GENETIC STUDIES OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FOR FARMING IN NORTHERN VIETNAM: GROWTH, SURVIVAL AND COLD TOLERANCE IN DIFFERENT FARM ENVIRONMENTS

Genetiske studier av Nil-tilapia (*Oreochromis niloticus*) for oppdrett I Nord-Vietnam: Tilvekst, overleving og kuldetoleranse i ulike oppdrettsmiljø

Philosophiae Doctor (PhD) Thesis

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ABSTRACT/SUMMARY

Luan, T. D. (2010). Genetic studies of Nile tilapia (*Oreochromis niloticus*) for farming in northern Vietnam: Growth, survival and cold tolerance in different farm environments. *Philosophiae Doctor Thesis* 2010:04 Norwegian University of Life Sciences.

The present work explores genetic parameters for growth, survival and cold tolerance of tilapia in different farm environments in northern Vietnam. The results are important for further development of selective breeding programs for *O. niloticus*. It was found that the GIFT strain grew significantly faster than the Viet strain in freshwater, but a smaller strain difference was observed in brackish water. The estimated genetic parameters and genotype by environment interactions were based upon; firstly, harvest body weight and survival recorded in fresh and brackish water (Paper I); secondly, harvest body weight recorded in two tank environments with controlled temperatures (O=optimum and L=low temperatures) and in a natural pond environment (N=natural) located in the mountain province (Paper II); and thirdly, cold tolerance recorded in short-term cold challenge tests, harvest body weight and growth during overwintering (Paper III). Genetic parameters for weight gain (WG), relative weight gain (RWG) and specific growth rate (SGR) were also estimated in this study (Paper II). Their estimates were similar to those determined for harvest body weight with respect to magnitude and direction.

The results indicate some genotype by environment interaction for harvest body weight and survival recorded in brackish- and freshwater. The genetic correlations between records in the two environments were low for both harvest body weight (0.45±0.09) and survival (0.42±0.05). Furthermore, relatively high genetic correlations between the two traits within each test environment were estimated (0.73±0.05 and 0.67±0.07 for fresh and brackish water environments, respectively). The genetic correlations between harvest body weight records in the different temperature environments were higher, (0.88±0.19 between O and L, 0.78±0.10 between L and N, and 0.61±0.05 between O and N), indicating a low genotype by environment interaction (Paper II). The estimated genetic correlation between harvest body weight (HW) and growth during overwintering (OW) was high (0.94±0.05) (Paper III), while low but favourable genetic correlations were estimated between HW and cold tolerance measured in the challenge test; 0.17±0.18 with cooling degree hours (CDH), -14±0.17 with temperature at death (TAD), 0.05±0.07 with survival (SUR) and 0.07±0.10 with survival at 50% mortality (SUR_{50}) respectively. Similarly, low and favourable genetic correlations were estimated between OW on one hand and CDH (0.23±0.26), TAD (-0.26±0.18), SUR (0.12±0.07), and SUR_{50} (0.12±0.09) on the other. The results indicate that selection for harvest body weight may result in a high and favourably correlated response in growth during overwintering, and a low but favourably correlated response in cold tolerance.

The heritability estimates for harvest body weight and growth were 0.24±0.04 for freshwater, 0.19±0.06 for brackish water, 0.31±0.08 for controlled O tanks, 0.19±0.04 for controlled L tanks, 0.26±0.09 for N pond environment and 0.18±0.06 for growth during overwintering. The estimated heritability for pond survival was 0.27±0.04 and 0.20±0.06 for freshwater and brackish water environments, respectively. The heritability estimates for traits measured in the cold tolerance test varied from 0.07 to 0.26. This study indicates that substantial additive genetic variation exists for the growth traits and pond survival in different farm environments that can be exploited through selective breeding. The heritabilities estimated for cold tolerance were lower and less accurate. They do, however, indicate a potential for using selective breeding to improve cold tolerance. It can also be concluded that selection for higher harvest body weight does not have any negative consequences for survival at harvest, growth during overwintering or cold tolerance. Additionally, a focus on improved husbandry practices and management will be important to improve cold tolerance performance of *O. niloticus*.

It is recommended that the ongoing breeding program for tropical freshwater farming of tilapia in Vietnam should include harvest body weight and survival. In addition, it is suggested that a separate breeding program be implemented in order to improve growth and survival in brackish water aquaculture. In order to support tilapia farmers in the highlands of northern Vietnam, one should also consider to include cold tolerance in the selection criterion for freshwater farming. It is further recommended that a sample of each full-sib group may be tested for growth and survival in a mountain pond in addition to family testing in the on-going breeding program for freshwater farming. This would make it possible to select broodstock from the best families in the mountain pond to then be disseminated to hatcheries in the highland areas. Further research is recommended to validate and possibly improve the cold challenge tests for genetic improvement of cold tolerance.

***Keywords**: Nile tilapia, GIFT, Oreochromis niloticus, harvest bodyweight, cold tolerance, heritability, genetic correlation, genotype by environment interaction.*

SAMMENDRAG

Arbeidet omhandler genetiske studier av vekst, overleving og kuldetoleranse hos tilapia i ulike oppdrettsmiljø i Nord-Vietnam. Resultatene har betydning for videre utvikling av avlsprogram for *O. niloticus*. Det ble dokumentert en raskere vekst hos GIFT-stammen sammenlignet med Viet-stammen. Genetiske parametre og samspillseffekter mellom genotype og miljø ble estimert for vekt ved slaktetidspunkt registret i ferskvann og brakkvann i studie 1, levendevekt ved slaktetidspunkt registrert i tank under kontrollerte temperaturbetingelser (O=optimal og L=lav temperatur) og i dam lokalisert i fjellområdet med naturlige temperatursvingninger i studie 2, og kuldetoleranse registrert i kultetest samt vekt ved slaktetidspunkt og vekst ved overvintring i studie 3. Genetiske parametre for tilvekst, relativ og spesifikk tilvekst ble også estimert (studie 2). Estimatene for disse egenskapene var imidlertid svært like estimatene for levende vekt mht størrelse og retning.

Vekt og overleving målt ved slaktetidspunkt i hht ferskvann og brakkvann viste signifikante samspillseffekter med genetiske korrelasjoner mellom de to miljøene på hhv 0.45±0.9 og 0.42± 0.05. Det ble funnet høye genetiske korrelasjoner mellom vekt og overleving i begge miljøene (0.73±0.05 og 0.67±0.07) (Artikkel I). Korrelasjonene var høyere mellom høstevekt (HW) målt i de ulike temperaturregimene (0.88±0.19 mellom O og L, 0.78±0.10 mellom L og N, og 0.61±mellom 0.05 mellom O og N) noe som indikerte lave samspillseffekter (Artikkel II). Genetisk korrelasjon mellom HW og vekst ved overvintring (OW) var høy (0.94±0.05), mens lave og gunstige genetiske korrelasjoner ble funnet mellom HW og ulike mål for kuldetoleranse registrert i kuldetest; hhv 0.17±0.18 for kuldetidgrader (timegrader) (CDH), -0.14±0.17 for temperatur ved død (TAD), 0.05±0.07 for overleving (SUR) og 0.07±0.10 for overleving ved 50% dødelighet (SUR_{50}) (Artikkel III). Likeens ble det funnet lave men gunstige korrelasjoner mellom OW og hhv. CDH (0.23±0.26), TAD (-0.26±0.18), SUR (0.12±0.07), og SUR_{50} (0.12±0.09). Resultatene indikerer at seleksjon for høstevekt kan føre til en gunstig respons i vekst ved overvintring, og en lav men gunstig respons i kuldetoleranse.

Arvegradene estimert for høstevekt i ferskvann og brakkvann var hhv 0.24±0.04 og 0.19±0.06, mens de var 0.31±0.08, 0.19±0.04 og 0.26±0.09 for temperaturregimene O, L og N, og 0.28±0.06 for OW. Arvegraden for overleving var hhv. 0.20±0.06 og 0.27±0.04 i brakkvann og ferskvann. For egenskaper målt i kuldetesten varierte arvegradene fra 0.07 til 0.26. Studiene

indikerer en betydelig additiv genetisk variasjon for vekst og overleving under ulike miljøforhold noe som gir mulighet for endring gjennom seleksjon. Arvegradene var noe lavere og mer usikre for kuldetolerense målt i test. De viser likevel et potensial for endring av kuldetolerense gjennom seleksjon. Det kan også konkluderes med at seleksjon for økt høstevekt ikke synes å ha negativ effekt på overleving ved høstetidspunkt, vekst ved overvintring og kuldetoleranse. Miljømessige forhold knyttet til drift vil, også være avgjørende for produksjon ved lave temperaturer.

Det anbefales at det pågående avlsprogrammet for tropisk ferskvannsproduksjon av *O. niloticus* i Vietnam bør fokusere på vekst og overleving. I tillegg foreslås det å introdusere eget avlsprogram for brakkvannsproduksjon. For å støtte produsenter i fjellområdene i nordre deler av Vietnam bør det vurderes å ta hensyn til kuldetoleranse i avlsprogrammet for ferskvannsoppdrett. Det er videre anbefalt at man for hver generasjon av avlsfisk tester et utvalg fra hver familie for testing i fjellområdene i tillegg til familietestingen som pågår i dagens i avlskjerne. På den måten vil fisk fra de beste familiene i fjellområdene kunne velges ut som avlsfisk og gjøres tilgjengelige for klekkerier nettopp i disse områdene. Mer forskning anbefales for å validere og videreutvikle metodikken for kuldetesting for å selektere for høyere kuldetoleranse.

1. INTRODUCTION

1.1. Aquaculture in Vietnam

** Roles of aquaculture for poverty reduction in Vietnam*

Vietnam is a predominantly agrarian country with 80 % of the total population living in rural areas and two-thirds of them dependent upon agriculture for a living (Tung, 2000). In addition to agriculture, aquaculture provides an important source of animal protein for the people of Vietnam. The current per capita consumption of aquaculture products is approximately 15 kg per year and this figure is expected to increase to 25 kg in 2015. This implies that aquaculture production currently has, and will continue to have an important role in meeting the expected demand for fish products. The development of aquaculture has been encouraged in many localities on a small-scale, as well as on a larger-scale, including inland, brackish and marine environments (MoFI, 1994). According to the statistical handbook of Vietnam (2008), the surface area of water for aquaculture has increased dramatically from 641.9 thousands ha with a total production of 589.6 thousand tons in 2000 to 1018.8 thousands ha with 2123.3 thousand tons in 2007 respectively, while the wild capture remains at approximately 2000 tons per year.

Aquaculture, especially small-scale culture systems, has contributed significantly to poverty reduction as well as to improved food security, nutrition, economy and employment. Approximately, 35% of the total animal protein intake of the nation comes from fish, and it is higher in remote areas such as the highlands and coastal areas. Small-scale culture systems are relevant to poor people due to the reduced need for investment and the potential for re-use of waste from agricultural activities. Thus, poor farmers can afford to develop their own aquaculture farms. Furthermore, large–scale culture systems or cooperatives of farmers are also encouraged to address the increasing demands of aquatic production and quality.

** Current status and potential for aquaculture development*

Vietnam, with a coastline of 3,260 km, and with 12 lagoons, 112 estuaries, numerous canals and thousands of islands scattering the coast, has a huge area of water available for aquaculture. Inland alone, there is approximately 1.7 million ha of water surface suitable for aquaculture (MoFI, 2005); out of which, the proportions of low-lying rice fields, reservoirs, perennial tidal flats, lagoons, small lakes and ponds account for 39.8, 28.7, 21.1, 6.2 and 4.2 %, respectively. Only one third of the available water surface is being used and exploited for aquaculture activities (MoFI, 2005). In inland aquaculture, more than 90% of the water surface area is occupied by small-scale culture systems with earthen ponds, while the remainder is integrated rice-cum-fish culture. In brackish water and marine areas, the water surface area currently used for aquaculture is much lower than their potential. This indicates that the potential has not been fully exploited for aquaculture and a lack of quality seed and productive technologies are constraints for future development (Thien, 1993).

The aquaculture systems and species are diversified according to geography and climatic conditions. The northern region is dominated by freshwater fish ponds, rice-cum-fish, brackish and marine cage culture, whereas the central regions concentrate on the intensive culture of giant tiger shrimp and marine cage culture for fin fish or lobster. The southern part of the country has the most diversified farming activities which include pond, fence and cage culture of catfish as well as several indigenous species, various intensification levels of giant tiger shrimp and freshwater prawn culture and integrated culture such as rice-cum-fish, rice-cum-prawn and mangrove-cum-aquaculture (Tuan, 2003). The catfish however, is commonly cultured in the Mekong River delta with production at approximately 1.2 million tons (Wilkinson, 2008). Aquaculture in Vietnam utilizes a wide range of species which provide significant potential for future development.

** Tilapia in aquaculture systems*

Tilapia was first introduced to Vietnam in 1951 with the Mozambique tilapia (*Oreochromis mossambicus*). This species has not been commonly used in culture systems due to its poor growth performance. Nile tilapia (*Oreochromis niloticus*) was subsequently introduced to Vietnam in 1973 and in comparison with *O. mossambicus*, it has exhibited better growth and shown better acceptance by farmers after its introduction. During the past few decades, it has lost its role in culture systems due to degradation and contamination of the seed quality. However, *O. niloticus* has started regaining its role in aquaculture in the region since 1994 after introduction of strains Thai and GIFT (Thien et al., 2001). *Oreochromis niloticus* has become an important culture species after the introduction of fish from the fifth generation of GIFT in 1997, and a selection program started with nationwide dissemination throughout the country.

Tilapia production estimated in 2005, which mainly originated from *O. niloticus*, was 54.5 thousand tons, with 60% and 30% of it taking place in the Mekong and Red River deltas, respectively, while the rest was in the central region (MoFI, 2006). The aquaculture area of this species is increasing in both small-scale and commercial systems. Tilapia is now gaining increasing importance in the output from freshwater farming and probably now constitutes 5-7% of this production. The target is that tilapia is to begin being exported, supplementing other important export products such as catfish from the south.

Most tilapia farming takes place in ponds, whereas smaller production takes place in cages in rivers or reservoirs. Thus, vast water areas are still not utilized for aquaculture. It has been recently suggested that tilapia is expected to replace low-valued carp species due to its advantages in both small and large-scale commercial levels, as well as for its higher market demand. According to the ambition of the ministry of fisheries, production should increase to 300,000–350,000 tons by 2015 (MoFI, 2006). Hence, the governmental investigation for research and dissemination of tilapia has been approved.

* *Characteristics of aquaculture in northern Vietnam*

In the north, small-scale fish pond culture is dominated by polyculture of local carps, indigenous and exotic species. The productivity is low and consumed in the local market only. It is therefore difficult to intensify culture systems and increase production. The culture period of carps may last for two years, with risks of disease or environment problems during overwintering resulting in low profits for farmers. In addition, the deterioration of genetic quality in several culture fish species since the late 1970s has been astounding (Thien, 1996). Changing from extensive to semi-intensive culture systems at a commercial scale requires high-value species and better seed quality. Hence, the introduction of improved strains to start new culture systems in the northern provinces is given priority by the local government. Furthermore, aquaculture is negatively affected by cold weather during the winter in the northern provinces. It requires relevant species, strains and culture techniques for such production conditions.

This study has targeted fish farmers in northern Vietnam, where traditional fish culture is currently based on polyculture of carps. Introduction of new species to the region is expected to replace traditional carps with effective species to culture systems. It is hoped that fast growing fish species can contribute to improved livelihood of poor farmers and provide initiative for more industrial, but sustainable aquaculture production using the potential areas for aquaculture to meet future demands.

1.2. Tilapias for diversification of aquaculture

Tilapias are among the most important warm water fish species used for aquaculture production and originate from Africa and the Middle East (Feryer and Iles, 1972). Tilapia farming is considered to be the fastest developing fish farming in term of areas and production (Fitzsimmons, 2000). One advantage of tilapia aquaculture is that they can feed on a wide

range of food from natural organisms to artificial pellets (Bowen, 1982; Jauncey and Ross, 1982). To-date, several tilapia species and hybrids have been widely distributed throughout the tropics, subtropics, and temperate continents for culture purposes (Eknath, 1995). Nile tilapia (*O. niloticus*) is the most important, constituting 90% of all tilapia cultured outside Africa (FAO, 2004). Most of the culturing of Nile tilapia in developing countries is carried out in polyculture systems with carps or shrimp, and production is on semi-intensive or small-scale levels. Recently, the production and areas of tilapia culture are increasing due to supplement of quality seed and relevant production techniques (Little, 2004). Large gaps in tilapia productivity between small-scale and commercial levels, as well as variation in production conditions, require different research approaches to meet the future demands.

Evaluation of the Nile tilapia strain was conducted at several locations after the introduction of tilapia. Macaranas et al. (1997) reported that the Chitralada strain of *O. niloticus* showed the best reproduction, growth and survival between four strains evaluated. The Egypt and Ivory Coast strains were more reproductive than the Victoria and Segana strains, but they showed similar growth performance (Osure and Phelps, 2006). After GIFT dissemination, this strain indicated a superior growth rate and it was recommended for aquaculture in Asian countries (Dan and Little, 2000a; Dey et al., 2000; Sifa et al., 1999). Most results of on-farm trials with GIFT showed higher growth rates than other strains (Dey et al., 2000). Moreover, new *O. niloticus* strains such as GET EXCEL, GSTs and GMT have served well in tilapia aquaculture (Tayamen, 2004; Zimmermann and Natividad, 2004). In general, Nile tilapia plays an important role in aquaculture systems. To optimise productivity and reduce costs, there needs to be studies to determine which strains are relevant for specific environments or locations.

A limitation of tilapia culture is the fish's sensitivity to low ambient temperatures which leads to poor growth and mass mortality during over-wintering (Chervinski and Lahav, 1976;

Tave et al., 1990). Depending on the geographic area, the restriction of grow-out period in these regions is normally about three months (Hofer and Watts, 2002). To optimise the production and grow-out season, fingerlings are usually produced indoor during the cold months, or alternatively, fingerlings are produced in autumn months and then over-wintered before being stocked during warmer summer months (Dan and Little, 2000a,b). The optimal temperature for growth of *O. niloticus* species is around 30^{o}C (Abdel-Fattah and Mamdouh, 2008; Azaza et al., 2008; Chervinski, 1982) and they cannot survive in temperatures less than 10-12^{o}C for more than a few days (Chervinski, 1982). Sifa et al. (2002) reported that the GIFT strain was less tolerant to low temperature than the Sudan 78 and Egypt 78 strains. Another study showed strain variation for growth of Nile tilapia in declining water (Rezk et al., 2002). Studies on cold tolerance of *O. niloticus* are limited, but overall, cold tolerance of tilapia is affected by both husbandry practices and genetics. Improvement of cold tolerance, and hence capability of growth in low temperatures as well as improved husbandry practices are considered essential to enhance the productivity and the adaptation of tilapia outside Africa.

Limitation of, and conflicts over freshwater resources, have encouraged an expansion of tilapia culture in marine environment. Because tilapia is an euryhaline fish, it can tolerate both high salinity as well as freshwater environments (Chervinski, 1982; Philippart and Ruwet, 1982; Suresh and Lin, 1992). Nile tilapia is considered to be a species which grows particularly well in freshwater, while other species such as *O. mossambicus*, *O. spilurus* and *Sarotherodon melanotheron*, hybrid red tilapia can tolerate higher salinity than others (Kamal and Mair, 2005; Nugon, 2003; Suresh and Lin, 1992; Villegas, 1990). A new strain of *O. niloticus* or hybrid for certain brackish water environment have been developed (Rosario et al., 2004a; Tayamen et al., 2002). Based on these efforts, the tilapia production in brackish water has increased from just 65,989 MT in 1996 to 190,176 MT in 2001, an increase from 8.1% to 13.7% of total global tilapia production (FAO, 2002). This was also related to the abandoned

monoculture in shrimp ponds or more recently, the expansion of polyculture with shrimp. This culture system has shown potential for improving the overall productivity in brackish water, disease control as well as increased shrimp yield in some cases (Fitzsimmons, 2001; Thien et al., 2004; Yi and Fitzsimmons, 2004).

1.3. Breeding programs of Nile tilapia

Genetic improvement programs have contributed to increased productivity of cultured aquatic species significantly (Dey and Gupta, 2000; Gjedrem, 2000b; Lymbery et al., 2000). Breeding programs for a number of species have been carried out, such as for carps, shrimps, oysters and other marine species. However, Nile tilapia has recently been investigated as a highly potential species in aquaculture in both tropical and subtropical regions. A number of selection experiments and testing programs that aimed to increase growth rate of tilapia culture in ponds have been conducted for *O. niloticus* (Bentsen et al., 1998; Bolivar and Newkirk, 2002; Brzeski and Doyle, 1995; Charo-Karisa et al., 2006; Eknath et al., 1993; Hulata et al., 1986; Luan et al., 2008; Ponzoni et al., 2005; Rezk et al., 2009; Rutten et al., 2005b). These selective breeding programs have typically been done in favorable environments where growth is expected to be high, and the results indicate an additive genetic variance that can be exploited through selective breeding programs. Therefore, several selective breeding programs have been implemented. Moreover, no evidence of genotype by environment interaction (GxE) has been reported for harvest body weight traits in different freshwater environments, except that a minor interaction of little practical importance was found for harvest weight by Bentsen et al. (1998). However, recently a GxE was clearly found for harvest body weight and survival of *O. niloticus* in fresh and brackish water environments (Luan et al., 2008).

Genetic parameters of salinity tolerance or selective breeding in brackish and marine environments have not been documented for *O. niloticus*, except those reported by Luan et al. (2008). Most research has been carried out to evaluate growth rate and to define suitable

culture species in different salinity levels (e.g. Kamal and Mair, 2005), or to develop a strain suitable for saline water (Rosario et al., 2004a; Tayamen et al., 2004). Furthermore, most studies consider hybrid red tilapia and its potential for marine farming. In fact, the less saline tolerant *O. niloticus* appear to grow as well as hybrid red tilapia in freshwater at moderate salinities (Suresh and Lin, 1992). Hence, testing and genetic improvement of growth performance and survival of *O. niloticus* in brackish water environment may be possible.

The genetic mechanism and control of cold tolerance is still poorly understood and little is known about the differences in cold tolerance within and between tilapia species and strains. Testing of cold tolerance for *O. niloticus* strains and hybrids have been conducted, but these have been mainly limited to evaluate the magnitude of genetic parameters and potential for selection program (Behrends et al., 1996; Charo-Karisa et al., 2005). Some studies however, have reported low heritabilities for this trait (<0.10) (Behrends et al., 1996; Charo-Karisa et al., 2005; Cnaani et al., 2000). Hence, cold tolerance of *O. niloticus* may be improved by both selective breeding and improvement of husbandry practices.

2. OBJECTIVES OF THE STUDY

The broad aim of this thesis project was to estimate genetic parameters and estimate genotype by environment interaction for growth, survival and cold tolerance of Nile tilapia (*O. niloticus*) under different farm environments. The results are intended to support the setup of selective breeding and the further development of a selection program in Vietnam. This is expected to contribute to the reduction of poverty in Vietnam in addition to contributions to the further development of commercial tilapia aquaculture.

The specific objectives were to:

- Study genotype by environment interaction of growth and survival of tilapia in different environments/production systems in northern Vietnam.
- Examine the need and potential for separate breeding programs for tilapia farming in brackish water and mountain areas.
- Examine possibilities for genetic improvement of cold tolerance and growth of tilapia at low temperatures.

3. THESIS OUTLINE

This work is based on three experiments that cover different evaluations of performance of tilapia in a range of aquaculture environments such as brackish and freshwater, different water temperatures, in addition to survival in cold tolerance challenge tests. These environments are representative of aquaculture farming conditions in northern Vietnam. A general introduction, materials and methods section, summary of results of the papers and a general discussion precede the papers. All discussions, conclusions and recommendations for further work are based upon results of the following three papers.

In paper I, the aim of the study was to estimate heritabilities and correlations for harvest body weight and survival of *O. niloticus* in brackish- (shrimp pond) and freshwater ponds. The genotype by environment interaction was also evaluated for the two farm environments. This analysis was based on data recorded from the test fish in the two environments for three years. Strain comparison (Viet and GIFT) was also included in this study. **In paper II**, an evaluation of the growth performance of *O. niloticus* in different controlled, as well as natural, temperature regimes is presented. The genetic parameters and genotype by environment interaction were estimated for fish tested in two controlled (optimum and low) temperature tanks and in an open pond located in the mountain province. The experiments were conducted in two different periods which illustrated late autumn and early spring. **In paper III**, a study with the purpose to estimate heritabilites of cold tolerance in short-term challenge test, as well as genetic correlation with harvest body weight and growth during overwintering is presented. Data for this paper were based on different experiments in three years. The results show the feasibility for improving harvest body weight and cold tolerance during the winter season with low temperature. Thus, the result aids in improving the understanding of tilapia performance in such climates.

4. MATERIALS AND METHODS

4.1 Tilapia strains and production of families

The first Nile tilapia (*O. niloticus*) strain was introduced to Vietnam in 1973 from Taiwan and is known as the Viet strain (Thien, 1993). This strain was maintained at the Research institute for aquaculture No.1 (RIA1) and was reproduced for testing in fresh and brackish water environments (Paper I). The GIFT strain was derived from the GIFT international foundation Inc., The Philippines. This strain was introduced to RIA1 in 1997 (ICLARM, 1998). It was reproduced for the breeding program and tested in all three papers.

Families were produced using a hierarchical mating design and natural mating through all generations. Each male was mated to two females in a hapa to simulate natural spawning. Swim-up fry were collected and transferred to individual fine mesh nursing hapas. Each full-sib family was reared separately in hapas until reaching the size required for tagging. The fish were tagged with electronic PIT tags and were then tested in different environments. About 60-80 identified fingerlings from each full-sib family were tested in each generation. The method applied for production of families is described in the manual of GIFT technology (WorldFish Center, 2004).

Production of families was carried out from April to June, and separate rearing of families continued until August. Experiments were typically carried out from August to December, with exception of the tests presented in Paper II. The fish were overwintered from December to March in hapas within the pond with sex separated (Paper III). This management and selection cycle was repeated in all years (2004-2007) in this study.

4.2 Test environments

Earthen ponds located on-site at RIA1 were used to test the growth performance of fish in freshwater. This location is representative of the vast majority of freshwater aquaculture environments in the Red river delta. The brackish water pond was located in Nghe An province, and was representative of coastal areas from where shrimp farming typically occurs in the northern provinces. A highland natural pond environment was selected in the mountain province, Lao Cai. This area is representative of the mountain provinces in northern Vietnam. Overall, these environments were chosen to represent mountain, delta and coastal aquaculture farming conditions. In addition, fish were also tested in two controlled (optimum and low) temperature environments at RIA1 in cement tanks. The cold tolerance test was conducted using an automatic temperature adjusting cooling system, where fish were evaluated for survival in declining water temperatures.

In growth experiments, harvest body weight, sex, pond, tank, environment and time of harvest were recorded for each individual. For the cold tolerance tests, the time and water temperature at death were recorded for each individual fish.

4.3 Data analysis

For estimation of the fixed effects of strain and environments, the data (for Paper I, II) were initially analyzed using the statistical software SAS (SAS Institute Inc., 2003). Genetic parameters were estimated using a restricted maximum likelihood method as implemented in the Asreml software package (Gilmour et al., 2002). Linear animal and threshold sire-dam models were used to obtain these genetic parameters and genetic correlations, for normally distributed and binary data, respectively (Paper I-III).

5. SUMMARY OF RESULTS

5.1 Paper I: Genetic parameters for harvest body weight and survival of Nile tilapia (*O. niloticus*) in brackish water and freshwater and studies of genotype by environment interaction

The objectives of this study were first to compare growth and survival performance of Vietnam and GIFT strains of Nile tilapia, and then to estimate genetic parameters for harvest body weight and survival of GIFT tilapia in freshwater and brackish water environments. The results from this study showed that the GIFT strain performed significantly better with respect to growth rate in both fresh and brackish water ponds compared to the Viet strain. Heritability estimates for harvest body weight in brackish- and freshwater were moderate (0.19-0.24). The common environmental effect due to separate rearing of families in hapas was substantial (0.09-0.10) for harvest weight in both test environments. Heritability estimates for survival at harvest were relatively high (0.20-0.27). Estimates of genetic correlations were rather low

between the traits recorded in the two environments. However, they were relative high between survival and body weight recorded in the same environment. The results indicate that substantial additive genetic variation exists for both harvest body weight and survival that can be exploited through selective breeding. However, considerable genotype by environment interactions exist for both traits, and hence separate breeding programs for tilapia farming in fresh and brackish water should be considered.

5.2: Paper II: Genetic parameters and genotype by environment interaction for growth of Nile tilapia (*O. niloticus*) in low and optimal temperatures

The objective of this study was to estimate genetic parameters to consider genotype by environment interaction for growth of *O. niloticus* in controlled temperatures (optimal=O and low=L) and natural pond (N) environments. The results showed high genetic correlations between harvest body weight (HW) recorded in O and L temperatures and between HW at L and N temperatures, while a slightly lower genetic correlation was estimated between O and N environments. Similarly, general moderate to high genetic correlations were estimated between test environments for weight gain (WG), specific growth rate (SGR) and relative weight gain (RWG), with one exception of low genetic correlation for RWG between O and N environments (r_g=0.46). The heritability estimates ranged from 0.19 to 0.31 for harvest body weight, 0.21-0.29 for weight gain, 0.19-0.39 for specific growth rate and from 0.16 to 0.42 for relative weight gain. The genetic parameters show that there is a substantial additive genetic variance for growth traits for the breeding population studied, and potential for selective breeding for higher body weight in low temperature environments. Some genotype by environment interaction was detected, but a low tilapia production volume and low ability to invest for small-scale fish farmers in the mountain and highland areas in Vietnam are reasons to question whether investments in a highland breeding program can be justified. Nevertheless, the results show that it may be important to continue the testing and selection in a natural pond

environment with natural temperature variation, in order to avoid the development of a more sensitive and less robust fish for inland aquaculture.

5.3: Paper III: Genetic parameters of cold tolerance and growth of Nile tilapia (*O. niloticus*)

The objective of this study was to estimate genetic parameters for cold tolerance, harvest body weight and growth during overwintering of *O. niloticus*. Cold tolerance was expressed as cooling degree hours (CDH), temperature at death (TAD), survival at 50% mortality (SUR_{50}) and survival (SUR). The estimated heritabilities were generally low for the cold tolerance traits studied. However, the heritability estimates were moderate for harvest body weight (HW) and growth during overwintering (OW). Genetic correlation between CDH and TAD was strongly negative, while positive and moderate to high genetic correlations between CDH and SUR_{50}, and CDH and SUR were obtained. The estimated genetic correlation between SUR and SUR_{50} was relatively high. A high genetic correlation between HW and OW was also estimated. The estimated genetic correlation between cold tolerance and growth traits were low but favourable. Our results indicate that selecting for higher harvest body weight will not give any unfavourable response on short-term survival of fish in cold water at $12^{o}C$ and below. Moreover, high survival rate, weight gain during overwintering and a high genetic correlation between harvest body weight and overwinter weight gain suggest a potential to extend the grow-out period in the cold season for tilapia farming to reach the marketable size of fish in northern Vietnam. The results indicate a potential for selection for both cold tolerance and growth during winter. Further research is however needed to more efficiently improve and apply selection for cold tolerance using cold challenge tests.

6. GENERAL DISCUSSION

The research in this thesis aimed to estimate genetic parameters and genotype by environment interactions of the current Nile tilapia population in northern Vietnam in response to their subsequent introduction to new environments. The different farm environments in which this research was undertaken varied from mountainous to coastal regions, with different aquaculture and climatic conditions. The test environments included freshwater, brackish water, and a natural pond in the mountain province, in addition to different temperature regimes in tank environments, including a cold tolerance test. The research focused on potentials for genetic improvement of harvest body weight, survival and cold tolerance. The knowledge obtained from the results of this thesis will be integral in supporting the further development and implementation of breeding programs for *O. niloticus*, in order to supply high quality genetic material of tilapia to different aquaculture farms in northern Vietnam.

6.1 Tilapia strains and environments

The growth performance of the GIFT strain was higher in both freshwater and brackish water compared to the Viet strain, although not significantly higher in brackish water (Paper I). This is concordant with other reports for this strain following its dissemination to different locations and when compared with other strains (Dan and Little, 2000a; Dey et al., 2000; Eknath and Acosta, 1998a) in freshwater. The low harvest body weight of the Viet strain may be explained by long-term poor broodstock management and contamination (Thien et al., 2001). Therefore, the GIFT strain is recommended as a base population for a breeding program and for dissemination of seed to farmers in Vietnam. However, to fully exploit the potential of this species for a breeding program, it needs to be tested and examined in new environments.

Genetic variation for salinity tolerance and growth rate has been documented for tilapia species and strains, with some studies reporting that a hybrid tilapia descending from *O.*

mossambicus and *T. zillii* parents are highly tolerant to saline water (Romana-Eguia and Eguia, 1999; Suresh and Lin, 1992; Watanabe et al., 1990). However, *O. mossambicus* shows lower growth rates when compared to *O. niloticus*; and consequently, *O. niloticus* has recently been recommended for stocking in freshwater environments. This is in agreement with a report concerning *O. niloticus* by the FAO (2004), where they considered *O. niloticus* to be an important species for farming, as it constitutes 90% of all farmed tilapia outside Africa. This species has also performed well in moderate salinity or brackish water ponds as concluded by Suresh and Lin (1992). However, there may be a potential to improve growth of *O. niloticus* in brackish water through selective breeding.

The GIFT strain is found less tolerant to low temperatures than other *O. niloticus* strains such as Sudan 78 and Egypt 88 as reported by Sifa et al. (2002). In addition, Rezk et al. (2002) found that *O. niloticus* performed better than *O. aureus* in cooler aquaculture conditions. Based on results from this thesis on growth and survival of fish in freshwater (Paper II), the GIFT strain has the potential to further adapt to subtropical climatic conditions and thus improve the production in these regions. Results from this thesis (Papers I, II, III) also suggest that the GIFT strain can meet the requirements of the farm conditions in northern Vietnam to match, and indeed perform better than, the competing strains, through the use of selective breeding.

6.2 Genetic parameters

The genetic parameter values estimated in the present study indicate additive genetic variation for the tilapia population for most traits in the environments under investigation. The most important function of the heritability is its predictive role and as an expression of the reliability of the phenotypic value as a guide to the breeding value (Falconer and Mackey, 1996). The size of the heritability is decisive for the choice of an appropriate breeding strategy. The heritability estimates for harvest body weight varied from moderate to high for fresh- and

brackish water, and for different temperature environments (Papers I, II, III). These estimates were in the range of most heritabilities reported for *O. niloticus* (Bentsen et al., 2003; Eknath et al., 2007; Ponzoni et al., 2005; Rezk et al., 2009; Rutten et al., 2005b), although lower than those reported by Charo-Karisa et al. (2006). The heritabilities estimated for survival in this study (Paper I), for both fresh and brackish water, were higher than those reported by Rezk et al. (2009). The heritability estimates for cold tolerance (Paper III) were generally low, but similar to the estimates reported for *O. niloticus* (Charo-Karisa et al., 2005). In another study, Behrends et al. (1996) reported a low realized heritability for cold tolerance of *O. niloticus* (-0.05), while it was 0.33 for *O. aureus* and 0.31 for their hybrids.

The estimated heritabilities in freshwater were higher than those in brackish water (Paper I) for both harvest body weight and survival traits. Unfortunately, no heritability estimates have been published to-date for harvest weight and survival of tilapia in brackish water. The highest heritability for harvest body weight and its related traits (weight gain and relative and specific weight gain) were obtained in the optimum temperature environment (Paper II), while lower heritabilities were estimated in the low temperature environment, the natural mountain pond and for growth during overwintering (Papers I, II, III). This shows that the heritabilities are lower in harsh environments due to larger environmental variance. Nevertheless, these results strongly indicate a substantial additive genetic variance for growth and survival that can be exploited through selective breeding. According to the obtained heritabilities, the expected genetic gain for cold tolerance traits measured in the cold challenge test is also promising, especially for cooling degree hours (CDH) and survival (SUR). There were substantial common environmental variance for the cold tolerance estimates, suggesting a significant effect of husbandry practices on these traits which is also suggested by others (Charo-Karisa et al., 2004; Dan and Little, 2000b). However, the common environmental variance can be reduced by maintaining uniform rearing environment and feeding. Rearing period can be

reduced by supplying high quality feed and using synchronised methods to obtain a sufficient number of families in a limited period of time. The introduction of DNA markers for individual tagging can provide possibilities to keep the fish in common rearing tanks very soon after hatching, which will reduce the variance due to common environment of full-sib families.

The genetic correlations between harvest body weight and survival within fresh and brackish water environments, representing the delta and coastal environments, were high, positive and significantly deviated from zero (Paper I). These results imply that selecting for growth rate will have a positive correlated response in the overall survival rate. The estimated genetic correlation between harvest body weight and survival was higher than those reported for *O. niloticus* in freshwater by (Rezk et al., 2009), but it was in the range reported for common carp by Nielsen et al. (Nielsen et al., 2010).

The genetic correlations between growth and cold tolerance presented in Paper III indicated a favorable, but low, correlation between the two traits. It indicated that selection for higher harvest body weight does not cause any reduction in cold tolerance. As mentioned previously, cold tolerance and survival may also be improved by environmental factors such as maternal effects (Tave et al., 1989), rearing condition (Charo-Karisa et al., 2004; Cnaani et al., 2003), and management during overwintering (Dan and Little, 2000b).

6.3 Genotype by environment interactions

The genetic correlation for harvest body weight or survival between recordings in fresh and brackish water environments was low (Paper I). This indicates that there is a genotype by environment interaction (GxE). To our knowledge, there are no corresponding published estimates for comparison. The genetic correlations of harvest body weight recorded in optimum and low temperatures and the natural pond, were medium to high (Paper II) and significantly different from zero. The results confirmed that selection for harvest weight in a

favorable environment will also improve growth performance in cooler environments. In addition, a high genetic correlation was estimated between harvest body weight in a favorable environment and growth during overwintering (Paper III). These results clearly indicate that farmers in different environments can benefit from the on-going selective breeding program for tilapia in Vietnam.

The estimated genetic correlations between records in the different environments for weight gain, specific growth rate and relative weight gain are similar to those estimated for harvest body weight (Paper II). Besides the GxE detected in Paper I, a low genetic correlation (<0.5) was also estimated for relative weight gain between optimal temperature and natural pond (Paper II). This indicates a GxE between the two environments for this trait. According to the conclusions by Mulder et al., (2006), separate selection programs are needed for optimising genetic gain when the genetic correlations between two environments is less than 0.7-0.8. However, the decision depends on how much the breeding goals differ for the different environments, the need for additional investments, as well as the production volume in question. Although a certain GxE interaction is documented in the present study (Paper II), a low tilapia production volume and low ability to invest for small-scale fish farmers in the mountain and highland areas in Vietnam are reasons to question whether investments in a highland breeding program can be justified.

The presence and magnitude of GxE is important when establishing a breeding program and when defining the breeding goal. In our study, both fresh and brackish water are very important culture environments, while tilapia production in the mountain provinces are not well-developed and consist mostly of small-scale production. According to suggestions by Mulder et al. (2006), where both environments were equally important and the genetic correlation was higher than 0.61, the highest average genetic gain was achieved with a single breeding program. Running two environment-specific breeding programs was found to be the

optimal situation for genetic correlations up to 0.7. However, this strategy was less appropriate for situations where one of the two environments had a relative importance below 10 to 20%. Based on this suggestion, two separate selection programs should be considered in order to optimize genetic gains of harvest body weight and survival for fresh and brackish water environments.

The ultimate goal of a selection program for *O. niloticus* in northern Vietnam is to obtain higher harvest body weights and to maximize survival in low or winter temperatures. The investment ability is limited in specific highland environments while reducing poverty is of significant importance. Increased aquaculture production is given priority among the freshwater tilapia farmers in the region. The challenge is thus to run a cost-efficient breeding program which will benefit these farmers. Based on the genetic parameters for growth performance of fish in different temperature regimes (Paper II), growth in favorable environments and during overwintering, as well as cold tolerance (Paper III), a combined breeding program can be considered. Testing of families could be conducted in both favorable temperature and mountain environments as well as for survival in cold tolerance tests, and combined in a selection index. Furthermore, selecting survivors within cold tolerant families from cold challenge tests may increase the selection response and genetic improvement in cold tolerance. Also, testing of families in highland areas may allow for selection and dissemination of broodstock and seed fit for tilapia farming in the mountain areas using optimal dissemination schemes as suggested by Skagemo et al. (2010). By this, fish fit for both semi-intensive and intensive tilapia freshwater culture systems can be obtained, which will also contribute to aquaculture development in the remote areas of northern Vietnam.

6.4 Selection response

The realised genetic gain for each trait in the breeding goal should be estimated to consider how much predicted genetic gain is actually being reached (Gjedrem and Baranski,

2009). The selection response can be estimated by different methodologies (Bolivar and Newkirk, 2002; Gall et al., 1993; Gjedrem and Baranski, 2009; Rye and Gjedrem, 2005), but most conclusions indicate agreement among the methods that have been used (Maluwa and Gjerde, 2007; Ponzoni et al., 2005; Rezk et al., 2009). According to Gjedrem and Baranski (2009), using a control population may be an appropriate method for the first two to three generations of selection, while average breeders or repeated mating approaches tend to be more appropriate for a long-term breeding program. However, a genetic trend analysis using mixed model methodology will be a good alternative when complete pedigree information is available for several generations and genetic ties are continuously produced between generations.

Preliminary and unpublished estimates of selection response for the on-going selection program in Vietnam based on least squares means of overlapping generations (2002, 2004 and 2006), showed that the average realized selection response was 10.5% per generation for harvest body weight, while a selection response of 4.0% was found for CDH in the cold tolerance test (Luan et al., in preparation). This estimated response of selection for harvest body weight is similar to most corresponding studies (8.4-20% per generation) reported for tilapia as well as other aquaculture species of per generation (Bolivar and Newkirk, 2002; Charo-Karisa et al., 2006; Eknath et al., 1998; Gjedrem, 2000b; Mai and Luan, 2008; Ponzoni et al., 2005), although it is higher than those estimated by Khaw et al. (2008), Maluwa and Gjerde (2007), Rezk et al. (2009). The variation in selection responses between studies may be due to differences in species/strain, experimental design and the methodology used for analysis. To-date, no estimated selection responses have been reported for cold tolerance of tilapia. In the study mentioned above, the estimate was approximately 4% per generation for CDH, while the genetic gain for TAD and survival was not significant and lower than expected. This may suggest that selection should focus on increasing harvest body weight and

survival. Further research and development may however increase the heritability and variance of cold tolerance measures by i.a. improving and developing the methodology for cold challenge testing. Hence, the prospects for selection for cold tolerance may be improved. The cold tolerance can also be improved by enhancing the rearing condition and husbandry practices as suggested in other studies (Atwood et al., 2003; Charo-Karisa et al., 2004; Cnaani et al., 2003). Overall, testing and selection should be performed in environments which reflect real farm environments, in order to develop a robust fish for different farming conditions in northern Vietnam.

6.5 Correlated responses and selection strategy

The number of traits to be included in a selective breeding program may be minimized to keep the breeding program focused and the costs low (Shultz, 1986). Indirect selection can be utilized when the genetic correlations between traits in the breeding goal are sufficiently high (Falconer and Mackey, 1996). The genetic change could be estimated by using correlated response of selection for other traits that are not included in the selection index. Estimates of direct and correlated response to selection are described by Falconer and Mackay (1996). The equation estimates are given by equation (1) and (2), below:

The direct response to selection of trait X: $R_X = ih_X\sigma_{AX}$ (1)

The correlated response of trait Y based on selection for X: $CR_Y = ih_Xh_Yr_A\sigma_{PY}$ (2)

where R_X and CR_Y is the direct and correlated response for trait X and Y, respectively; h_X and h_Y are the square root of heritabilities of traits X and Y; σ_{AX} is the standard deviation of additive genetic variation for trait X; σ_{PY} is the phenotypic standard deviation for the correlated trait Y; and i is the intensity of selection.

This approach can be used to compare direct and correlated selection responses for the traits in the present study. Estimated direct and correlated responses based on equations 1 and

2 are presented in Table 1. Based on results from paper I, the direct response to selection for growth in brackish water is estimated to 14.3 g per generation, while the correlated response is only the half, 7.2 g when selection is based on recording in freshwater. Direct selection for growth in low temperature will result in a lower response when compared to indirect selection based on recordings in optimum temperature conditions (Table 1). Direct selection for growth under natural pond conditions in mountain areas will result in a 42% higher response than indirect selection based on recordings under optimum temperature conditions (Paper II). Hence, the response achieved by direct versus indirect selection varies, and needs to be considered when defining breeding strategies for different environments. As we see, the biggest reduction in response when applying indirect selection is for HW in brackish water, and hence a separate breeding program for brackish water should be given priority.

Table 1: Estimated direct and correlated response to selection for harvest weight based on genetic parameters in Paper I and II and using equations 1 and 2 (Falconer and Mackey 1996).

Environment	Source of genetic parameters	Direct selection response (g)	Correlated selection response (g)	Trait selected for regarding correlated response
Brackish water	Paper I	14.3	7.2	HW in freshwater
Low temperature	Paper II	10.4	11.7	HW in optimum
Mountain pond	Paper II	18.5	13.0	temperature

* *Normal selection intensity*: 15% selected, i=1.55 *applied as in the on-going breeding program in Vietnam*

7. CONCLUSIONS

In conclusion, the results of this thesis assist in defining the breeding strategy and breeding goal corresponding to realistic tilapia production conditions as well as future perspectives in northern Vietnam. The main findings obtained in research paper I-III are:

- The GIFT strain from the on-going selection program is recommended for tilapia farming in both fresh and brackish water environments in Vietnam. This strain performed well with respect to both survival and harvest body weight.

- There is substantial genetic variation for harvest body weight and survival in the population of *O. niloticus* studied, which can be exploited via selective breeding in all test environments. There is significant genetic variation for CDH and survival in the cold tolerance test. In general, there seems to be potential for improving cold tolerance through selective breeding. However, high standard errors of estimates and relatively high common environmental variance make further development of the challenge test necessary. Furthermore, the challenge testing needs to be validated to mimic subtropical farm environments during the winters in northern Vietnam.

- There is substantial evidence of GxE for harvest body weight and survival of *O. niloticus* in fresh and brackish water and for relative weight gain in controlled optimum temperature and the natural pond in the mountain region. Based on the genetic correlation for harvest body weight, survival in brackish and freshwater, and the importance of both these environments, separate breeding programs for fresh and brackish water environments are recommended.

- The on-going tilapia breeding program in northern Vietnam should continue with testing and selection in real farming conditions with temperature variation during the year, in order to develop a robust fish for both small-scale and commercial large-scale aquaculture. Additional testing of families in highland areas may allow for selection and dissemination of broodstock and seed fit for tilapia farming in mountain areas.

8. FUTURE PROSPECTS

The improved strains from the on-going tilapia breeding program should be disseminated throughout the country to replace the fish that were disseminated prior to the introduction of the GIFT strain to Vietnam. The improved fish should first be disseminated to national broodstock centers for freshwater and provincial freshwater hatcheries. These hatcheries should continue dissemination to other hatcheries or farmers. Replacement plans should be conducted every second year and associations between the selection program and hatcheries will ensure the highest quality of seed supply for aquaculture farmers as well as high quality management of broodstock.

Improvement of the harvest body weight in brackish water environment is needed, and as concluded in the present study, selective breeding will be an efficient tool to enable this. However, crossbreeding may also be considered to add certain characteristics to the breeding population. These strains must however be sufficiently productive.

A main focus should be to improve growth rate in mountain environments through selective breeding by testing fish in both tropical and mountain environments. The genetic correlation for survival trait between studied environments has not yet been exploited. It should be examined together with survival in cold tolerance test. Moreover, improved cold tolerance test methodologies to control and reduce the common environmental effects are needed to estimate more accurate genetic parameters for cold tolerance in tilapia. The realized genetic gain should be properly estimated using BLUP methodology, which will allow for separation of the genetic and environmental trends in the data accumulated over generations of selection. Finally, disease outbreaks cause serious problems in many locations. To reduce the risk of disease outbreak, future genetic studies of tilapia should assess genetic variation of disease resistance before introducing such traits into the breeding program.

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List of papers:

Paper I: Genetic parameters for harvest body weight and survival of Nile tilapia (*Oreochromis niloticus*) in brackish and fresh water and studies of genotype by environment interaction.

Paper II: Genetic parameters and genotype by environment interaction for growth of Nile tilapia (*Oreochromis niloticus*) in low and optimal temperatures.

Paper III: Genetic parameters of cold tolerance and growth of Nile tilapia (*Oreochromis niloticus*).

Paper I

Genetic parameters for harvest body weight and survival of Nile tilapia (*Oreochromis niloticus*) in brackish water and freshwater and studies of genotype by environment interaction

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Abstract

Experiments to evaluate the Viet and GIFT strain performance and estimate genetic parameters of GIFT tilapia (*Oreochromis niloticus*) were conducted at Research Institute for Aquaculture No.1 (RIA1) in northern Vietnam during 3 years from 1999. The fish were reproduced and stocked in fresh and brackish water (previous shrimp farming) earthen ponds. Strain comparisons were based on least squares means of harvest weight data for three year classes/generations. Genetic parameter estimates for harvest body weights and survival of GIFT tilapia were obtained from 13,464 fish of a total of 261 full-sib families from three generations. The results from this study show that the GIFT strain performed significantly better with respect to growth rate in both fresh and brackish water ponds compared to the Viet strain. Heritability estimates for harvest weight in brackish and fresh water were moderate (0.19±0.06 and 0.24±0.04, respectively). The common environmental effect due to separate rearing of families in hapas was substantial for harvest weight in both test environments (0.09 - 0.10 of total phenotypic variance). Heritability estimates for survival at harvest were relatively high (0.27±0.04 and 0.20±0.06 in fresh and brackish water, respectively). Estimates of genetic

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correlations were rather low between the same trait in different environments (0.45±0.09 and 0.42±0.05 for harvest body weight and survival, respectively). However, they were rather high between survival and body weight measured within the same environment (0.73±0.05 and 0.67±0.07 for fresh and brackish water, respectively). The study indicates that substantial additive genetic variation exists for both traits that can be exploited through selective breeding. However, considerable genotype by environment interactions exist for both traits, and separate breeding programs for tilapia farming in fresh-, brackish water should thus be considered.

Keywords: Nile tilapia; Oreochromis niloticus; GIFT; Harvest weight; Survival; Heritability; Genetic correlations; Fresh water; Brackish water

1. Introduction

Tilapias are widely recognized as one of the most important fish species for freshwater aquaculture in a range of production systems (Pullin, 1985). The tolerance of tilapias to a range of environments and intensification systems have resulted in a rapid expansion of tilapia farming as well as dissemination worldwide, in which Asian countries are the largest producers (FAO, 2004; Pullin, 1997). Among the variety of tilapias, Nile tilapia (*Oreochromis niloticus*) is the most common in aquaculture. Tilapias are euryhaline fish, so they can tolerate a high salinity level as well as freshwater, whereas some tilapia species tolerate a wider range of salinity than others (Chervinski, 1982; Philippart and Ruwet, 1982).

The published research on growth and survival of tilapias in brackish water and salt water show different results. Nile tilapia (*O. niloticus*) is considered to be a species which grows particularly well in fresh water, but has a low tolerance to salinity. Meanwhile, species such as *O. mossambicus*, *Sarotherodon melanotheron* and *O. spilurus* have lower growth rate, but higher salinity tolerance compared to other tilapia species (Nugon, 2003; Villegas, 1990). Different experiments for testing and comparing salinity tolerance (growth) have been reported

with focus on stock performances at different salinity concentrations (Jonassen et al., 1997; Kamal and Mair, 2005; Lemarie et al., 2004). The increasing demand for tilapia and the availability of vast brackish water in some regions have encouraged attempts to develop new strains which are more suitable for growth and cultivation in such areas (Manuegl Garcia-Ulloa, 2001; Rosario et al., 2004b; Tayamen et al., 2002). Therefore, the tilapia production has increased dramatically in brackish water as well as in freshwater. Besides farming in increasing intensification of culture systems, tilapias are good candidates for polyculture farming with shrimp (Thien et al., 2004; Yi and Fitzsimmons, 2004). Polyculture of shrimp and tilapia in brackish water is beneficially with respect to productivity as well as to reduce waste discharge to the environment and to prevent shrimp diseases (Thien et al., 2004).

Genetic improvement programs have contributed to increase the productivity of cultured aquatic species significantly (Gjedrem, 2000a; Lymbery, 2000). In order to implement and maintain a breeding program for improving economically important traits, knowledge about the genetic parameters, such as heritability and genetic correlations among the traits under selection are required. Tilapia-breeding programs have almost exclusively focused on growth rate in freshwater. Several estimates of heritability, for harvest weight, fillet yield and growth rate in fresh water have been published. The reported heritability estimates of these traits vary from 0.11 to 0.55 (Bolivar and Newkirk, 2002; Ponzoni et al., 2005; Rutten et al., 2005b). The estimates for the tropical Nile tilapia are of the same magnitude as reported for cold water fish species in a review by Gjedrem (2000a).

The heritability estimates for survival of tilapias in different environments are limited. Low heritabilities (0.04-0.09) were reported for survival in early life in Atlantic salmon estimated by linear models and sire variance components (Rye et al, 1990), while higher estimates (0.34) on the underlying liability scale were presented by Nielsen et al. (submitted) for pond survival during the second growth season for common carp. Furthermore, genetic analysis of survival

in disease challenge tests have been reported for Pacific White Shrimp and Atlantic salmon (Gitterle et al., 2005; Hung, 2005; Odegard et al., 2006). The moderate genetic correlations (0.23 and 0.37) between survival and growth at early fresh water stages for Rainbow trout and Atlantic salmon were reported (Rye and Lillevik, 1990) whereas higher genetic correlation (0.65) was estimated between pond survival (second growth season) and harvest weight for common carp (Nielsen et al., submitted).

Genetic parameters are functions of the specific population and environment studied (Falconer and Mackey, 1996). The estimates of these parameters need to be obtained by using information for each species and population under the actual farming conditions. By estimating the genetic correlation between performances in different environments, genotype by environment interaction (GxE) can be analyzed. A high genetic correlation between the same traits measured in different environments indicate non-significant GxE, as the same genes affect the trait in both environments. Contrary, a low genetic correlation implies significant GxE, as performance in the two environments actually are two different traits. Low genetic correlations and thus significant GxE may reduce genetic gain, and in extreme cases a separate breeding program is required for each type of farm environment, implying higher costs. Several studies of genotype by environment interactions in aquaculture species are reported in the literatures (Bentsen et al., 1998; Eknath et al., 1993; Maluwa et al., 2006; McKay et al., 1984; Sylven et al., 1991; Wangila and Dick, 1998). However, genetic parameters can vary over time and for different test environments. Hence, the estimates of genetic parameters and GxE should be studied for any (new) breeding program and (new) farm environments.

The main objective of this study was to estimate genetic parameters and the magnitude of GxE for harvest weight and survival of Nile tilapia (*O. niloticus*) in fresh and brackish water (coastal areas) in Northern Vietnam. Furthermore, we wanted to compare harvest weight of two Nile tilapia (*O. niloticus*) strains, Viet and GIFT. Genetic parameters and the effect of the

test environment on growth and survival of Nile tilapia are also discussed with respect to selection strategy for tilapia breeding in Vietnam.

2. Materials and methods

2.1. Original Nile tilapia strains

The genetic analyses were based on data of harvest weight and survival of two strains (Viet and GIFT strain) of Nile tilapia (*O. niloticus*) in two environments, freshwater and brackish water ponds. The Viet strain has been maintained in Northern Vietnam since its introduction from Taiwan, via Southern Vietnam, in 1977 (ICLARM, 1998). The GIFT strain was derived from GIFT International foundation Inc., Philippines. A total of 106 families from the fifth generation selected for growth rate in the project, "Genetic Improvement of Farmed Tilapia" (GIFT) were imported to Research Institute for Aquaculture No.1 (RIA1) in 1997 (ICLARM, 1998). The two strains were reared and reproduced in fresh water ponds at RIA1 for this study.

2.2. Mating design and seed production

The reproduction and management schedules applied in 1999 – 2001 are shown in Table 1. During early spring (March-April), all selected broodstock were removed from overwintering hapas, transferred to rearing hapas for conditioning before they were assigned to breeding hapas. When females were ready to spawn, they were placed individually in 3 m^3 mating hapas with one male for natural spawning. Family groups of swim-up fry were collected and stocked in separate nursing hapas.

For the GIFT strain, hierarchical mating design was applied. Each male was mated with two females in a mating hapas for natural spawning. Seven days after stocking, mating hapas were inspected twice every day. Swim-up fry resulting from the successful mating were transferred to individual fine mesh nursing hapas and the spawned females were removed to

allow the male to mate with a second female for production of a second batch of fry. The methodology used is described in the manual of GIFT technology (WorldFish Center, 2004).

The broodstock of the Viet strain were mass spawned in three 25m^3 fine mesh hapas. A total of 50 pairs of males and females were placed in each hapa. Groups of swim-up fry were collected twice per week during the same time as collection of GIFT families. A total of 6-8 groups were produced each year for testing (Table 2).

Each full sib family/group was reared separately in a nursing hapa at the same density, feeding regime and in the same pond until tagging with electronic AVID PIT tags (average body weight of 12-15 g). A total of 60 fingerlings from each full-sib family/group were individually identified with electronic PIT tags.

2.3. Test environments and acclimation

Following tagging, a random sample of 40 fish from each of the families/groups was stocked in earthen freshwater ponds at RIA1 located near Hanoi in Northern Vietnam. The remaining 20 fish in each family/group was acclimatized and stocked in an earthen brackish water pond at a shrimp farm in Nghe An province, 300 km South of Hanoi (Middle of North Vietnam). This area is representative for coastal farm environments with a salinity level similar to most shrimp farms in Vietnam. The salinity level increased approximately from 8.0 ppt. in August to 20.0 ppt. in December during each of the 3 years of the experiment (Fig.1). All individually tagged fish were kept in fine net hapas for 5-7 days to recover after the tagging stress, mortality control and re-injecting of lost PIT tags in order to keep the same number of fish in each family/group. Average temperature during the grow-out period was about 28°C with a range from 25°C to 34°C. The temperature recorded in the brackish water was slightly higher than in the fresh water ponds (Table 1) and with little variation between different years.

Acclimation was conducted at RIA1 before transferring fingerlings to the brackish water farm. Salt was mixed with the freshwater in tanks and then added to the fish tanks to increase salinity level gradually to the level of the brackish water pond. The daily increase of salinity level was 2 ppt. Other environmental factors such as temperature, dissolved oxygen (DO) and pH were monitored regularly. Aeration was supplied to maintain DO level in the tanks.

For each test environment and pond, the fish were stocked at a density of 2–3 fish per m^2. In fresh water, two ponds with the size of 1,200 m^2 were used to stock the fish for the first two years. In the third year, they were replaced by one bigger pond of 1,800 m^2. For testing the fish in brackish water, a pond of 1,000 m^2 was used. The fish were fed with pellet feed containing 22% crude protein at a rate of 2-3% of the fish biomass per day. The water level in the ponds was maintained at 1.2 m (Table 1). Other environmental factors such as temperature, DO, pH and salinity were monitored and recorded daily.

2.4. The fish and data

The data structure and mating design with number of sires, dams and progeny tested for each strain, environment and different generation/year are shown in Table 2. The data of harvest weight and survival were obtained from the three selected generations (F1, F2 and F3) of *O. niloticus* from the tilapia breeding program at RIA1. A total of 261 full-sib families were produced including 90 families in both F1 and F2 and 81 families in F3 were produced (Table 2). Individual body weight at harvest was recorded for the fish that survived. Selection was conducted from F1 to F3 based on ranking with respect to estimated breeding values for harvest weight in fresh water ponds. The data for harvest weight of the Viet strain was recorded at the same time as recording of harvest weight of GIFT fish. Viet strain was only selected for the first year based on high breeding value, and then they were randomly selected based on phenotype in the years later during harvest time.

Descriptive statistics for harvest weight and survival from tagging to harvest for each strain and environment are given in Table 3. Here, the number of observations, means, standard deviations, coefficients of variation, sex ratios and survival rates are presented. Table 3 shows that the mean harvest weight varied substantially between strains and environments, and that harvest weights were higher in freshwater compared to brackish water. The Viet strain performed poorer than GIFT fish, especially in brackish water. The different sex ratios in different environments and strains as well as mean harvest weight of males and females are also given. A higher survival rate was obtained in fresh water (82.0 and 91.6 % for Viet and GIFT strain, respectively) compared to brackish water (77.6 % for Viet and 87.0 % for GIFT strain). The coefficient of variation for harvest weight varied according to environment and strain and was particularly high for the Viet strain in brackish water.

Survival was scored as binary (0/1) trait, where fish was recorded as survived (1) if harvest weight was recorded, and recorded as dead (0) otherwise.

2.5. Data analyses

Weight and survival of fish at harvest in both environments were analysed using different models as given below.

2.5.1 Strain and environment comparison using a fixed effect model

For strain and environment comparison, a Proc Mixed procedure (SAS Institute Inc., 2001) was used to analyze the data with the following Model 1, while taking into account heterogeneous variances within sexes and year/generation:

$$y_{ijmnklg} = \mu + E_i + S_j + L_k + G_m + P_n(E_i) + (E*S)_{ij} + (E*L)_{ik} + (G*S)_{mj} + A_l(E_i) + e_{ijmnklg}$$

where $y_{ijmnklg}$ is harvest weight of the g^{th} individual, μ is overall mean, E_i is the fixed effect of the i^{th} test environment (fresh or brackish water), S_j is the fixed effect of the j^{th} sex (female or

male), L_k is the fixed effect of the k^{th} strain (Viet or GIFT strain), G_m is the effect of the m^{th} year/generation (m = 1,2 or 3), $P_n(E_i)$ is the effect of the n^{th} pond within the i^{th} environment, $(E*S)_{ij}$ is the interaction effect between test the i^{th} environment and the j^{th} sex, $(E*L)_{ik}$ is the interaction effect between the i^{th} test environment and the k^{th} strain, $(G*S)_{mj}$ is the interaction effect between the m^{th} year/generation and the j^{th} sex, $A_l(E_i)$ is the covariate of the l^{th} age within the i^{th} environment and $e_{ijkmnglg}$ is the random residual error for the g^{th} individual.

For the analysis of variance for survival a model comparable to Model 1 was applied. The covariate of the age and the pond nested within test environment were not significantly different. Further, sex was not recording for the dead fish. Hence, those effects were not included in the model. However, we found significant effects of generation/year by strain and environment by generation/year interactions, and hence those effects were included in the model.

During the analysis, the non-significant two- and three-way interactions among the varieties were removed from the model by a stepwise procedure. Least squares means of body weights at harvest and survival were estimated for each strain, environment and strain by environment.

2.5.2. Estimation of genetic parameters

Due to insufficient pedigree records of the Viet fish resulting from mass spawning, genetic parameters were estimated using data of GIFT fish only. For estimation of variance components, heritability and genetic correlations, the ASReml software (Gilmour et al., 2002) was used.

2.5.2.1. Analysis of harvest weight

Variance components for the random effects of harvest weight in freshwater and brackish water were obtained from the mixed animal model. Both models used for harvest weights had the following general characteristics:

$\boldsymbol{y = Xb + Z_1a + Z_2c + e}$.

where $\boldsymbol{y}$ is a vector of the observed weight (g) at harvest for either fresh- or brackish water, $\boldsymbol{b}$ is a vector of fixed effects (sex, pond, generation and their significant interactions), including a covariate effect of age at harvest, **a** is a vector of random additive genetic effects for each individual fish, $\boldsymbol{c}$ is a vector of random common environment effect (effects due to mouth incubation of dams and other possible maternal effects as well as separate rearing of full-sib families in hapas until tagging), $\boldsymbol{e}$ is a vector of random residual errors, and $\boldsymbol{X}$, $\boldsymbol{Z_1}$ and $\boldsymbol{Z_2}$ are known design matrices assigning observations to levels of $\boldsymbol{b}$, $\boldsymbol{a}$ and $\boldsymbol{c}$, respectively.

A full pedigree for individual fish provided the additive genetic relationships among the fish recorded and analysed. Unfortunately, sex was not recorded for individual fish in brackish water at the second year. Hence, the genetic analyses of harvest weight in brackish water were based on data for only two years/generations (1999 and 2001). However, pedigree for all generations was utilized.

Two analyses using animal models were carried out; a univariate analysis (Model 2) for harvest weight in each test environment (including a fixed generation, sex, pond and generation by sex interaction effects), and a bivariate analysis (Model 3), where harvest weight in fresh and brackish water were treated as two different traits (Falconer, 1952). The residual covariance between the harvest weights in brackish and freshwater was set to zero because each fish was only recorded in one test environment. Covariate of age (days) was included in these statistical models.

For each trait, heritability was estimated as: $h^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_c^2 + \sigma_e^2)$, where σ_a^2, σ_c^2 and σ_e^2 are estimated additive genetic, common environmental and residual variance components,

respectively. The variance ratio of common environmental effect was further estimated as: $c^2 = \sigma_c^2 / (\sigma_a^2 + \sigma_c^2 + \sigma_e^2)$ for each trait. The genetic correlation between harvest weight in fresh and brackish water was estimated as $r_{a(i,j)} = \sigma_{a(i,j)} / \sigma_{a(i)}\sigma_{a(j)}$, where $\sigma_{a(i,j)}$ is the additive genetic covariance between two traits, $\sigma_{a(i)}$, $\sigma_{a(j)}$ are additive genetic standard deviations (square root of the additive genetic variances) of the i^{th} and j^{th} traits, respectively. Correspondingly, the correlation due to the common environment was estimated as $r_{c(i,j)} = \sigma_{c(i,j)} / \sigma_{c(i)}\sigma_{c(j)}$.

2.5.2.2. Analysis of survival

Survival of fish in fresh and brackish water was classified as binary traits, based on whether or not the individual survived until harvest. Survival was coded as '1' if the fish was alive at harvest and '0' if record of harvest weight was missing. Information about time of death during grow-out period was not available. Variance components for survival at harvest in different test environments were estimated by the threshold model (Gianola and Foulley, 1983); assuming a normal underlying liability variable, λ, determining categorical outcomes of survival such that $\lambda_{ijl} \leq 0$ corresponds with $Y_{ijk}=1$. Residual variance of λ was assumed to be 1. The model can be written as:

$$Pr(Y_{ijkl} = 1) = Pr(\lambda_{ijk} > 0) = \Phi(\mu + s_j + d_k + c_{jk})$$

where Y_{ijk} is survival at the end of testing period (0 = dead, 1 = alive) for fish l, in full-sib family jk, with sire j and dam k, μ is the overall mean (fixed effects were fitted to the models as explained above), s_j is the random additive genetic effect of sire j, d_k is the random additive genetic effect of dam k, c_{jk} is the random common effect of full-sib family jk, and $\Phi(.)$ is the cumulative standard normal distribution.

As for harvesting weights, genetic parameters were estimated in univariate models, analyzing survival in fresh- and brackish water separately (Model 4). Further, genetic and common environmental correlations were estimated in bivariate models, either including

survival for the two environments as separate traits (Model 5) or bivariate threshold linear sire-dam models for harvesting weight and survival within the same environment (Model 6), the model specified above was used for the harvest weights. The heritability was calculated as $h^2 = 4\sigma_{sd}^2 / (2\sigma_{sd}^2 + \sigma_c^2 + \sigma_e^2)$, where σ^2_{sd} is additive genetic sire-dam variance, and the other parameters are as defined above.

3. Results

3.1. Strain comparison, growth and survival performance

Results from the analysis of variance for harvest body weight and survival using Model 1 are shown in Tables 4 and 5. Model 1 explained 46.1% of the total variation in harvest body weight (Table 4) and 14.3% of the total variation for survival (Table 5). Based on marginal increases in R^2, Table 4 shown the main sources of variation in harvest weight were the effects of years/generations and pond, followed by sex, and age within test environment. Most effects were highly significant ($P<0.001$). The main sources of variation for survival were the effects of environment by generation/year, followed by strain and generation/year (Table 5). Most of effects were highly significant ($P<0.001$), except for test environment and strain was not significant ($P>0.05$). Furthermore, the effect of interaction between strain and environment was significant for both traits in this analysis.

Least squares means (LSM) of harvest weight and survival for the two strains and environments are shown in Table 6. The GIFT strain performed significantly better with respect to growth and survival than the Viet strain irrespective of environment ($P<0.05$), and both strains performed significantly better in fresh than in brackish water ($P<0.05$). However, harvest weight of the GIFT strain was higher than the Viet strain in brackish water, but not significantly different ($P>0.05$). Survival of the GIFT strain in freshwater was higher than in brackish water and the GIFT strain performed also better than Viet strain with respect to

survival in brackish water, but it was not significantly different (*P>0.05*). Survival of the GIFT strain in brackish water was similar as survival of the Viet strain in freshwater (Table 6).

3.2. Heritability and common environmental effect for harvest weight and survival

The estimates of heritability and variance due to common environment for harvest weight and survival in each test environment (Model 2) are shown in Table 7. The heritability estimates for harvest weight in both environments were moderate. The heritability estimated for harvest weight in fresh water (0.24±0.04) was slightly higher than for the same trait obtained in brackish water (0.19±0.06). The estimated variance due to common environment for full-sibs was substantial for harvest weight and significantly different from zero in both fresh- and brackish water.

The heritability estimates for survival in both environments were also moderate and significantly different from zero (*P<0.05*). The estimate of heritability for survival in fresh water (0.27±0.04) was slightly higher than the same trait in brackish water (0.20±0.06) (Table 7). The variance due to common environment estimated for survival in both environments were low. However, the effect was still included for survival due to biological reasons for such effects on survival and results from other genetic studies on tilapia (Maluwa et al., 2006; Martínez et al., 1999; Ponzoni et al., 2005; Rutten et al., 2005b).

3.3 Correlations

Estimates of genetic and common environmental (family) correlation between and within the two test environments are shown in Table 8. The estimated genetic correlation between harvest weights in fresh and brackish water (0.45±0.09) was relatively low (Table 8). This indicates that growth in fresh and brackish water environments are clearly distinct traits. The same was the case for the survival analysis; the corresponding estimate of genetic correlation

for survival in fresh and brackish water (0.42±0.05) was also relatively low, and significantly different from zero as well as from unity ($P<0.05$).

The genetic correlation between harvest weight and survival in each test environment are given in Table 8. The genetic correlations between harvest weight and survival in both freshwater (0.73±0.05) and brackish water (0.67±0.07) were relatively high (Table 8), while the estimated common environmental correlations between fresh and brackish water environments was relative low, 0.36±0.13 and 0.47±0.11 for harvest weight and survival, respectively.

Within environment, the common environmental correlations between the two traits were high; 0.93±0.03 and 0.89±0.03 for fresh and brackish water, respectively (Table 8).

4. Discussion

4.1 Strain and environmental interaction effects

An objective of the Vietnamese government is to increase polyculture with tilapia and shrimp to reduce stress-associated disease problems in intensive shrimp farming. Moreover, suitable strains of tilapia for better growth in brackish water environment are needed to utilize effectively the large potential of areas along the coastal basin. Therefore, the question of which tilapia strain is the best choice (quality of stocks) for use in brackish water is addressed here. Further, more knowledge is needed about genetic parameters of relevant traits in order to outline efficient breeding programs and to determine whether separate breeding programs are needed for tilapia farming in fresh- and brackish water environments in Vietnam.

For fish breeding and farming, high genetic variation and good performance with respect to growth and survival in real farming conditions is imperative. The Viet strain was introduced from Taiwan ca 30 years ago, and was considered favourable in freshwater ponds. However, GIFT performed better with respect to both growth and survival in this study. A similar

observation for body weight and survival was reported earlier by ICLARM (1998) and Dan and Little (2000a). These results are probably and mainly due to the five generations of selection for growth of the GIFT population (Dey and Gupta, 2000; Eknath and Acosta, 1998b), for which a genetic gain from 6.2% to 19.1% in harvest weight over five selected generations was reported. Furthermore, the poor performance of the Viet strain may be due to poor management (i.e. inbreeding) of the broodstock for a long time (Thien et al., 2001). Also, a relatively low number of Viet family/groups were tested in this study, potentially sired by a limited number of males, and may thus not be fully representative for the Viet population in Vietnam. The fifth selected generation of GIFT showed better harvest weight and survival, and thus it should be a natural choice as a base population for breeding and farming in Vietnam.

The significant strain by environment interaction effect on harvest body weight and survival, but the low marginal R^2 increase of the same interaction (Tables 4 and 5) showed that the strains performed relatively different in both test environments. Consequently, both growth and survival of GIFT were higher compared to Viet strain in both environments, however, growth and survival of the GIFT in brackish water was not clearly higher than Viet strain (Table 6). Bentsen et al. (1998) found a significant, but low, interaction effect between strains and test environments (1.8%) on harvest weight in *O. niloticus*, while Maluwa and Gjerde (2006) found no significant GxE for harvest weight in *O. shiranus* strains. Neither of the two latter reports did however consider brackish water environments.

4.2 Heritabilities of harvest weight and survival

The achievements of the GIFT strain has been reported by Eknath and Acosta (1998a) and Bentsen et al. (1998). Furthermore, (Ponzoni et al., 2005) found that there was still additive genetic variance in the GIFT population after five generations of selection. The heritability estimates for harvest weight in fresh water (0.24) and in brackish water (0.19) (Table 7) are in

accordance with several previous findings in Nile tilapia *O. niloticus* (Gall and Bakar, 2002; Luan et al., 2008; Rutten et al., 2005b; 2005a) and *O. shiramus* (Maluwa et al., 2006). However, they are lower than the estimates for *O. niloticus* reported by some other authors (Bolivar and Newkirk, 2002; Kronert et al., 1989; Ponzoni et al., 2005). Charo-Karisa et al. (2006) found much higher heritability of harvest weight for *O. niloticus* reared in low-input earthen pond. Moreover, our estimates of heritabilities were comparable with those reported for other species such as Pacific White shrimp (Gitterle et al., 2006), but lower than those estimated for tiger shrimp (Kenway et al., 2006), cold-water fish species (Gjedrem, 2000a; Su et al., 1996) and seabass (Saillant et al., 2006). Unfortunately, no heritability estimates are published for harvest weight of tilapia in brackish water.

The estimate of common environmental variance (10%) for harvest weight of fish in fresh water in this study is similar to those reported from most genetic studies of tilapia (Maluwa et al., 2006; Ponzoni et al., 2005; Rutten et al., 2005b; Rutten et al., 2005a), Atlantic salmon (Rye and Mao, 1998) and in rainbow trout (Pante et al., 2002). In this study, the rearing of families in separate hapas for ca 50-60 days seem to cause significantly different environmental conditions for the different families. The results therefore suggest that reduced age at tagging and earlier communal stocking may be used to reduce the effects of early separate rearing. Rutten et al. (2005a) showed that the common environmental effects on harvest weight diminished over time (from 100 to 365 days to recording), thus increasing the culture period after communal stocking may reduce the variance of common environment. Most rearing periods until harvesting for tilapia vary between 180 and 220 days. In our study, the range of rearing period was 220 – 230 days.

There are few published estimates of genetic parameters for survival at harvest of farmed tilapia. The present heritability estimates for survival in fresh (0.27±0.04) and brackish water (0.20±0.06) ponds were moderate (Table 7). These estimates are much higher than reported by

Charo-Karisa et al. (2006) for *O. niloticus* in low input fresh water ponds (0.03 - 0.14). However, a linear model was used in their study, and the estimated heritability is thus expected to be lower than with the threshold model used in the present study. As for harvest weights, no reported estimates of heritability for survival of tilapia in brackish water is published. However, for Nile tilapia (*O. niloticus*) the mortality usually increases at higher salinity level as reported by different authors (Doudet, 1992; Kamal and Mair, 2005; Lemarie et al., 2004; Watanabe et al., 1985). In this study, the salinity level increased naturally from stocking to harvesting (Fig. 1) in brackish water pond, and thus increasing mortality is expected during the culture period. Also, a higher mortality was observed in brackish water. Time of death for the individual fish were not recorded during the rearing period due to practical reasons, and variation in survival time could thus not be taken into account in the genetic analysis of survival. However, Gitterle et al. (2006) and Ødegård et al. (2006,2007) showed that simple linear or non-linear (threshold) models were robust, at least when using data from well designed challenge tests with specific diseases. Our heritability estimates for survival were comparable with others reported for other aquaculture species, such as Atlantic salmon (Jonasson, 1993; Ødegård et al., 2006; Standal and Gjerde, 1987), rainbow trout (Rye et al., 1990) and white shrimp (Gitterle et al., 2005), but much higher than those obtained from less domesticated and juvenile Atlantic cod (Gjerde et al., 2004).

4.3 Correlations

The estimates of within-test environment genetic correlations between harvest weight and survival were 0.67 in brackish and 0.73 in fresh water (Table 8). The sign and the magnitude of the genetic correlation between two traits found in this study indicate that selecting for growth will cause a positive correlated response in overall survival in both environments. No estimates of genetic correlation between harvest weight and survival of tilapia are reported so far.

However, our estimates between the two traits were higher than corresponding estimates in early life of Atlantic salmon (0.37) and rainbow trout (0.23) (Rye et al., 1990). Gitterle et al. (2005) reported a correlation between *P. vannamei* full-sib family breeding values of harvest weight and survival of around 0.4. However, our estimates were closer to the genetic correlation between pond survival during second growth season and harvest weight (0.65 ± 0.15) for common carp, reported by Nielsen et al. (submitted). The common environment correlation between harvest weight and survival in both test environments estimated in the present study was relatively high.

The low genetic correlation between harvest weights (Table 8) in the fresh and brackish water test environments suggests that GxE is relatively high, which implies that growth in the two environments can be considered as two different traits affected by different sets of genes or that the same genes have different pleiotropic effects. From studies on GxE based on genetic correlations between harvest weight of tilapias in a range of different farm environments, positive and moderate to high genetic correlations are reported. Some of them were high and not significantly different from unity in fresh water (Eknath et al., 1993; Maluwa et al., 2006), whereas others were lower (Bentsen et al., 1998). A moderate to high slaughter weight correlation (0.58-0.86) reported for rainbow trout in fresh- and brackish- and salt water environments (Sylven et al., 1991). Similarly, a relatively low genetic correlation between survival in the two environments (0.42) was estimated in this study, suggesting substantial GxE also for the latter trait. Rye et al. (1990) found low to medium genetic correlations between survival of Atlantic salmon at different life stages (0.2–0.5), while Gitterle et al. (2005) reported a highly positive genetic correlation between survival in ponds and tanks of *P. vannamei*. Although the GxE, analysed by Model 1 as strain by environment interaction, was not significant, the resulting genetic correlations strongly indicate substantial GxE within the GIFT population for both harvest weight and survival in the fresh and brackish water ponds.

5. Conclusions

The findings in this study indicate that the GIFT strain performed better than the Viet strain with respect to growth and survival, especially in freshwater environment. The GIFT strain is thus a good candidate for tilapia farming, especially in freshwater in Vietnam. Despite several generations of selection, the GIFT population still shows high additive genetic variance of both growth and survival in both fresh and brackish water. For both environments, growth showed a highly favourable genetic correlation with survival. Hence, further genetic improvement of both traits is therefore possible within both fresh- and brackish water environments. However, the across-environment genetic correlations were low, albeit positive, for both traits. This suggests that separate selection programs should be considered for tilapia to be farmed in fresh and brackish water environments.

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Table 1: Physical and environmental conditions and management of farming ponds with fresh and brackish water during the performance tests of three generations of Nile tilapia and the scheme of family production and testing.

Pond management data	Test environments	
	Freshwater	Brackishwater
Test location	RIA.1	Nghe An province,
Fish stocking density (fish m^{-2})	2 - 3	2
Pellet feeding rate (% BW day^{-1})	2-3	2-3
Pond water dept (m)	1.2 – 1.4	1.2
Selection of broodstock & conditioning	March – April	March – April
Mating	April - June	April - June
Nursing of fry in hapas	May - June	May - June
Rearing of fingerlings in hapas	June - July	June - July
Tagging	Late August – Sept.	Late August – Sept.
Fish per full-sib family at start of test	40	20
Harvest	Late December	Early December
Temperature (^{0}C)	25-34	27-36
Salinity (ppt.)	0	8-20

Table 2: Data structure including number of sires/groups, dams and progeny (N) by strain and generation/year class and environments

Generation/year class	Strain	Sires	Dams	Progeny		
				Freshwater	Brackish water	Total
1	GIFT	50	90	3600	1800	5400
2	GIFT	50	90	3600	1800	5400
3	GIFT	46	81	3240	1620	4860
Sub-total		*146*	*261*	*10440*	*5220*	*15660*
1	Viet	8 groups*		320	160	480
2	Viet	6 groups*		240	120	360
3	Viet	8 groups*		320	160	480
Sub-total				*880*	*440*	*1320*
Total				*11320*	*5660*	*16980*

* batch of fry collected in different hapas and date

Table 3: The number of recorded fish (N), mean, coefficient of variation (CV) of harvest weight, sex ratio and survival rate from tagging to harvest for GIFT and Viet strains tested in fresh and brackish water ponds.

Environment	N	Mean (g)	CV (%)	Sex ratio (M/F)	Survival (%)
Freshwater					
Viet	653	144.36	36.36	0.95	82.0
GIFT	9144	179.56	31.33	1.14	91.6
Brackish water					
Viet	297	111.05	45.51	1.27	77.6
GIFT	4320	160.19	38.58	0.97	87.0
Across strains					
Freshwater	9797	177.57	31.89	1.12	86.8
Brackish water	4617	157.53	42.40	0.98	82.3

Table 4: Results of the analysis of variance of body weight at harvest in Nile tilapia (*O. niloticus*) by using Model 1. Degrees of freedom (*df*), marginal sum of squares (Type III) and marginal increase in the proportion of the total variance explained by the model (R^2) associated to each effect.

Effect	df	Marginal (Type III) sum of squares	Marginal R^2 increase x 100
Test environment	1	933.4	0.02*
Sex	1	395727.5	7.13***
Strain	1	11062.8	0.20***
Generation	2	1567085.6	28.22***
Pond(Environment)	2	679386.5	12.23***
Environment x Sex	1	966.9	0.02*
Environment x Strain	1	3855.7	0.07***
Generation x Sex	2	11805.6	0.21***
Age(Test environment)	2	145393.4	2.62***
Error	12632	2992578.9	
Model	13	2561144.1	46.12

Test level of significant from zero (*** *P < 0.001 and* **P<0.05*)

Table 5: Results of the analysis of variance of survival in Nile tilapia (*O. niloticus*) by using Model 1. Degrees of freedom (*df.*), marginal sums of squares (Type III) and marginal increase in the proportion of the total variance explained by the model (R^2) associated to each effect.

Effect	df.	Marginal (Type III) sums of squares	Marginal R^2 increase x 100
Test environment	1	0.05	0.021^{ns}
Strain	1	0.17	0.073^{ns}
Generation	2	3.66	1.580***
Environment x Strain	1	1.92	0.829***
Environment x Generation	2	14.63	6.313***
Generation x Strain	2	4.17	1.798***
Error	16605	198.74	
Model	9	33.09	14.27

Test level of significant from zero (*** $P < 0.001$), ns is not significant ($P>0.05$)

Table 6: The least squares means (LSM) for harvest weight (g ± se) and survival (%±se) trait for different strains in fresh and brackish water ponds and different GxE combinations of Nile tilapia (*O. niloticus*)

Trait	Environment	Strain	LSM*
Harvest weight	Freshwater	Viet	153.75 ± 2.11^{b}
		GIFT	179.27 ± 0.51^{a}
	Brackish water	Viet	134.01 ± 4.02^{c}
		GIFT	140.58 ± 1.07^{c}
Survival	Freshwater	Viet	82.41 ± 0.34^{e}
		GIFT	87.62 ± 1.36^{f}
	Brackish water	Viet	81.36 ± 0.48^{e}
		GIFT	82.50 ± 2.01^{e}

* LSM with the same subscript are not significantly different (*P>0.05*).

Table 7: Estimates of heritability (h^2 ± se) and common environmental effect (c^2 ± se) for harvest weight and survival of GIFT tilapia farmed in fresh and brackish water ponds.

Environment	h^2 ± se	c^2 ± se
For harvest weight [a]		
Freshwater	0.24 ± 0.04	0.10 ± 0.02
Brackish water	0.19 ± 0.06	0.09 ± 0.05
For survival [b]		
Freshwater	0.27 ± 0.04	0.00 ± 0.00
Brackish water	0.20 ± 0.06	0.01 ± 0.01

[a] estimated by a linear animal model

[b] estimated by a threshold sire-dam model

Table 8: Genetic correlations ($r_g \pm se$) and common environmental correlations ($r_c \pm se$) between and within body weight at harvest (HW) and survival (S) of Nile tilapia in two test environments.

Correlation	$r_g \pm se$	$r_c \pm se$
Between test environment		
HW in fresh- and brackish water	0.45 ± 0.09	0.36 ± 0.13
S in fresh- and brackish water	0.42 ± 0.05	0.47 ± 0.11
Within test environment		
HW vs. S in freshwater	0.73 ± 0.05	0.93 ± 0.03
HW vs. S in brackish water	0.67 ± 0.07	0.89 ± 0.03

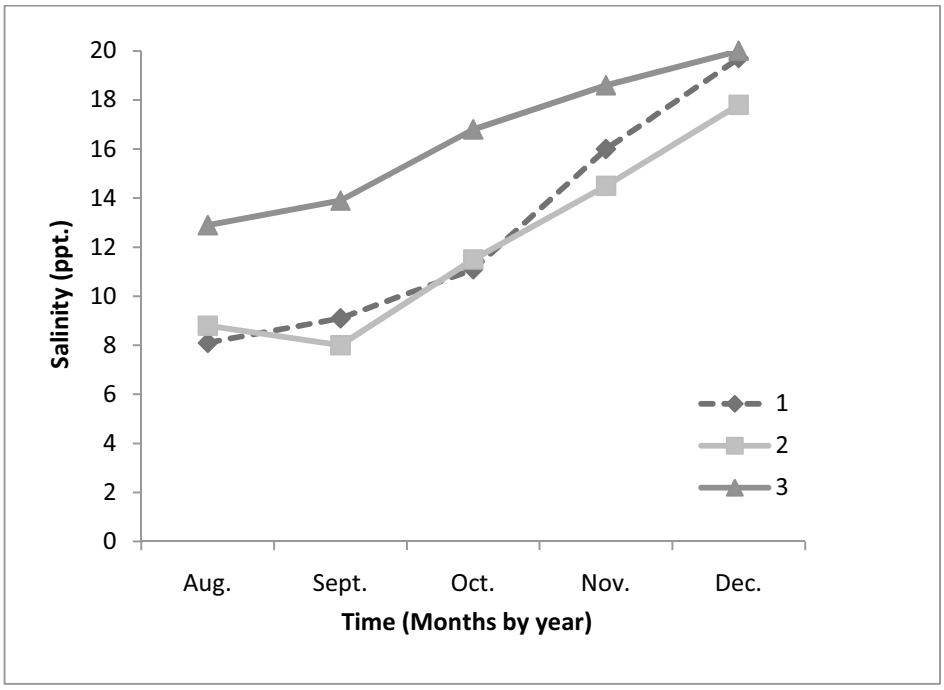

Figure 1: A plot of mean salinity level (ppt.) in the brackish water pond for different months and years.

Paper II

Genetic parameters and genotype by environment interaction for growth of Nile tilapia (*Oreochromis niloticus*) in low and optimal temperatures

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Abstract

The objective of this study was to estimate genetic parameters to consider genotype by environment (*GxE*) interaction for growth of *Oreochromis niloticus* at optimal (~30^{o}C), low (~21^{o}C) and natural (subtropical) temperature environments as a basis for establishing a breeding strategy for tilapia in Vietnam. The fish were reproduced and stocked in tanks for testing in three different environments; two types of tanks with controlled temperature environments (O=optimal and L=low temperature), and one natural subtropical environment conducted in an open pond located in the mountain province, Lao Cai. The experiment was carried out in two batches (spring and autumn), and each batch lasted for three months. Growth performance was recorded on 7,053 fish from 102 full-sib families originating from the GIFT population. Linear animal models (univariate and multivariate) were applied for genetic analyses. High genetic correlations were estimated between harvest body weights (HW) recorded in O and L environments (0.88±0.19) and between harvest body weights at L and N environments (0.78±0.10), while a slightly lower genetic correlation (0.61±0.05) was estimated between optimal and natural environment. Similarly, generally moderate to high genetic

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correlations were estimated (0.61-0.90) between test environments for weight gain (WG), specific growth rate (SGR) and relative weight gain RWG) with one exception (r_g=0.46 between O and N environments for RWG). The heritability estimates ranged from 0.19 to 0.31 for HW, 0.21 to 0.29 for WG, 0.19 to 0.39 for SGR and from 0.16 to 0.42 for RWG. The estimated heritabilities were higher in the O than in the L tanks, while the estimate for N pond environment was intermediate. It is concluded that there was a substantial additive genetic variance for growth traits in the breeding population studied. Estimated genetic correlations suggest that there is some *GxE* interaction for growth traits in controlled optimal temperature and natural subtropical mountain pond environments. However, the genetic correlation for growth between the two environments indicate that a moderate and favorable correlated selection response can be obtained for growth in natural pond environments when selecting and testing in optimal temperature conditions. It is recommended that testing and selection should be carried out in natural tropical ponds to improve growth and robustness of fish to be fitted for farming in different commercial environments of Vietnam. However, additional samples of families may be tested in a subtropical pond in the mountain area to allow for some selection and dissemination of broodstock and/or seeds that are more fit for mountain farming.

Keywords: *Oreochromis niloticus*; Genetic parameters; Genotype by environment interaction; Temperature; Harvest weight.

1. Introduction

Tilapia is an economically important species cultivated mostly in tropical countries. They are primarily cultured in a wide range of production systems, from simple small-scale waste-fed fresh water ponds or rice paddy fields, to highly intensive culture systems. The adaptability and tolerance of this species to diverse environments and systems has resulted in a rapid expansion of tilapia farming not only among fish farmers in Asia, but also an increasing

interest in other parts of the world (Pullin, 1996). Nile tilapia (*Oreochromis niloticus*) is well suited for farming in a range of environments. It is important to ensure farmers in the Northern part of Vietnam access to robust fish material which can produce under their harsh environmental conditions.

Water temperature is recognized as one of the most important factors influencing growth. The optimal temperature for growth of most tilapias range from 26^{o}C to 30^{o}C, while the lethal temperature is 10^{o}C. Activity and feed utilization will decrease when temperature falls below 20^{o}C (Chervinski, 1982). Therefore introduction of Nile tilapia *O. niloticus* to subtropical regions, will lead to poor growth and massive mortality during over-wintering (Chervinski and Lahav, 1976; Tave et al., 1990).

Selective breeding has contributed significantly to faster domestication of tilapia worldwide (ADB, 2005). Growth rate is one of the most economically important traits in fish breeding programs. Several estimates of heritability for harvest body weight of Nile tilapia are reported in the literature, ranging from 0.12 to 0.65 (Bolivar and Newkirk, 2002; Charo-Karisa et al., 2006; Kronert et al., 1989; Luan et al., 2008; Oldorf et al., 1989; Ponzoni et al., 2005; Rutten et al., 2005b). Estimates of heritability for *O. mossambicus*, *O. hornorum* and *O. shiranus* were in the range of 0.20 - 0.32 (Gall and Bakar, 1999,2002; Maluwa et al., 2006). However, none of these estimates are based on growth in temperate environment. Cold tolerance may be considered as an important trait for developing a more robust fish. A few studies have documented genetic variation for cold tolerance between tilapia species or strains (Behrends et al., 1990; Cnaani et al., 2000; Rezk et al., 2002; Sifa et al., 2002). However, the limited estimates of heritability reported for cold tolerance in *O. niloticus* were based on survival data from cold tolerance tests and were below 0.10 (Behrends et al., 1996; Charo-Karisa et al., 2005). Furthermore, Cnaani et al. (2000) suggested that there was a large dominance genetic component for cold tolerance. Standardized short term cold tolerance

challenge tests were applied in these studies. However, performance and robustness of tilapia in temperate climates may also be improved by increasing growth rate and pond survival at low temperatures. This will contribute to increased production of tilapia by either extending the growth season or by increasing the number of crops per year.

Genetic parameters for growth at low or suboptimal temperature has so far not been reported, and the potential for genetic improvement of growth during periods with decreased temperature or in highland areas has neither been explored nor exploited.

Northern Vietnam has a subtropical climate with lower temperature from December to March compared to the summer. Occasionally, water temperature drops to 20^{o}C for a period during the winter. This is considered as an important limitation for growth of farmed Nile tilapia.

A selective breeding program for tilapia was implemented in the North Vietnam and has improved growth significantly in natural ponds under favorable (optimal) temperature or tropical conditions (Luan et al., 2008). The improved strain has become popular among fish farmers and the fish has been disseminated all over the country. However, the performance at lower temperature such as during the winter and fall also needs to be improved. Although the tilapia production volume and market value of sales in the mountain areas is not high (> 5,000 tones), the production may still have relatively high social importance for the small and poor households and communities in these areas. However, justifying the relatively high costs of an additional breeding program implies that the *GxE* interaction must be large and contribute to a considerably lower realized (correlated) selection response through a significantly lowered correlation between growth in optimal and e.g. cooler mountain environment. Mulder et al. (2006) indicated that a genetic correlation between performances in different farm environments of 0.7-0.8 or lower would justify separate breeding programs for the two farm environments. Hence, the main objective of the present study was 1) to investigate the

potential for improving growth under low temperature conditions by estimating genetic parameters for growth traits at different temperature regimes of *O. niloticus*, and 2) to explore the need for region specific breeding programs by studying the possible degree of *GxE* interaction.

2. Materials and Methods

2.1 Experimental design and test environment

The on-going selective breeding program of Nile tilapia at Research Institute for Aquaculture No.1 (RIA1) provided the experimental fish. A total of 102 family groups were produced and nursed in accordance with the GIFT guidelines (WorldFish Center, 2004).

The schedule of reproduction and management of the experiment is presented in Table 1. Families were produced in breeding hapas using a hierarchical design, where every sire was stocked with two different dams. However, high water temperature often prevented mating of males with the second female, resulting in a lower number of half-sib families than planned. The first batch consisted of 40 full-sib families produced during autumn 2006 by 38 sires and 40 dams. The second batch consisted of 62 full-sib families produced during spring 2007 by 51 sires and 62 dams. In each batch, 300 swim-up fry were collected from each full-sib family in the breeding hapas. They were reared separately in 1 m^3 hapas in 30 days before being transferred to a 3 m^3 hapas with a total of 150 fingerlings, and hence a lower stocking density. All hapas were kept in a 1,200 m^2 earthen pond. Fish was randomly chosen from each full-sib family and tagged with electronic PIT tags. They were further stocked in hapas for recovering and for checking of tag losses. Initial body weights were recorded before an equal number of fish from each family were stocked together in the experimental tanks and pond.

The first batch of full-sib families (Batch 1) were produced from end of September to end of October, 2006, while the test period for these fish was carried out from February until June

2007 at RIA1 and in the Lao Cai province (Table 1). Note that the rearing period of Batch 1 corresponds to early seed stocking season in the North of Vietnam. The second batch of full-sib families (Batch 2) were produced between May and June, 2007, while the test period for these fish was carried out from October 2007 until early January 2008 (Table 1). Note that the rearing and test period of Batch 2 corresponds to winter time in North Vietnam.

The fish was tested in three temperature regimes; in an on-station trial with controlled temperature in four tanks holding 30^{o}C (Optimal = O) and four tanks holding 21^{o}C (Low = L), and in an on-farm trial in the mountain province (Lao Cai) where the fish were kept in an earthen pond without any control of the temperature (Natural pond = N). A total of 8 cement tanks, each with the dimension of 25 m^2 and water depth of 1.2 m were used for the on-station test with controlled temperature in each batch.

Stocking density in each tank was 12.4-14.0 fish per m^2. Water temperature was monitored in each tank every day. In the four tanks with optimal temperature, the temperature was maintained at approximately 30^{o}C by using electric heating. In the remaining tanks, low water temperature was maintained at approximately 21^{o}C by using a cooling system. Each tank was constantly aerated using five air-stones connected to an air pump. Dissolved oxygen (DO) and pH were measured every morning, while total ammonia, nitrate and nitrite were measured every week, using a DO meter (YSI model 58), digital pH meter (model 1290) and HACH kits respectively. Tanks were also cleaned by a siphon once a day to remove feces. Water lost during cleaning was compensated with clean pre-treated water. The pH, DO, total ammonia, nitrite and nitrate was kept at acceptable levels for the experimental fish (Table 1).

The on-farm trial was conducted in a natural household pond of 500 m^2 in the mountain province (Lao Cai), 350 km North of Hanoi and more than 1,200 m above the sea level. The pond was managed at a water depth of 1.2 m and continuously exchange of water by irrigated

water from the mountain. Tagged fish was stocked at a density of 2.5-2.8 fish per m^2. Water temperature was measured every morning.

All experimental fish were fed with floating pellet feed (20–25% crude protein) at a rate of 3.0% of body weight per day during the first month, and 2.0% of body weight per day from the second month until the end of the experiment. Feeding rate adjustments were conducted every second week. Body weight was recorded for all fish at stocking and harvesting.

2.2 Traits included in the analysis

Individual body weight was recorded at tagging (IW) shortly before the fish were transferred to the experimental facilities, and at the end of the test period or harvest (HW). To account for the considerable individual variation in IW at the start of the experiment, the following derived variables related to growth were calculated: body weight gain (WG, g) = HW - IW; relative weight gain (RWG, %) = (WGx100)/IW; and specific growth rate (SGR, $g.day^{-1}$) = (LnHW– LnIW)/(t_2-t_1) where t_1 and t_2 were the recording date of IW and HW, respectively, t_2-t_1 is number of days from tagging to harvesting.

Detailed information about sex, age, batch of test, test management between environments are presented in Table 1. The IW and HW as well as survival rate for each batch and test environment are presented in Table 2. The IW ranged from 38.2 g to 43.3 g for batch 1, while lower weights were observed at stocking for batch 2 (Table 2). Table 2 shows that the mean HW ranged between 128.1 to 207.8 g and 142.5 to 221.1 g for the batch 1 and batch 2, respectively. The survival rate was high in all test environments, except the survival rate in the natural pond in the first batch, which was relatively low (78%) due to stress after long transportation and many escapees during exchange of water. Consequently, survival was not included in the analysis. The coefficient of variation was high for both IW and HW (Table 2).

2.3 Statistical analyses

The least squares means (LSM) were estimated for all growth traits and batches and test environments by using the Mixed procedure of SAS (SAS Institute Inc., 2003). The fixed effects included in the statistical model were sex, batch, tank/pond nested within test environment and their significant interactions, while taking into account heterogeneous variances within sexes. Age (days) at harvest was included in the model as a covariate. The regression coefficient of harvest body weight on body weight at tagging was obtained for each environment and sex in a separate simple regression analysis.

Genetic and environmental variances and co-variances was estimated based on a linear animal model using the Asreml software (Gilmour et al., 2002). In matrix notation, the model can be written as:

$$\boldsymbol{y} = \mathbf{X}\boldsymbol{b} + \mathbf{Z}\boldsymbol{a} + \mathbf{W}\boldsymbol{c} + \boldsymbol{e}$$

where $\boldsymbol{y}$ is a vector of body weight or growth observed for each fish in different test environments and batches; $\boldsymbol{b}$ is the vector of fixed effects of tank/pond, batch, sex and batch by sex interaction, and the linear and quadratic covariate effects of age at end of test nested within test environment and batch; $\boldsymbol{a} \sim (\mathbf{0}, \mathbf{A}\sigma^2_a)$ is the vector of random additive genetic effects for each fish; $\boldsymbol{c} \sim (\mathbf{0}, \mathbf{I}\sigma^2_c)$ is the vector of random effects common to full-sibs (i.e., environmental effect caused by the separate rearing of each full-sib family until communal stocking after tagging, non additive genetic and/or maternal effects); and $\boldsymbol{e} \sim (\mathbf{0}, \mathbf{I}\sigma^2_e)$ is the vector of random residual effects. **X**, **Z** and **W** are known incidence matrices assigning the observations to the fixed effects, the random additive genetic effects of the fish and the residual random effects, respectively. Six generations of pedigree information was included in the analysis.

A univariate animal model was used to estimate genetic parameters for each test environment. Common full-sib effect (c^2) was successfully included in this univariate analyses. A multi-trait animal model was used to obtain (co)variance components for body weight and

derived growth variables for different test environments and across batches. The genetic correlation between body weights recorded in different test environments was calculated as $r_{a(i,j)} = \sigma_{a(i,j)}/\sigma_{a(i)}\sigma_{a(j)}$ where $\sigma_{a(i,j)}$ is the additive genetic covariance component between the pairs of body weights in different test environments and $\sigma_{a(i)}$, $\sigma_{a(j)}$ are the additive genetic standard deviations for body weights in the *i*th and *j*th test environments, respectively. The heritability for body weights was calculated as $h^2 = \sigma_a^2/(\sigma_a^2 + \sigma_c^2 + \sigma_e^2)$ and the common full-sib effect was calculated as $c^2 = \sigma_c^2/(\sigma_a^2 + \sigma_c^2 + \sigma_e^2)$, where σ_a^2 is the additive genetic variance, σ_c^2 is the variance due to common rearing of full-sibs, non-additive genetic and maternal effects, and σ_e^2 is the residual variance.

The residual covariances between the traits recorded in the different test environments was set to zero in multi-trait analysis, because the trait was recorded on different fish in the different environments. Unfortunately, only genetic and phenotypic correlation between IW and HW were obtained from a model including the common full-sib effects, while the other genetic correlations between environments were estimated without including common full-sib effects due to convergence problems for these multi-trait models analyses.

3. Results

3.1 Growth performance

Least squares means for IW, HW and the derived variables are shown in Table 3. As expected, IW did not differ between the three test environments ($P>0.05$). HW recorded in the optimal environment (215.85 g) was significantly higher than in the other two test environments, while lower HW was recorded (150.56 g) in the natural pond and the last was HW recorded (136.96 g) in low temperature environment, and significant differences were estimated ($P<0.05$). Similarly, WG was significantly higher in the optimal temperature

(179.83 g) compared to lower in the natural conditions (115.84 g) and in the low temperature (100.41 g) (Table 3).

The SGR ($g.day^{-1}$) was significantly ($P<0.05$) lower at low temperature (1.28 g) compared to optimal temperature (1.73 g) and natural pond conditions (1.75 g), while no differences were found between SGR at optimal temperature and in natural pond environment (Table 3). RWG differed significantly between all environments ($P<0.05$). Optimal temperature resulted in the highest RWG (100*WG/IW), while intermediate RWG was found in natural pond and lowest at low temperature environment.

Estimated linear regression coefficients of WG on IW within test environment and sex were all positive for both males and females and significantly different from zero ($P<0.001$) (Table 4). The males had significantly higher regression coefficients than the females. This regression coefficient was larger in optimal temperature and natural pond (Table 4).

3.2 Heritabilities

Estimates of the heritability (h^2) and common full-sib effects (c^2) for different traits in each test environment across batches are shown in Table 5. The h^2 estimates for HW was lowest at low temperature (0.19±0.04), and highest at optimal temperature (0.31±0.08). Similarly, the h^2 estimates for WG was 0.29±0.08, 0.21±0.04 and 0.28±0.07 for optimal and low temperatures and natural pond environment, respectively (Table 5). The c^2 for the two traits, HW and WG, were relatively low and not significantly different from zero ($P>0.05$). The heritability estimates for IW (0.32±0.12) was relatively high. However, the standard errors (SE) of this estimate and the estimate of c^2 (0.18±0.09) were also high.

The h^2 estimated for SGR and RWG at optimal temperature was 0.39±0.11 and 0.42±0.07 respectively, while it was lower for the other environments (Table 5). In general, the estimated h^2 for SGR and RWG were higher than for HW and WG. The estimates of c^2 were relatively

low and varied from 0.03 to 0.12, with relatively high SE (Table 5). They were however lower than the SEs estimated for the heritabilities in this study. The h^2 of IW was 0.32±0.18 and a high c^2 was estimated (0.18) for this trait reflecting a substantial common environmental effects of full-sib families during the nursing period.

3.3 Correlations

Estimated genetic and phenotypic correlations between IW and HW within each environment were positive and ranged from medium to high (Table 6). A high genetic correlation (0.86±0.03) was estimated for low temperature environment, while relatively lower correlations, 0.80±0.09 and 0.75±0.04 respectively, were estimated for optimal temperature and natural pond environments.

The genetic correlations between pairs of test environments for HW, WG, SGR or RWG were generally high (Table 7) and significantly different from zero ($P<0.05$). The correlations ranged from 0.61 to 0.88 for HW, from 0.65 to 0.90 for WG, from 0.62 to 0.87 for SRG, and from 0.46 to 0.85 for RWG (Table 7). The highest correlations were found between the controlled optimal and low temperature environments. The correlations between optimal and natural temperature environments were lower than between optimal and low, and was particularly low for RWG (0.46±0.03).

4. Discussion

4.1 Growth performance in different environments

An optimal temperature range of 26-32^{o}C is recommended for Nile tilapia (El-Sayed and Kawanna, 2008; Likongwe et al., 1996). In a selective breeding program for tilapia in North Vietnam the main aim has been to improve growth performance in favorable temperature farm environments (Luan et al., 2008). The question is whether low temperature regions will benefit

from using improved fish from the on-going selective breeding programs. The present study aimed at answering this question, by examining growth traits in controlled optimal and low temperature tanks, as well as a natural pond in a mountain province to reflect different temperature conditions during winter or the harsh conditions in highland areas.

The levels of HW, WG, SGR and RWG increased with increasing temperature (Table 3). This is in accordance with Azaza et al. (2008) who found maximum growth of *O. niloticus* at 30^{o}C as well as high performance for other production traits. Others have also reported that 28-30^{o}C was optimal for growth of tilapia at fry and fingerlings stages (Baras et al., 2001; El-Sayed and Kawanna, 2008). Although the average recorded morning temperature in the natural pond was not significantly higher than the controlled low temperature environment (Table 1), a higher performance was detected in the natural pond. This may be explained by a higher temperature during day time in the pond, which was not reflected when temperature was recorded in the morning. The results may suggest that tilapia farming in the North Vietnam can be extended to periods with short-term temperature drops to 20^{o}C during the winter, which is also supported by Maluwa et al. (2006).

The positive regression coefficient of WG on IW indicates that initial weight at stocking has a highly significant and positive effect on WG in both sexes at harvest. The variation in stocking weight is caused by the variation in time of the production and the separate nursing of families as well as genetic effects. The age of fingerlings at stocking varied from 74 to 120 days in this test and may also explain some of the individual variation in IW. Similar results were found by Eknath et al. (2007) and Longalong et al. (1999), who also reported that increased stoking body weight resulted in an increased average harvest body weight. However, Maluwa and Gjerde (2007) found no significant relationship between harvest weight and stocking weight for females of *O. shiranus*. This may due to differences between species and/or management in these studies.

4.2 Genetic parameters and GxE interactions

Heritability estimates were moderate for all traits presented in Table 5. The h^2 estimated for HW, WG, SGR and RWG in this study varied for the different environments. Generally, h^2 estimated for optimal temperature and natural environments were higher than in low temperature environment. This is expected as increasing the water temperature to optimal level increase the growth rate of tilapia and the genetic potential of the fish for growth may be expressed better (Azaza et al., 2008; Baras et al., 2001; El-Sayed and Kawanna, 2008). The estimated h^2 for HW (0.19-0.31) in this study were within the range of estimates published for *O. niloticus* by Gall and Bakar (1999,2002), Luan et al. (2008), Ponzoni et al. (2005) and Rutten et al., (2005) and for *O. shiranus* by Maluwa et al. (2006). Our estimates of h^2 for HW are slightly lower than the estimate reported by Charo-Karisa et al. (2006) for growth of fish in low input environment. However, estimates of h^2 for WG, SGR and RGW is not reported earlier in the literature. The magnitude of h^2 estimates for growth traits show that the population has sufficient additive genetic variance for growth to be utilized in selective breeding programs. So far, most studies have focused on cold tolerance of tilapias using cold challenge test survival data (Behrends et al., 1996; Charo-Karisa et al., 2005; Sifa et al., 2002), and in some studies, a low heritability for cold tolerance was found (Behrends et al., 1996; Charo-Karisa et al., 2005). The present study indicates, however, a large potential for improving production in cold climates by selecting for growth in low temperatures and in mountain pond environment. Expanding tilapia farming to subtropical and temperate regions is thus possible and beneficial for the farmers if such testing and selection is implemented in the breeding program as also concluded by Rezk et al. (2002).

Estimated genetic correlations between IW and HW were relatively high and varied from 0.75 to 0.86 for each test environment (Table 6). This suggests a potential for indirect selection

of HW based on IW. However, the separate rearing of full-sib families may also increase the proportion of common environment effects in early weight and reduce the level of the heritability. Due to high common environmental rearing of families effects, thus improved management and husbandry practices will reduce this effect considerably.

In the present study, the genetic correlations between HW recorded in different environments were moderate to high and significantly different from zero. A certain degree of *GxE* interaction between optimal temperature and natural pond in mountain areas is indicated through a moderate genetic correlation. Other studies report similar results of *GxE* interaction for HW in different test environments (Bentsen et al., 1998) and between "low and high input" pond environments (Khaw et al., 2009). The estimated genetic correlations were similar and lower than reported earlier between cage and pond for harvest weight of *O. niloticus* (Eknath et al., 2007), but much higher than found for harvest weight of *O. shiranus* at different farm environments (Maluwa et al., 2006), and between brackish and fresh water in *O. niloticus* (Luan et al., 2008). According to the conclusions by Mulder et al., (2006), separate selection programs is needed for optimising genetic gain when the genetic correlations between two environments is less than 0.7-0.8. However, the decision depends on how much the breeding goals differ between the environments, the need for additional investments as well as the production volumes in question. Although a certain *GxE* interaction is documented in the present study, a low tilapia production volume and low ability to invest among small-scale fish farmers in the mountain and highland areas in Vietnam are reasons to question whether investments in a highland breeding program can be justified. However, the results show that it may be important to continue the testing and selection in a natural pond environment with natural temperature variation in order to avoid that a more sensitive and less robust fish is developed.

Estimates of the genetic correlations between different environments for WG, SGR and RWG in tilapia are previously not reported. However, the estimated genetic correlations between environments for WG, SGR and RWG in the present study were similar to the estimates for HW between different environments. The estimated genetic correlation for HW, WG, SGR and RGW may be biased upwards because common full-sib effects were not included in the statistical model, and should therefore be treated with care.

Due to limitations in the mating design implying relatively few half-sib families produced and the fact that only one generation with data was included, the common environmental effect could not be included in the multi-trait analysis due to convergence problems. Although, there were six generations of pedigree information, this may have caused biased correlation estimates. However, in the univariate analyses the estimates of common environmental effects (c^2) were generally less than 10%. This is lower than found in several other studies (Charo-Karisa et al., 2006; Khaw et al., 2009; Ponzoni et al., 2005; Rutten et al., 2005b), but within the same range as reported by Maluwa et al. (2006).

5. Conclusion and implications

The present study shows a potential for improving growth of tilapia in subtropical farm environment, which will benefit farmers in the mountain areas of North Vietnam. This can be achieved either through direct selection for performance in the natural cold climate ponds or to a smaller extent through indirect selection for performance in optimal temperature ponds. Although there is an evidence of *GxE* interaction, this is considered to be of less practical importance in tilapia breeding and farming in Vietnam. Moreover, the magnitude of genetic variation indicated a large potential for further improvement by selective breeding in both low and optimal temperature farming environment. Consequently, one selection program in optimal temperature environment will benefit farming of tilapia in other and cooler freshwater

environments in North Vietnam. Anyhow, testing and selection should be based on growth performance in farm pond environments with natural variation in temperature to develop a robust fish for commercial farming. Furthermore, additional testing in a subtropical pond in the mountain area to allow for some selection and dissemination of broodstock and/or seeds that are more fit for mountain farming.

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LIST OF TABLES

Table 1: Schedule of reproduction and management of experiments

Experimental management	Test temperature environments		
	Optimal [a]	Low [a]	Natural pond [b]
Test location	On-station	On-station	On-farm
Pellet feeding rate (% BW day^{-1})	3.0 - 2.0	3.0 - 2.0	3.0 - 2.0
Sex ratio (M/F)	1.40	1.46	1.14
1st batch (2007)			
Test duration	Feb.-June	Feb.-June	March-July
Fish stocking density (fish m^{-2})	14.0	14.0	2.8
Production of family in hapas & nursing	Nov. - Feb.	Nov. - Feb.	Nov. - Feb.
No. stocked per family	35	35	35
Temperature (^{0}C)	30.1 (28.1-32.7)	20.9 (18.5-22.5)	23.5 (19.0-27.5)
pH	7.5 (6.7-8.0)	7.7 (7.2-7.8)	-
DO (mg L^{-1})	4.1 (3.6-6.1)	3.47 (2.9-4.8)	-
2nd batch (2007 – 2008)			
Test duration	Oct. - Jan.	Oct. - Jan.	Oct. - Jan.
Fish stocking density (fish m^{-2})	12.4	12.4	2.5
Production of family in hapas & nursing	April - Sept.	April - Sept.	April - Sept.
No. stocked per family	20	20	20
Temperature (^{0}C)	29.2 (27.8-31.1)	21.1 (18.6-22.0)	22.1 (18.0-25.2)
pH	7.7 (7.0-8.5)	7.9 (6.8-8.5)	-
DO (mg L^{-1})	5.4 (4.1-6.0)	5.0 (3.0-6.0)	-

[a] cement tanks at RIA-1, [b] pond in mountainous province. Max. & min. values in brackets

Table 2: Number of fish recorded at harvest (N), mean body weight (g) and coefficient of variation (CV) of tagging and harvest body weight and survival at each test environment and batch

Batch	Test Environment *	N	Initial body weight		Final body weight		Survival rate (%)
			Mean	*CV*	*Mean*	*CV*	
1	O	1260	41.8	49.1	207.8	28.9	99.6
	L	1209	43.3	46.2	128.1	33.1	95.7
	N	916	38.2	56.8	173.1	24.9	77.9
2	O	1243	29.6	49.5	221.1	31.4	98.3
	L	1234	28.2	50.0	142.5	39.3	97.6
	N	1191	30.7	48.3	158.8	34.1	94.2

** O is optimal; L is low and N is natural temperature regime of the test environment*

Table 3: Least squares means for traits measurement (±*se*) recorded in each test environment of *Oreochromis niloticus*

Trait	Environments*		
	O	L	N
Body weight at tagging (IW)	35.88±0.62[a]	37.66±0.57[a]	35.50±0.71[a]
Harvest body weight (HW)	215.85±5.17[c]	136.96±4.11[a]	150.56±7.33[b]
Weight gain (WG g)	179.83±3.68[c]	100.41±2.97[a]	115.84±5.12[b]
Specific growth rate (SGR g.day^{-1})	1.73±0.05[b]	1.28±0.05[a]	1.75±0.09[b]
Relative weight gain (RWG %)	634.56±5.56[c]	345.07±5.26[a]	429.96±6.33[b]

[*] *O is optimal; L is low and N is natural temperature regime of the test environment*

Values followed by the same letter within row is not significantly different (P>0.05)

Table 4: The linear regression coefficients (β±se) of body weight gain (WG) on tagging body weight (IW) within each test environment and sex of *Oreochromis niloticus*.

Test environment	Sex[a]	β±se[b]
Optimal temperature (O)	M	0.44±0.09
	F	0.25±0.09
Low temperature (L)	M	0.38±0.08
	F	0.23±0.07
Natural temperature pond (N)	M	0.50±0.06
	F	0.32±0.06

[a] *M male and F female*

[b] *all significant deviation from zero (P<0.001)*

Table 5: Estimates of the heritability ($h^2 \pm s.e.$) and the common full-sib effect ($c^2 \pm s.e.$) for weight measurement of *O. niloticus* for each test environment

Variable	Optimal temp.		Low temp.		Natural pond temp.	
	h^2±s.e.	c^2±s.e.	h^2±s.e.	c^2±s.e.	h^2±s.e.	c^2±s.e.
Harvest weight (HW)	0.31±0.08	0.03±0.05	0.19±0.04	0.04±0.05	0.26±0.07	0.06±0.05
Weight gain (WG)	0.29±0.08	0.04±0.05	0.21±0.04	0.05±0.05	0.28±0.07	0.04±0.05
Specific growth rate (SGR)	0.39±0.11	0.11±0.06	0.27±0.05	0.08±0.04	0.30±0.06	0.09±0.04
Relative weight gain (RWG)	0.42±0.07	0.04±0.04	0.34±0.07	0.12±0.05	0.34±0.06	0.06±0.12

Table 6: Genetic ($r_g \pm se$) and phenotypic ($r_p \pm se$) correlation between initial body weight (at tagging) and harvest body weight of *Oreochromis niloticus* at different environments.

Environments*	r_g	r_p
O	0.80±0.09	0.63±0.05
L	0.86±0.03	0.61±0.02
N	0.75±0.04	0.68±0.03

* *O is optimal; L is low and N is natural temperature regime of the test environment*

Table 7: Genetic correlation (±*se*) for traits recorded of *Oreochromis niloticus* between the pairs of test environments for different growth variables.

	Environment *		
Trait	O *vs.* L	O *vs.* N	L *vs.* N
HW	0.88±0.19	0.61±0.05	0.78±0.10
WG	0.90±0.17	0.65±0.05	0.83±0.12
SGR	0.87±0.19	0.62±0.05	0.77±0.09
RWG	0.85±0.17	0.46±0.03	0.61±0.04

** O is optimal; L is low and N is natural temperature regime of the test environments*

Paper III

Genetic parameters of cold tolerance and growth of Nile tilapia (*Oreochromis niloticus*)

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Abstract

The objective of this study was to estimate genetic parameters for cold tolerance, harvest body weight and growth rate during overwintering of Nile tilapia (*Oreochromis niloticus*) in North Vietnam. A total of 323 full-sib families were produced by mating each sire with two dams in three subsequent years/generations (2004-2006). Swim-up fry of each family were collected and nursed in separate hapas until tagging with PIT tags. The individually tagged fingerlings were tested for cold tolerance as well as harvest body weight and growth during overwintering after communal rearing. The cold tolerance were expressed as cooling degree hours (CDH), temperature at death (TAD), survival at 50% survival for the test population as a whole (SUR_{50}) and survival (SUR) at the end of the test. The estimated heritabilities with standard errors ($h^2 \pm se$) were 0.15±0.07 for CDH, 0.07±0.03 for TAD, 0.26±0.13 for SUR and 0.11±0.02 for SUR_{50}. The heritability estimates were 0.22±0.06 and 0.18±0.06 for harvest body weight (HW) and growth during overwintering (OW), respectively. Genetic correlations between CDH and TAD (-0.85±0.03), between TAD and SUR_{50} (-0.35±0.08) and between TAD and SUR (-0.69±0.05) were all strongly negative, while strongly positive genetic correlations were estimated between CDH and SUR_{50} (0.89±0.09) and

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between CDH and SUR (0.50±0.08). Furthermore, the estimated genetic correlation between SUR and SUR_{50} (0.79±0.08) was high. A high genetic correlation (0.94±0.05) between harvest body weight and growth during overwintering was also estimated. Although not significantly different from zero, low genetic correlations were estimated between CDH, SUR or SUR_{50} on one hand and HW or OW on the other. Our results indicate that selecting for harvest body weight will not cause any negative consequences in cold tolerance. Moreover, one may increase the possible benefit from extending culture period into the winter season when selecting for harvest body weight. The results indicate a potential for selection for both cold tolerance and growth during winter as well as the summer. Further research is however needed to more efficiently improve and apply selection for cold tolerance using cold challenge tests.

Key worlds: Cold tolerance; Harvest body weight; Overwintering; *Oreochromis niloticus*; Genetic parameters, Nile tilapia

1. Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the most important culture species, constituting 90% of all tilapia cultured outside Africa (FAO, 2004). However, tilapia is highly sensitive to low ambient temperatures leading to poor growth and mass mortality during winter (Chervinski and Lahav, 1976; Tave et al., 1990). Depending on the climatic region, the grow-out period is normally restricted to three months (Hofer and Watts, 2002). To optimise the production and grow-out season, fingerlings are usually produced indoor during the cold spring months. Fingerlings may also be produced during the late autumn and then over-wintered (Dan and Little, 2000) before stocking for grow-out during warmer summer months. The optimal temperature for growth of *O. niloticus* species is around 30°C (Abdel-Fattah and Mamdouh, 2008; Azaza et al., 2008; Chervinski, 1982) and they cannot survive in a temperature below 10-12°C for more than a few days (Chervinski, 1982). Improved

management during overwintering of tilapia fry to improve harvest body weight has been demonstrated (Behrends et al., 1990; Cruz and Ridha, 1994; Dan and Little, 2000). This may however be impossible or too costly in many regions.

In North Vietnam, seasonally low temperature during winter months between December and March affects both growth rate and survival of tilapia. The water temperature is then normally around 20°C , but will frequently drop to a minimum of 11°C. Suitable production time for growing tilapia is between April and November, but it may be challenging for many farmers to obtain marketable size of fish during this short culture period.

Improving growth in optimal and cold temperature as well as improving cold tolerance (survival) through selective breeding can reduce cost and losses associated with over-wintering as suggested by several authors (Behrends et al., 1990; Charo-Karisa et al., 2005; Sifa et al., 2002). However, only a few genetic studies of cold tolerance in tilapia have been carried out (Charo-Karisa et al., 2005; Luan et al., submitted[b]). Cnaani et al. (2000) concluded that a large component of the cold tolerance variance was due to dominance effects for some tilapia species and their hybrids. Other studies suggested a relatively low heritability of -0.05–0.09 for cold tolerance (Behrends et al., 1996; Charo-Karisa et al., 2005). Genetic correlations estimated between body weight at cold challenge testing on one hand and CHD and TAD on the other were 0.72 and -0.68, respectively (Charo-Karisa et al., 2005).

A breeding program for tilapia has been implemented at Research Institute for Aquaculture No. 1 (RIA1), Vietnam, to improve performance of a Nile tilapia strain in a range of environmental conditions in North Vietnam. The purpose of the present paper is to provide knowledge about genetic parameters for growth and cold tolerance traits to consider the outcomes of different breeding strategies. The study is based on genetic analysis of survival data from three years of challenge testing for cold tolerance in addition to harvest body weight

in the on-going breeding program for Nile tilapia and weight gain during overwintering these years.

2. Materials and methods

2.1 Fish material and production of families

The brood fish population was obtained from an on-going breeding program of Nile tilapia at RIA1 (Luan et al., submitted[a]). The objective of the breeding program included cold tolerance, and selection was based on breeding values using both survival records from cold challenge tests as well as harvest weight since 2003. Full-sib families were produced at RIA1 during the spring breeding season (April – June) each year from 2004 to 2006. Body weight was recorded at harvest time (December) and after overwintering (March). Detailed information about the family production and fry collection is given in Table 1. Mating design, fry collection and nursing of families was carried out in accordance with the GIFT guidelines (WorldFish Center, 2004).

The pairs mating gave 106 full-sib families (63 sires and 106 dams) in 2004, 103 full-sib families (64 sires and 103 dams) in 2005 and 114 full-sib families (74 sires and 114 dams) in 2006. Swim-up fry were produced during a period of max 50 days per year (Table 1). In each generation, 300 swim-up fry from each full-sib family were randomly collected. They were reared in separate $1m^3$ hapas for 30 days, before being transferred to a 3 m^3 hapas with 150 fingerlings until tagging size (ca 10-12 g). All spawning hapas were installed in a 1.200 m^2 earthen pond. Supplemental powder feed containing 25-30% crude protein was fed at a rate of 6-8% of fish biomass. Fingerlings of 70 – 120 days of age were randomly chosen from each full-sib family and injected with electronic PIT tags for grow-out test in ponds or challenge testing. Two weeks after tagging, the cold challenge test was carried out. Detailed information

about each test is presented in Table 2 and Table 3. Data from three generations were analysed together with six generations of pedigree information.

2.2 Cold challenge test

After 5-7 days recovering from the stress of tagging (PIT tags), 20 healthy fingerlings from each family were transferred to the cooling tank for cold tolerance challenge testing. The test was conducted by using an automatically temperature adjustment cooling system. Water was supplied in a 6.0 m^3 tank located indoor. Water temperature was adjusted every hour according to the schedule given in Figure 1. Dissolved oxygen (DO) was supplied by constant aeration using 10 air-stones connected to an air pump to maintain a DO of minimum 5 mg/l. Total ammonia, nitrate, nitrite and pH were measured twice per day by using HACH kits and pH meter. These parameters were maintained at a level considered safe for the fish. Water and tank was cleaned by a recirculation system during the challenge test. The tank bottom was cleaned daily by using a siphon.

The fingerlings were kept at a temperature of around 30°C before being transferred to the tank for challenge testing. As shown in Figure 1, the temperature was first reduced from 30°C to 20°C at a rate of 1°C per 2.5 hours, and then reduced from 20°C to 15°C at a rate of 1°C per 5 hours. From 15°C to 12°C, water temperature was reduced at a rate of 1°C per 8 hours. Finally, the temperature was reduced at a rate of 0.1°C per 3 hours until the end of the test. The water temperature was monitored and controlled every hour during the test. All fish were kept in the same tank during the cold challenge test. The test was stopped at 85-90% mortality. Feed was provided when the water temperature was above 20°C, whereas no feed was supplied when the temperature was below 20°C. The fish was confirmed dead if they lost balance, fell on their side, generally ceased movements and lost response to external stimuli. Dead fish were removed from the tank every hour with a scoop net, and tag number and temperature at death

(TAD) was recorded. Furthermore, survival at the end of the test (SUR) was recorded as '1' for survived and '0' for dead individuals. Similarly, SUR_{50} expresses survival when 50% of the test fish was dead. The fish started to die at 12°C , and cooling degree hours (CDH) was calculated for each dead fish as the sum of hours the fish survived after the temperature passed 12°C multiplied by the difference between 12°C and individual temperature at death. Surviving fish had missing lethal temperature and CDH. General information and raw means of traits recorded in the challenge tests is presented in Table 3. Overall mean body weight was 15.8 g, and the age of fingerlings in the test was 107 days on average. Overall mean CDH and TAD was 107.7 and 10.3, respectively. Survival rate at the end of test was 10.4 % for overall three test years, which varies from 8.8% to 15.0% for each year.

2.3 Grow-out and overwintering of fish

A total of 40 individually tagged fish were randomly selected from each full-sib family and stocked together in 20 m^2 fine mash hapas after 5-7 days recovering from tagging. The grow-out period was from August to December in a pond of 1800 m^2 with water temperature of 26-35°C. The fish were fed twice a day with pellet feed containing 22% crude protein. Total daily feed corresponded to 2-3% of the fish biomass. The water level in the pond was maintained at 1.2-1.4 m. Other environmental factors such as temperature, DO and pH were monitored and recorded every morning. The feeding level was adjusted every two weeks. All the fish were collected for recording of body weight, sex, date after more than 200 days of grow-out.

The fish overwintered in hapas from December to March, where males and females were kept separate. A total of 10 hapas (6x4x1.5 m) were installed in a 1,200 m^2 pond where water level was maintained at minimum 2.0 m. The pond was cleaned and disinfected with agricultural lime before the hapas were installed. Sufficient level of dissolved oxygen was

maintained by two aerators. The fish were randomly distributed in ten hapas; i.e. five for each sex. Stocking density was 14-17 fish per m^2. During overwintering, fish were fed a daily amount of 1% of fish biomass during day time when the water temperature was above 20^oC. The initial body weight, sex, date at harvest and weight after overwintering were recorded and are presented in Table 2. The overall average HW was 218.3 g, while average growth during overwintering (OW) was 83.4 g.

2.4 Statistical analysis

Variance components of additive genetic and common full-sib effects (other than additive genetic effects) for CDH, TAD, harvest body weight and growth during overwintering were estimated by restricted maximum likelihood, using a single trait animal model (Model 1). In matrix notation the model can be written as:

$$\boldsymbol{y = Xb + Za + Wc + e} \qquad (1)$$

where $\boldsymbol{y}$ is the vector of observations of CDH, TAD, harvest body weight or growth during over-wintering; $\boldsymbol{b}$ is the vector of fixed effects including year, sex and year by sex (for growth traits), and year (for CDH and TAD) and fixed regression for first and second order polynomials of age; $\boldsymbol{a}$ is the vector of random additive genetic effects for each individual; $\boldsymbol{c}$ is the vector of random effects common to full-sibs caused by factors other than additive genetic effects (i.e., environmental effect caused by the separate rearing of each full-sib family until tagging); $\boldsymbol{e}$ is the vector of residuals; **X**, **Z** and **W** are known design matrices assigning the observations to the fixed effects, the random additive genetic effect of the individual animal and the common full-sib effects included in the model, respectively. All non-significant effects were eliminated from the model by a stepwise procedure. Unfortunately, effect of overwintering hapa was not included model analyzing growth during overwintering due to missing recording.

A corresponding threshold model (Model 2) was used to analyse the traits coded as binary data (SUR and SUR_{50}) (Odegard et al., 2006). Variance components were estimated by the threshold model, assuming a normally distributed underlying liability variable, *l*, determining categorical outcomes of survival such that $l_{ijk} \leq 0$ correspondents with $Y_{ijk}=0$ and $l_{ijk}>0$ correspondents with $Y_{ijk}=1$. Residual variance of *l* was assumed to be 1. In matrix notation this model can be written as:

$$Pr(Y_{ijk}=1) = Pr(l_{ijk}>0) = \Phi(\mu + s_i + d_j + c_{ij}) \qquad (2)$$

where Y_{ijk} is survival recorded (SUR or SUR_{50}) for fish k^{th} in the test , in full-sib family *ij* (sire *i* and dam *j*), μ is overall mean (fixed effects were fitted to the model as described in Model 1), s_i is the random additive genetic effect of sire *i*, d_j is the random additive genetic effect of dam *j*, c_{ij} is the random common effect of full-sib family *ij*, and $\Phi(.)$ is the cumulative standard normal distribution. Pedigree information of seven generations was included in the analysis to account for the relationship between all animals.

Genetic correlations between responses to cold tolerance and harvest body weight and growth during overwintering were obtained using the additive genetic components of (co)variance estimated by fitting a multivariate linear animal model (Model 1) for all traits including the binary recorded survival traits (SUR and SUR_{50}). The model included both the additive genetic (animal) and common full-sib effects (dam), except for the analyses of SUR and harvest body weight, which included only random additive genetic effects, because of convergence problems. The residual covariance between cold tolerance traits (CDH, TAD, SUR_{50}, SUR) and growth traits (HW, OW) was set to zero, because the traits were recorded on different fish. Variance components obtained from the univariate analyses were used as starting values in the multivariate analyses.

The data was analyzed using the Asreml software package (Gilmour et al., 2002). The heritability (h^2) estimates based on Model 1 was calculated as $h^2=\sigma^2_{a(i)}/(\sigma^2_{a(i)}+\sigma^2_{c(i)}+\sigma^2_{e(i)})$ and

the common full-sib effect (c^2) as $c^2=\sigma^2_{c(i)}/(\sigma^2_{a(i)}+\sigma^2_{c(i)}+\sigma^2_{e(i)})$, where $\sigma^2_{a(i)}$, $\sigma^2_{c(i)}$, and $\sigma^2_{e(i)}$ are the additive genetic, common full-sib and residual variance for trait *i* respectively. The h^2 estimates for the binary traits based on Model 2 was calculated as $h^2=4\sigma^2_{sd}/(2\sigma^2_{sd}+\sigma^2_{c}+\sigma^2_{e})$, where σ^2_{sd} is the additive genetic sire-dam variance, σ^2_{c} is the common environment variance and σ^2_{e} is the residual variance (set to 1 in this model). The genetic correlation ($r_{a(i,j)}$) between traits was estimated as $r_{a(i,j)}=\sigma_{a(i,j)}/\sigma_{a(i)}\sigma_{a(j)}$, where $\sigma_{a(i,j)}$ is additive genetic covariance component between two traits and $\sigma_{a(i)}$ and $\sigma_{a(j)}$ are the genetic standard deviations of the i^{th} and j^{th} traits, respectively. Phenotypic correlation was also estimated using corresponding phenotypic covariances and standard deviations.

3. Results

3.1 Cold tolerance challenge test

The survival curves for different test years are presented in Figure 2. They show that the mortality started at approximate 12.0°C, and increased dramatically between 11.0 and 10.0°C. Overall mortality at the end of the tests was 85-90% and occurred at 8.8°C (Table 3). The responses to cold tolerance challenge test varied between years. The fish in 2006 seemed to have a slightly higher cold tolerance compared to 2005 and 2004 (Figure 2). In 2005, the fish died earlier than the other years, and had higher mortality at higher temperature. Figure 3 illustrates the variation in survival rate between families in 2006. The survival rate at the end of the test varied from 0% to 75% for the 114 families tested in 2006 (Figure 3).

Estimated heritability for responses to the cold challenge test are given in Table 4. When using the threshold model, a moderate h^2 was estimated for SUR (0.26±0.13), while a lower heritability was estimated for SUR_{50} (0.11±0.02). The h^2 estimates for CDH and TAD were 0.15±0.07 and 0.07±0.03 respectively. The h^2 estimate for SUR had a high standard error (Table 4), but all other heritability estimates were significantly different from zero. The

variance due to common full-sib effects (c^2) varied from 1 to 13% for the cold tolerance traits (Table 4).

3.2 Performance of growth and overwintering

The estimated heritabilities for HW and OW are given in Table 4. All heritabilities were significantly different from zero ($P<0.05$). A slightly, but not significantly lower h^2 was estimated (0.18±0.06) for OW compared to HW (0.22±0.06). The estimated common full-sib effects (c^2) were also high and the highest for HW (Table 4).

3.3 Correlations

The estimated genetic correlations for responses to the cold challenge test, HW and OW are presented in Table 5. The genetic correlations between TAD on one hand and CDH, SUR and SUR_{50} on the other were strongly negative (-0.35 to -0.85). Similarly, estimates of phenotypic correlations between these traits were also negative (Table 5). The genetic correlation estimate between CDH and SUR_{50} were strongly positive (0.89±0.09), while it was weaker between CDH and SUR (0.50±0.08). Furthermore, relatively high genetic and phenotypic correlations were estimated between SUR and SUR_{50} (0.79±0.08 and 0.52±0.01, respectively). The genetic and phenotypic correlation between CDH and body weight of fingerlings at test was 0.44±0.13 and 0.14 ±0.02, respectively.

The estimated genetic correlation between harvest body weight and growth during overwintering was high (0.94±0.05), while the phenotypic correlation was somewhat lower (0.81±0.01) (Table 5).

A low and non-significant genetic correlation was estimated between SUR on one hand and HW or OW on the other. The genetic correlation between CDH and HW was 0.17±0.18, while genetic correlation was 0.23±0.26 between CDH and OW (Table 5). The estimated

genetic correlations were generally very low between TAD, SUR and SUR_{50} on one hand, and HW and OW on the other (-0.26 to 0.12) (Table 5). Anyhow, the genetic correlations between cold tolerance and growth indicate a favourable relationship. The estimates of phenotypic correlations were similar to the genetic correlations in magnitude and direction.

4. Discussion

Through selective breeding, growth performance of the GIFT strain in Vietnam is continuously improved for farming in the new environment. However, it is important to know how the current selection program affects the tolerance to the lower temperatures occurring in North Vietnam during the winter and the monsoon. Hence, in this study we tested fish in real winter conditions and for short term cold challenge test mimicking short term monsoon and winter conditions.

4.1 Responses to cold challenge test and heritabilities

Temperature at death (TAD), cooling degree days (CDD) and cumulative degree hours (CDH) are described in the literature as ways of expressing cold tolerance (Atwood et al., 2003; Behrends et al., 1990; Charo-Karisa et al., 2006; Cnaani et al., 2000; Khater and Smitherman, 1988). Other measures such as number of days until death (Tave et al., 1989) or the lowest lethal temperature of 50% individuals (LD_{50}) has also been used (Atwood et al., 2003; Sifa et al., 2002). Analysing survival at the end of cold challenge test or survival at 50% overall mortality has to our knowledge not been done in previous studies of cold tolerance of tilapia. However, survival data have been analysed for disease challenge tests in other aquaculture species (Gitterle et al., 2006; Ødegård et al., 2006). Survival and CDH may be more precise for performing genetic analysis and selective breeding compared to TAD and CDD. The hourly recording of survival by using CDH provides the possibility to distinguish

between fish that die at the same temperature but at different time. For instance in the last generation (2006), there were 78 out of 114 tested families with surviving fish at the end of the test. Furthermore, there were 36 families with no survivors as well as a few highly cold tolerant families with 75% survival rate at the end of the test (Figure 3). CDH, which accounts for survival time as well lethal temperature may reveal a higher proportion of the genetic variation for cold tolerance among the test fish. Furthermore, the level of the heritability of SUR is on the underlying scale, and is expected to be higher than on the observable scale. Hence, the lower heritability of CDH and TAD may be more comparable and closer to the heritability for SUR on the observable scale or if estimated by a linear model. However, survival data does not include censored records (missing observations of survivors) and may therefore cover more variance including i.a. the survivors in some highly tolerant families. Also, survival at the end of the test seemed to show more genetic variation than SUR_{50} with lower mortality rate. The coefficients of variation of the cold tolerance traits are also low (Table 3), and were particularly low in 2006 for TAD. This may be due to the fact that the fish died over a shorter period, and that they were older and heavier compared to the other years of testing. The low variation may in fact limit the prospects for selection response for these traits, and improvement of the challenge test as well as reducing the common environment effects may be needed.

Some estimates of genetic parameters for cold tolerance in tilapia have been investigated. Variation in cold tolerance between species and strains were conducted (Behrends et al., 1990; Hofer and Watts, 2002; Khater and Smitherman, 1988; Sifa et al., 2002), and heritability estimates were reported (Behrends et al., 1996; Charo-Karisa et al., 2005). However, low heritability estimates were reported for *O. niloticus* in these studies.

The heritability estimates for cold tolerance of *O. niloticus* fingerlings in the present study varied from low to moderate and were all significantly different from zero ($P<0.05$). The

estimated heritability for CDH (0.15) is somewhat higher than the estimate of 0.08 reported for *O. niloticus* in low-input environment (Charo-Karisa et al., 2006) probably due to the fact that the fish was heavier in the present study (15 versus 5 g). It is reported that body weight, dietary level and other environmental influences will have an impact on cold tolerance of tilapia (Atwood et al., 2003; Charo-Karisa et al., 2004). The estimated heritability of 0.07 for TAD in the present study is however close to the estimate reported by Charo-Karisa (2006). Behrends et al. (1996) reported a higher realised heritability estimate of CDH (0.31) for a tilapia hybrid (*O. aureus x O. niloticus*), and for *O. aureus* (0.33), but as low as -0.05 for *O. niloticus*. Their estimates may be affected by the crossing in hybrids, and the use of the control population to estimate selection response and realised heritability. Heritability estimated for SUR is at a similar level as the estimates found for survival at harvest in brackish- and fresh water ponds using threshold models (0.20 and 0.27) (Luan et al., 2008). Rezk et al., (2009) reported lower estimated heritability (0.12) for survival of *O. niloticus* in ponds using a linear model. Our estimates are lower than the estimated heritabilities reported for upper thermal tolerance test (>0.4) for rainbow trout (Perry et al., 2005).

The heritability estimates for harvest body weight and growth during overwintering are in accordance with previous studies of the same population (Luan et al., submitted[a, b]). Heritability estimates reported earlier for *O. niloticus* have also been in a the range of 0.2-0.4 (Eknath et al., 2007; Gall and Bakar, 2002; Ponzoni et al., 2005; Rutten et al., 2005). However, other studies have shown higher estimates (Oldorf et al., 1989; Rezk et al., 2009). These results suggest that selective breeding for HW as well as OW can contribute to extend the culture period and the body weight during the winter. Furthermore, results in this study show that there is a significant additive genetic variance for cold tolerance such as CDH and survival in addition to growth rate in the population under investigation, that can be exploited through selective breeding programs.

There was a substantial common environmental variance estimated for CDH and survival in cold tolerance test. This may be caused by the size of body weight, rearing period and diet. Hence, heritability of cold tolerance may increase if the common environmental effects can be reduced. Testing of smaller fish may make the test closer to the real farming conditions. Recently, molecular marker can make it possible to rear families in common tanks and challenge test fish at a smaller size. This may eliminate the variation due to separate rearing of full sibs. However, production of a certain number families in a sufficiently short time is then needed to keep low variation in the size of the fish to be communally reared. Hence, improvement of the family production technique is needed and will require more research in tilapia reproduction.

However, the design of a cold tolerance test should be close to real conditions of farming or with the water temperature changes as during the winter conditions in northern Vietnam. In fact, the size of our test fish were bigger than fingerlings produced at autumn that farmers stock for overwintering or fry produced at early spawning season (early and cool spring). The size of the test fish in this cold tolerance study were also bigger than those reported by Charo-Karisa et al. (2005).

4.2 Correlations

In general, studies on genetic correlations between expressed cold tolerance variables are limited. Our genetic correlations between CDH and TAD are in agreement with those reported by Charo-Karisa et al. (2005). High and negative correlation (-0.85) indicate that these are nearly inverse traits. A moderate and positive genetic correlation (0.50) was estimated between CDH and survival. Hence, these two traits express partly different aspects of cod tolerance. CDH was more genetically correlated to OW than SUR indicating that CDH reflects the natural conditions during overwintering to a higher degree. The genetic correlation between

body weight at test and CDH was 0.44±0.13 in this study, which was lower than the estimate (0.72±0.81) by Charo-Karisa et al. (2005). This may be due to differences between the studies in body weight of fish at test, suggesting that bigger fish may less susceptibility to low temperature. Cnaani et al. (2000) reported even lower genetic correlation between size of fish and CDD (>0.3) for *O. mossambicus* and *O. aureus*. Rearing conditions can have impacts on cold tolerance of tilapia (Atwood et al., 2003; Charo-Karisa et al., 2004; Cnaani et al., 2003), and hence, both genetics and husbandry practice should be considered in studies of cold tolerance of this species.

A high genetic correlation between HW and OW was found. No corresponding estimates are published for such growth traits. However, correlations between repeated measurements have been reported. Rutten et al. (2005) found a high genetic correlation (>0.8) between body weights at different ages (100–326 days) for *O. niloticus*. Corresponding high genetic correlations (~0.8) between harvest body weights at optimum and low temperature and between low and natural temperatures were found, while lower genetic correlation was found between HW in optimum temperature and natural pond in the mountain (0.61) (Luan et al., submitted[b]). These genetic correlations suggest that the fish that grow fast during grow-out period also tend to grow faster during the winter.

Favourable but low correlations between cold tolerance traits on one hand and HW and OW on the other were found in the present study (Table 5). There are no other reports on corresponding estimates for these traits in *O. niloticus*. Cnaani et al. (2003) found significant association between two QTLs for cold tolerance and body weight within the same linkage group (LG23) but the two QTLs were approximately 22cM apart. However, the genetic correlation between growth and cold tolerance should be interpreted with care due to their relatively high standard errors. Anyhow, results from the present study suggest that selection for higher harvest body weight does not have any negative effects on cold tolerance. The

results show that growth and cold tolerance may also be improved simultaneously in a selective breeding program, which will cause significant benefits for tilapia production in North Vietnam.

5. Conclusions and implications

The present study show significant additive genetic variance for cold tolerance (CDH and survival) and growth which can be exploited through selective breeding. The current breeding program with a main focus on harvest body weight will also have a positive effect on growth performance during the winter and may allow for an extended culture period for tilapia in North Vietnam. Further, the existing selection program for tilapia in northern Vietnam does not have a negative effect on cold tolerance, and further selection for better cold tolerance using cold challenge test and harvest body weight should be considered.

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LIST OF TABLES

Table 1: Data structure: number of sires, dams and progeny tested and period of swim-up fry collection by year

Spawning season	Sires	Dams	Progeny tested		Fry collected period*
			Grow-out	Cold tolerance	
2004	63	106	4,240	2,120	30 Apr. – 15 Jun.
2005	64	103	4,120	2,060	29 Apr. – 9 Jun.
2006	75	114	4,560	2,280	27 Apr. – 15 Jun.
2004-2006	202	323	12920	6,460	

** Date swim-up fry collected of the first and last full-sib families*

Table 2: The number of recorded fish (N), mean harvest body weight (HW) and gain in body weight (WG), coefficient of variation (CV) and survival rate (SR) from tagging to harvest and during over-wintering and sex ratio (M/F) by year

Spawning season	Grow-out				Over-wintering				Sex ratio M/F
	N	HW (g)	CV (%)	SR (%)	N	WG (g)	CV (%)	SR (%)	
2004	3,812	209.1	35.4	89.9	3,759	62.16	47.47	98.6	1.34
2005	3,659	213.1	27.9	88.8	3,502	80.22	48.89	95.7	0.99
2006	4,059	231.9	28.0	89.0	3,978	114.80	56.99	98.0	1.10
2004-2006	11,530	218.3	30.3	89.3	11,239	83.41	51.18	97.5	1.14

Table 3: Overall means and coefficient of variation (%) of body weight (IW), age, temperature at death (TAD) and cooling degree hours (CDH) of *Oreochromis niloticus* fingerlings under cold tolerance challenge test according to different years and across years

Trait	Year	Mean	Coefficient of variation	Maximum	Minimum
IW (g)	2004	10.4	36.2	39.2	6.6
	2005	15.7	41.7	45.6	7.0
	2006	21.4	42.5	61.7	8.1
	Across	15.8	28.3	61.7	6.6
Age (days) [a]	2004	93.0	13.7	112.0	66.0
	2005	108.3	13.4	133.0	73.0
	2006	119.6	9.6	153.0	81.0
	Across	107.0	10.2	153.0	66.0
TAD (^{o}C)	2004	10.2	6.8	12.0	8.8
	2005	10.4	6.0	11.9	9.5
	2006	10.1	3.8	12.0	9.5
	Across	10.3	6.7	12.0	8.8
CDH	2004	122.56	22.76	313.6	0.0
	2005	96.10	32.49	285.0	0.3
	2006	115.61	23.84	265.5	0.0
	Across	107.73	35.69	313.6	0.0

[a] *number of days from swim-up fry collected to tagging*

Table 4: Heritability estimates ($h^2 \pm se$) and common full-sib/environmental effect ($c^2 \pm se$) for harvest body weight, growth during overwintering, cooling degree hour (CDH), temperature at death (TAD), survival at 50% death and at end of test and total phenotypic variance (σ^2_P)

Trait	$h^2 \pm se$	$c^2 \pm se$	σ^2_P
Cold tolerance			
Cooling degree hours (*CDH*)	0.15±0.07	0.13±0.04	211.37
Temperature at death (*TAD*)	0.07±0.03	0.03±0.01	0.41
Survival (*SUR*)*	0.26±0.13	0.12±0.06	1.44
$Survival_{50}$ (SUR_{50})*	0.11±0.02	0.01±0.01	1.07
Harvest body weight (HW)	0.22±0.06	0.23±0.04	4427
Growth during overwintering (OW)	0.18±0.06	0.14±0.02	1964

** Threshold sire-dam model (Model 2)*

Table 5: Genetic correlations (below the diagonal) and phenotypic correlation (above the diagonal) with standard errors between responses to cold tolerance challenge traits, harvest body weight (HW) and growth during overwintering (OW).

Trait	CDH	TAD	SUR	SUR_{50}	HW	OW
CDH	-	-0.92±0.01	0.41±0.02	0.75±0.01	0.14±0.20	0.29±0.24
TAD	-0.85±0.03	-	-0.42±0.02	-0.66±0.01	-0.02±0.02	-0.03±0.02
SUR	0.50±0.08	-0.69±0.05	-	0.52±0.01	ns	ns
SUR_{50}	0.89±0.09	-0.35±0.08	0.79±0.08	-	0.01±0.02	0.01±0.02
HW	0.17±0.18	-0.14±0.17	0.05±0.07	0.07±0.10	-	0.81±0.01
OW	0.23±0.26	-0.26±0.18	0.12±0.07	0.12±0.09	0.94±0.05	-

Note: CDH = cooling degree hours; TAD = temperature at death

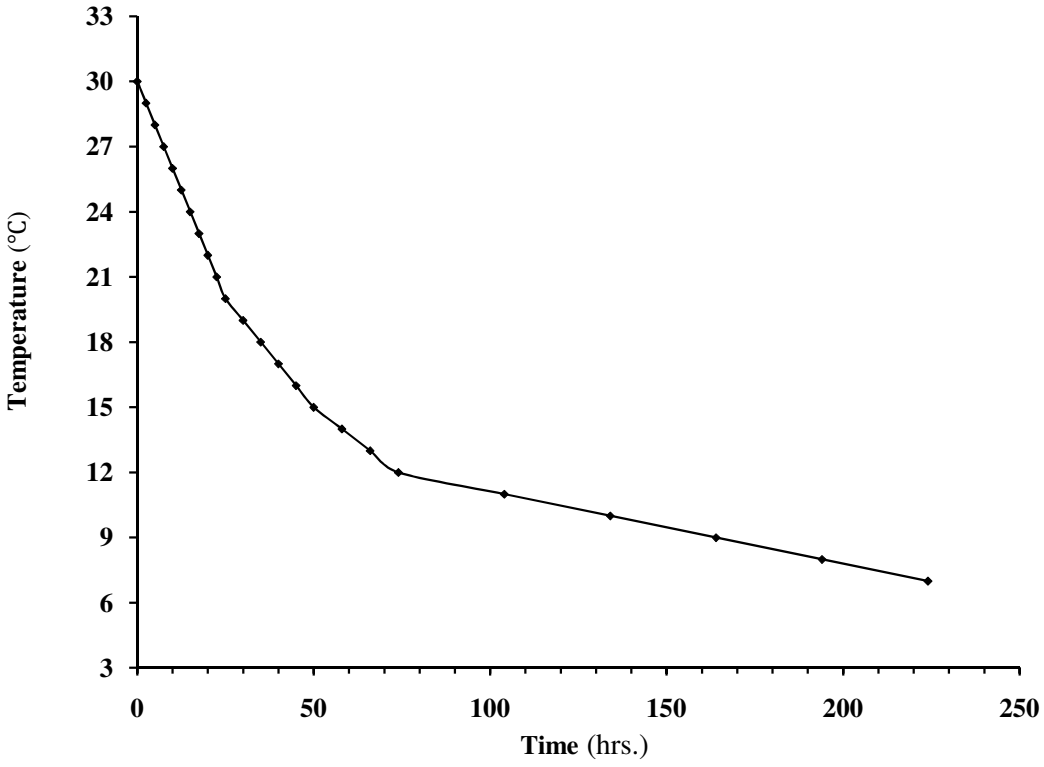

Figure 1: Schedule of temperature adjustment during cold tolerance test

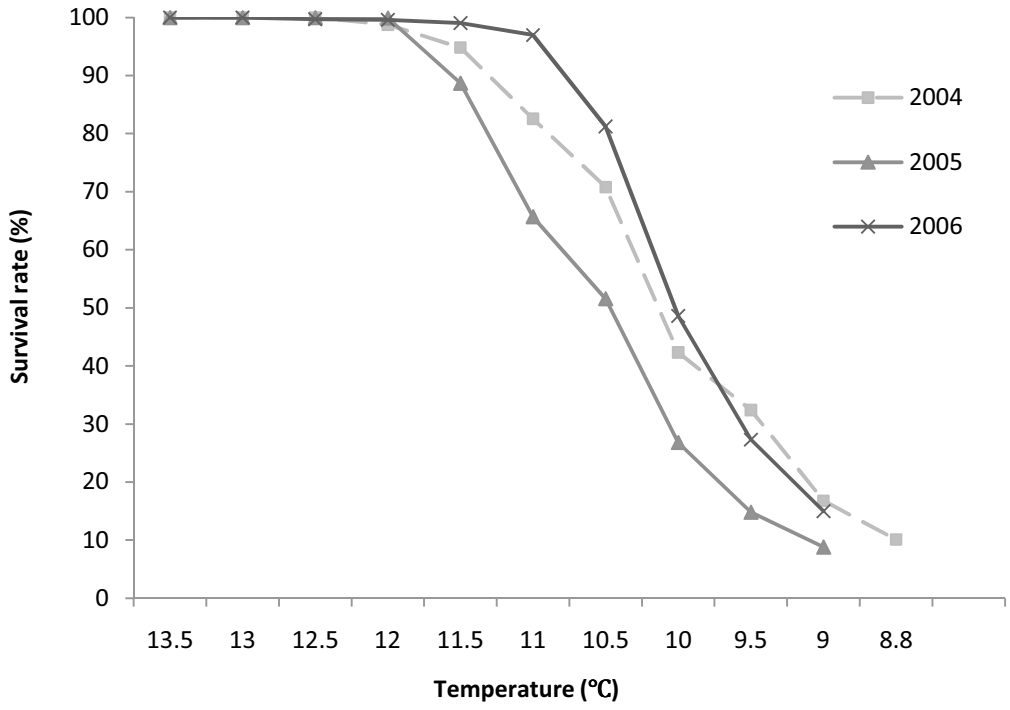

Figure 2: Survival rate (%) of *O. niloticus* fingerlings in different test years at different temperature during the cold challenge test

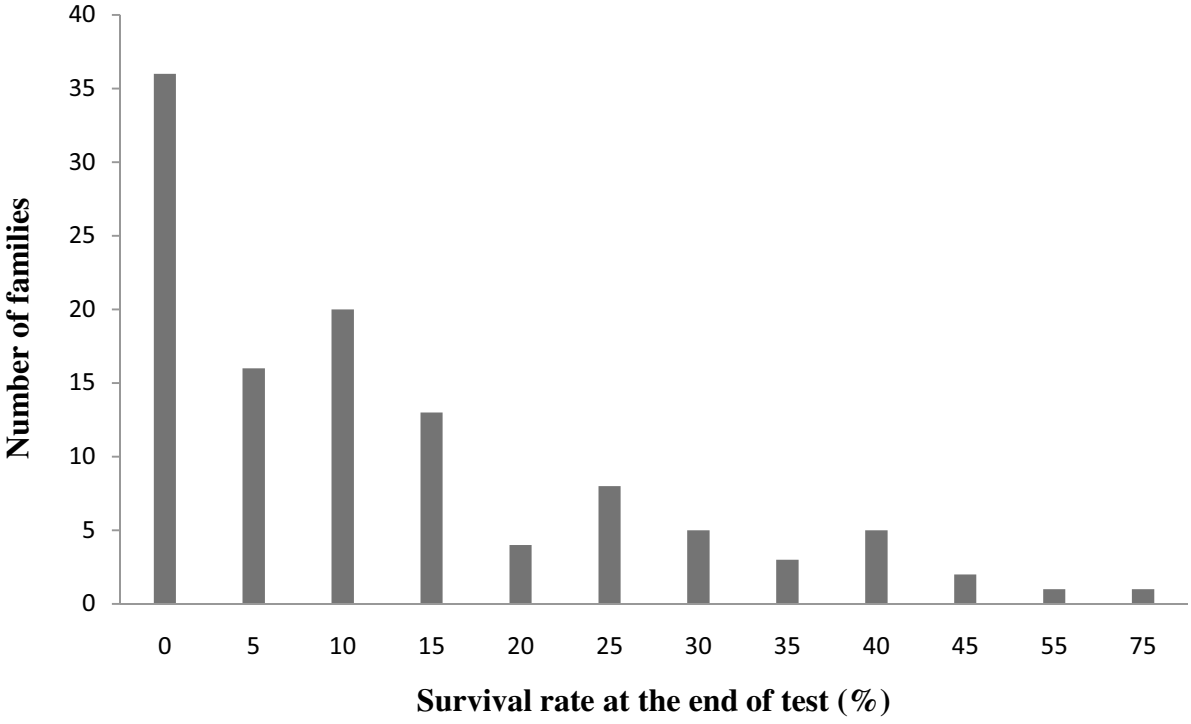

Figure 3: Number of families with different survival rate (%) at the end of the cold challenge test in 2006