in brackish water (17 ppt) and seawater (34 ppt) than in freshwater (Liao and Chang 1983). A wider range of tolerance (15 – 42 ppt) was reported in a similar cross of MO x NI (Villegas 1990).

This study found results similar to those reported previously (Villegas 1990), in which purebred MO and the cross MO x NI were more salinity tolerant ($P < 0.05$) than purebred NI. In

addition, the present study agreed with reports where purebred MO was more tolerant than MO x NI (Kamal and Mair 2005) even though both studies reported different maximum ST for each species. Parental variety MO in both trials were derived from the same African strains and NI (in Kamal and Mair 2005) had gone through rigorous selection, unlike the A-E variety used in this study.

Although certain crosses in this study appear to be good candidates for commercial production in brackish water testing is needed to determine food conversion ratios (FCR), growth and survival in commercial settings. Six of the 36 crosses produced in this study were previously evaluated for growth and survival in freshwater and brackish water (23 ppt) in outdoor mesocosms using fish from the same populations (Paz 2004). In addition, Paz (2004) evaluated the following crosses: MC, FL, BL, BL x NI, BL x FL and MO x NI. In freshwater, FCR, growth and survival followed the pattern: FL > BL x FL > MO x NI > BL > MC > BL x NI. Survival ranged from 80% (BL x FL, MO x NI) to above 90% (all other crosses). In contrast, FCR and survival in brackish water were as follows: FL (6/ 100%), BL x FL (>10/ 29%), MO x NI (>10/ 19%), BL (>10/ 12%), MC (>10/ 2%), and BL x NI (0 / 0%). Low survival in brackish water tests was attributed to the fact that fingerlings were less salinity tolerant than larger fish (Villegas, 1990).

Even though some of the same fish populations were used in the research conducted for this study as in Paz (2004), differences in protocols should be mentioned. Paz conducted feeding trials in outdoor pools, while in this study trials were conducted in indoor recirculating systems. Paz increased salinity at 3 ppt/d, while in this thesis salinity was increased at 7 ppt/d. Tilapia could adapt better to salinity increases of 7 ppt/d than 3 ppt/d because rapid salinity increases would reduce the metabolic cost of osmoregulation when compared to gradual increases.

Standard Errors

Among parental varieties standard errors were between 1.19 and 1.39. The lowest SE was exhibited by BL, while the highest were exhibited by FL and MC. Commercial varieties such as MC and FL exhibited very similar SE, 1.38 and 1.39, respectively, but differed significantly in MST ($P < 0.05$) (Figure 2.1). Parental varieties NI and RE exhibited almost identical SEs of 1.27 and 1.28, respectively and exhibited no significant difference ($P > 0.05$). Small differences in SE estimates among parental varieties denoted that sample sizes were probably large enough to detect meaningful differences among the varieties tested.

Among all crosses, SE estimates were between 1.16 and 3.14. The largest SE was exhibited by FL x NI in which only 10 fish were available for evaluation. If FL x NI were not considered, the range of SE would have been from 1.16 to 1.84.

Best Linear Unbiased Predictors (BLUPs)

Best linear unbiased predictors (BLUPs) were useful in the estimation of maternal and paternal influences of purebred varieties on offspring ST. In addition, BLUPs allowed the estimation of MST in the missing crosses NI x FL and NI x MO. Based on these estimates, it could be inferred that the missing crosses would have exhibited CS similar to those of MC x FL and RE x FL (56 - 63 ppt) and to those of MC x MO and RE x MO (70 ppt), respectively.

BLUP-based estimates for NI x MO, however, were below those reported by Mateo et al. (2004) who estimated an MST of 112.5 ppt for O.niloticus x O. mossambicus crosses. BLUPs were also essential in the estimation of genetic effects, as the two missing crosses were required for calculations in the full diallel analysis. The usefulness of BLUP estimates as selection tools for commercial improvement of traits has been recognized in aquaculture and particularly in tilapia (Gjerde 2005, Charo-Karisa 2006).

Cumulative Survival

Cumulative survival curves were useful in identifying potential heterosis and maternal effects among purebreds and crosses. In the absence of a software program to statistically determine genetic effects and heterosis, plotting and comparing survival curves may also provide an indication of maternal, line and reciprocal effects.

Projected CS values (Table 2.5) yielded information which could be applicable in commercial production and environmental conservation. Cumulative survival at 85% (CS_{85}) was predetermined as acceptable loss for farming operations requiring use of saline waters. However, these values should be considered only as estimates, as the long-term effect of exposure to a particular salinity level would likely influence survival and growth.

Genetic Effects Influencing ST

Net variety effect followed similar patterns in dams and sires. Most importantly, net variety effect of dams and net crossing effects followed an identical pattern. Lines (varieties) with the highest CGAs would also exhibit highest net crossing effects (Eisen et al. 1983).

Estimation of net cross effect ($\bar{y}*_{ij}$) presents the opportunity to test crosses for the potential development of synthetic varieties (Gregory et al. 1978) based on the averages of one sire variety against all dam varieties and vice versa. In this regard, an aquaculture facility capable of conducting a simple 3 x 3 diallel design may obtain information to improve a particular trait based only on statistical means. Based on the breeding values of dams and sires (Table 2.8), it is reasonable to expect that varieties FL, MO, BL would be the candidates to produce a salinity tolerant synthetic.

Inclusion of purebred varieties in the diallels allowed estimation of direct line effects (Gardner and Eberthard 1966). Direct line effects of FL, MO, and BL accounted for the high ST

of offspring when these varieties were used. On the contrary, the other three parental varieties reduced ST in offspring.

Maternal effects played an important role in ST of the crosses produced in the diallel crosses. Although NI and RE varieties were both O. niloticus, offspring of RE were more salinity-tolerant than those of NI due to their highly significant maternal effects.

General combining ability (GCA) estimates, either positive or negative, provide a point of comparison among parental varieties used in the diallel crosses. Highest GCAs were exhibited by FL and MO and to a lesser extent to BL. These three varieties would influence the parental means of VCs and therefore the overall ST of offspring produced.

The combination of specific heterosis (S_{ij}) and $\bar{h}$ significantly contributed towards increasing cross heterosis, even when parental varieties exhibited no significant or even negative direct heterosis. This was the case in BL-MC, BL-NI, BL-RE, FL-NI, MC-MO and MO-RE. In other cases, cross heterosis was produced even when parental varieties exhibited negative h_i and S_{ij} such was the case of BL-MO, FL-MC, FL-MO and MC-NI.

The two main causes of heterosis are generally recognized as partial to complete dominance within loci, and diverging allele frequencies between parental populations (Falconer and Mackay, 1996; Hallauer and Miranda, 1988). Based on that, choosing a particular crossbreed for exploitation of a trait should not be based only in a specific genetic effect, but in the interaction of all contributing genetic effects as a whole.

Cross heterosis was significant ($P < 0.05$) for FL-NI, and highly significant ($P < 0.01$) for MC-MO, FL-RE and BL-NI suggesting that these VCs may have the highest potential as crosses to be used in commercial applications. Ranked according to IM and CS_{85}, best VCs were MC-MO, BL-NI, and FL-RE. Best IM and CS_{85} were exhibited by MC-MO which presented equal

IM of 21 ppt and CS_{85} of 28 ppt for reciprocal crosses. The following best was VC BL-NI, NI x BL exhibited IM of 14 ppt and CS_{85} of 21 ppt, while BL x NI exhibited 7 ppt more tolerance in both indexes. Finally, FL-RE, exhibited differences between crosses. Cross FL x RE exhibited IM at 7 ppt, while IM in RE x FL was 28 ppt.

Reciprocal effects denoted maternal influences on offspring ST. Despite exhibiting positive h_i, ST of MC- RE crosses were both below those of the parental varieties. This was due to highly significant negative maternal effects observed in MC dams (in the case of the MC x RE) and to the highly significant negative line effects expressed in both varieties.

In RE-BL, RE dams contributed towards increasing ST, denoting the influence of maternal genotype on their offspring. In contrast, in NI-RE, RE dams decreased ST in their offspring (Figure 2.16). Negative reciprocal effects exhibited by BL-NI were the result of NI dams reducing ST in NI x BL. Similarly, in FL-MC, offspring produced by MC dams exhibited a 22.8 ppt difference below the MST of offspring from the reciprocal cross. The fact that certain maternal varieties contributed more than others toward salinity tolerance of their offspring may be the result of the adaptation of maternal effects to environmental evolutionary changes (Mousseau and Fox 1998) particularly to salinity tolerance.

Negative reciprocal effects demonstrated that FL dams would contribute more toward ST in their offspring than MC mothers would in the reciprocal cross. Similarly, offspring produced by NI dams in NI-FL would be highly less salinity tolerant than offspring produced by BL dams. In addition, in FL-RE, RE dams contributed greatly to the improvement of ST over fish produced by FL dams. Similarly, in BL-FL, FL dams improved ST of offspring over fish incubated by BL dams.

Specific reciprocal effects (as cytoplasmatic effects) were found in to improve ST in FL-MC, MC-MO and MC-RE, and BL-NI. As described by Tave et al. (1990b) difference between

MC and RE egg cytoplasm resulted in offspring with superior ST in RE x MC. Similarly, differences between MC egg cytoplasm and RE egg cytoplasm would increase ST in MO x MC offspring compared to the reciprocal cross. The ST deficiency of MC egg cytoplasm was also exhibited in FL-MC. The negative line and maternal effects of MC dams are probably related to cytoplasm factors. In the case of BL-NI, BL were shown to be more salinity tolerant than NI. These results corroborate numerous previous reports. Highly significant specific reciprocal effects exhibited by BL-MO were the result of maternal effects of MO dams on their offspring. Similarly, the significant r_{**ij} exhibited by FL-RE crosses was the result of highly significant ($\underline{P} < 0.01$) maternal effects of both lines.

Conclusions

To date, most research on salinity tolerance in tilapia has been conducted on varieties of known high tolerance or combinations of high salinity-tolerant varieties and other varieties. In most cases, such research has not accounted for individual genetic effects influencing ST due to lack of available facilities or statistical procedures.

From a commercial perspective, the development of 36 crosses allowed the determination of MST in crosses not previously reported, such was the case of combinations of FL or MC with other varieties. In addition, the high ST of certain varieties and their offspring provides the opportunity for consideration of further study under commercial settings.

From a scientific perspective, estimation of genetic effects provided clear evidence of the importance of additive and dominance effects influencing ST in tilapia varieties and crosses. Parental varieties were influenced by additive inheritance therefore, selective breeding to utilize V_A among the most tolerant individuals should be conducted within varieties FL, MO and BL. Besides selective breeding, the combined used of hybridization (V_D) and backcrossing could be used to improve ST in certain varieties as well as crosses (Behrends and Smitherman 1984).

Similarly, the influence of maternal effects in the offspring derived from FL, MO, and particularly of RE is noteworthy. In addition, the estimation of specific heterosis and cross heterosis provided in all VCs presented information on the importance of considering dominance effects when developing crosses. Cross heterosis significantly influenced ST in 10 of 15 variety combinations denoting a large degree of dominance effects in the expression of ST in tilapia. Further trials are necessary to evaluate the use of such highly tolerant crosses in commercial applications, with an emphasis on growth, feed conversion and disease resistance.

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CHAPTER 3
COLD TOLERANCE

Research on cold tolerance (CT) in tilapia is driven by both commercial production and ecological considerations. There is interest in cold-tolerant tilapia that could be cultured in subtropical and temperate regions or at high elevations (Behrends et al. 1990; Lutz 1998). In the southeastern USA, for example, the growing season for outdoor tilapia pond production ranges between 120 and 150 d (Hargreaves 2000). Such a short growing period results in the need for overwintering fish indoors. Improvement of CT in tilapia could extend the growing season, lower mortality during overwintering and reduce production costs (Sifa et al. 2002).

Cold tolerance research in tilapia often takes into account visible thresholds. Such thresholds are lack of opercular movement, lack of response to touch, loss of balance (10 – 12 C), and comatose state (5 – 10 C) (Yashouv 1960; Starling et al. 1995; Hargreaves 2000). Knowledge of lower lethal temperatures has allowed the eradication of invasive O. aureus in power plant cooling ponds in Texas and Pennsylvania by exposing fish to sufficiently low temperatures (Ippolito 1985; Stauffer et al. 1988). Many studies with tilapia (Yashouv 1960; Chervinski and Lahav 1976; Shaftland and Pestrak 1982; Behrends and Smitherman 1984; Lahav and Ra'anan 1997) have described the lowest lethal temperature (LT) withstood before death as CT. In this chapter, lethal CT is defined as the lowest temperature at which a particular variety or cross of tilapia reaches 100% mortality and its dependent on exposure time and cooling rate.

Cold tolerance in tilapia is a quantitative trait affected by a variety of factors such as genetics, exposure time, and rate of temperature decrease (Behrends and Smitherman 1984; Lahav and Ra'anan 1997, Paz 2004). Variance in quantitative traits can be often partitioned into two distinct genetic effects: additive or dominance. Additive genetic effects are linked to the heritability of traits, and consequently are the basis for selection. Choosing the organisms

presenting the best performing traits as broodstock would result in the overall improvement of the offspring (Lutz 2001). On the contrary, dominance genetic effects are based on combinations of specific alleles, and consequently combinations of individuals, strains or species which carry those alleles.

Dominance genetic effects are expressed as heterosis, an increase in the overall fitness of hybrids or crossbreds over that of the parental varieties, or as inbreeding depression. In addition, maternal effects are occasionally important in early development of teleosts and are described as impacts made by female genotype or size, age, and condition at spawning on the offspring (Dunham 2004).

Reported CT for some of the major tilapia species was presented in Table 1.1. Published literature reviews (Wohlfarth and Hulata 1983; Hargreaves 2000) have shown a broad range of studies and variation in results. Some authors (Behrends et al. 1990) have generalized the ranking of CT in the main cultured tilapia species, with O. aureus being the most tolerant, O. mossambicus the least and O. niloticus exhibiting intermediate tolerance. However, strains within species may exhibit different CT.

Latitude of origin (and, accordingly, natural selection) has been described to have an influence on CT of Nile tilapia strains (Khater and Smitherman 1988) (Table 2.1). The O. niloticus strains studied and their origins of latitude were: Ghana (6°N), Ivory Coast (10°N) and Egypt (31°N). Ghana strain exhibited LT_{50} (temperature at which 50% of the population survived) of 14.1 C and survived for 18.6 d, while the Egyptian strain exhibited of LT_{50} of 10 C and survived 40 d.

Components of the genetic variance, such as additive genetic effects, maternal genetic effects, and heterosis were determined for CT in Egypt (E) and Ivory Coast (IC) strains of O. niloticus exposed to ambient winter temperatures in Alabama (Tave et al. 1990a). Based on non-

significant heterosis for F1, F2, and backcross hybrids and the fact that mean viabilities of F1 hybrids were intermediate to those of parental strains, it was concluded that viability was mainly influenced by additive rather than dominance genetic effects although maternal heterosis effects were evident. In both strains, selection rather than crossbreeding was the recommended breeding technique to improve viability under ambient winter conditions (Tave et al. 1990a.

Similar recommendations of selection as a breeding strategy to improve CT in O. niloticus were given by Sifa et al. (2002). Lack of differences in temperature tolerance between Sudan 78 and Egypt 88 strains was attributed to the possibility that both strains had reached their biological CT limits (Sifa et al. 2002). Conversely, the poorer performance of the GIFT strain was attributed to the possibility that O. mossambicus ancestry may have been present due to introgression in some of the O. niloticus strains used when the GIFT line was developed (Macaranas et al. 1997). Because O. mossambicus is regarded as the least cold-tolerant of the commercial Oreochromis species (Sifa et al. 2002), introgression could have resulted in reduced CT in the GIFT strain.

No heterosis for CT was exhibited in O. aureus x O. niloticus hybrids (Lee 1979). The authors suggested that CT in O. aureus and hybrids derived from it exhibited incomplete dominance, with a strong maternal component (Lee 1979). Similarly, Behrends et al. (1990) suggested that CT in O. aureus, was controlled by additive genetic effects with a strong maternal component. In contrast, one study on the genetic basis of CT in O. mossambicus, O. aureus and their hybrids suggests that CT is influenced by a dominance component, based on the similarity between F1 hybrids and the O. aureus parent (Cnaani et al. 2000).

Efforts to develop a cold-tolerant population of red tilapia were conducted in ambient winter temperatures in Alabama in 1982 (Behrends and Smitherman 1984). Heterosis was observed in the superior CT and survival of the F1 backcross hybrid. Introgressive hybridization

(hybridization and backcrossing) was an effective method for combining red coloration and CT traits of O. mossambicus and O. aureus in their hybrids (Behrends and Smitherman 1984). Other red tilapia hybrids whose ancestry includes O. mossambicus have limited CT. Florida red tilapia and the Taiwanese red tilapia grow poorly in temperatures below 22 C (Lovshin 1997).

Apart from genetic effects, other factors have been reported to affect CT of tilapia, such as size, age, acclimation time, and thermal scheduling (Behrends and Smitherman 1984). Research on effect of size and weight on cold tolerance is controversial. McBay (1961) found O. aureus juveniles less cold-tolerant than adults. Similarly, genetically male O. niloticus fingerlings (2- 20 g) exposed to drastic temperature shocks from 20 - 12 C (Hofer et al. 2001) suggested that smaller juveniles (mean = 5.8 g) were significantly less tolerant than larger fingerlings (mean = 9.6 g). Similarly, the effect of fish size, photoperiod, and diet on survival of O. niloticus fingerlings exposed to a temperature decrease of 0.5C/ 24 h was studied by Atwood et al. (2003). Smaller fish (136 mm) were less cold-tolerant than larger fish (220 mm). No photoperiod effect was apparent. In addition, diet had little effect on the ability of O. niloticus to tolerate low temperatures. In contrast, no correlation between weight and lethal temperature at time of death among parental O. mossambicus, O. aureus and the F1 backcrossed hybrid were determined (Behrends and Smitherman 1984, Zale and Gregory 1989). Similarly, Behrends et al. (1990) found no correlation between CT and fish size (range 2 -90 g) in O. niloticus, O. aureus or O. mossambicus and their hybrids. Similarly, no correlation between fish size and CT were found in fish size ranging from 23-105 mm SL in O. aureus and O. mossambicus and their hybrids (Cnaani et al. 2000).

Some discrepancies among CT reports in tilapia may be due to differences in acclimation rate or thermal scheduling (Zale and Gregory 1989). Pre-exposure to 20 C for 96 h improved survival in genetically male Nile tilapia fingerlings (originally maintained at 28 C) and reduced

LT_{50} by approximately 2 C (Hofer et al. 2001). In contrast, exposure of O. niloticus fingerlings to an overnight drop in temperature from 9 C to 5 C resulted in 99 % mortality (Yashouv 1960). A temperature decline from 19 C to 13 C over 2 weeks resulted in only 30% mortality of O. aureus fingerlings in Alabama (Behrends and Smitherman 1984).

Behrends et al. (1990) conducted short-term CT tests on O. aureus, O. niloticus, and O. mossambicus over 8-12 h, (-1.5 to - 3.5 C / h), and long-term tests over 11 d (-1 C/ d). The authors reported no differences in CT and survival ranking of species in short-term or long-term temperature reduction. Highest to lowest CT was exhibited by O. aureus, O. niloticus and O. mossambicus

Significant temperature declines (e.g. 1 C / h) may result in osmoregulatory failure in tilapia (Zale and Gregory 1995; Ross 2000). However, abrupt temperature shocks (from 20.5C and 17.0 C to 9.5 C) for one hour were not lethal to O. niloticus (Yashouv 1960). Fish became comatose but were able to recover when temperature was increased to 17 C. In the same study, an overnight reduction in ambient temperature from 9 C to 7 C resulted in 82% mortality, with an additional 12% the following day and 5% on the third day.

Fernandes and Rantin (1986) found that drastic temperature changes affected respiration rates of O. niloticus resulting in decreased metabolism, followed by several days of acclimation to reach a steady metabolic state. When the temperature shock was from 20 C to 30 C, recovery time was 4 to 5 d. However, when the temperature shock was from 30 C to 20 C, the recovery period was 7 to 14 d. Similarly, a thermal reduction rate of -5 C/ 24 h from an initial 20 C acclimation temperature was suggested as a method for precipitating a large scale die-off of O. aureus populations in power plant thermal effluents of the Susquehanna River, Pennsylvania (Stauffer et al. 1988). This conclusion was reached after a variety of laboratory experiments exposed O. aureus to various thermal schedules.

Cold tolerance in O. niloticus, O. aureus, and two commercial varieties, the Mississippi commercial strain and the Florida red tilapia, was evaluated using three different temperature reduction rates (Paz 2004). Temperature reduction rates were rapid (-0.5 C/5 h), moderate (-1 C/24 h), and gradual (-1 C/48 h). Fish exposed to rapid reduction (-0.5 C/5 h) were able to reach lower lethal temperatures than fish exposed to the moderate or gradual reduction rates. Extended exposure time to thermal stress resulted in higher lethal temperatures for all varieties.

Cold tolerance in tilapia may also be influenced by salinity by aiding in osmorregulation at isosmotic salinities (Allanson et al. 1971). Exposure of O. mossambicus to fresh water and brackishwater (5 ppt) at 11 C resulted in fish reaching a comatose state only in freshwater. Similarly, mortality of O. aureus exposed to 11 C was twice as high in freshwater as at 5 ppt (Chervinski and Lahav, 1976). Zale and Gregory (1989) evaluated the effect of salinity on CT of juvenile O. aureus using a thermal schedule of 1 C/ 24 in an isosmotic media (11.6 ppt) and at other salinities. Salinity influenced temperatures at which fish stopped feeding, lost equilibrium and died, and fish at isosmotic salinity survived lower temperatures than fish exposed to lower or higher salinities.

Paz (2004) evaluated the effect of salinity (0, 5 and 10 ppt) on CT in tilapia fingerlings (O. niloticus, O. aureus, Mississippi commercial strain and Florida red tilapia). No significant differences were found at a reduction rate of 1 C/ 24 h and salinity did not improve survival. Conversely, Allanson et al. (1971) and Zale and Gregory (1989) reported that CT in O. mossambicus, O. aureus, and O. niloticus improved at 5 – 12 ppt compared to freshwater or seawater (36 ppt). Al Amoudi et al. (1996) applied temperature shocks to O. mossambicus and O. aureus x O. niloticus hybrid by transferring the fish from 25 C to 15, 20, 30, and 35 C in freshwater and saltwater (26 ppt). Fingerlings exposed to freshwater shocks became comatose at

15C. Saltwater shock had no effect on survival of O. mossambicus, however, in hybrids survival was lowest at 15C and 35C exhibiting 6.7% and 59.7%, respectively.

The goal of the research described in this chapter was to evaluate genetic effects influencing tilapia CT using a diallel mating design including six parental varieties with differing CT. Specific objectives were to: (1) determine CT in all varieties and their crosses, and (2) estimate genetic effects influencing CT. To accomplish these objectives, the following hypotheses were tested:

1) Mean temperature tolerance (MTT = μ) is equal among all varieties and their crosses.

H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6$

H_1: $\mu_1 \neq \mu_2 \neq \mu_3 \neq \mu_4 \neq \mu_5 \neq \mu_6$

2) Crosses between parental varieties exhibit no heterosis

H_0: $V_{1x}\ V_2 = V_{1x}\ V_3 = V_{1x}\ V_3 \ldots V_{5x}\ V_6$

H_1: $V_{1x}\ V_2 = V_{1x}\ V_3 = V_{1x}\ V_3 \ldots V_{5x}\ V_6$

3) Reciprocal crosses present no differences in CT

H_0: $r_{ij} = r_{ji}$

H_1: $r_{ij} \neq r_{ji}$

4) Maternal effects (m_i) are no different among dams

H_0: $m_i = m_j$

H_1: $m_i \neq m_j$

Materials and Methods

Parental Varieties and Mating Design

Five purebred and one crossbred tilapia varieties maintained at the Aquaculture Research Station (ARS), Louisiana State University Agricultural Center (LSU Agcenter), were used in this research. The methodology used for their breeding was previously described in Chapter 2.

Cold Tolerance (CT) Trials

An indoor, temperature-controlled recirculating system containing four 280-L circular insulated fiberglass tanks, two pumps, a 360-L sump, and three in-line titanium chillers was used in these experiments (SOP #4). Prior to the beginning of each trial, water quality was adjusted to pH 7-8, alkalinity 220 ± 50 mg/l as $CaCO_3$, total hardness 280 ± 50 mg/l as $CaCO_3$, and 300- 400 mg/l chlorides.

Samples of 10 to 20 fish (1.5 – 6.5 g) from each cross were placed in separate 3.8-L polyethylene jars (Consolidated Plastics Co. Inc., Ohio). If less than 10 fish were available from a cross, all fish were used. The mouth of each jar was fitted with a 0.15 m x 0.15 m piece of soft knotless 4 mm mesh nylon netting secured by two rubber bands. Ten to 12 jars each were immersed in three of the four fiberglass tanks. Fish were acclimated to the system at 24 C for 24 h before the beginning of each trial. After the initial acclimation period, temperature was decreased at a stepwise rate of 2 C per day at 24 ± 1h intervals (between 11:00 and 13:00 h daily). Temperature was monitored by two submersible probes placed in the system sump. In addition, a hand held thermometer was kept in one of the 280-L tanks. To minimize temperature fluctuations, all four 280-L tanks were covered with an opaque plastic sheet (3.65 m x 2.75 m).

Each jar was checked for mortalities prior to each daily temperature reduction. Mortality was determined on the basis of loss of equilibrium, lack of opercular movement (no respiration), and failure to respond to gentle probing with a net (Behrends et al. 1990). Dead fish were removed from jars and mortality recorded for each cross at each temperature interval.

Statistical Analysis

Data analyses for CT were conducted as described for salinity tolerance in Chapter 2. In addition, mean temperature tolerance (MTT) was defined as the temperature at which 50% of fish in a variety or cross died.

Results

The first diallel crosses (summer 2004) resulted in 27 of 36 possible crosses, whereas the second (summer 2005) resulted in 34 of 36. A total of 2227 fish were tested for CT over two years (272 and 1955 fish in 2004 and 2005, respectively).

Among parental varieties, MTT ranged from 8.9 C (FL) to 6.1 C (BL) (Table 3.1), with BL differing significantly ($\underline{P} < 0.01$) from all other varieties. Mean temperature tolerance of BL was 1.5 C lower than NI and 2.8 C lower than FL.

Among crosses, MTT ranged from 10.2 C to 5.7 C. Mean temperature tolerance of offspring produced by dams crossed to various sires exhibited the following pattern of CT: BL > RE > MO = NI > MC > FL. Crossing of BL dams with sires of all other varieties resulted in offspring exhibiting heterosis. Improvement in CT was found in offspring produced by BL or MO dams with sires other than RE. Similar improvement in CT of offspring was found when FL dams were crossed with sires other than NI. Crossing of MC dams with NI, FL or BL sires improved CT in their offspring. NI dams only improved CT in offspring when crossed with MC sires. Crossing of RE dams with any sire variety improved MTT of their offspring. Analysis of variance indicated highly significant ($\underline{P} < 0.0001$) dam and sire effects for CT (Table 3.2).

Tukey-Kramer analysis of all matings allowed the partitioning of dam and sire effects on MTT (Tables 3.3 and 3.4). Non-estimable contributions were due to missing crosses required to evaluate all potential variety combinations.

Highest MTT was exhibited by offspring of FL dams (8.7 C). Lowest MTT was exhibited by those of BL dams (6.8 C). BL dam offspring were significantly different ($\underline{P} < 0.05$) than those of MO, MC and FL dams. Offspring of BL sires were the most tolerant at 7.1 C, but were not significantly different than those of MC or NI sires. Offspring of RE sires were not significantly different ($\underline{P} < 0.05$) than those produced by all other sires.

Table 3.1. Mean temperature tolerance (MTT) of tilapia F1 offspring resulting from 2004 and 2005 diallel crosses (n = 2227) (Tukey-Kramer). Purebred parental varieties are in italics.

Dam x	Sire	N	MTT	SE	Mean days/death	Letter Group
FL	NI	11	10.2	0.72	7	ABC
FL	*FL*	*73*	*8.9*	*0.28*	*8*	*AD*
FL	MC	42	8.9	0.37	8	ABCDE
MC	RE	92	8.8	0.25	8	ABD
FL	BL	57	8.5	0.32	8	ABCDEF
MC	MO	75	8.2	0.27	9	ABCDEF
BL	RE	52	8.1	0.33	9	ABCDEFGH
MO	RE	75	8.1	0.27	9	ABCDEFG
FL	RE	43	8.0	0.36	9	ABCDEFGH
FL	MO	57	8.0	0.32	9	ABCDEFGH
NI	RE	76	7.8	0.27	10	ABCDEFGH
MO	*MO*	*82*	*7.8*	*0.26*	*10*	*ABCDEFGH*
RE	*RE*	*71*	*7.7*	*0.28*	*10*	*ABCDEFGH*
MC	*MC*	*86*	*7.6*	*0.26*	*10*	*ABCDEFGH*
NI	*NI*	*83*	*7.6*	*0.26*	*10*	*ABCDEFGH*
MO	FL	75	7.5	0.27	10	ABCDEFGHI
MC	NI	73	7.5	0.28	10	ABCDEFGHI
RE	FL	86	7.4	0.26	10	BCEFGHIJ
MO	MC	40	7.4	0.38	10	ABCDEFGHIK
RE	MO	75	7.3	0.27	10	CEFGHIJ
RE	BL	69	7.3	0.29	10	CEFGHIJK
NI	MC	66	7.2	0.29	10	CEFGHIJK
MO	BL	86	7.2	0.26	10	EFGHIK
BL	MO	60	7.1	0.31	10	EFGHIK
MO	NI	75	7.0	0.27	10	FGHIK
BL	NI	29	7.0	0.44	10	DEFGHIJK
RE	MC	59	6.9	0.31	11	FGHIK
NI	BL	84	6.9	0.26	11	FGHIK
BL	FL	55	6.9	0.32	11	FGHIK
RE	NI	8	6.8	0.84	11	ABCDEFGHIK
MC	FL	85	6.7	0.26	11	GHIK
MC	BL	88	6.7	0.25	11	HIK
BL	*BL*	*72*	*6.1*	*0.28*	*11*	*IK*
BL	MC	67	5.7	0.29	12	K

Varieties and crosses with the same letters were not significantly different ($\underline{P} > 0.05$).

Table 3.2. Analysis of variance of dam and sire variety contributions to CT in tilapia varieties.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
Dam	5	2193	16.41	<.0001
Sire	5	2193	8.35	<.0001
Dam*Sire	23	2193	3.07	<.0001

Table 3.3. Estimated maternal effects for CT (Mean ± SE).

Dams	Estimate (C)	SE	Letter group
FL	8.7	0.17	A
MC	7.6	0.11	B
MO	7.5	0.12	B
RE	7.2	0.18	BC
BL	6.8	0.14	C
NI	N/E	N/E	-

Crosses with the same letters are not significantly different ($\underline{P} > 0.05$)
N/E = non estimable due to missing crosses in the diallels

Table 3.4. Estimated maternal effects for CT (Mean ± SE)

Sire	Estimate (ppt)	SE	Letter group
RE	8.1	0.12	A
NI	7.7	0.21	AB
MC	7.3	0.13	B
BL	7.1	0.11	B
FL	N/E	N/E	-
MO	N/E	N/E	-

Crosses with the same letters are not significantly different ($\underline{P} > 0.05$)
N/E = non estimable due to missing crosses in the diallels

Cumulative Survival

Cumulative survival (CS) of parental varieties indicated that BL was the most cold-tolerant (surviving to 6.1 C), followed by NI, MC, RE, MO (7.7C – 7.6 C), and FL (8.9 C) (Figure 3.1). Approximately 50% of the FL population died between 12 and 10 C, while at the same temperatures CS of the other purebreds remained above 75%. MO reached 50% mortality at 7.8 C closely followed by RE at 7.7 C, MC at 7.6 C and NI at 7.6C. BL reached 50% mortalities at 6.1 C. Three distinct survival patterns were observed, including FL alone, MO, RE, MC and NI together and BL alone. All three groupings were significantly different ($P < 0.01$) from each other. Cumulative survival curves began at the point where first mortalities occurred in each cross.

Cumulative survival of BL and FL purebreds and their reciprocal hybrids is presented in Figure 3.2. Cumulative survival of FL purebred was significantly lower ($P < 0.01$) than that of BL. Cumulative survival of FL x BL was significantly lower than that of the reciprocal cross. Approximately 50% of FL died at 10 C, while BL x FL exhibited CS above 75% at the same temperature. In addition, FL x BL and purebred BL exhibited CS greater than 90% at 10 C. Crosses exhibited CS intermediate from the parental varieties. Cumulative survival of the purebred FL was the lowest of all four groups, reaching complete mortality at 8 C. Maternal effects were apparent due to differences in CT between reciprocal crosses.

Cumulative survival of BL and MC varieties and their reciprocal crosses is presented in Figure 3.3. Among parental varieties, BL was more cold-tolerant than MC. Cross BL x MC was more cold-tolerant than its reciprocal. Cross MC x BL exhibited the lowest MTT of all 28 crosses produced over the two years of research. However, it exhibited non-significant differences ($P > 0.05$) in MTT from BL or MC x BL. Apparent heterosis and maternal effects were observed as CS of both crosses exceeded the parental varieties.

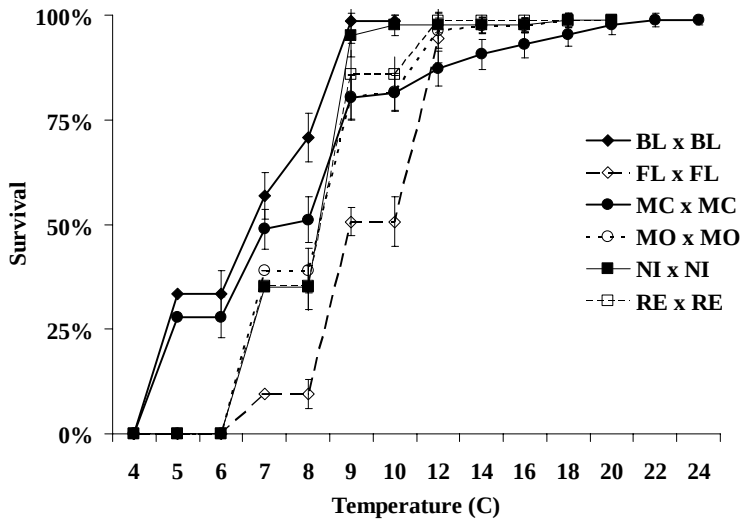

Figure 3.1. Cumulative survival of tilapia varieties exposed to decreasing temperatures (Mean ± SE).

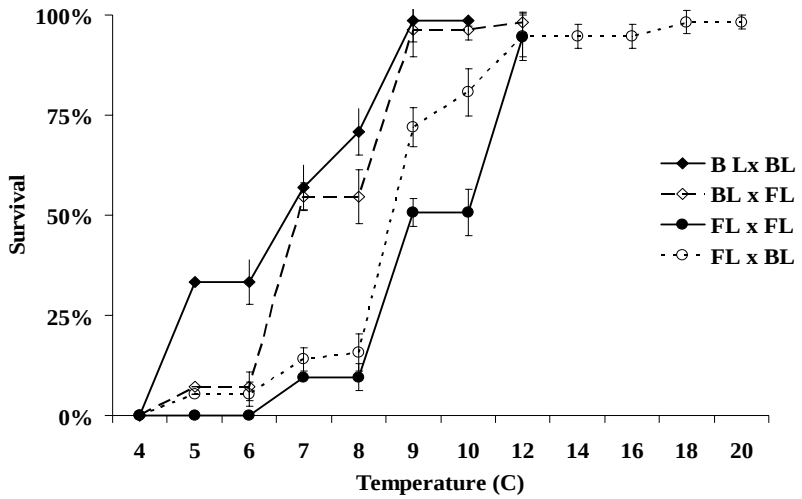

Figure 3.2. Cumulative survival of BL and FL varieties and their crosses (Mean ± SE).

Cumulative survival of BL and MO and their reciprocal crosses (Figure 3.4) indicated that purebred MO and MO x BL followed a similar CS pattern, reaching complete mortality before BL x MO and BL. BL x MO exhibited higher CS than the reciprocal MO x BL at 5 C. No heterosis was apparent as both crosses were intermediate from that of the parental varieties.

Cumulative survival of BL and NI varieties and their reciprocal crosses was presented in Figure 3.5. No apparent heterosis was observed, as cross performances were intermediate of those of the parental varieties.

Cumulative survival of varieties BL and RE and their reciprocal crosses (Figure 3.6) indicated that CS of all groups remained above 80% above 9 C. At 8 C, CS dropped to: 71% (BL), 60% (BL x RE), 45% (RE x BL), and 35% (RE). Purebred BL was the only group able to tolerate temperatures below 6 C. Cumulative survival curves denote an apparent degree of heterosis and possibly maternal effects.

Cumulative survival curves of FL and MC and their reciprocal crosses (Figure 3.7) indicated similar survival of both crosses to that of their maternal varieties. Cross FL x MC exhibited CS intermediate to both parents, but lower than its reciprocal (MC x FL). Maternal effects and heterosis were apparent.

Cumulative survival of FL and MO and their reciprocal crosses (Figure 3.8) indicated that among these groups, purebred FL was the least cold-tolerant. Cumulative survival of FL x MO was intermediate to both parents, but lower than its reciprocal cross. Maternal effects and heterosis were apparent.

Cumulative survival of FL, NI and FL x NI is presented in Figure 3.9. Purebred NI exhibited higher CS than the other two groups. Cross FL x NI exhibited the lowest CT among all groups. Heterosis was apparent.

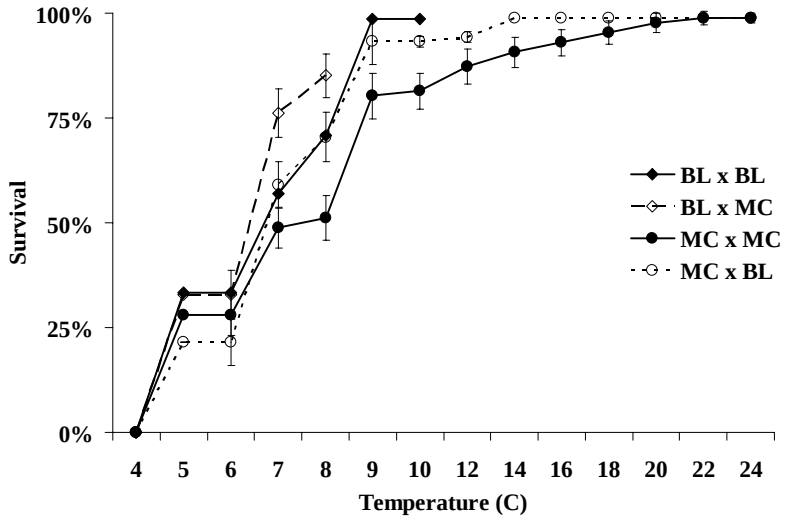

Figure 3.3. Cumulative survival of BL and MC varieties and their crosses (Mean ± SE).

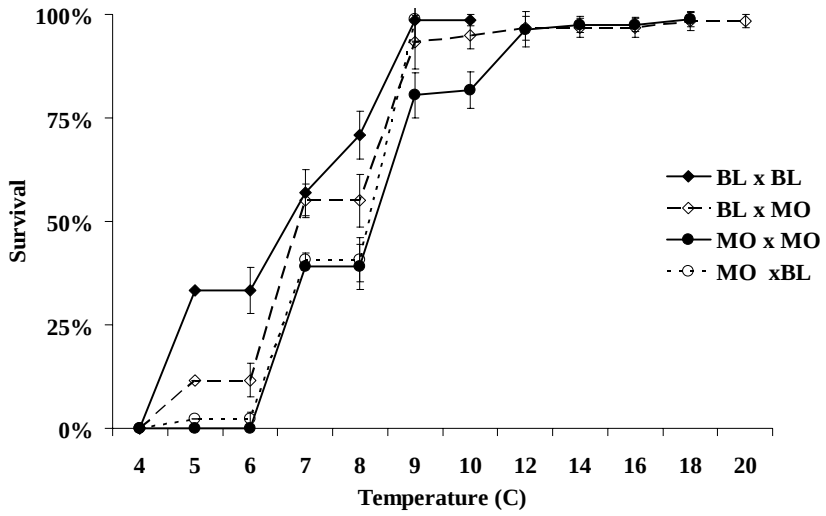

Figure 3.4. Cumulative survival of varieties BL and MO and their crosses (Mean ± SE).

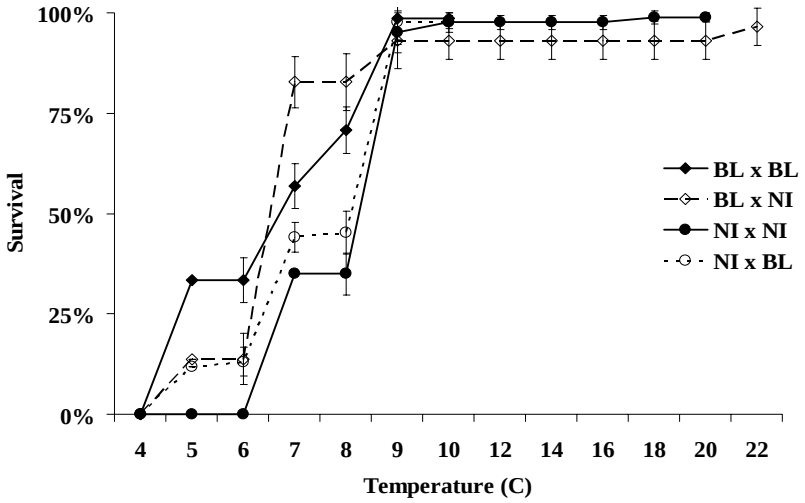

Figure 3.5. Cumulative survival of BL and NI varieties and their hybrids (Mean ± SE).

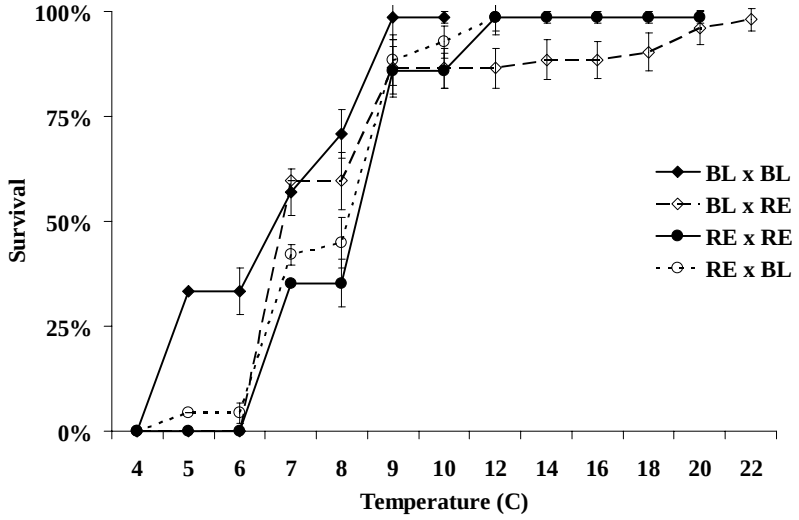

Figure 3.6. Cumulative survival BL and RE varieties and their crosses (Mean ± SE).

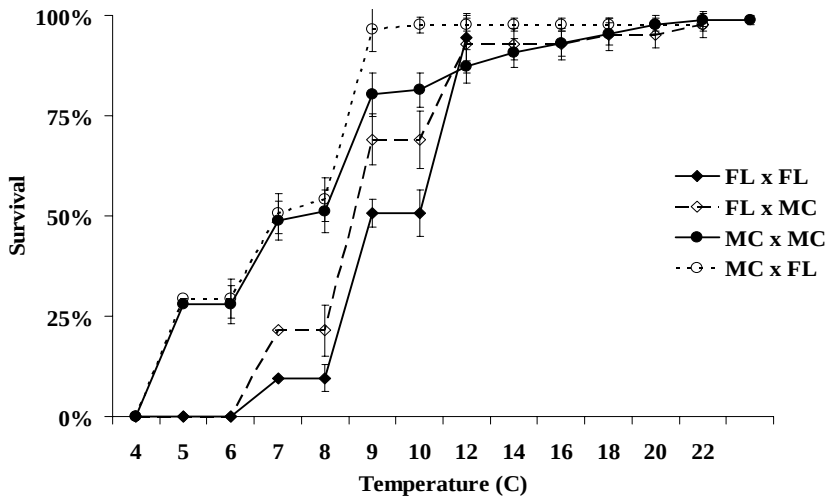

Figure 3.7. Cumulative survival of FL and MC varieties and their hybrids (Mean ± SE).

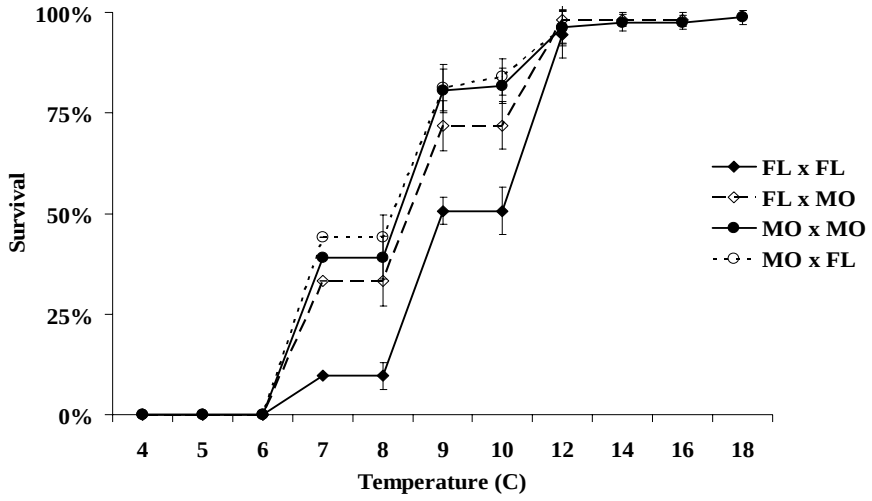

Figure 3.8. Cumulative survival of varieties FL and MO and their hybrids (Mean ± SE).

Among FL, RE, and their reciprocal crosses, purebred FL exhibited the lowest CS and CT (Figure 3.10). Cumulative survival curves suggested no differences among reciprocals and purebred RE. Potential heterosis was apparent.

Cumulative survival of MC and MO and their reciprocal crosses (Figure 3.11) indicated that the highest MTT was tolerance was exhibited by purebred MO. At temperatures between 10 and 7 C MO x MC was more cold-tolerant than other groups. Maternal effects and heterosis could each be inferred in the CS curve of the MO x MC cross.

Among MC, NI and their reciprocal crosses purebred MC exhibited the highest tolerance and NI exhibited the lowest tolerance (Figure 3.12). Reciprocal crosses exhibited CS closely resembling that of purebred NI. Maternal influences and possible heterosis were apparent.

Purebred RE and MC x RE exhibited lower CS than MC and RE x MC (Figure 3.13). Between 9 C and 10 C maternal effects and heterosis were suggested, but further analysis would be required for their determination.

Purebred MO and NI (Figure 3.14) exhibited lower CS between 7 C and 9 C than MO x NI. No significant differences ($\underline{P} > 0.05$) were found among groups. Lowest CS was exhibited by NI closely followed by MO, and all groups reached 0% survival at 6 C.

Purebreds MO and RE and their reciprocal crosses exhibited no significant differences in CS (Figure 3.15). MO exhibited higher tolerance than RE, and above 9 C, MO x RE exhibited lower temperature tolerance than their reciprocal crosses. Conversely, below 9 C, both parental varieties were less tolerant than their reciprocal crosses. Some degree of maternal effects and heterosis was apparent.

Cumulative survival of NI, RE and their reciprocal crosses indicated initial mortalities at 16C (Figure 3.16). Between 9 C and 7 C, CS of parental varieties and NI x RE drastically declined from a combined average of 85 % to 35%. Conversely, at 7 C, CS in RE x NI was 75%.

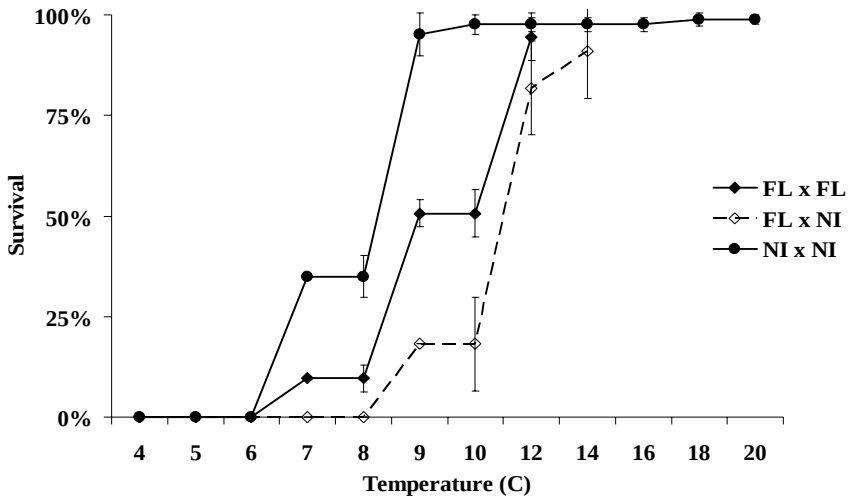

Figure 3.9. Cumulative survival FL and NI varieties and their cross (Mean ± SE).

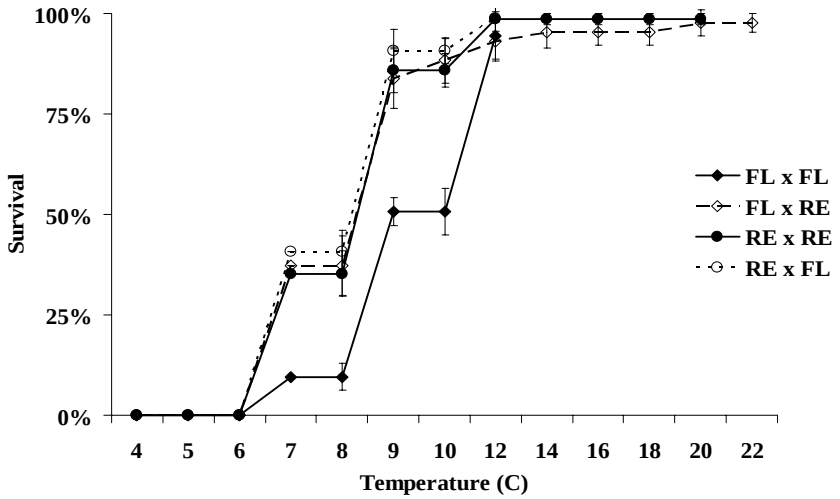

Figure 3.10. Cumulative survival of FL and RE varieties and their crosses (Mean ± SE).

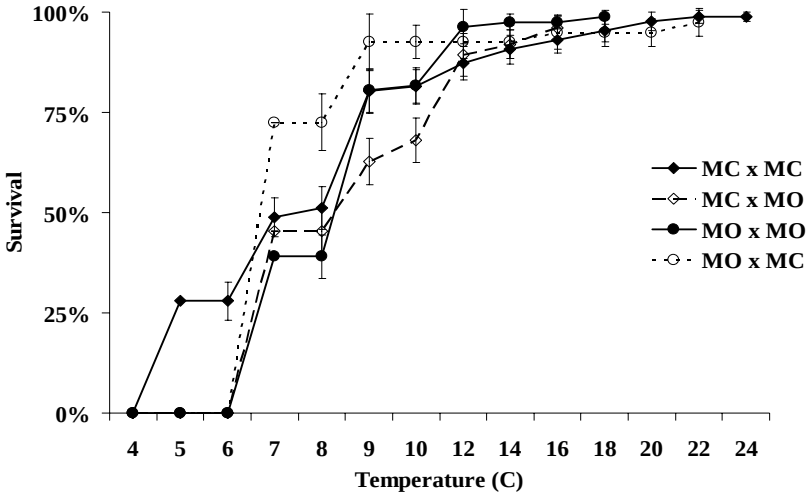

Figure 3.11. Cumulative survival MC and MO varieties and their crosses (Mean ± SE).

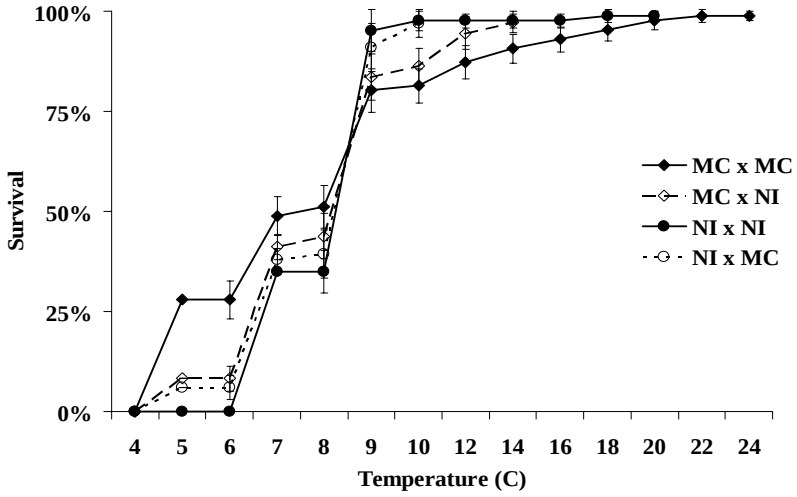

Figure 3.12. Cumulative survival of MC and NI varieties and their crosses (Mean ± SE).

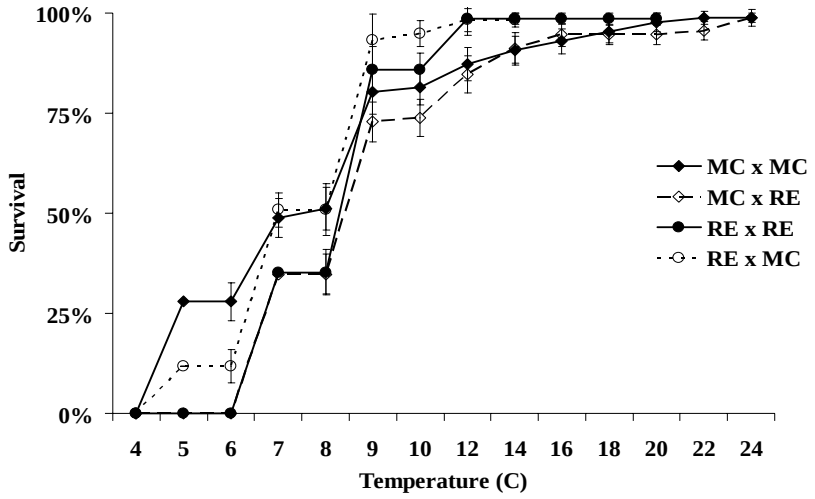

Figure 3.13. Cumulative survival of varieties MC and NI and crosses (Mean ± SE).

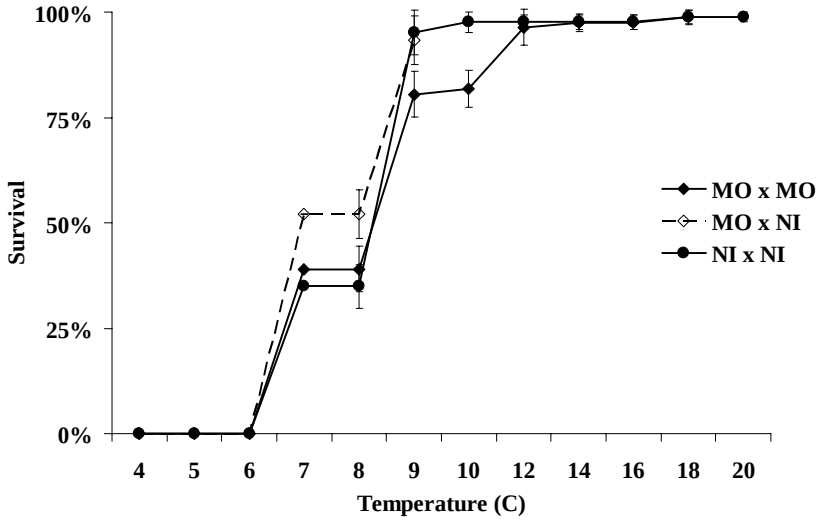

Figure 3.14. Cumulative survival of varieties MO and NI and their crosses (Mean ± SE).

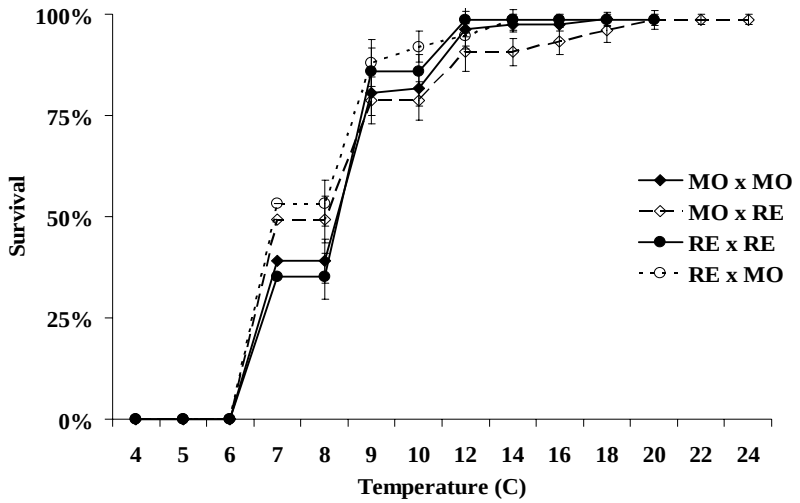

Figure 3.15. Cumulative survival MO and RE varieties and their crosses (Mean ± SE).

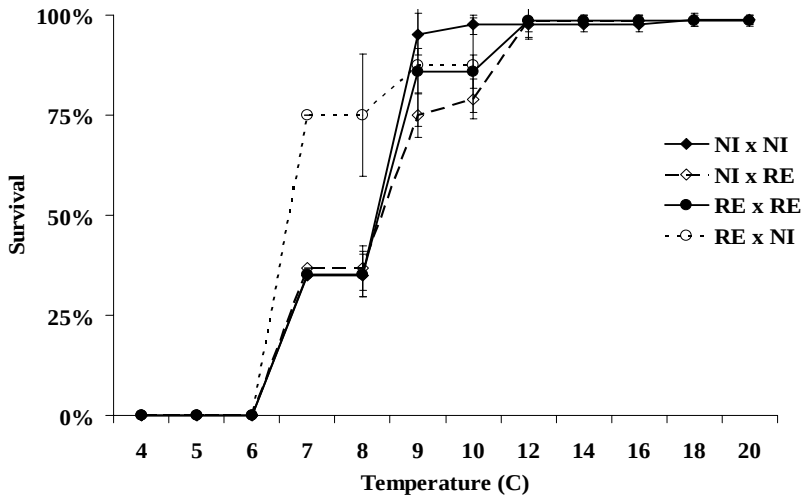

Figure 3.16. Cumulative survival NI and RE varieties and their crosses (Mean ± SE).

Best Linear Unbiased Predictors (BLUPs)

BLUPs (Table 3.5 and 3.6) indicated that crossing of MC dams with BL or MC sires produced offspring more cold-tolerant ($\underline{P} < 0.01$) than those than those produced by other dams and sires. Estimated values of two missing crosses were calculated using BLUPs. These estimates required information for NI, FL, and MO varieties, therefore, BLUPs based on NI (Table 3.5) were cross-referenced with BLUPs based on RE (Table 3.6). Estimated MTT for NI x FL and NI x MO were 7.7 C and 7.9 C, respectively. Values were appended to the original data set to conduct the diallel analysis.

Diallel Analysis

Mean temperature tolerance (MTT) of parental varieties and their crosses (Table 3.7) denoted that overall MTT and parental variety mean were both 7.6 C, indicating that most crosses exhibited negligible heterosis. Among purebreds, FL exhibited the highest MTT, and BL the lowest. No significant statistical differences ($\underline{P} > 0.05$) were found among NI, MC and RE, but were different from both FL and BL ($\underline{P} < 0.01$).

Genetic Effects

Net variety effect of sires ($\bar{y}*_i$) exhibited CT ranking of MC>FL>BL>NI>MO>RE.

Estimates of $\bar{y}*_i$ denoted that MC,FL and BL sires, if crossed individually with dams of other varieties would produce offspring with CT greater than 7.2 C. Variety ranking according to breed effect of dams ($\bar{y}*_j$) was BL>RE>MO>NI>MC>FL. Crossing BL dams to sires of all other varieties except BL to produce a synthetic variety would result in offspring with predicted MTT of 7 C. Ranking varieties according to their net cross effect (Equation 5) produced the following pattern: BL>MC>MO>NI>RE>FL.

Table 3.5. Best linear unbiased predictors for CT using the NI variety as an intercept.

Effect	Dam	Sire	Estimate		SE
Intercept			7.5663	***	0.2611
Dam	BL	--	-0.6007		0.513
Dam	Fl	--	2.6156	***	0.7631
Dam	MC	--	-0.1142		0.3816
Dam	MO	--	-0.5396		0.3789
Dam	RE	--	-0.8163		0.8805
Dam	NI	--	0	.	.
Sire	--	BL	-0.6615	**	0.3681
Sire	--	Fl	0.6453		0.8791
Sire	--	MC	-0.339		0.3922
Sire	--	MO	0.5167		0.8846
Sire	--	RE	0.2364		0.3776
Sire	--	NI	0	.	.
Dam*Sire	BL	BL	-0.2207		0.6396
Dam*Sire	BL	Fl	-0.7381		1.0348
Dam*Sire	BL	MC	-0.8952		0.6583
Dam*Sire	BL	MO	-0.4322		1.0353
Dam*Sire	BL	RE	0.9135		0.6681
Dam*Sire	BL	NI	0	.	.
Dam*Sire	Fl	BL	-1.0642		0.8654
Dam*Sire	Fl	Fl	-1.9231	*	1.1681
Dam*Sire	Fl	MC	-0.9857		0.896
Dam*Sire	Fl	MO	-2.6985	**	1.1815
Dam*Sire	Fl	RE	-2.3717	***	0.8879
Dam*Sire	Fl	NI	0	.	.
Dam*Sire	MC	BL	-0.1315		0.5266
Dam*Sire	MC	Fl	-1.3915		0.9575
Dam*Sire	MC	MC	0.48		0.5451
Dam*Sire	MC	MO	0.2713		0.9672
Dam*Sire	MC	RE	1.0616	**	0.5306
Dam*Sire	MC	NI	0	.	.
Dam*Sire	MO	BL	0.786		0.526
Dam*Sire	MO	Fl	-0.1253		0.9611
Dam*Sire	MO	MC	0.6623		0.6088
Dam*Sire	MO	MO	0.2493		0.9628
Dam*Sire	MO	RE	0.8436		0.5417
Dam*Sire	MO	NI	0	.	.
Dam*Sire	RE	BL	1.1724		0.9615
Dam*Sire	RE	Fl	0	.	.
Dam*Sire	RE	MC	0.5212		0.9782
Dam*Sire	RE	MO	0	.	.
Dam*Sire	RE	RE	0.7319		0.964
Dam*Sire	RE	NI	0	.	.
Dam*Sire	NI	BL	0	.	.
Dam*Sire	NI	MC	0	.	.
Dam*Sire	NI	RE	0	.	.
Dam*Sire	NI	NI	0	.	.

* $\underline{P} < 0.10$, ** $\underline{P} < 0.05$, *** $\underline{P} < 0.01$; Dots (.) are part of the BLUP calculation related to the intercept value.

Table 3.6. Best linear unbiased predictors for CT using the RE variety as an intercept.

Effect	Dam	Sire	Estimate		SE
Intercept			7.7183	***	0.2823
Dam	BL	--	0.3971		0.4341
Dam	FL	--	0.3282		0.4596
Dam	MC	--	1.0317	***	0.3757
Dam	MO	--	0.3884		0.3938
Dam	NI	--	0.08432		0.3926
Dam	RE		0	.	.
Sire	--	BL	-0.4574		0.4021
Sire	--	FL	-0.323		0.3814
Sire	--	MC	-0.7861	*	0.419
Sire	--	MO	-0.4516		0.3938
Sire	--	NI	-0.9683		0.887
Sire	--	RE	0	.	.
Dam*Sire	BL	BL	-1.5746	***	0.5908
Dam*Sire	BL	FL	-0.9197		0.5976
Dam*Sire	BL	MC	-1.5979	***	0.6072
Dam*Sire	BL	MO	-0.6137		0.5985
Dam*Sire	BL	NI	-0.1816		1.0443
Dam*Sire	BL	RE	0	.	.
Dam*Sire	FL	BL	0.8671		0.6264
Dam*Sire	FL	FL	1.1806	**	0.5954
Dam*Sire	FL	MC	1.5967	***	0.6647
Dam*Sire	FL	MO	0.4051		0.6212
Dam*Sire	FL	NI	3.1036	***	1.1969
Dam*Sire	FL	RE	0	.	.
Dam*Sire	MC	BL	-1.6335	***	0.5361
Dam*Sire	MC	FL	-1.7212	***	0.523
Dam*Sire	MC	MC	-0.3709		0.5503
Dam*Sire	MC	MO	-0.05836		0.5404
Dam*Sire	MC	NI	-0.3296		0.9621
Dam*Sire	MC	RE	0	.	.
Dam*Sire	MO	BL	-0.4981		0.5503
Dam*Sire	MO	FL	-0.237		0.5443
Dam*Sire	MO	MC	0.02944		0.6264
Dam*Sire	MO	MO	0.1377		0.5473
Dam*Sire	MO	NI	-0.1117		0.9683
Dam*Sire	MO	RE	0	.	.
Dam*Sire	NI	BL	-0.4404		0.5508
Dam*Sire	NI	MC	0.2107		0.5794
Dam*Sire	NI	NI	0.7319		0.964
Dam*Sire	NI	RE	0	.	.
Dam*Sire	RE	BL	0	.	.
Dam*Sire	RE	FL	0	.	.
Dam*Sire	RE	MC	0	.	.
Dam*Sire	RE	MO	0	.	.
Dam*Sire	RE	NI	0	.	.
Dam*Sire	RE	RE	0	.	.

* $\underline{P} < 0.10$, ** $\underline{P} < 0.05$, *** $\underline{P} < 0.01$; Dots (.) are part of the BLUP calculation related to the intercept value.

Table 3.7. Mean temperature tolerance (MTT) of parental varieties and crosses produced in two diallel crosses (n = 2229 includes data from two missing crosses). Shaded cells denote purebred crosses.

Sire	Dam							
	BL	FL	MC	MO	NI	RE	$\bar{y}_i$	$\bar{y}^*_i$
BL	6.1	8.5	6.7	7.2	6.9	7.3	7.1	7.3
FL	6.9	8.9	6.7	7.6	7.7	7.4	7.5	7.2
MC	5.8	8.9	7.6	7.4	7.2	6.9	7.3	7.2
MO	7.0	8.0	8.2	7.8	7.9	7.3	7.7	7.7
NI	7.0	10.2	7.5	7.0	7.6	6.8	7.7	7.67
RE	8.1	8.1	8.8	8.1	7.8	7.7	8.1	8.2
$\bar{y}_j$	6.8	8.8	7.6	7.5	7.5	7.2		
$\bar{y}^*_j$	7.0	8.7	7.6	7.5	7.5	7.1		

Parental mean = 7.56 $\bar{y}_i$ = mean of sire line *i*; $\bar{y}^*_i$: Net variety effect of sires *i*

Overall mean = 7.61 *** $\bar{y}_j$ = mean of dam line *j*; $\bar{y}^*_j$: Net variety effect of dams *j*

Estimates of direct line effects (l_i) and maternal genetic effects (m_i) are presented in Table 3.8. Line effects estimates indicate BL offspring were significantly more cold-tolerant ($P < 0.01$) than those of all other varieties. The BL offspring were able to tolerate temperatures 1.3 C lower than the mean of the parental varieties. On the contrary, RE offspring were significantly ($P < 0.05$) less cold-tolerant than those of other varieties, tolerating 0.98 ± 0.33 C more than the mean of all parental varieties.

Maternal effects estimates (m_i) indicated that RE dams would significantly ($\underline{P} < 0.01$) contribute towards improving the CT of their offspring. To a lesser extent than RE dams, BL dams would also contribute ($\underline{P} < 0.10$) towards offspring CT. The FL variety dams would significantly ($\underline{P} < 0.01$) decrease CT of their offspring.

Estimates of general combining ability (GCA) and direct heterosis (h_i) are presented in Table 3.9. Estimates of GCA indicated that among all parental varieties, BL would provide the most significant ($\underline{P} < 0.05$) contributions in combinations for CT. Similarly, MC would also improve CT ($\underline{P} < 0.05$) in its offspring. On the contrary, the RE variety would significantly ($\underline{P} < 0.05$) reduce CT when used to produce crosses with other varieties. Mean heterosis ($\bar{h}$) for CT was not significant ($\underline{P} > 0.05$), indicating that overall crosses were not different than the parental mean. No significant heterosis (h_i) was exhibited among varieties; however, varieties with negative h_i estimates (FL, MC and MO) contributed to cross heterosis (Equation 2).

Table 3.8. Least squares estimates of direct line genetic effect (l_i) and maternal effects (m_i) in tilapia varieties (Estimate ± SE.)

Variety	l_i			m_i		
BL	-1.24	± 0.30	***	-0.28	± 0.16	*
FL	0.07	± 0.50		1.22	± 0.44	***
MC	-0.30	± 0.28		0.28	± 0.15	**
MO	0.39	± 0.49		-0.21	± 0.42	
NI	0.10	± 0.64		-0.15	± 0.60	
RE	0.98	± 0.33	**	-0.87	± 0.20	***

* $\underline{P} < 0.10$, ** $\underline{P} < 0.05$, *** $\underline{P} < 0.01$

Table 3.9. Least square estimates of general combining ability (GCA), and line heterosis (h_i) in tilapia varieties (Estimate ± SE.)

Variety	GCA		h_i	
BL	-0.39 ± 0.19	**	0.23 ± 0.22	
FL	-0.07 ± 0.42		-0.11 ± 0.28	
MC	-0.34 ± 0.20	*	-0.19 ± 0.21	
MO	0.12 ± 0.42		-0.07 ± 0.27	
NI	0.13 ± 0.22		0.07 ± 0.33	
RE	0.56 ± 0.19	**	0.07 ± 0.22	
$\bar{h}$	--	--	-0.06 ± 0.17	

* $\underline{P} < 0.10$, ** $\underline{P} < 0.05$, *** $\underline{P} < 0.01$

In Tables 3.10 and 3.11 crosses are combined as variety combinations (F1 and reciprocal crosses together). For example, the variety combination (VC) of MO-BL would include the F1 MO x BL (Dam x Sire) and its reciprocal cross BL x MO. Statistical comparisons were done only within VCs and not among VCs.

Estimates of specific heterosis (S_{ij} = SCA) and cross heterosis (h_{ij}) and percent cross heterosis (h_{ij} %) are presented in Table 3.10. Negative and significant S_{ij} ($\underline{P} < 0.01$) was found only in BL-MC, indicating that this variety combination contributed to improving cross heterosis despite non-significant direct heterosis found in the parental varieties. Similarly, the specific combination of FL-RE would improve CT in their offspring. On the contrary, BL-RE would not contribute ($\underline{P} < 0.01$) to increase CT in the crosses involved in that VC.

Estimates of h_{ij} ranged from -9.36% to +11.45%. Four of the fifteen VCs exhibited significant h_{ij} ($\underline{P} < 0.05$), and three of those (BL-MC, FL-MO, and FL-RE) exhibited improved

CT of 6.8 – 9.3% over their parental varieties. The highest observed positive h_{ij} was significant ($\underline{P} < 0.05$) for BL-RE, indicating that this VC would yield crosses with little CT improvement.

Table 3.10. Estimation of cross heterosis (h_{ij}), specific heterosis (S_{ij}), derived from a diallel cross in six varieties of tilapia.

Variety combinations[1]	h_{ij} ± SE.		h_{ij} %	S_{ij} ± SE.	
BL-FL	0.17 ± 0.30		2.34	0.12 ± 0.28	
BL-MC	-0.64 ± 0.27	***	-9.36	-0.62 ± 0.24	***
BL-MO	0.16 ± 0.27		2.38	0.07 ± 0.27	
BL-NI	0.11 ± 0.32		1.61	-0.13 ± 0.32	
BL-RE	0.79 ± 0.29	***	11.45	0.56 ± 0.25	***
FL-MC	-0.47 ± 0.29		-5.58	-0.11 ± 0.28	
FL-MO	-0.58 ± 0.28	**	-6.83	-0.33 ± 0.31	
FL-NI	0.70 ± 1.26		8.50	0.80 ± 0.77	
FL-RE	-0.59 ± 0.30	**	-7.04	-0.48 ± 0.22	*
MC-MO	0.10 ± 0.30		1.37	0.42 ± 0.28	
MC-NI	-0.24 ± 0.26		-3.17	-0.06 ± 0.31	
MC-RE	0.19 ± 0.28		2.42	0.37 ± 0.24	
MO-NI	-0.23 ± 1.21		-2.99	-0.17 ± 0.75	
MO-RE	-0.07 ± 0.27		-0.84	0.00 ± 0.27	
NI-RE	-0.37 ± 0.49		-4.84	-0.44 ± 0.38	

* $\underline{P} < 0.10$, ** $\underline{P} < 0.05$, *** $\underline{P} < 0.01$ [1] Includes reciprocal hybrids

Reciprocal effects (r_{ij}) and specific reciprocal effects (r^{**}_{ij}) are presented in Table 3.11. Negative r_{ij} values indicated that in FL-MC, MC dams produced offspring of superior CT than those produced by FL dams. Highly significant and negative r_{ij} ($\underline{P} < 0.01$) were exhibited by FL-MC as well as MC-RE. Differences in MTT of 2.15 C were observed in FL-MC, and in MC-RE, using RE dams instead of MC dams resulted in a reduction of 1.82 C in MTT of the offspring.

Table 3.11. Estimation of reciprocal effects (rij) and maternal specific reciprocal effects (r**ij) influencing cold tolerance trials in six varieties of tilapia.

Variety combinations[1]	rij ± SE.			r**ij ± SE.			
BL-FL	0.79	±	0.22 ***	0.04	±	0.27	
BL-MC	0.46	±	0.19 **	0.18	±	0.16	
BL-MO	0.05	±	0.20	0.01	±	0.26	
BL-NI	-0.03	±	0.25	-0.10	±	0.35	
BL-RE	-0.43	±	0.22 **	-0.13	±	0.19	
FL-MC	-1.08	±	0.22 ***	-0.61	±	0.27	**
FL-MO	-0.23	±	0.21	0.49	±	0.33	
FL-NI	-1.25	±	1.24	-0.56	±	0.86	
FL-RE	-0.33	±	0.22	0.72	±	0.28	**
MC-MO	-0.45	±	0.24 **	-0.20	±	0.27	
MC-NI	-0.11	±	0.20	0.10	±	0.34	
MC-RE	-0.91	±	0.20 ***	-0.33	±	0.17	*
MO-NI	0.42	±	1.20	0.39	±	0.84	
MO-RE	-0.42	±	0.19 **	-0.09	±	0.26	
NI-RE	-0.53	±	0.44	-0.17	±	0.42	

* $\underline{P} < 0.10$, ** $\underline{P} < 0.05$, *** $\underline{P} < 0.01$ [1] Includes reciprocal hybrids

Significantly and negative r_{ij} ($\underline{P} < 0.05$) were exhibited by BL-RE, MC-MO and MO-RE. In BL-RE, RE dams produced more cold-tolerant offspring than BL dams. Similarly, in MO-RE, MO dams produced more cold-tolerant offspring. Significant and positive r_{ij} ($\underline{P} < 0.05$) was only exhibited by BL-FL, in which the MTT of the offspring produced by BL x FL parents was 1.58 C more than in offspring produced by FL x BL parents.

Significant negative r^{**}_{ij} ($\underline{P} < 0.05$) was exhibited in FL-MC. In this VC, MC dams may have contributed more of a cytoplasmatic effect on offspring CT than FL dams (Eisen et al. 1983). On the contrary, significant and positive r^{**}_{ij} ($\underline{P} < 0.05$) were only exhibited by BL-FL.

Discussion

Differences Among Parental Varieties

Results from both diallels demonstrated that five of the six parental varieties were not statistically different in MTT. Lethal lower temperature for the BL variety was between 4 and 5 C. This value was lower than previous reports for O. aureus of 6 C (Zale and Gregory 1989), 7.3 C (El Gamal 1987), 8.9 C (McBay 1961), 9C (Chervinsky and Lahav 1976), and 10.9 C (Starling et al. 1995). In addition, MTT of BL (6.08 C) was much lower than the MTT value of 11.6 C described for O. aureus (Behrends and Smitherman 1984). Lethal temperature for MC was between 4 and 5 C, and MTT was 7.59C. These values were in close agreement with the MTT of 7.5 C reported for the same population of Mississippi commercial strain fish (Paz 2004) exposed to a moderate temperature reduction rate (-1 C/24 h). Lethal temperature for FL in this trial was between 6 and 7 C, while the observed MTT was 8.9 C, lower than the 9.5 C value reported by Paz (2004) for the same variety. No previous reference can be given regarding the lethal temperature of the crossbred RE. In this study, lethal temperature of the varieties RE and NI ranged between 6 and 7 C, while MTTs were 7.72 C and 7.57 C respectively. Phenotypic differences in color between RE and NI were not related to CT. Similarly, no statistical

differences in CT between normal and red phenotypes of F1 hybrids of O. aureus and O. mossambicus have been reported (Behrends and Smitherman 1984).

Lethal temperature for NI (A-E line) was between 6 and 7 C, similar to the 6.8 C value reported for O. niloticus (Atwood et al. 2003). Lethal temperature of NI in this study was lower than reported in various O. niloticus strains, such as: Sudan 78 (8.2 C), Egyptian (8.3 C), Egypt 88 (8.2 – 8.5 C), GIFT (8.8 – 9 C), Auburn-Egypt (10 C), Ivory Coast (12.2 C), and Ghana (14.1 C) (El Gamal 1987; Khater and Smitherman 1988; Sifa et al. 2002). Mean temperature tolerance of NI (7.57 C) in this study was similar to a reported value for O. niloticus of 7.4 C (Behrends et al. 1990), but higher than the MTT of 5C reported by Yashouv (1960) in outdoor ponds.

It is possible that exposure of parental varieties used in this study to outdoor conditions up to late October with air temperatures ranging from 2.7 C to 25 C (LSU Agriclimatic Station), and subsequent overwintering in recirculating systems at the ARS has contributed towards improvement in CT over time within the populations. Similar improvement over time has been reported for O. niloticus (Sifa et al. 2002). Parental varieties NI and RE did not show significant differences in CT. However, in combination with other varieties, their offspring exhibited a wide range of CT due to maternal and line effects.

Lethal temperature for MO in this study was between 6 and 7 C. This value was lower than lethal temperatures previously reported for O. mossambicus of 8C (Chimits 1957; Popper and Lichatovich 1975), and 9.5 C (Shafland and Pestrak 1982; Behrends et al. 1990). In addition, MTT (7.8 C) was lower than previous reports of 9.5 C for O. mossambicus (Behrends et al. 1990), although their strain and the O. mossambicus used in this research (thesis) both originated from South Africa.

Differences Among Crosses

More than 50% of crosses exhibited MTT lower than, but not significantly different (P< 0.05) form the parental mean (7.6 C). These relatively small variations are often genetically based (Cnaani et al. 2000) and may denote the influence of additive genetic effects in the parental varieties tested. The fact that crosses produced using FL dams had MTT above 8.00 C may reflect that the FL was developed for salinity tolerance rather than cold tolerance. On the contrary, MC offspring may have generally improved CT due to the many generations of pond production and overwintering in temperate climates.

Two variety crosses, NI x MO and NI x FL did not produce offspring over two consecutive years. Unsuccessful production of the NI x MO has been previously reported (Mateo et al. 2004, Kamal and Mair 2005. Our estimations using BLUPs indicated that in the absence of significant specific heterosis, both NI x MO and NI x FL would have been 0.08 C and 0.17 C above the parental mean, respectively.

Genetic Effects Influencing CT

Inclusion of parental varieties in the diallel allowed estimation of line effects (Gardner and Eberthard 1966). The superior CT of BL over other species has been widely reported (see previous sections), while reports on CT of MC are limited (Paz 2004). Although the line effects of RE were inferior to the other five parental varieties, RE maternal effects were significant, increasing CT of their offspring by 0.9C from the parental mean.

Maternal effects have been described for reproductive traits and growth in tilapia (Siraj et al. 1983, Yapi-Gnaore 1996), but little information is available on maternal effects influencing CT. In this study, the influence of maternal effects on CT was similar to previous reports in O. niloticus (A-E and Ivory Coast strains) where additive genetic effects largely determined cold tolerance in parental varieties (Tave et al. 1990). Maternal effects in many species have been

reported to decrease with offspring age (Siraj et al. 1983, Heath et al. 1999), however, in tilapia the maternal effects on offspring temperature tolerance may last until the second hemoglobin type is developed (at 50 d) and fish are able to better maintain osmoregulation when exposed to stressful temperatures. The fish used for in CT trials were older than 50 d at the time of testing.

Specific heterosis (S_{ij}) was a determining genetic component in crosses exhibiting higher CT than their parental varieties. For example, in BL-MC dominance effects were obvious, improving CT of the crosses. Additional examples were FL-MO and FL-RE, in which more than half of cross heterosis resulted from S_{ij} and not from h_i; hence, the importance of S_{ij} in development of cold-tolerant crosses with potential commercial exploitation.

Reciprocal effects were potentially beneficial for CT in 10 of the 15 VCs, however, they were significant only in BL-RE, FL-MC and MC-RE. A similar situation was apparent in MC-RE. Benefits of using RE dams in cold-tolerant crosses may not only be justified by their maternal effects, but also by their specific reciprocal effects. In FL-MC, MC dams contribute a more cold-tolerant genotype to their offspring than FL.

Specific reciprocal effects were found to improve CT in MC-RE and FL-MC. Differences between substitution of MC egg cytoplasm and RE egg cytoplasm in MC-RE resulted in offspring with superior CT in RE x MC. Benefits of crossbreeding in RE Nile tilapia may have contributed through cytoplasmatic effects to improvement of CT in its offspring. Similar results were also reported in Egypt and Ivory Coast strains of O. niloticus (Tave et al. 1990). In addition, differences in FL egg cytoplasm and MC egg cytoplasm improved CT in MC x FL over the reciprocal cross. The cytoplasmatic origin of specific reciprocal effects may be a result of mitochondrial DNA (mtDNA), however, further studies are needed to determined its influence on traits of economic importance such as cold tolerance.

Conclusions

Based on the results of this study, it may be possible to improve commercial tilapia production under low temperatures by taking advantage of additive genetic variance (V_A) in selecting the most cold-tolerant fish in the BL variety. In addition, RE dams should be considered due to their contribution towards offspring CT. Besides selection, the combined used of hybridization would exploit dominance variance (V_D) along with backcrossing to improve CT in certain varieties as well as crosses. Best CT would be expected in BL-MC and FL-RE.

Findings presented in this study could be used to assist in estimating the potential range of expansion of tilapia production in temperate and high elevation areas. It is possible to infer that certain crosses would be highly cold-tolerant and perhaps could develop populations in areas where temperatures decline below 10 C for short periods. It is recommended that biosecurity protocols be developed and conducted to prevent the potential environmental impact that wild tilapia populations may cause on natural fish populations.

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CHAPTER 4
SUMMARY AND CONCLUSIONS

Interest in developing salinity-tolerant and cold-tolerant tilapia has increased over the last two decades due to the socio-economic impact of their culture worldwide. To date, little scientific research has taken into account the genetic effects influencing the phenotypic expression of these traits. Some breeding programs have tried to improve growth under a wide range of salinity environments (Bentsen et al. 1998, Tayamen et al. 2002) and cold tolerance under winter temperatures (Behrends and Smitherman 1984).

Diallel crosses and their interpretation were essential in the determination of the genetic effects influencing salinity and cold tolerance. Based on the information presented in Chapters 1 and 2, salinity tolerance (ST) and cold tolerance (CT) in tilapia may be improved by taking advantage of additive genetic effects through inbreeding and dominance genetic effects by hybridization or crossbreeding. The combined use of selection along with hybridization and backcrossing could be used to improve either ST or CT in tilapia (Figure 4.1). In Figure 4.1, the goal is to develop a salinity-tolerant synthetic using varieties with high GCAs, such as FL and MO. Initially, selection is applied on parental varieties (individuals or families, depending on the breeding program) to take advantage of any potential additive genetic effects (V_A) in parental varieties. In cases where there is little additive genetics variance, it is expected that ST would be highly influenced by dominance effects (V_D), therefore, hybridization of FL dam and MO sires would be necessary. FL dams would be selected due to their maternal effects and NI variety due to their commercial potential. Hybridization would result in F1s with a certain level of cross heterosis (Equation 2, Figure 2.5 and Table 2.11). To continue the improvement of ST in these crosses, F1 fish would be backcrossed to BL dams to take advantage of their maternal effects. Breeding techniques have been previously used in tilapia research (Behrends and Smitherman

1984, Mateo 2004). Such breeding programs will involve extensive planning, human and economic resources and knowledge of the biology of each species (Tave 1993, Gjedrem 2005, Gjerde 2005).

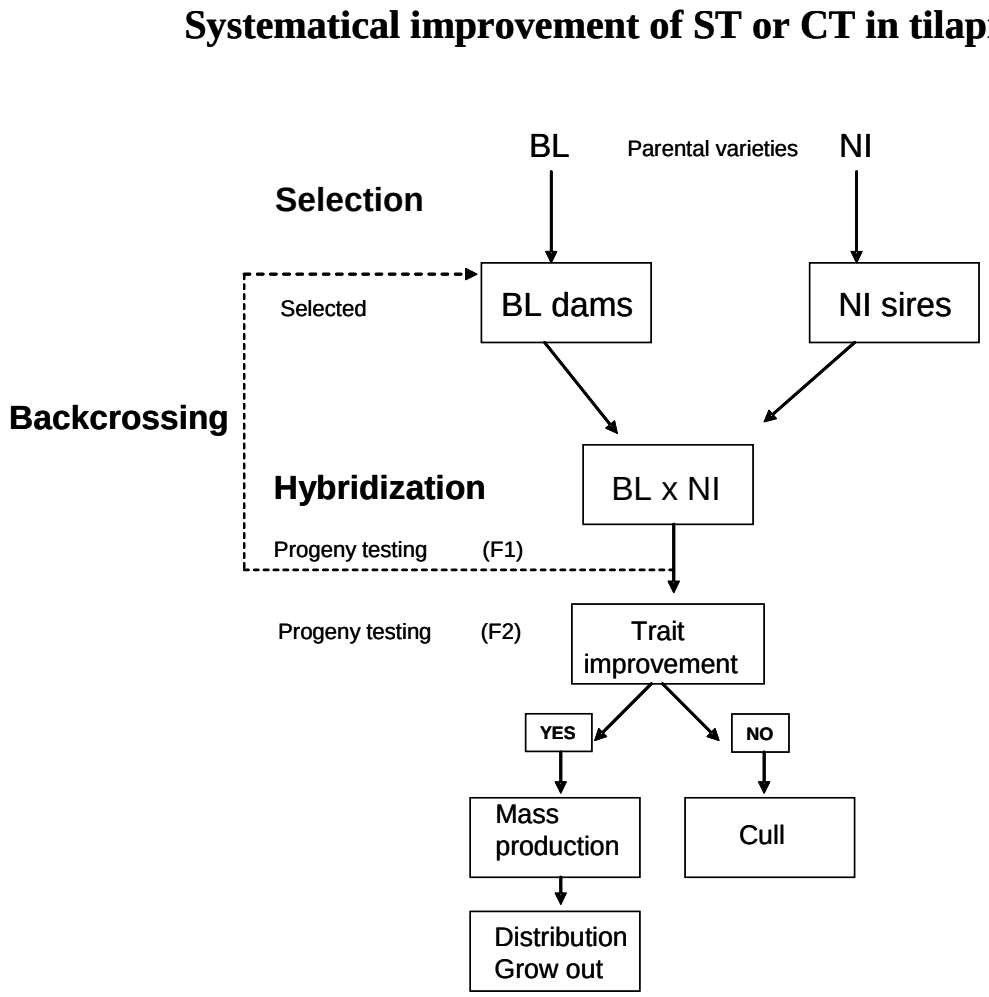

Figure 4.1. Systematic improvement of ST or CT in tilapia.

The objectives of this research produced valuable information regarding genetic influences over ST and CT in tilapia varieties and their hybrids. Trials resulted in: 1) the evaluation of ST and CT in six parental varieties and their reciprocal crosses, 2) development of Best linear unbiased predictors (BLUPs) to estimate parental contributions to offspring ST and

CT, 3) estimation Mean Salinity Tolerance (MST) and Mean Temperature Tolerance (MTT) in two missing crosses, 4) and estimation of the relative influences of additive, dominance, and maternal genetic effects on ST and CT.

Salinity Tolerance Trials

The most tolerant parental varieties were MO and FL, exhibiting CS_0 of 84 and 77 ppt, respectively (Figure 2.1). From the initial salinity tolerance analysis, it was apparent that MO and FL dams (Figure 4.2) could improve ST in their offspring, a conclusion which was subsequently corroborated and quantified in the diallel analysis. Data represented in Figure 4.2 does not include information on the interaction between salinity and temperature and the influence of both factors on fish biology, particularly in osmoregulation; therefore inferences should be carefully formulated.

Specific heterosis and maternal effects were important influences on ST. Among crosses, NI x RE exhibited the lowest MST (25.2 ppt) and maximum lethal salinity at 49 ppt. Perhaps more important from an environmental standpoint, the highest maximum lethal salinities were exhibited by MO x BL and MO x FL (both tolerating up to 84 ppt), and FL x MO and FL x MC (tolerating up to 77 ppt) (Table 2.5).

Genetic line effects exhibited by parental varieties indicated that varieties FL, MO and BL were the most tolerant, in close agreement with MST and survival curves. Although RE exhibited a highly negative line effect, it also presented potential to improve ST of offspring when used as the female in crosses due to maternal effects.

This study further illustrated that choice of dams was not only important in providing maternal effects when crossing varieties, but also in influencing ST through reciprocal and specific reciprocal effects. Reciprocal effects were highly significant in CVs such as BL-MO (Figure 2.4), BL-NI (Figure 2.5) and FL-MC (Figure 2.7).

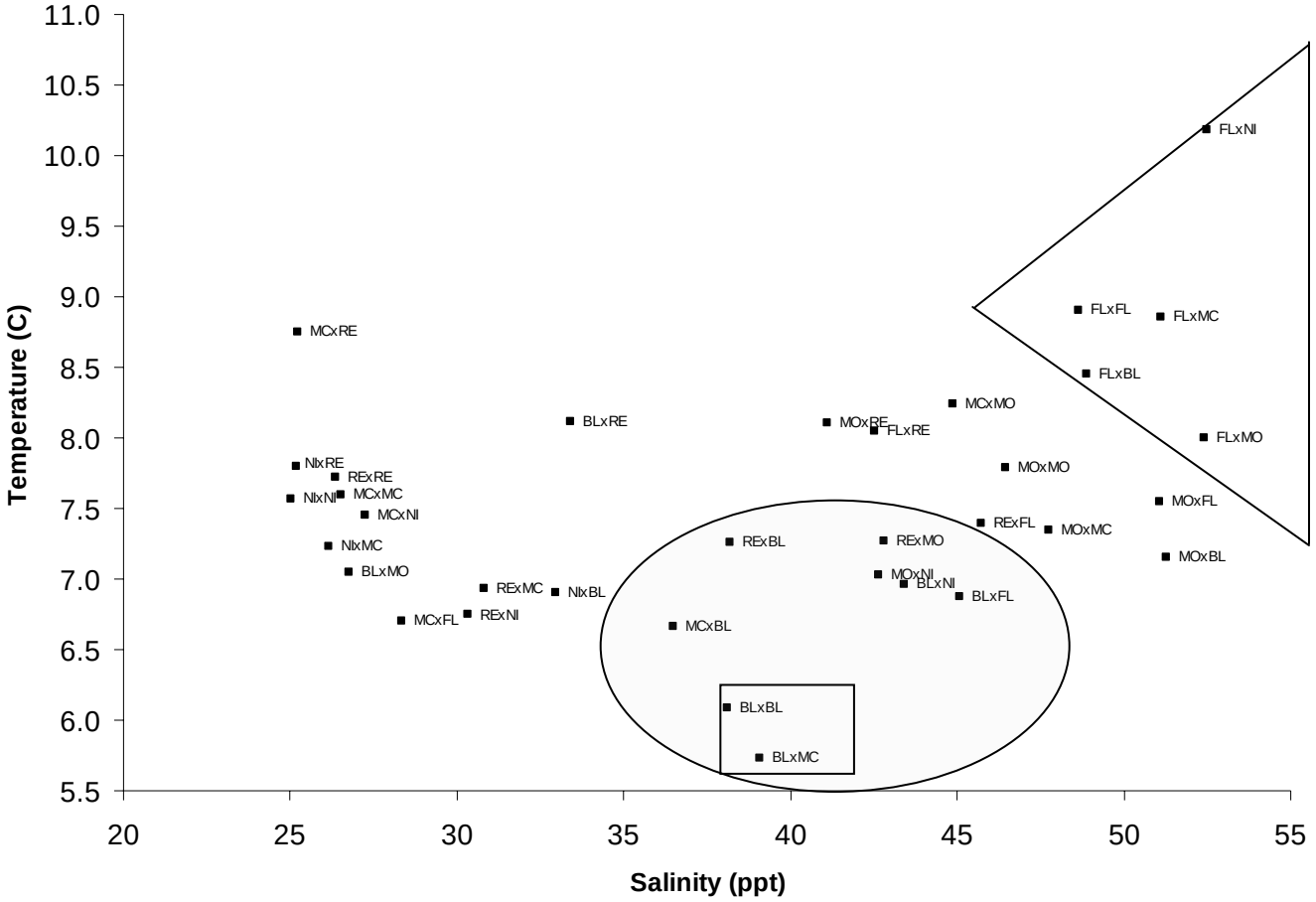

Figure 4.2. Combined ST and CT of parental varieties and crosses developed in this research (Mean). Most salinity tolerant crosses (inside triangle), most cold tolerant (inside square). Potentially most ST and CT crosses (inside oval).

Environmental and economic salinity thresholds (Table 2.5) were valuable measures in addition to minimum and maximum salinity tolerance among parental varieties and crosses. These values set a precedent for researchers to produce comparable information which can be readily applied in commercial, environmental or regulatory decision making.

From a commercial perspective, Table 2.5 could be used to infer that certain crosses may have the potential to be cultured in shrimp ponds either as primary culture stocks or as rotational crops, but the impact of salinity tolerance in tilapia goes beyond the production of revenue for the high end producer. Most shrimp culture is practiced in developing countries where tilapia polyculture may contribute towards the development of local communities through employment, nutrition and economic impact.

From an environmental perspective, there is a risk that if produced in commercial settings, some of these varieties or their crosses may escape and reproduce in natural waters. This could have detrimental effects in aquatic environments, as tilapia would compete for food sources and spawning grounds with native fish populations (Chapter 2). In this regard, Table 2.5 may provide a point of reference for regulatory agencies in the development of policies and bio-security protocols to assess and prevent the risk of such invasions in areas with specified salinity ranges. Although tilapia may readily become established in freshwater and brackish water, they may not gain establishment in coastal marine areas as easily due to salinity fluctuations, disruption of nest building and reproduction in inter-tidal zones or the presence of predatory marine fishes in such areas (Watanabe et al. 2006). However, tilapia populations have already been reported in marshes in Southern Mississippi in areas surrounding thermal effluents (Peterson et al. 2004, 2005).

Cold Tolerance Trials

Breeding programs could be developed to increase CT and take advantage of available genetic effects. Hybridization in this study produced BL x MC which exhibited lower cold tolerance than the parental BL variety, but was not significantly different from it. In addition, contribution of heterosis was non significant for CT, therefore, recurrent selection is suggested to method to improve this trait. In this study, phenotypic variance (V_P) was mainly determined by the limited genetic variance (V_G) exhibited by the standard errors of parental varieties. Such small SE (0.26 to 0.28, Table 3.1) would not allow the improvement of the population mean. Evaluation of genetic effects presented in this thesis may provide an insight to commercial operations and environmental agencies regarding points to be considered before cold tolerant crosses are developed.

From an environmental perspective, there is the potential that some of these crosses may escape culture conditions and reproduce in natural waters. On the negative side, there is the potential that cold tolerance of such species improves over time due to the additive genetic effects controlling this trait. On the positive side, however, the limited phenotypic variance exhibited by parental varieties would reduce the potential for adaptation and improvement of CT in the wild. In addition, the range of geographic distribution would be determined by the specific CT of each varieties or cross. Results obtained in these trials suggest that potential for escaped tilapia to adapt to natural environments in temperate climates is very low because of small additive effects, limited phenotypic variance, and large dominance exhibited by these varieties and hybrids.

The potential for the development of crosses exhibiting simultaneous ST and CT should be considered in temperate regions with abundant brackish water, where outdoor growing seasons are less than 150 d /year. The plotting of the crosses produced in this study could be

used as a point of reference for the potential development of a cool and brackish water tolerant cross (Figure 4.2). Crosses presented in the ovals could be considered as potential candidates for cool water and brackish water culture.

Two selection methods could be used to simultaneously improve ST and CT in tilapia. The first one, independent culling requires the fish pass a predetermined minimal standard in both traits being selected (Tave 1993, Lutz 2001). Fish should excel in both traits not only in one. In addition, this type of selection will reduce variability in the population potentially influencing genetic drift and inbreeding. The second selection method, selection index is more efficient. Selection index requires the input of all phenotypic information for each fish, then all data for each desirable trait are calculated resulting in an index. Individual fish are selected based on their numerical score (Tave 1993).

When considering the production of such crosses, the combined estimates of each genetic effect for ST and CT should be considered, such as GCAs (Figure 4.3), line effects (Figure 4.4), maternal effects (Figure 4.5) and direct heterosis (Figure 4.6). Similar to Figure 4.2, data presented in Figures 4.3 to 4.6 exhibited simultaneous data plots; however, these data do not reflect interaction between salinity and temperature on the fish tested.

The evaluation of ST and CT in these trials was conducted in controlled laboratory conditions with no feeding. If some of the crosses developed were to be considered as potential candidates for commercial production, further research will be needed to determine their growth, survival, FCR, disease resistance, and the effects of genotype x environment interactions.

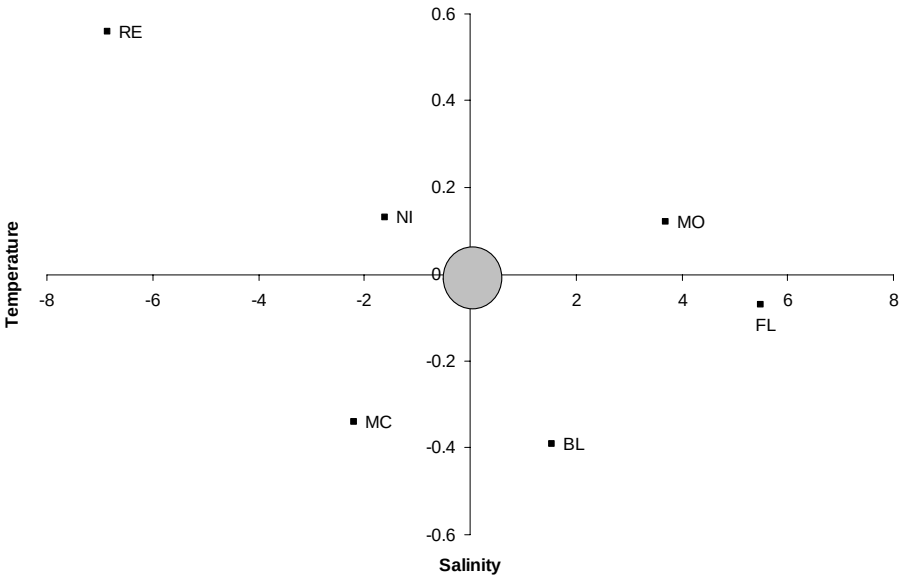

Figure 4.3. Combined GCAs estimates for ST and CT exhibited by parental tilapia varieties (Means). Parental means for ST and CT are represented in the shaded circle.

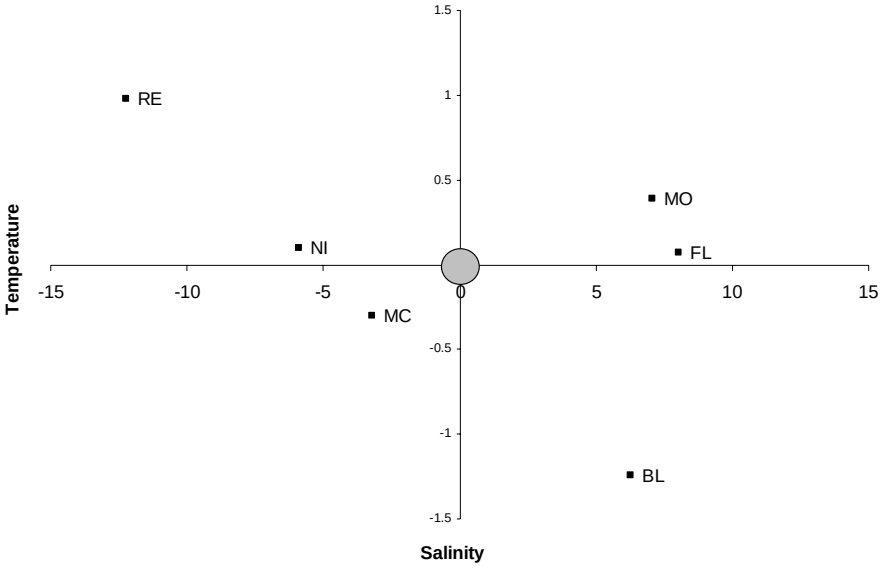

Figure 4.4. Combined line effects estimates for ST and CT exhibited by parental tilapia varieties (Means). Parental means are represented in the shaded circle.

Figure 4.5. Combined maternal effect estimates for ST and CT exhibited by parental tilapia varieties (Means). Parental means are represented in the shaded circle.

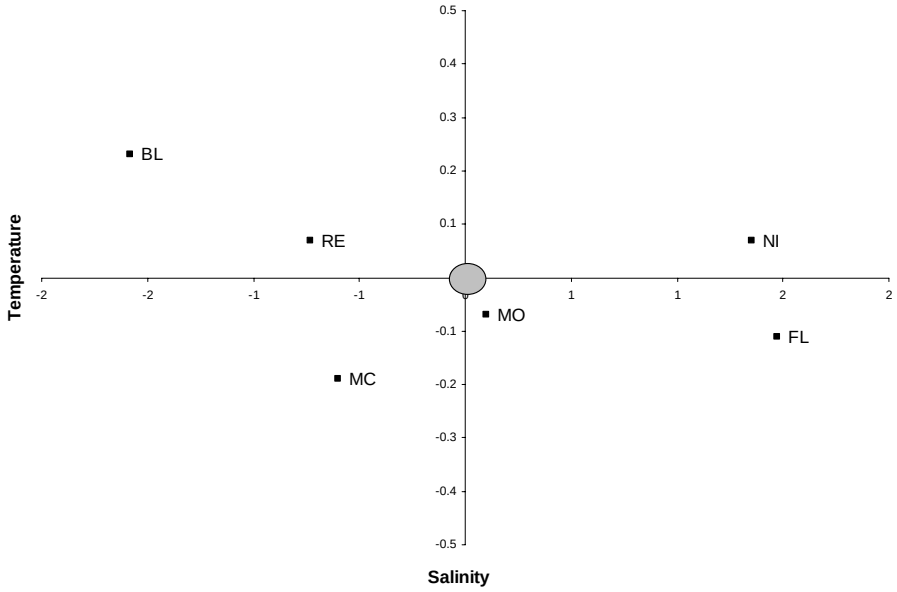

Figure 4.6. Combined direct heterosis estimates for ST and CT exhibited by parental tilapia varieties (Means). Parental means are represented in the shaded circle.

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APPENDIX A – ABBREVIATIONS USED IN THIS THESIS

Salinity

ST	salinity tolerance
ppt	parts per thousand
MST	Mean salinity tolerance

Temperature

CT	cold tolerance
C	degrees Celsius
MTT	Mean temperature tolerance

Survival

CS	Cumulative survival
CS_0	Cumulative survival 0% mortality
CS_{15}	Cumulative survival 15% mortality
CS_{100}	Cumulative survival 100% mortality

Tilapia varieties and crosses

BL	Blue tilapia - Oreochromis aureus
FL	Florida red tilapia
MC	Mississippi commercial strain
MO	Mosambique tilapia - Oreochromis mossambicus
NI	Nile tilapia - Oreochromis niloticus
RE	Red Nile tilapia - Oreochromis niloticus (crossbred)
BL x MC	Cross of BL (dam) by MC (sire)
VC	Variety combination = Combination of a cross and its reciprocal
BL - MC	Variety combination of BL x MC and reciprocal cross

Variance

V_P	Phenotypic variance
V_G	Genetic variance
V_E	Environmental variance
V_{G-E}	Genetic-environmental interaction variance
V_A	Additive variance

V_D	Dominance variance
V_I	Epistatic variance

Genetic effects

GCA	Genetic combining ability
$\bar{h}$	Average heterosis
h_i	Direct line heterosis
h_{ij}	Cross heterosis
h_{ij}%	Percent cross heterosis
S_{ij}	Specific heterosis (Specific combining ability)
l_i	Direct line effects
m_i	Maternal effects
r_{ij}	Reciprocal effects
r_{**ij}	Specific reciprocal effects
$\bar{y}_i$	Mean of sire line *i*
$\bar{y}_j$	Mean of dam line *j*
$\bar{y}*_i$	Net variety effect of sires *i*
$\bar{y}*_j$	Net variety effect of dams *j*

Others

BLUPs	Best linear unbiased predictors
CC	Chloride cells
SE	Standard error
SOP	Standard operating procedures

APPENDIX B – MAIN DEFINITIONS USED IN THIS THESIS

Breed	Fish of common origin but presenting characteristics that make them different to other groups.
Crossbred	Offspring produced by the mating of two or more pure breeds.
Genetic effects	The influence (genetic) that maternal or paternal genotype has on the phenotype of their offspring. Parents transmit their genes, not their genotype to their offspring.
Hybrid	Offspring of genetically homozygous parents. However, the term has been misused or adapted to include offspring of species, crosses, inbred lines. Dunham (2004) suggested that the term should only be used for inter-specific crosses.
Inbreeding	The mating of close relative organisms in a population.
Line	A population or group developed through a breeding program by means of inbreeding or selection to enhance specific traits usually of commercial importance.
Strain	A group of fish of specific origin or produced by an specific breeding program.
Synthetic variety	a population produced crossing genotypes which are known to improve one or more traits.
Variety	In this thesis is used to describe pure species, crossbreds and lines of commercial importance.

Suggested reading materials:

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APPENDIX C – STANDARD OPERATING PROCEDURES (SOP)

SOP-1. Breeding of tilapia in outdoor pools

Materials needed:

Fiberglass pools (diameter 2.45 m x height 0.5 m)
0.35 m^3 soil per pool
2.3 m^3 well water per pool
Rubber tubing (size)
Airstones (2.5 x 2.5 x 5 cm)
Nets to cover pools
Dipnets
Tilapia broodstock, 25 cm total length or larger
20-L plastic buckets
28% protein floating feed

Procedure:

1. Determine experimental crosses to be conducted and the number of breeding pools required.
2. Fill pools with pond water two weeks prior to the beginning of the experiment. Check for leaks and potential problems (broken pipes, faulty tubing, missing airstones).
3. Clean airstones by placing in 10% bleach solution for 24 h. Rinse airstones thoroughly before placing them in the pools. Replace airstones as needed.
4. To decrease stress, do not feed fish for 1-2 days prior to transporting to new pools.
5. Collect broodstock and place three to four fish in a 20-L bucket with tank water. Separate fish by sexes.
6. Rapidly transport and place fish to breeding pools. (Ensure that ambient temperature is similar to laboratory temperatures).
7. Place broodstock in pools at a ratio of approximately 3:1 (dams: sires).
8. Cover pools with netting to prevent predation by birds and other animals.
9. Feed 30 – 50 g. of feed twice per day. After 30 minutes, remove excess feed with a small net.

SOP-2. Collection and maintenance of tilapia fingerlings

Materials needed:

Plastic tubing (size)
Airstones
Dipnets
20-L plastic buckets
25-L conical tanks
525-L sump
Pump
Bead filter
Duct tape
Permanent markers
28% protein feed

Note: One week prior to collection of fingerlings, condition water in recirculating system. In addition, label all tanks. Use a permanent marker on duct tape to prevent any confusion.

Procedure:

1. Remove stand pipe from fiberglass pool (SOP.-1).
2. Cover drain with a small net to prevent escape of fish from pool.
3. Allow 70% of water to drain.
4. Half fill 20-L bucket with pool water.
5. Avoid mistakes by transferring only one group of fish at a time.
6. Collect fingerlings with a small dipnet and place them in the bucket.
7. Refill breeding pools if necessary.
8. Transfer fingerlings into tanks in the recirculating systems.
9. Cover tanks to avoid fish jumping into adjacent tanks.
10. Remove dead fish as needed.
11. Maintain water quality parameters in system; back flush biofilter twice weekly.

SOP-3. Salinity tolerance trial system

Materials needed:

20-L plastic buckets
Dipnet
Plastic tubing (size)
Airstones
Four 280-L tanks
Permanent marker
Hand-held refractometer
Rubber bands
Tilapia fingerlings (average size 1.5 - 6.5 g)

NaCl (Diamond Crystal® Solar Salt, Cargill™ Minneapolis, MN, USA)
3.8-L wide-mouth polyethylene plastic jars (Consolidated Plastics Co. Inc., Ohio, USA)
Soft knotless nylon netting (16 mm^2)

Note:
Four days before the beginning of an experiment, adjust water quality as described in materials and methods.
Based on the existing water volume in the system, estimate amount of salt needed to raise salinity in the system 7 ppt.
Using refractometer, ensure that initial salinity in the system is 0.2 ppt.

Procedure:

1. Place airstones in each tank, adjacent to the stand pipe to avoid tangling of tubing with jars.
2. Mark each jar with information regarding the fish it will contain.
3. Add approximately 2-L of tank water to each plastic jar.
4. Collect 10 – 20 fingerlings with a small net and place them in the corresponding.

5. Secure a piece of 15 cm^2 of soft knotted nylon mesh over the mouth of the jar with two rubber bands.
6. Record stocking of fish on a separate sheet of paper.
7. Place jars into three of the four tanks to allow for jar rotation during daily monitoring.
8. Allow jars to float and rotate freely in the tanks to maintain water quality in jars.
9. Raise salinity 7 ppt/day by adding salt into sump every 24 h.
10. Check for target salinity with hand-held refractometer.
11. Maintain fish at target salinity for 24 h.
12. Remove dead fish (if any) and record data for each group of fish tested.
13. Raise salinity 7ppt by adding required salt.
14. Continue with steps 12 – 14 until all fish are dead.

SOP-4. Cold tolerance trial system

Materials needed:

20-L plastic buckets
Dipnets
Plastic tubing (size)
Airstones
Floating bead filter
Four 280-L tanks
Permanent marker
0.5 hp submersible pump
Styrofoam packing peanuts
Thermometer probe
Rubber bands
Tilapia fingerlings (average size 1.5 - 6.5 g)
Three in-line titanium chillers
3.8-L wide mouth plastic jars (Consolidated Plastics Co. Inc., Ohio, USA)
Soft knotless nylon netting (16 mm^2)
Large opaque plastic sheet (3.65m x 2.75 m)

Notes:
Four days before the beginning of an experiment, adjust water quality as described in materials and methods. Test and set chiller temperature controls two days prior to the beginning of the experiment.

Procedure:

1. Place submersible pump in sump and connect to one of the in-line titanium chillers.
2. Connect outlet of the last chiller to pipes and valves to supply water to all four 280-L tanks.
3. Place packing peanuts in sump to prevent surface warming of water from the system.
4. Check chiller temperature settings.
5. Place airstones adjacent to stand pipes to avoid tangling of tubing with jars.
6. Mark each jar with information regarding the fish it will contain.
7. Add approximately 2-L of tank water to each plastic jar
8. Collect 10 – 20 fingerlings with a small net and place them in the corresponding jar.
9. Secure a piece of 15 cm^2 of soft knotted nylon mesh over the mouth of the jar with two rubber bands.
10. Record stocking of fish on a separate sheet of paper.
11. Place jars into three of the four tanks to allow for jar rotation during daily monitoring.

12. Repeat process and distribute jars evenly among three tanks
13. Allow jars to float and rotate freely in the tanks to maintain water quality in jars.
14. Decrease temperature at a rate of -2C/day.
15. After temperature drops to 16 C, cover all tanks with large plastic sheet to avoid heat gain.
16. Repeat procedure for each group of fish tested.
17. Maintain fish at target temperature for 24 h.
18. Remove dead fish (if any) and record data for each group of fish tested.
19. Re-set chiller to desired temperature.
20. Continue with steps 16 – 18 until all fish are dead.

SOP-5. Estimation of cold and salinity tolerances using BLUP tables.

1. Choose the appropriate table for salinity or temperature estimation.
2. Select the BLUP table (e.g. Select Cold tolerance BLUP for O. aureus).
3. Choose the breed which you want to estimate cold or salinity tolerance (e.g. FLxNI)
4. Remember that in the breed the female parent goes first, then the male.
5. Find the effects as described in the following table:

Effect	Table shows	Estimate (C)
Intercept	Intercept	6.0833
Dam	FL	2.3728
Sire	NI	0.8822
Interaction (dam*sire)	FL* NI	0.8435
Estimated MTT		10.1818

6. Estimated value will be the Mean temperature tolerance (MTT) or Mean salinity tolerance (MST) depending of the BLUP used.

SOP-6. SAS Data Manipulation in MS-Excel and MS-Word.

The following instructions will aid the transferring of data from SAS to Excel.

SAS® to MS-Excel®

1. Open SAS and load your program (e.g. tilapia.sas).
2. From the Tools menu, select Options → Preferences.
3. Once in the Preference submenu, select the Results Tab.
4. Check the boxes for: (a) Create listing, (b) Create HTML, (c) see results as they are generated.
5. Select a folder (browse or create a new folder) where the HTML files will be placed.
6. Run program (tilapia.sas).
7. Besides your regular output window, a new window called Results window – SAS output will be created.
8. From this new window, select the page that you would like to export into Excel.

9. Highlight the area, and use the Copy command.
10. Open a new workbook in Excel and Paste the contents of the clip board.
11. At this point, modify the file as necessary.
12. A copy of all your PROCs with their results will be archived in destination folder in case the files are needed later on.

The following instructions will aid the transferring of data from SAS to MS-Word.

SAS® to MS-Word®

1. Open SAS and load your program (e.g. tilapia.sas)
2. Run program
3. Go to the Output window
4. Right click from your mouse.
5. Go File → Save as ….(select lst , e.g. tilapia.lst).
6. Open Word
7. Select Open file (tilapia.lst)
8. Fix margins to make the output fit the required margins.
9. Choose font Courier 10.

APPENDIX D – MORTALITY OF TILAPIA VARIETIES AND CROSSES EXPOSED TO INCREASING SALINITY (DIALLELS 2004 AND 2005)

Crosses	Salinity (ppt)													Total
	0.2	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**	n
BL x BL	0	0	0	2	28	20	6	19	7	1	0	0	0	83
BL x FL	0	0	0	1	5	9	14	16	10	4	0	0	0	59
BL x MC	0	0	0	6	18	14	17	11	8	3	0	0	0	77
BL x MO	0	1	1	15	18	1	1	2	1	0	0	0	0	40
BL x NI	0	0	0	3	3	7	3	9	10	0	0	0	0	35
BL x RE	0	0	0	2	36	7	0	1	2	5	0	0	0	53
FL x BL	0	0	0	4	4	1	6	20	11	6	4	0	0	56
FL x FL	0	0	0	0	6	11	5	19	6	8	4	2	0	61
FL x MC	0	0	0	0	4	1	7	13	9	3	5	1	0	43
FL x MO	0	0	1	3	7	0	4	11	14	14	9	1	0	64
FL x NI	0	0	0	0	4	0	1	0	0	2	5	0	0	12
FL x RE	0	1	0	2	15	5	5	8	15	4	0	0	0	55
MC x BL	0	1	1	6	21	21	10	10	9	0	0	0	0	79
MC x FL	0	0	4	14	48	13	0	3	1	0	0	0	0	83
MC x MC	0	0	0	23	33	4	0	2	0	0	0	0	0	62
MC x MO	0	0	0	3	9	5	15	23	12	2	2	0	0	71
MC x NI	0	0	1	25	42	4	1	0	2	1	0	0	0	76
MC x RE	0	1	3	38	41	2	0	2	1	0	0	0	0	88
MO x BL	0	0	0	0	0	9	20	11	13	11	3	3	1	71
MO x FL	0	0	0	1	1	10	4	32	16	1	3	4	2	74
MO x MC	1	0	0	1	6	2	7	4	16	8	0	0	0	45
MO x MO	0	0	1	7	8	12	13	11	9	4	1	9	2	77
MO x NI	0	1	2	2	18	11	6	17	11	2	7	0	0	77
MO x RE	0	0	0	3	18	12	6	13	16	1	0	0	0	69
NI x BL	0	0	2	13	34	11	7	11	3	1	0	0	0	82
NI x MC	0	0	0	24	42	0	1	0	1	0	0	0	0	68
NI x NI	0	0	5	27	37	3	0	1	0	0	0	0	0	73
NI x RE	0	1	5	18	45	0	0	1	0	0	0	0	0	70
RE x BL	0	0	0	5	24	7	9	23	4	0	0	0	0	72
RE x FL	0	0	0	0	16	9	9	18	26	5	0	0	0	83
RE x MC	0	0	0	9	30	13	6	0	2	0	0	0	0	60
RE x MO	0	0	0	2	15	11	17	14	13	4	1	0	0	77
RE x NI	0	0	0	1	28	4	0	3	0	0	0	0	0	36
RE x RE	0	0	0	19	51	2	0	0	0	0	0	0	0	72
Total	1	6	26	279	715	241	200	328	248	90	44	20	5	2203

APPENDIX E – MORTALITY OF TILAPIA VARIETIES AND CROSSES EXPOSED TO DECREASING TEMPERATURE (DIALLELS 2004 AND 2005)

Crosses	Temperature (C)														Total
	4	**5**	**6**	**7**	**8**	**9**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	n
BL x BL	24	0	17	10	20	0	1	0	0	0	0	0	0	0	72
BL x FL	4	0	26	0	23	0	1	1	0	0	0	0	0	0	55
BL x MC	22	0	29	6	10	0	0	0	0	0	0	0	0	0	67
BL x MO	7	0	26	0	23	1	1	0	0	1	0	1	0	0	60
BL x NI	4	0	20	0	3	0	0	0	0	0	0	1	1	0	29
BL x RE	0	0	31	0	14	0	0	1	0	1	3	1	1	0	52
FL x BL	3	0	5	1	32	5	8	0	0	2	0	1	0	0	57
FL x FL	0	0	7	0	30	0	32	4	0	0	0	0	0	0	73
FL x MC	0	0	9	0	20	0	10	0	0	1	0	1	1	0	42
FL x MO	0	0	19	0	22	0	15	0	0	1	0	0	0	0	57
FL x NI	0	0	0	0	2	0	7	1	1	0	0	0	0	0	11
FL x RE	0	0	16	0	20	2	2	1	0	0	1	0	1	0	43
MC x BL	19	0	33	10	20	0	1	4	0	0	0	0	0	1	88
MC x FL	25	0	18	3	36	1	0	0	0	0	0	0	2	0	85
MC x MC	24	0	18	2	25	1	5	3	2	2	2	1	0	1	86
MC x MO	0	0	34	0	13	4	16	2	3	3	0	0	0	0	75
MC x NI	6	0	24	2	29	2	6	2	2	0	0	0	0	0	73
MC x RE	0	0	32	0	35	1	10	6	3	0	0	1	3	1	92
MO x BL	2	0	33	0	50	1	0	0	0	0	0	0	0	0	86
MO x FL	0	0	33	0	28	2	9	3	0	0	0	0	0	0	75
MO x MC	0	0	29	0	8	0	0	0	1	0	0	1	1	0	40
MO x MO	0	0	32	0	34	1	12	1	0	1	1	0	0	0	82
MO x NI	0	0	39	0	31	5	0	0	0	0	0	0	0	0	75
MO x RE	0	0	37	0	22	0	9	0	2	2	2	0	0	1	75
NI x BL	10	1	26	1	44	0	2	0	0	0	0	0	0	0	84
NI x MC	4	0	21	1	34	4	2	0	0	0	0	0	0	0	66
NI x NI	0	0	29	0	50	2	0	0	0	1	0	1	0	0	83
NI x RE	0	0	28	0	29	3	15	0	0	1	0	0	0	0	76
RE x BL	3	0	26	2	30	3	4	1	0	0	0	0	0	0	69
RE x FL	0	0	35	0	43	0	7	1	0	0	0	0	0	0	86
RE x MC	7	0	23	0	25	1	2	0	1	0	0	0	0	0	59
RE x MO	0	0	40	0	26	3	2	3	1	0	0	0	0	0	75
RE x NI	0	0	6	0	1	0	1	0	0	0	0	0	0	0	8
RE x RE	0	0	25	0	36	0	9	0	0	0	0	1	0	0	71
Total	164	1	826	38	868	42	189	34	16	16	9	10	10	4	2227

APPENDIX F –PROGRAM CODES (DEVELOPED IN SAS®) USED IN THIS RESEARCH

This appendix contains links to SAS® program files found along with this thesis. Click on the link to open or download the file. Program files may be obtained from the author at the following e-mail address: aarmas@lsu.edu. Note: You'll need to have SAS® 9.1 to run the programs.

Salinity Tolerance Files

Mean Salinity Tolerance and BLUPs.
This file contains the program and data to run the mean salinity tolerance (MST), best linear unbiased predictors (BLUPS), and estimation of cumulative survival.

Full Diallel Analysis
This file contains the program to run a full diallel analysis of salinity data.

Survival Analysis
This file generates the data tables used to produce survival curves.

Cold Tolerance Files

Mean Temperature Tolerance and BLUP
This file contains the data and code to run the mean temperature tolerance (MTT), best linear unbiased predictors (BLUPS), and estimation of cumulative survival.

Full Diallel Analysis
This file contains the program to run a full diallel analysis of cold tolerance data.

Survival Analysis
This file generates the data tables used to produce survival curves.

Others

Mean separation and into letter grouping requires the use of the macro **PDMIX800.**

VITA

Alvaro Manuel Armas-Rosales was born on February 6, 1968. In 1985 he enrolled at Universidad Centro Americana in Managua, Nicaragua, where he studied ecology and natural resources management for three years. In 1988, he was awarded a Fulbright-CAMPUS scholarship and transferred to the University of Wisconsin-Eau Claire, Wisconsin. In 1990, he took summer courses at the Gulf Coast Research Laboratory in Ocean Springs, Mississippi. In 1991 he received a Bachelor of Science degree with a double major in biology and geography. The same year his son Manuel was born. After completion of his studies he returned to Nicaragua and worked for the Nicaraguan Ministry of Fisheries for a year. In 1992 he was awarded a scholarship by the European Union Fisheries development program-PRADEPESCA (Programa Regional de Apoyo al Desarrollo de la Pesca en Centro America) to conduct graduate research at the Institute of aquaculture, University of Stirling, Scotland, UK. In 1995, he received a Master of Science degree (by research) in Aquaculture nutrition and bioenergetics of Penaeus monodon. At the end of his studies he returned to Nicaragua and worked for a small aquaculture consulting firm. In 1996, he and his family moved back to Wisconsin where he married Tonya. Subsequently, he worked as a web site developer and English-Spanish translator. In 2000, he worked as yellow perch fish farm manager in Southern Wisconsin. In 2001, his daughter Anamaria Esperanza was born. He was a dedicated stay-at-home dad for the next 18 months. In 2003, he returned to graduate school at Louisiana State University to pursue a degree in fisheries science specializing in tilapia culture. He is currently a candidate for a Master of Science degree in Fisheries with specialization in aquaculture.