Optimisation of selective breeding program for Nile tilapia (*Oreochromis niloticus*)

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This research was conducted under the auspices of the Graduate School of Wageningen Institute of Animal Sciences (WIAS).

Optimisation of selective breeding program for Nile tilapia (*Oreochromis niloticus*)

Trịnh Quốc Trọng

Thesis

submitted in fulfilment of the requirements for the degree of doctor at
Wageningen University
by the authority of the Rector Magnificus
Prof.dr. M.J. Kropff,
in the presence of the
Thesis Committee appointed by the Acadamic Board
to be defended in public
on Wednesday June 19, 2013
at 1.30 p.m. in the Aula

T. Q. Trọng,
Optimisation of selective breeding program for Nile tilapia (*Oreochromis niloticus*).

PhD thesis, Wageningen University, the Netherlands (2013)
With references, with summaries in English and Dutch

ISBN 978-94-6173-544-7

Abstract

T.Q., Trọng (2013). Optimisation of selective breeding program for Nile tilapia (*Oreochromis niloticus*). PhD thesis, Wageningen University, the Netherlands

The aim of this thesis was to optimise the selective breeding program for Nile tilapia in the Mekong Delta region of Vietnam. Two breeding schemes, the "classic" BLUP scheme following the GIFT method (with pair mating) and a rotational mating scheme with own performance selection and natural group spawning, were investigated. In the latter scheme, the aim was to mimic natural spawning conditions of Nile tilapia to reduce the time for family production; however reconstruction of pedigrees using DNA markers to monitor inbreeding is required. Parental assignment using microsatellites and SNPs showed that exclusion- and likelihood-based methods are equally good for parental assignment, provided that good marker sets with high exclusion power, such as SNPs, are available and that all parents are sampled. Prolonged family production is problematic in BLUP breeding value estimation and could be a consequence of selection for harvest weight in Nile tilapia. Using a natural mating design with single males mated to multiple females in groups, 85% of the successful spawns were collected within 20 days. Genetic correlations between harvest weight and spawning success ranged from 0.48 to 0.52, provided that the mating period is limited to 20-32 days. We conclude that Nile tilapia favour mating in groups, and that selection for harvest weight in GIFT should improve spawning success of Nile tilapia. Moreover, harvest weight and body weight at spawning have favourable genetic correlations with number of eggs, relative fecundity, and number of swim-up fry, which are the desired characteristics for Nile tilapia seed production. High-input cages and low-input ponds are the dominant production systems for tilapia in the Mekong Delta. We show that selection in nucleus ponds will produce desired correlated responses in Nile tilapia grown in river-cages. Moreover, they are expected to develop a more rotund and thicker body shape at the same length compared to fish grown in ponds. In conclusion, we recommend the use of the 'single male, multiple females' mating as this will reduce the generation interval by 2 months, thereby increasing genetic gain by about 20%. A rotational mating scheme, with at least 4 cohorts, can be incorporated into the GIFT selection scheme to further reduce inbreeding, to estimate pond effects and to secure the breeding material. Finally, a reliable multiplier system is important to sustain the current Nile tilapia breeding program, which can provide sufficient improved fry (>50 million per year) for the whole Mekong Delta Nile tilapia production.

Contents

1

General introduction

1.1 Introduction

Nile tilapia

Tilapia is the common name used to classify three groups of *Cichlidae* fish: *Tilapia*, *Sarotherodon*, and *Oreochromis*. Among these, the Nile tilapia (*Oreochromis niloticus*) is the most cultured species (FAO, 2012). In Vietnam, Nile tilapia is the second most important freshwater species, after the pangasius catfish (*Pangasianodon hypophthalmus*) (Merican, 2011). The total production of Nile tilapia was estimated to be 20,000 tonnes in 2010 (personal communication). The Mekong Delta region in the South of Vietnam is the major tilapia production area of the country. Nile tilapia is cultured in three production environments: in river cages, in monoculture in ponds and in low-input integrated poly-culture in ponds with a mix of other fish species and livestock species (VAC[1]). The majority of Nile tilapia production however is conducted in cages in the Mekong river (see e.g. Merican, 2011). Production from VAC ponds is mainly for household consumption and the domestic market.

Selective breeding in Nile tilapia and the GIFT project

There have been several selective breeding programs for Nile tilapia (review by Ponzoni *et al.* (2011). They are the 'Genetic Improvement of Farmed Tilapias' (GIFT), GET-EXCEL (Tayamen, 2004), FaST (Bolivar, 1998), GST (GenoMar Supreme Tilapia) (Zimmermann and Natividad, 2004), and Hainan Progift (Thodesen *et al.*, 2011). Among these projects, the GIFT project is the best documented one (Bentsen *et al.*, 2012; Gjedrem, 2012; Ponzoni *et al.*, 2011). The 10-year GIFT project was initiated in 1988 (Pullin *et al.*, 1991), jointly by Akvaforsk (Institute of Aquaculture Research, Norway) and the International Center for Living Aquatic Resources Management (ICLARM, now renamed the WorldFish Center). The GIFT project was funded, first by the United Nation Development Programme (UNDP), and thereafter co-funded by the Asian Development Bank (ADB). The National Freshwater Fisheries Training and Research Center in Munoz, Nueva Ecija, Philippines, was selected as the location for the project. The GIFT project which was terminated in 1997, produced a vast amount of data and knowledge about tilapia breeding. To this date, not all results from this project have been published (Gjedrem, 2012). At the end of 2000, the WorldFish Center (WFC) teamed up with

[1] Acronym for 'vườn', 'ao' and 'chuồng' meaning garden, pond and livestock pen.

the Malaysian Department of Fisheries, took over the 6th generation of GIFT, and has continued further selection to this date. In 2006, fifty full-sib families of generation 10 were transferred to the Research Institute for Aquaculture No. 2 (RIA2), to initiate the breeding program for GIFT in the Mekong Delta of Vietnam that is described in this study.

In GIFT, harvest weight has been the main trait of interest (Gjedrem, 2012; Ponzoni *et al.*, 2011), with genetic gains for harvest weight ranging from 10 to 15 per cent per generation over 6 generations (Ponzoni *et al.*, 2011). In addition to harvest weight, other traits have been studied in different subsets of GIFT generations including body dimension (Nguyen *et al.*, 2007), fillet yield (Nguyen *et al.*, 2010a), and flesh composition (Nguyen *et al.*, 2010b).

The breeding scheme of the GIFT project is based on Best Linear Unbiased Prediction (BLUP) breeding value estimation using individual information (own performance) and information from relatives (full-sibs, half-sibs, and progeny). The BLUP selection scheme builds on controlled single pair mating to produce full- and half-sib families, and reliable pedigree identification via tagging (Gjerde, 2005).

Reproduction in the GIFT breeding program

While the GIFT breeding program resulted in considerable genetic gain, reproduction remained problematic. The GIFT breeding program applies single pair mating, that is, one male and one female are stocked into a spawning unit ('hapa' or tank). This single pair mating prolongs the time required for the production of full- and half-sib families. for GIFT generation 1 to 5, the time for family production ranged from 40 to 101 days in the Philippines (Bentsen *et al.*, 2012), for GIFT 6 to 13 at the WorldFish Center in Penang, Malaysia it was 60 to 180 days (Ponzoni *et al.*, 2011), and for GIFT 11 to 13 in Vietnam (this study) it ranged from 105 to 136 days. The prolonged time for family production increases the time for family rearing in hapas, because tagging can only be conducted when fingerlings in the last produced family reach tagging size. By the time of tagging, the differences in ages and thereby in sizes of fingerlings between- and within-families can be substantial.

For harvest weight, the main selected trait in GIFT, prolonged time for family production reduces accuracy of estimated breeding values (EBV), and increases the

impact of environmental effects common to full-sibs (c^2) (Bentsen *et al.*, 2012). In addition, prolonged time for family production increases the generation interval by 3 to 4 months, which reduces genetic gain per generation.

It has been theorised that selection for harvest weight might lead to undesirable correlated responses in spawning success, fecundity, and fertility traits of GIFT Nile tilapia. In many livestock species, long-term selection for high production efficiency resulted in physiological, immunological and reproductive problems (Rauw *et al.*, 1998). Typical reproductive problems are defective eggs and poor semen quality in chicken, delayed age at puberty and farrowing in pigs, and low success rates after insemination in dairy cattle (Rauw *et al.*, 1998). However, in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), there seems to be no strong unfavourable relationship between growth rate and age at maturity (Gjerde, 1986).

Biologically, it can be argued that the difficulty to produce full- and half-sib families within a reasonable time-span is a consequence of the natural mating and spawning behaviour of Nile tilapia. In Nile tilapia, natural spawning behaviour resembles that of other lekking animals (Turner and Robinson, 2000), that is, groups of males occupy a spawning area and each male defends a "nest" as a site for mating and oviposition. Females enter the spawning area when they are ready to ovulate and mate with one or more males. Fessehaye *et al.* (2006) showed that mating systems in Nile tilapia are diverse, including not only single pair mating but also polygamous mating. The GIFT mating of one male to one female is clearly very different from the group mating condition of the species. In other words, a female is left with little choice when confronted with a single male in a spawning hapa. Yet Nile tilapia is known as a frequent spawner. Ponzoni *et al.* (2007) estimated from literature that the inter-spawning interval of Nile tilapia females ranges from 18 to 27 days, which is relatively short, although smaller/younger females are known to spawn more frequently than older/larger ones (Guerrero and Guerrero, 1985).

In commercial Nile tilapia seed production, group mating is normally used. The stocking sex ratio is often 1 male to 2 females (Barman and Little, 2006), 1 to 3 or even 1 to 4 (Mires, 1982). Today many small-scale tilapia seed production systems use a ratio of 1 male to 2 females (Barman and Little, 2006; Bhujel, 2000). In the Mekong Delta of Vietnam, Nile tilapia hatcheries normally use a stocking ratio of 1 male to 4 females or 1 to 5, and reproduction is normally allowed for 21 days. The fact that group mating for 21 days is sufficient to produce large numbers of fry suggests that single pair mating is perhaps not optimal for the production of

offspring, and that group mating designs could be more successful. For a GIFT breeding program, the use of group mating requires modification of the breeding scheme, because the parentage of sires is unknown, rendering complete pedigree tracking impossible. To implement a "classic" GIFT breeding program with group mating, pedigrees would need to be re-constructed by e.g. using molecular markers, which requires all individuals (parents and offspring) to be genotyped. This is very time-consuming, costly and practically difficult, because individuals still need to be physically identified (by means of e.g. tagging) or held separately.

In this thesis we tested an alternative breeding scheme, which is based on mass selection on harvest weight and uses natural mating in groups to produce offspring. In this scheme, rotational mating is used to control inbreeding. Rotational mating is a mating scheme that aims to maintain the rate of inbreeding at an acceptable level in a closed population (Nomura and Yonezawa, 1996). With rotational mating, a population is first divided into a number of groups or sub-populations (cohorts). Thereafter individuals are exchanged between groups in a systematic way. Based on the pattern of exchange, the schemes can be categorized as circular or cyclical mating. To monitor the rate of inbreeding, only the selected sires and dams are genotyped in each generation. The advantage of such a scheme is in the decreased generation interval and high genetic gain with low rates of inbreeding. The disadvantage is obviously the fact that selection can be on only a single trait, e.g. harvest weight.

In GIFT, most estimates for genetic parameters have focused on harvest weight. However, Nile tilapia on-growers in the Mekong Delta are more concerned about growth rate during the grow-out period, because high growth rate is associated with higher feed efficiency (Henryon *et al.*, 2002) and reduced grow-out time. It has also been observed that the shape of Nile tilapia seems to differ between rearing environments, that is, fish grown in cages are thicker than those grown in ponds. On-growers, consumers, and processors prefer thicker fish, because they look nicer and give higher meat percentage. Consumers are willing to pay higher prices for well-shaped fish, which is especially true for live fish and un-gutted fish. Recently, Blonk et al (2010) reported for common sole (*Solea solea*) that shape could be defined as ellipticity. The heritability of ellipticity was 0.34, and the genetic correlation with harvest weight was −0.44. As harvest weight is currently the only selection trait in GIFT, knowing the heritability and genetic correlations of this trait with growth rate and shape would be of added value for the breeding program.

The GIFT breeding program is conducted by the Research Institute for Aquaculture No. 2 (RIA2) in the Mekong Delta of Vietnam. Fish are selected from nucleus ponds at the station, but the major production is conducted in cages and low input VAC ponds. Therefore knowledge on a possible genotype by environment interaction (G×E) is required, not only for harvest weight, but also for growth rate and for shape. In European seabass (*Dicentrarchus labrax*), Dupont-Nivet *et al.* (2010) found substantial genotype by environment (G×E) interaction for growth rate (daily growth coefficient, DGC), while no G×E was found for harvest weight. The explanation was that a prolonged pre-tagging rearing period, when fish are reared in the same environment, increases genetic correlations of harvest weight between grow-out environments, if not properly corrected for. On the other hand, DGC accounts for only the growth period, therefore allows more accurate estimates of G×E. In Nile tilapia, various estimates for G×E for harvest weight have been reported, depending on the magnitude of differences among environments. Eknath *et al.* (2007) reported genetic correlations (r_g) of 0.76–0.99 for within ponds and 0.99 within cages, but 0.36–0.82 between ponds and cages. Bentsen *et al.* (2012) on the other hand reported that G×E interactions were not important across the pond, rice fish and extensive cage environments tested, but substantial G×E interactions occurred in the cages that used commercial pelleted feed compared to other test environments. G×E interaction was found to be unimportant for harvest weight in Nile tilapia in China (Thodesen *et al.*, 2011) and in Malaysia (Khaw *et al.*, 2012). In Egypt, the genetic correlation for harvest weight of Nile tilapia divergently selected for high or low input environments was 0.77–0.84 (Khaw *et al.*, 2009). Finally, substantial G×E was found for harvest weight and survival of GIFT grown in brackish water and in freshwater (r_g = 0.45 for harvest weight and 0.42 for survival).

1.2 Aim and outline of the thesis

The aim of the research described in this thesis was to optimise the selective breeding program for Nile tilapia in the Mekong Delta region of Vietnam (Figure 1.1). The “classic” BLUP scheme followed the GIFT method as proposed by the WorldFish Center (WorldFish Center, 2004), and was conducted for four generations from G10 to G13 (Figure 1.1). An alternative breeding method, which was based on own performance selection, natural group spawning and rotational

(cyclical) mating (Nomura and Yonezawa, 1996), was investigated for three generations (from R10 to R12, Figure 1.1).

The aim of rotational mating scheme was to mimic natural spawning conditions in Nile tilapia, thereby reducing the time for family production. In this method, reconstruction of the pedigree to monitor inbreeding is required. In **chapter 2**, we compared and evaluated two different methods to re-construct the pedigree for generations R10 and R11, using two types of molecular markers, namely microsatellites and Single Nucleotide Polymorphisms (SNPs) (Figure 1.1).

Results from natural mating in groups showed that reproduction time could be shortening to 28 days. However, reconstruction of pedigree proved difficult due to missing parents. In chapter 3 and 4, we therefore explored alternatives to the single pair mating scheme of GIFT. Two mating schemes were compared in terms of female reproductive success: one scheme in which a single male was stocked with 10 females, and one scheme in which 7 males were stocked together with 15 females. We also estimated genetic parameters for female reproduction performance in these mating schemes. In **chapter 3**, spawning success, defined as spawn/no spawn, was investigated. In **chapter 4**, genetic parameters for fecundity, number and size of eggs spawned, and fertility traits were investigated. Furthermore, in **chapter 3** and **4** we estimated genetic correlations between reproductive traits and harvest weight.

Growth rate and fish shape are traits of economic importance for Nile tilapia culture in the Mekong Delta of Vietnam. In **chapter 5**, using fish from G13, we estimated heritability and phenotypic and genetic correlations for harvest weight, growth rate (daily growth coefficient), and shape, defined as ellipticity in the breeding nucleus. The magnitude of G×E between the nucleus and the two main production environments, river cage and VAC, was also investigated for these traits.

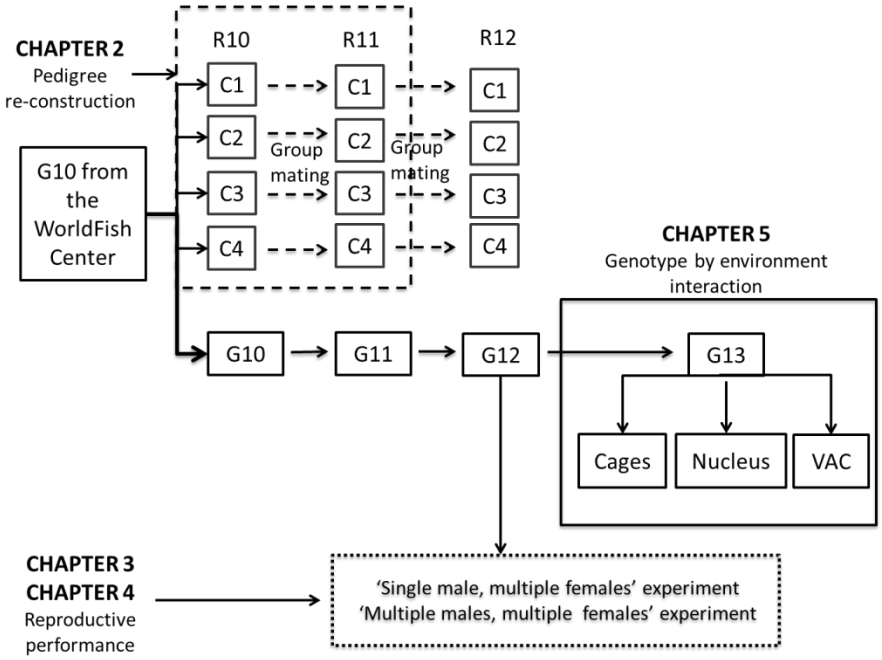

Figure 1.1 Diagram of the study.
R = Rotational mating, C = cohorts in R, G = GIFT breeding program. Numbers following R and G indicate generations. Numbers following C indicate cohort number.
G10 was the base population from the WorldFish Center, Penang, Malaysia.

The thesis work was a collaboration initiative between Wageningen University, WFC and RIA2 in Vietnam. The project received fish material (G10) from WFC, Penang, Malaysia as the base population, and was partly funded by the WFC from 2007 to date.

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2

A comparison of microsatellites and SNPs in parental assignment in the GIFT strain of Nile tilapia (*Oreochromis niloticus*): the power of exclusion

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Aquaculture 388–391 (2013): 14–23

Abstract

In this study, parental assignment was studied in the 10th generation of a pedigreed selected Nile tilapia (*Oreochromis niloticus*) population (GIFT) and their offspring, by comparing two types of molecular markers, microsatellites and SNPs, using an exclusion-based (Vitassign) and a likelihood-based (Cervus) method. For the experiment, G10 parents were divided in 4 groups (cohorts) and allowed to produce offspring by natural group mating. In total 173 offspring were tested against 238 parents, using either 12 microsatellites (PIC = 0.639; exclusion power 68.0%) or 122 SNPs (PIC = 0.341; exclusion power 99.9%). In this study, more than half of the candidate parents were either full- or half-sibs with other parents. Furthermore, 13.8% of the parents died before being sampled for DNA.

When offspring were assigned to parents in the same cohort, using Vitassign, for microsatellites, allowing up to 2 mismatches, 37.6% offspring got unique assignments, 45.1% got multiple assignments, and 17.3% were not assigned; for SNPs with up to 15 mismatches allowed, 83.8% offspring got unique assignments while 13.9 % got multiple assignments. Only 2.3% were not assigned. Using Cervus, for microsatellites, the mean 'strict' (>95% CF) assignment rate across the 4 cohorts was 18%, the 'relax' (80–95% CF) assignment rate was 43%, and 39% were not assigned; for SNPs, 39% 'strict' assignments were obtained (mean across 4 cohorts); the remaining offspring were not assigned. In general assignment rates were higher when cohort offspring were assigned to all parents combined, irrespective of method (Vitassign or Cervus) or marker used. However, consistency of assignments between microsatellites and SNPs was low: 28% with Vitassign and 16% with Cervus. Consistency of assignments between Cervus and Vitassign was high with SNPs (65%), but was low with microsatellites (31%). We conclude that missing parents and relatedness among candidate parents resulted in low assignment rates. Furthermore, low exclusion power of the microsatellite set resulted in low assignment rates and multiple parent pair assignments irrespective of method used. Exclusion methods and likelihood-based methods can be equally good for parental assignments, providing that good marker sets with high exclusion power are available.

Key words: microsatellites, SNPs, parental assignment, exclusion power.

2.1 Introduction

In aquaculture, selective breeding programmes improve performance of many important farmed species such as Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), Nile tilapia (*Oreochromis niloticus*) and shrimp (Gjedrem, 2005). The two most popular selection methods used are based on either (i) own performance or (ii) BLUP (Best Linear Unbiased Prediction) estimation of breeding values (Gjerde, 2005). The first method is based solely on own performance of selection candidates and only requires pedigree information to control rate of inbreeding (Falconer and Mackay, 1996). The second method requires pedigree to construct the additive genetic relationship matrix, which allows estimation of genetic parameters, rate of inbreeding and breeding values (Gjedrem, 2005; Pemberton, 2008). However, in aquatic species, pedigree recording requires a costly, systematic tagging system. In addition, tagging is possible only when the animals reach a certain size, which requires additional investment in family rearing facilities. Equally important is that the separate rearing procedure might introduce systematic common environmental effects. For natural mating species like e.g. Nile tilapia, sole (*Solea solea*), seabream (*Sparus aurata*) or Atlantic cod (*Gadus morhua*), tagging of progeny is not an option as the identities of either one parent or both parents are unknown (Blonk *et al.*, 2010; Fessehaye *et al.*, 2006; Herlin *et al.*, 2007). In these situations, pedigree can only be reconstructed by parental assignment, i.e. comparing marker information from parents with offspring.

The marker-based parental analysis system reconstructs pedigree using genotyping data. In aquaculture, fisheries and aquatic conservation, microsatellites are still the (molecular) marker of choice for parental assignments and pedigree reconstruction, owing to their properties: highly polymorphic, co-dominant, and PCR-based. However, microsatellites are also sensitive to genotyping error, particular in automated multiplex systems (Pompanon *et al.*, 2005). In recent years, Single Nucleotide Polymorphisms (SNPs) are becoming increasingly popular (Anderson and Garza, 2006; Hauser *et al.*, 2011; Jones *et al.*, 2010; Pemberton, 2008). The main reasons are the possibility for high-throughput screening, their low genotyping error rate (<0.1%) and the fact that they are easier and cheaper to standardise between labs compared to microsatellites (Anderson and Garza, 2006). SNPs are bi-allelic which gives them lower resolving power compared to multi-allelic microsatellites. However, this can be compensated for by genotyping animals for a larger number of markers (Haasl and Payseur, 2011; Hess *et al.*, 2011; Wang and Santure, 2009).

There are two major approaches for parental analysis, namely exclusion and likelihood-based methods. The principle of exclusion, which checks compatibility of offspring and parental genotypes with Mendelian inheritance, is simple and straightforward. According to this, a parent and an offspring will share at least one allele per locus for a co-dominant marker, so that a putative parent is rejected as a true parent if both alleles at one locus mismatches with that of an offspring. Frequently used exclusion-based computer software packages for microsatellite genotypes in aquaculture species are FAP (Taggart, 2007) and Vitassign (Vandeputte *et al.*, 2006). Likelihood-based methods employ Mendel's laws quantitatively to calculate the likelihoods of different candidate relations among a set of individuals, and choose the relations that have the maximum likelihood as the best inference (Wang, 2012). The method calculates a LOD score, which determines the likelihood of an individual (or pair of individuals) being the parent (or parents) of a given offspring divided by the likelihood of these individuals being unrelated. Offspring are assigned to the parent (or parental pair) with the highest LOD score. Parentage remains ambiguous when multiple parent–offspring relationships obtain equally high likelihood. Offspring are not assigned when all parent-offspring relationships have zero likelihood. The most frequently used likelihood-based software packages are Cervus (Marshall *et al.*, 1998), Colony (Wang, 2004) and PAPA (Duchesne *et al.*, 2002).

In aquaculture and fisheries, microsatellite-based parental analysis has been carried out in a wide range of both freshwater and marines species, including Nile tilapia (Fessehaye *et al.*, 2006), sole (Blonk *et al.*, 2010), sockeye salmon (*Oncorhynchus nerka*) (Hauser *et al.*, 2011), Atlantic cod (Herlin *et al.*, 2007), rainbow trout and common carp (Vandeputte *et al.*, 2006; Vandeputte *et al.*, 2011), Pacific shrimp (Dong *et al.*, 2006), crayfish (Jerry *et al.*, 2004), (Jerry *et al.*, 2006) and molluscs (Hedgecock *et al.*, 2004; Slabbert *et al.*, 2009). These studies involved wild, hatchery and selected populations. Microsatellite markers were also used to investigate genetic change between hatchery and wild Atlantic salmon (Skaala *et al.*, 2006). Single Nucleotide Polymorphisms (SNPs) markers, in contrast, have mainly been used in population genetics studies (Haasl and Payseur, 2011; Hess *et al.*, 2011; Morin *et al.*, 2009; Smith and Seeb, 2008), and to the knowledge of the authors, only one study used SNP markers for parentage analysis in fish (Hauser *et al.*, 2011). According to Hauser *et al.* (2011), eighty SNPs resulted in higher assignment rates than 11 microsatellites in parental assignment for a wild sockeye salmon population.

The aim of the present study was to compare the efficiency of 12 microsatellites and 122 SNPs in parental assignment for Nile tilapia. Nile tilapia (*Oreochromis niloticus*) is a widely farmed fish species in Vietnam. Farming of Nile tilapia depends strongly on availability of genetically improved seed (El-Sayed, 2006; Ponzoni *et al.*, 2010). As part of a regional programme in the South of Vietnam, we have been testing a breeding scheme that is based on natural mating and individual selection, in combination with rotational mating to counteract inbreeding. The purpose of the present study was to perform a parental allocation to four groups of progeny that had been obtained by natural mating and reproduction of pedigreed parents of Nile tilapia. Microsatellites and SNPs were compared in terms of (i) assignment rate, (ii) power of assignments expressed as level of confidence of assignments and (iii) consistency of assignments, using an exclusion-based program (Vitassign) and a likelihood-based program (Cervus).

2.2 Materials and methods

2.2.1 Experimental fish

Fish of 10th generation (hereafter G10) of the GIFT strain (Genetically Improved Farmed Tilapia, Ponzoni *et al.* (2010)) were supplied by the WorldFish Center (WFC in Penang, Malaysia) to the Research Institute for Aquaculture No. 2 (RIA2), Vietnam in July 2006. Fish were from 50 different families with full pedigrees (Ponzoni *et al.*, 2010). Males and females were selected from the 25% fish with highest EBVs for body weight in their sex group and were randomly assigned to four cohorts, labeled R1 to R4. In each cohort, 12–14 male and 20–25 female fish were stocked into one 50 m^2 nylon spawning hapa, and allowed to spawn naturally. Eggs and fry were collected from mouth-brooding females at four day intervals. Candidate parents that died were replaced by new G10 fish. All candidate parents, including the substituted ones, were replaced by new fish after two months. The total number of G10 candidate parents used in each cohort is presented in Table 2.1. In total, 276 G10 fish were used to produce G11 offspring. At the end of the spawning period, all candidate parents were blood-sampled for DNA collection. Candidate parents that died before DNA sampling were recorded as missing parents (Table 2.1).

In total, 192 G11 offspring batches were collected, 48 from each cohort. Fry from these batches were pooled by cohort and approximately 5,000 randomly selected swim-up fry were nursed in a 50 m^2 hapa (100 fry m^{-2}). Fry were fed fine powder feed (35% crude protein) *ad libitum*, three times per day. After two months, 1000

G11 fingerlings from each cohort were stocked into one of four 1,000 m^2 earthen ponds (one for each cohort) and fed a commercial floating pellet (20% crude protein) for five months. After harvest at the age of seven months, the largest males and females in each cohort pond were selected and blood-sampled for genotyping, giving a total of 42 male and 131 female offspring (Table 2.1).

2.2.2 DNA Sampling

Blood samples were taken from the peduncle vena, using a 3 mm sterile syringe that was pre-rinsed with a solution of 100 mg/ml EDTA (Merck), and stored in a 0.5 ml Eppendorf tube, containing 200 µl EDTA at −35°C. DNA was extracted from blood using the PUREGENE kit (Gentra Systems, Minneapolis, MN, USA) following the manufacturer's instructions for non-mammalian blood and finally diluted to 10 ng/µl.

2.2.3 Microsatellites

All animals were genotyped for 12 microsatellites: UNH146, UNH160, UNH203, UNH211, UNH212, UNH222, UNH123, UNH169, UNH178, UNH231, UNH208 and UNH214. Primer information for these microsatellites was obtained from the NCBI database (http://www.ncbi.nlm.nih.gov). Nine of the microsatellites had previously been used in a parental assignment study of Nile tilapia (Fessehaye *et al.*, 2006), while the three others (UNH146, UNH169 and UNH222) were chosen based on their low genotyping error (based on results from Pedant, Johnson and Haydon (2007)) and high Polymorphic Information Content (PIC) (Table 2.2). Polymerase Chain Reaction (PCR) was performed at: 5 minutes at 95°C, 35 cycles of 30 s at 95°C, 45 s at annealing temperature (45 – 60°C), and 90 s at 72°C, followed by a final elongation step of 4 minutes at 72°C. The intensity of the PCR-amplicon of each marker was measured on a 1.5% agarose gel in order to determine the amount of each PCR product to be used for pooling. Amplified products were combined in two multiplex sets with 5 and 6 markers, diluted 10 times with MQ and 1 µl of the pool was transferred to a barcoded plate which contained 9 µl of formamide mix (a mixture of 1000 µl formamide and 5µl Liz 500 (Applied Biosystems). PCR products were analyzed on the ABI 3730 DNA Analyzer (Applied Biosystems). GeneMapper 4.1 (Applied Biosystems) was used for the analysis of the genotyping results.

Table 2.1 Number of candidate G10 parents and G11 offspring sampled in each generation for each cohort, and numbers of missing G10 parents.

	Female					Male					Total
	R1	R2	R3	R4	Total	R1	R2	R3	R4	Total	
G10	40	35	35	37	147	23	21	26	21	91	238
G10 missing	7	8	7	7	29	4	2	1	2	9	38
G11	39	34	27	31	131	10	12	11	9	42	173

R1, R2, R3, R4: cohort number; **G10**: generation 10 of GIFT; **G11**: offspring generation of G10; **G10 missing**: Candidate G10 parents that died before sampling.

Table 2.2 Microsatellite loci, basic statistics and genotyping error rate. Outputs from Cervus.

Locus	k	N	HExp	F_{IS}	PIC	F(Null)	NE-PP	E1	E2	HW
UNH146	5	401	0.509	-0.014	0.431	-0.012	0.621	0.000	0.000	NS
UNH160	9	383	0.645	0.105	0.614	0.060	0.361	0.011	0.000	NS
UNH203	7	389	0.84	0.207	0.818	0.114	0.153	0.000	0.006	***
UNH211	10	399	0.822	0.009	0.797	0.002	0.174	0.006	0.006	NS
UNH212	6	334	0.611	0.216	0.568	0.118	0.435	0.000	0.000	***
UNH222	6	376	0.334	-0.027	0.32	-0.031	0.67	0.000	0.000	NS
UNH123	11	411	0.8	-0.031	0.776	-0.015	0.186	0.013	0.000	***
UNH169	15	400	0.794	-0.039	0.771	-0.023	0.186	0.007	0.000	NS
UNH178	7	411	0.633	-0.030	0.592	-0.020	0.405	0.000	0.000	NS
UNH231	7	411	0.552	-0.058	0.507	-0.030	0.501	0.011	0.000	NS
UNH208	10	409	0.742	0.001	0.707	0.004	0.278	0.045	0.008	NS
UNH214	14	406	0.791	-0.047	0.768	-0.027	0.189	0.007	0.000	NS
Average	9	394	0.673	0.024	0.639	0.012	0.347	0.008	0.002	

k: Number of alleles, **N**: number of animals genotyped, **HExp**: Expected heterozygosity, **FIS**: $(H_{expected}-H_{observed})/H_{expected}$; **PIC**: Polymorphic Information Content, **F(Null)**: Estimated null allele frequency, **NE-PP**: Average non-exclusion probability for a candidate parent pair, **E1**: allelic dropout (Pedant), **E2**: False allele (Pedant), **HW**: Significance of deviation from Hardy-Weinberg equilibrium. **NS**: Not significant, *******: Significant at the 0.1% level.

2.2.4 SNPs

The studied fish were genotyped for 384 SNPs in a multiplex assay using the GoldenGate assay (www.illumina.com). The origin and development of the SNPs is described elsewhere (Van Bers *et al.*, 2012). A subset of 122 SNPs was selected from the 384 SNP set, based on a minor allele frequency > 0.2 and a SNP call rate >

95% (highest call rate and with the most distinct clustering of the three different genotype classes AA, AB and BB). The dbSNP accession numbers of the SNPs are given in Table 2.7 of the Appendix A section.

2.2.5 Parentage assignment

Two hundred thirty-eight candidate parents (G10) and 173 offspring (G11) were used for parental assignment using microsatellites or SNPs. For microsatellites, all individuals had more than 75% of loci typed and were used for analysis. For SNPs, all individuals had a call rate > 0.8. Microsatellites genotyping error (error rate per allele) was estimated by genotyping 48 individuals twice at all 12 loci. Estimation was carried out using the software Pedant v 1.0 (Johnson and Haydon, 2007). The program uses a Bayesian procedure that distinguishes (i) allelic drop out and (ii) false allele errors. Error rate is calculated as the sum of these two. Unlike microsatellites, repeat genotyping was not carried out for SNPs. Instead, for all SNP runs the error rate was assumed to be less than 1%. This is a conservative estimate and in line with the normal genotyping error rate for laboratories (Pompanon *et al.*, 2005).

First, we assigned offspring from each cohort to parents from the matching cohort ('within cohort assignments'). Parental analysis was first done using Vitassign, an exclusion based method (Vandeputte *et al.*, 2006). For microsatellites, parental assignment was based on 0 – 2 mismatches, which is typical for microsatellites (Vandeputte *et al.*, 2006) while for SNPs, a range of 0 to 15 mismatches was allowed, as the number of SNPs were ten times those of microsatellites. The power of exclusion was calculated for each set of markers by simulating 2000 offspring from the parents and calculating the theoretical assignment rate.

Next, parental analysis was carried out using Cervus v 3.0 (Kalinowski *et al.*, 2007; Marshall *et al.*, 1998). The analysis included three steps. First, allele frequencies and simple statistics were calculated. Second, simulation of parentage analysis in Cervus was done using the following parameters: (i) 10,000 simulated offspring, (ii) proportion of candidate parents sampled accounting for missing parents (see Table 2.1), (iii) mean error rate 0.00643 (microsatellites, from Pedant) or 0.01 (SNPs, assuming), and (iv) minimum loci typed was half of total number of loci (default value). Third, parental analysis using actual genotypes was performed. Only parent pair (pp) assignments were considered, with confidence levels of 95% ('strict') or >80 – 95% ('relax'). We used the latter confidence range instead of the Cervus

default 'relax', because Cervus defines assignments with confidence level from 80 – 95% (inclusive) as 'relax'. In a few cases Cervus output gives 'most likely but unassigned' parent pairs. In the analysis, these pp were considered 'not assigned'.

Third, we evaluated the reliability of exclusion- and likelihood-based methods, by looking at the consistency of assignments using either method in combination with microsatellites or SNPs. For this evaluation, we first assigned cohort offspring using all parents as candidates. Next we compared offspring that were assigned a unique pp with Vitassign and a 'strict' assignment by Cervus, by comparing the sires and dams assigned by each method. Comparisons were done in two ways: 1) comparing Vitassign and Cervus using either microsatellite assignments or SNP assignments, and 2) comparing microsatellite and SNP based assignments using either Vitassign or Cervus.

2.3 Results

2.3.1 Markers

Table 2.2 shows summary statistics of the microsatellite genotyping. The number of alleles per locus ranged from 5–15, with an average of 9. The number of individuals successfully typed at each locus ranged from 334–411 (81–100%). The expected heterozygosity ranged from 0.334–0.840, with an average of 0.673. The polymorphic information content (PIC) ranged from 0.320–0.818, with an average of 0.639. Loci UNH203, 212 and 123 showed significant deviation from Hardy-Weinberg equilibrium at the 0.1% confidence level. The estimated null allele frequency was found to be lower than 0.05, which is the recommended level by Cervus, except for loci UNH160, UNH203, and UNH212.

Figure 2.1 shows the distribution of the minor allele frequency over 122 SNPs tested, which were already selected based on minor allele frequency (MAF) > 0.2. The average MAF was 0.346. The average expected heterozygosity and F_{IS} were 0.440 and −0.055, respectively (see Table 2.8, Appendix A). There were 15 SNPs that showed a significant deviation from Hardy-Weinberg equilibrium, 6 at 5%, 4 at 1% and 4 at 0.1% confidence levels, respectively.

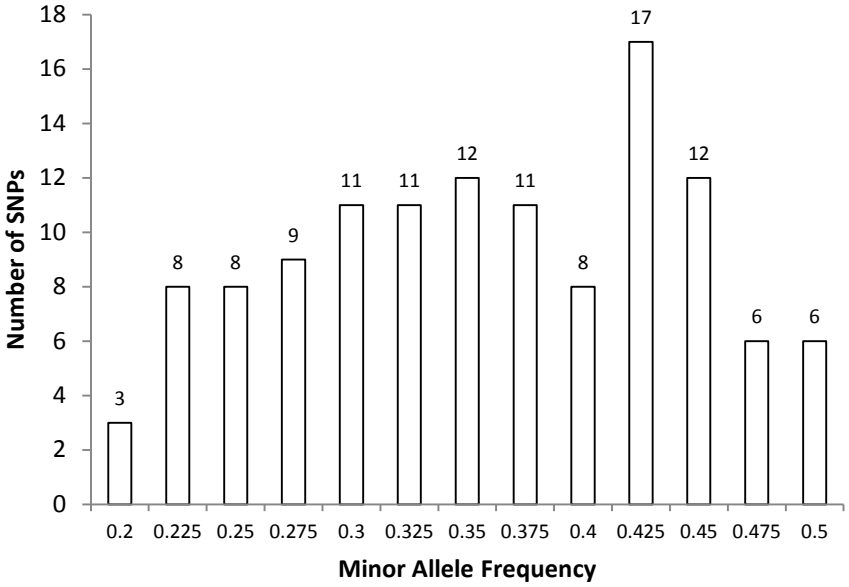

Figure 2.1 Minor allele frequency of 122 SNPs used for analyses.

2.3.2 Within cohort assignments

2.3.2.1 Parental assignments with Vitassign

Figure 2.2 shows the assignment results for microsatellites, obtained with Vitassign. The exclusion power of the microsatellite set was 68%. Results are the sum of each cohort offspring – cohort parents analysis (R1 through R4). The total number of unique parent pairs (pp) assigned, based on 0–2 mismatches, was 65 (37.6%). The number of unique assignments with zero mismatch was 24 (13.9%). There were 78 offspring (45.1%) for which multiple parent pairs (2 to 33 pp) were identified. Of these, 53 offspring were assigned to 2–4 parent pairs. In a few cases (8 offspring) the number of assigned parent pairs was higher than 10 (11–33 pp). Thirty offspring (17.3% of the total offspring) were not assigned to any parent pair, even with 2 mismatches allowed.

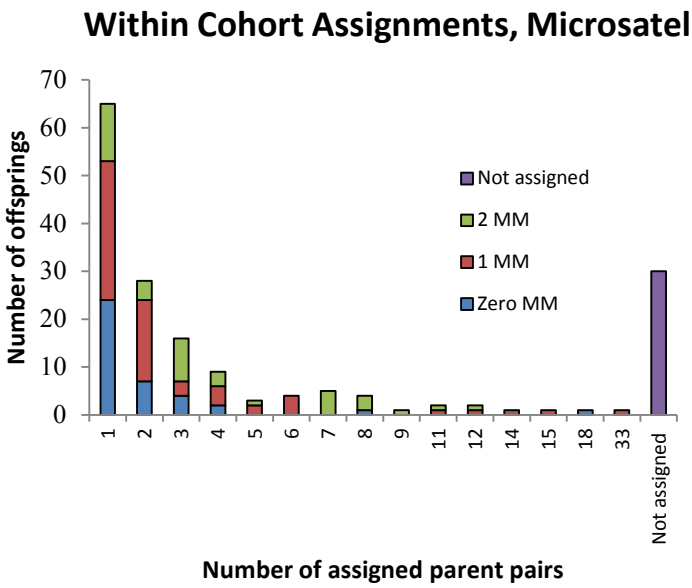

Figure 2.2 Number of offspring assigned to one or more parent pairs, using Vitassign. Assignments were based on zero (zero MM), one (1 MM) or 2 mismatches (2 MM). Results are the sum of each cohort offspring – cohort parents analysis (R1 through R4).

Figure 2.3 shows the cumulative assignment rates for SNPs, with increasing number of mismatches allowed. The exclusion power of the set was 0.999. Results are the sum of each cohort offspring–cohort parents analysis (R1 through R4). The rate of unique assignment was very low (2%, 3 offspring) when no mismatches were allowed. Increasing the number of mismatches increased the number of unique assignments. With more than 10 mismatches allowed, the number of offspring that were assigned more than one parental pair increased as well. When increasing the number of mismatches to 15, 145 (83.8%) offspring got unique assignments; there were 19 (11%) offspring assigned to 2 pp, 4 (2.3%) to 3 pp, and 1 (0.6%) to 4 pp. There were only 4 offspring (2.3%) that were not assigned to any parent pair, even with 15 mismatches allowed. We estimated the genotyping error rate for SNPs by calculating the ratio of cumulative number of mismatches over the total number of successful genotypes (i.e. excluding missing SNPs genotypes). For all unique assignments obtained with 5–10 mismatches, the estimated genotyping error rate was 2.21–3.78%.

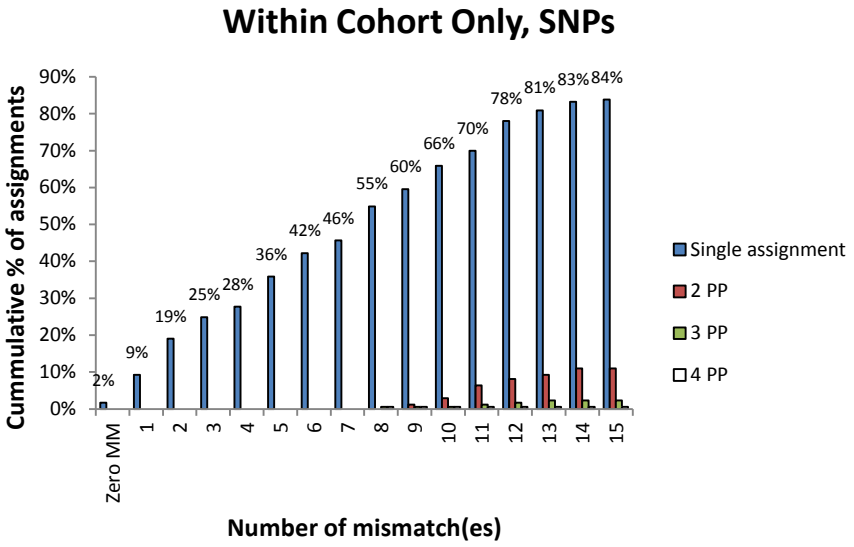

Figure 2.3 Cumulative percentage of parent pair assignments using Vitassign, with increasing number of mismatches allowed. Results are the sum of each cohort offspring – cohort parents analysis (R1 through R4). Single assignment- one parent pair assigned; 2 PP- 2 parent pairs assigned; 3PP- 3 parent pairs assigned; 4PP- 4 parent pairs assigned.

2.3.2.2 Parental assignment with Cervus

Table 2.3 shows the number and percentage of parental assignments at each confidence level ('strict' and 'relax') for microsatellites and for SNPs, obtained with Cervus. For microsatellites, when assigning offspring to parents in the same cohort, 'strict' assignment rate was low, ranging from 11% (in R2) to 29% (in R3). 'Relax' assignment rates were higher, ranging from 30% (R4) to 51% (R3). Many offspring remained unassigned (21–55%).

Table 2.3 Numbers (#) and rates (%) of assignment for microsatellites and SNPs when assigning offspring to candidate parents originating from the same cohort, using Cervus. Mean values for each category (Strict, Relax, and NA) are averages across four cohorts (R1 to R 4).

		Microsatellites		SNPs	
		#	%	#	%
R1 offspring	Strict	9	18	34	69
	Relax	25	51	0	0
	NA	15	31	15	31
Total R1		49	100	49	100
R2 offspring	Strict	5	11	9	20
	Relax	19	41	0	0
	NA	22	48	37	80
Total R2		46	100	46	100
R3 offspring	Strict	11	29	11	39
	Relax	19	50	0	0
	NA	8	21	27	71
Total R3		38	100	48	100
R4 offspring	Strict	6	15	11	27
	Relax	12	30	0	0
	NA	22	55	29	73
Total R4		40	100	40	100
Mean	Strict	8	18	16	39
	Relax	19	43	0	0
	NA	17	39	27	64

Strict: assignments at 95% confidence level, **Relax**: assignments from 80 – 95% confidence level, **NA**: assignments that have confidence levels lower than 80%.

For SNPs, when assigning offspring to parents in the same cohort, strict assignment rate ranged from 20–69% in different cohorts. Again many offspring remained unassigned, ranging from 31–80% in different cohorts.

2.3.3 Cohort offspring assigned to all parents

2.3.3.1 Parental assignment with Vitassign

For microsatellites, assignment rates for unique assignments ranged from 16 to 45% (Table 2.4). The rates for multiple assignments were similar in R4 (48%) but higher in the other three cohorts (52–68%). There was 7–13% offspring that were not assigned to any parent pair in different cohorts, even when 1–2 mismatches were allowed.

Table 2.4 Assignment rates (%) for microsatellites and SNPs when assigning offspring to candidate parents from all cohorts combined, using Vitassign and Cervus. Mean values for each category (Strict/UA, Relax/MA, and NA) are averages across four cohorts (R1 to R4).

		Microsatellites		SNPs	
		Vitassign	Cervus	Vitassign	Cervus
R1 offspring	Strict / UA	35	12	90	94
	Relax / MA	57	51	8	0
	NA	8	37	2	6
R2 offspring	Strict / UA	35	17	78	78
	Relax / MA	52	42	20	4
	NA	13	41	2	2
R3 offspring	Strict / UA	16	16	84	84
	Relax / MA	68	42	13	0
	NA	8	42	3	16
R4 offspring	Strict / UA	45	17	73	75
	Relax / MA	48	63	0	23
	NA	7	20	27	2
Mean	Strict / UA	33	16	81	83
	Relax / MA	56	50	10	7
	NA	9	35	9	7

Strict: Cervus assignments at 95% confidence level, **UA**: Vitassign unique assignment, **Relax**: Cervus assignments from 80 – 95% confidence level, **MA**: Vitassign multiple assignments, **NA**: not assigned offspring.

For SNPs, with up to 15 mismatches allowed, assignment rates for single assignments were 2–4 times higher than those of microsatellites, ranging from 73–90%. The rates for multiple assignments were 2–8 times lower than those of

microsatellites, ranging from 8–20%, and were even zero for R4 offspring. There was only 2–3% offspring that were not assigned to any pp (15 mismatches allowed), except for R4 in which 27% offspring were not assigned to any pp.

2.3.3.2 Parental assignment with Cervus

For microsatellites, when assigning offspring to all candidate parents, 'strict' assignment rates increased for offspring in R2 and R4, and decreased for offspring in R1 and R3. 'Relax' assignment rates stayed the same in R1 (51%) and R2 (41 to 42%), decreased in R3 (50 to 42%) and increased in R4 (30 to 63%). The rate of unassigned animals was 20–42% in different cohorts.

For SNPs, when assigning offspring to all parents combined, assignments for offspring in each cohort increased to 73–94%. All assignments were at 95% confidence level. There were only two offspring (4%) in R2 that were assigned at 80–95% confidence level (Table 2.4).

2.3.4 Consistency of parental assignment between microsatellites and SNPs

2.3.4.1 Assignments in Vitassign

Consistency of Vitassign assignments between microsatellites (up to 2 mismatches) and SNPs (up to 15 mismatches) is presented in Table 2.5. Of the total 173 offspring, 50 were uniquely assigned using both microsatellites and SNPs. Of these, 34% were assigned to the same pp, 20% were assigned to the same sires but different dams, and 14% were assigned to the same dams but different sires. The remaining 46.8% were assigned to different pp (Table 2.5).

Table 2.5 Consistency of unique assignments (all offspring to all parents) using microsatellites and SNPs, with Vitassign. Fifty (28.9% of the total 173 offspring) unique assignments from Vitassign for both types of markers are compared.

Compare sire	Different dam		Same dam		Total	
/dam	#	%	#	%	#	%
Different sire	16	32.0	7	14.0	23	46.0
Same sire	10	20.0	17	34.0	27	54.0
Total	26	52.0	24	48.0	50	100.0

Different sire: assigned sires using microsatellites and SNPs are different, **Different dam**: assigned dams using microsatellites and SNPs are different, **Same sire**: assigned sires using

microsatellites and SNPs are the same, **Same dam**: assigned dams using microsatellites and SNPs are the same.

2.3.4.2 Assignments in Cervus

Consistency of Cervus assignments between microsatellites and SNPs is presented in Table 2.6. Of the total 173 offspring, 28 were assigned at 'strict' confidence level, using both microsatellites and SNPs. Of these 28, 17 (60.7%) were assigned to the same pp, 3 (10.7%) were assigned to the same sires but different dams, and 3 (10.7%) were assigned to the same dams but different sires. The remaining 5 (17.9%) were assigned to different pp (Table 2.6).

Table 2.6 Consistency of assignments (all offspring to all parents) using microsatellites and SNPs, with Cervus. Twenty eight (16.2% of the total 173 offspring) 'strict' assignments (95% confidence) from Cervus are compared.

Compare sire	Different dam		Same dam		Total	
/dam	#	%	#	%	#	%
Different sire	5	17.9	3	10.7	8	28.6
Same sire	3	10.7	17	60.7	20	71.4
Total	8	28.6	20	71.4	28	100.0

Different sire: assigned sires using microsatellites and SNPs are different, **Different dam**: assigned dams using microsatellites and SNPs are different, **Same sire**: assigned sires using microsatellites and SNPs are the same, **Same dam**: assigned dams using microsatellites and SNPs are the same.

2.3.5 Consistency of parental assignment between Cervus and Vitassign

For microsatellites, there were 54 (31% of the total 173 offspring) unique parent pair assignments in Vitassign which were also assigned a single pp at the 'strict' or 'relax' confidence levels in Cervus. Of these, 43% (13 with zero mismatch, 7 with 1 mismatch, and 3 with 2 mismatches) were assigned the same parent pair at 'strict' level. Twenty-nine unique pp (12 with zero mismatch, 12 with 1 mismatch, and 5 with 2 mismatches) were assigned the same parents at 'relax' level only. One pp (1 mismatch) was assigned with the same dam but with different sire, and one pp (2 mismatches) was assigned to a completely different pp.

The comparison between Vitassign and Cervus when using SNPs is shown in Table 2.7. There were 113 (65% of the total 173 offspring) unique assignments in Vitassign that had the same pp assigned in Cervus, at 'strict' level. The majority (109) of these assignments were with 8 mismatches or less.

Table 2.7 Consistency of assignments[a] for SNPs in Cervus, with corresponding unique assignments in Vitassign when allowing for increasing numbers of mismatches (MM, 1 through 13). In total 130 offspring with significant assignments in Cervus were used.

# MM	Different sire		Same sire	
	Different dam	Same dam	Different dam	Same dam
0				3
1		1		20
2				23
3				16
4				9
5			1	14
6	1			13
7	1		2	5
8				6
9	2		1	2
10		1	1	1
11	3		1	
12			2	
13				1
Total	7	2	8	113

[a] at 'strict'and 'relax' levels in Cervus.

 # MM: number of mismatch(es) allowed in Vitassign, **Different sire**: assigned sires in Cervus and in Vittasign are different, **Different dam**: assigned dams in Cervus and in Vittasign are different, **Same sire**: assigned sires in Cervus and in Vitassign are the same, **Same dam**: assigned dams in Cervus and in Vitassign are the same.

2.4 Discussion

2.4.1 Comparison of microsatellites and SNPs

Within the mating scheme, i.e. cohort offspring–cohort parents analysis, Cervus 'strict' assignment rates were low for microsatellites (7–21%), and low to moderate (20–69%) for SNPs. For SNPs, all assignments were at 'strict' level. For microsatellites, the rates at 'strict' level were low and many assignments were at 'relax' level. With Vitassign, allowing for up to 2 microsatellites mismatches resulted 37.5% unique assignments, but also in multiple parent pair assignments (Figure 2.2). The rate of multiple parent pairs assigned with microsatellites in our study was 45.1% (78 offspring). For SNPs, allowing up to 8 mismatches still resulted in only unique assignments (58%, 95 offspring). However, allowing up to 10 mismatches resulted in 19 new unique assignments (and thus a total of 68% unique assignments), and 4 multiple pp assignments. Allowing for more mismatches did not increase the rate of unique assignments (83–84% when 14–15 mismatches were allowed) (Figure 2.3).

The number of SNP markers (122) was 10 times that of microsatellites (12) in this study. Earlier simulation work had shown that one requires approximately 10 times more SNPs than microsatellites to get roughly the same power for parental assignment (Wang and Santure, 2009). However, assignment rates using 12 microsatellites were much lower than expected based on this comparison. This result was surprising as the nine microsatellites (UNH160, UNH203, UNH211, UNH212, UNH123, UNH178, UNH208, UNH214, and UNH231) had been shown to be highly polymorphic and informative in a previous study in a Nile tilapia population in Egypt (Fessehaye *et al.*, 2006). Three other microsatellites (UNH146, UNH169 and UNH222) were selected based on low genotyping error rates and high PIC (Table 2.2).

In this study, more than half of the candidate parents were either full- or half-sibs with other parents. This was problematic because Cervus assumes no relationship between candidate parents. Olsen *et al.* (2001) reported reduced assignment success due to closely related candidate parents, that is, full- and half-sibs among both candidate parents. According to Vandeputte *et al.* (2006), in Vitassign, related parents can cause loss of assignment power, therefore negatively affecting assignment success. The main reason for this is that related candidate parents have more alleles in common than randomly selected candidate parents (Matson *et al.*, 2008; Villanueva *et al.*, 2002). Fung *et al.* (2002) showed that, in general, given the

genotype of the dam and offspring, the power of excluding a relative as the true sire reduces with a proportion equal to half the proportion of alleles IBD between the relative and the true parent. In other words, it is more difficult to exclude a relative from paternity when they are full or half-sibs.

We believe that in our study, the presence of related candidate parents affected the exclusion power of the microsatellites and resulted in multiple parental pair assignments with Vitassign, and low, strict assignment rates with Cervus. The parents in the study by Fessehaye *et al.* (2006) were themselves offspring from parents produced from all possible diallele crosses between four Egyptian strains of *O. niloticus*, i.e. the inbreeding coefficient among these candidate parents can be assumed as zero. In this study, the candidate parents were selected based on their estimated breeding values for body weight at harvest from a group of 50 families. Those fifty families originated from the 10^{th} generation of selected GIFT, for which the average coefficient of kinship (co-ancestry) was 0.0261 (Ponzoni *et al.*, 2010). However, the average kinship in the present candidate parents is higher than 2.6% because a) the Vietnamese population represents a smaller subset of GIFT 10, and b) parents were randomly selected from a group of animals that had been selected on BLUP EBVs only. Selection on BLUP breeding values will result in selection of relatives when no additional measures are taken, such as optimal contribution selection (Meuwissen, 1997). In our study, selection of relatives was expected to be not problematic as we employed a rotational mating design which will reduce the rate of inbreeding. Multiple parent pairs assigned were also observed, though at lower rate, when using microsatellites in Atlantic cod (21.6% before correction and 8.6% after correction for the markers used) (Herlin *et al.*, 2007) and in different generations of selection in common carp (14–34%) (Vandeputte *et al.*, 2008). When all parents were considered as candidates, the rate of multiple parent pairs assigned in our study (45.1% , 78 offspring, Figure 2.2), using Vitassign for microsatellites, was in good agreement with Vandeputte *et al.* (2006) who found 150 (38.5%, out of total 390) multiple assignments with 2 mismatches allowed for a set of 5 microsatellites loci. We conclude that the number of alleles and high PIC are not sufficient criteria to select microsatellites. When many closely related candidate parents exist, leading to many ambiguous assignments, as observed in this study, it is generally recommended to increase the number of loci to remedy the correctness of assignments (Olsen *et al.*, 2001; Vandeputte *et al.*, 2011). In our study this would mean that all animals should be typed for more informative microsatellite loci in order to get a good exclusion power, comparable to that of

the present SNP set. In such situations the cost-benefit ratio of using microsatellites instead of SNPs will change in favour of SNPs.

2.4.2 Genotyping error

Genotyping error has long been recognised as a major factor affecting assignment rates, as it causes erroneous paternity exclusion (Gagneux *et al.*, 1997) and has a negative impact on parentage assignment (Hoffman and Amos, 2005; Jones *et al.*, 2010; Pompanon *et al.*, 2005). A genotyping error occurs when the observed genotype does not correspond to the true genotype. An error can be detected when comparing a given set of genotypes against a reference that was obtained from high-quality genotype or from multiple repeats. Error can also be detected from field observations such as pedigree data. The effect of genotyping errors has increasingly raised concerns (Jones *et al.*, 2010; Pompanon *et al.*, 2005), and several efforts have been made to accommodate errors in parental assignment (Kalinowski *et al.*, 2007). In this study the Johnson and Haydon (2007) approach was used to determine genotyping error for microsatellites. This approach was used because neither allelic dropout nor false alleles are detectable just by comparing mismatches between repeat genotypes (Johnson and Haydon, 2007). In this study, microsatellites genotyping error was estimated based on repeated genotyping of 48 individuals, which equaled to 11.7% of the total number of individuals in the dataset. This was also the recommended rate of blind typing of 10% (Pompanon *et al.*, 2005). The estimated microsatellites error rate per locus was 0.6%, which was lower than the common rate of laboratories of 1.0%. However, even genotyping error rate lower than 1% can cause problems to parental assignment. Simulation in Cervus showed that success of paternity assignment can still decline rapidly even when genotyping error rate was lower than 1% (Marshall *et al.*, 1998). In a study on Antarctic fur seal (*Arctocephalus gazelle*), a genotyping error rate as low as 0.01 per locus resulted in >20% of false paternity exclusion, and it increased the numbers of offspring that matched to more than one candidate sire (Hoffman and Amos, 2005). In other words, genotyping error reduced the assignment power of the loci set. In this study, microsatellites genotyping error rates (per loci) were not uniformly distributed across loci. Locus UNH208 showed the highest allelic drop-out of 0.045. The remaining loci with genotyping error > zero were those with highest PIC (Table 2.2). In general, microsatellites with high variability, that is, high power for parentage, are also more prone to genotyping error than microsatellites with lower variability (Hoffman and Amos, 2005). Those loci with genotyping error could have

contributed to the low assignment rate of microsatellites in this study. However, trials in which these loci were removed did not improve assignment rates (data not shown).

Microsatellites differ from SNPs in level of genotyping error. Highly polymorphic microsatellites are prone to genotyping errors, and the error rate is relatively high (Hoffman and Amos, 2005; Pompanon *et al.*, 2005) and rarely below 1% (Kalinowski *et al.*, 2007). In addition, genotyping and allele-calling of microsatellites are semi-automated, which might introduce errors that contribute to mistyping. It is recommended to check a proportion of the microsatellite scores by eye, for cross-validation.

In contrast, SNP genotyping uses a highly automated method, which should result in a much lower genotyping error rate (Groenen *et al.*, 2009; Slate *et al.*, 2009). However, the estimated genotype error in this population based on mismatches was 2.2 – 3.8%, assuming that 5 – 10 mismatches are needed to assign all animals to their parents. Unfortunately the precise number of mismatches needed to obtain 100% allocation could not be determined as some parents were missing. It is clear, however, that this genotyping error is higher than expected, and higher than the value found in a SNP set by Hauser *et al.* (2011), which was 0.34% per genotype. The reasons for this discrepancy are not clear. The SNPs were selected based on their performance in a previous analysis (Van Bers *et al.*, 2012). When detecting the SNPs we used a high quality reference sequence of only nucleotides with a sequencing quality score of at least 20 which corresponds to a probability of >0.99 that the nucleotide is correctly called. The consensus quality score at the SNP position was 30, which corresponds to a probability of 10^{-3} that the consensus genotype is incorrect. Furthermore, 388 out of 411 study animals had a call rate > 0.9 and the rest (23) a call rate from 0.8–0.9 (data not shown). Finally, the minor allele frequency of all 122 SNPs was >0.2, a level below which SNPs lose their power in parental assignment (Anderson and Garza, 2006).

The most likely explanation for the high genotyping error rate is in the presence of null alleles, which is non- or less efficient-amplification of alleles (Pompanon *et al.*, 2005). A null allele can be considered as a type of systematic allele dropout (Jones *et al.*, 2010). Null alleles can cause false exclusion when heterozygotes are scored incorrectly as homozygotes. Null allele frequencies were calculated in Cervus, but the program has no formal procedure to handle null alleles in its analysis. Instead, Cervus detects null allele frequency per loci and leaves it to the users to decide

which loci should be in the analysis. In this study, four SNP loci with high null allele frequencies were detected. Preliminary analysis with those loci removed resulted in a reduction of the assignment rates. The possible reason might be that when the loci were removed, less genetic information was available for the analysis because those removed loci were highly informative. Nevertheless, null alleles might have contributed to the relative high number of mismatches in this study.

2.4.3 Assignment rates and the effect of missing parents

Missing parents is a major concern in molecular parental assignment, when not all parents are sampled, some offspring may not be assigned while some may be assigned incorrectly (Jones and Ardren, 2003; Jones *et al.*, 2010; Pemberton, 2008). Missing parents is the most difficult part of a parental analysis study (Jones *et al.*, 2010). In Cervus, the total number of candidate parents is important for assessment of confidence in actual assignments (Marshall *et al.*, 1998). More specifically, using allele frequencies from the sampled population, Cervus simulates populations of candidate parents and offspring with a user-specified proportion of parents sampled, in order to determine a cut-off critical LOD or delta value that gives the desired level of confidence in the actual assignments. Therefore, results from Cervus are sensitive to the user-defined proportion of parents sampled. In general, simulations in Cervus have shown that the success rate in paternity assignment never exceeds the proportion of candidate sires typed, even when all candidate dams are sampled (Marshall *et al*., 1998). When missing candidate dams exist, the paternity assignment rate is expected to be even lower. In the same study, paternity assignment reduced to less than half when all candidate dams were assumed un-sampled (Marshall *et al.*, 1998). For parent *pair* assignment, the problem is exacerbated with missing genotyped parents. This is so because a missing animal can be the parent of multiple offspring. In Vitassign, true but missing parents would result in no assignments (Vandeputte *et al.*, 2006).

In this study, the proportion of missing parents in each cohort ranged from 14.9 to 18.6% for candidate dams and from 3.7 to 14.8% for candidate sires (Table 2.1). For example, in R1 cohort offspring–cohort parent assignment, the missing proportions were 14.8% for sires and 14.9% for dams. Assuming random mating and equal contributions of parents to offspring, only 72.5% of the offspring can correctly be assigned to both sampled dams and sires, that is, offspring from the mating of genotyped dams with genotyped sires. Furthermore, 2.2% of the offspring will not have compatible parents at all (that is, offspring from the mating of missing dams

with missing sires), 12.7% offspring will have no compatible dam but a compatible sire (offspring from the mating of missing dams with existing sires) and 12.6% will have no compatible sire but a compatible sire (offspring from the mating of existing dams with missing sires). Similarly, the proportion of offspring that can theoretically be assigned to both an existing dam and sires in R2, R3 and R4 were 74.3, 80.2 and 76.8%, respectively. For Cervus cohort offspring–cohort parents analysis, the actual assignment rates of both microsatellites and SNPs, except for results of SNPs in R1 (69% at 'strict' level), were much lower than the expected values in all four cohorts, if only 'strict' confidence level was considered. In contrast, unique assignment rates for SNPs with Vitassign were close to the expected values, if 11–12 mismatches were allowed (76–79%). With this number of mismatches allowed, only 8% (12 offspring) multiple assignments were observed.

2.5 Conclusions

In the present study, assignment rates were low. The main causes for this low assignments rate were missing parents and relatedness among candidate parents. Both microsatellites and SNP markers can equally be good for parental assignments, providing that all parents are sampled, and that the degree of relatedness between parents is not too high. Furthermore, it is imperative that good marker sets with high exclusion power are available. We recommend estimating exclusion power of the microsatellites set in a subset of parents from a (future) population under study. If the exclusion power is low, it is necessary to improve the marker set by investigating new markers. Comparing Vitassign and Cervus in terms of consistency of assignments revealed that neither program performs well when the exclusion power of the marker set used is low, as was observed for microsatellites in our study. Cervus and other likelihood-based methods can be useful to resolve exclusion-based multiple parent pair assignments.

Acknowledgements

The authors thank Dr. Raul W. Ponzoni and Dr. Nguyễn Hồng Nguyên of the WorldFish Center, for supplying the GIFT 10^{th} generation, financial support for field work during the period 2007 to date, and DNA genotyping. We are grateful to Marc Vandeputte and an anonymous reviewer for their constructive comments which helped us to improve the paper. We thank Hamed Rashidi and Atal Saha for their

help with genotyping, and Prof. Johan van Arendonk for his constructive comments. Trọng thanks the tilapia team at RIA2 for their field work.

Appendix A

Table 2.8 SNP loci used and population basic statistics.

Locus	MAF	N	HExp	FIS	PIC	F(Null)	HW	Locus	MAF	N	HExp	FIS	PIC	F(Null)	HW
ss244316522	0.475	409	0.499	-0.082	0.374	-0.040	NS	ss244316492	0.253	407	0.380	-0.042	0.307	-0.021	NS
ss244316622	0.409	410	0.483	-0.025	0.366	-0.013	NS	ss244316495	0.211	403	0.327	-0.092	0.274	-0.044	NS
ss244316652	0.329	406	0.445	-0.090	0.346	-0.044	NS	ss244316657	0.318	411	0.431	-0.016	0.338	-0.008	NS
ss244316521	0.462	409	0.498	-0.227	0.374	-0.103	***	ss244316528	0.421	407	0.486	0.039	0.368	0.020	NS
ss244316460	0.469	403	0.498	0.092	0.374	0.048	NS	ss244316604	0.318	407	0.436	-0.009	0.341	-0.005	NS
ss244316467	0.222	398	0.350	-0.134	0.289	-0.063	NS	ss244316498	0.418	409	0.488	0.133	0.368	0.070	NS
ss244316649	0.314	411	0.437	0.087	0.341	0.044	NS	ss244316603	0.306	399	0.422	0.043	0.333	0.022	NS
ss244316539	0.466	358	0.499	-0.315	0.374	-0.137	***	ss244316563	0.364	406	0.464	-0.019	0.356	-0.010	NS
ss244316463	0.262	409	0.384	0.044	0.310	0.023	NS	ss244316458	0.292	408	0.413	0.128	0.327	0.067	NS
ss244316519	0.217	408	0.342	0.096	0.283	0.050	NS	ss244316607	0.332	400	0.444	-0.009	0.345	-0.005	NS
ss244316623	0.405	379	0.481	-0.131	0.365	-0.062	NS	ss244316560	0.302	408	0.426	0.073	0.335	0.038	NS
ss244316530	0.483	396	0.500	-0.090	0.375	-0.044	NS	ss244316609	0.275	401	0.397	-0.060	0.318	-0.030	NS
ss244316544	0.406	408	0.484	-0.008	0.366	-0.005	NS	ss244316608	0.333	388	0.444	-0.068	0.345	-0.033	NS
ss244316672	0.333	409	0.447	-0.045	0.347	-0.023	NS	ss244316501	0.410	344	0.483	-0.066	0.366	-0.033	NS
ss244316449	0.209	397	0.325	0.148	0.272	0.079	NS	ss244316561	0.365	406	0.462	-0.126	0.355	-0.060	NS
ss244316572	0.344	353	0.448	-0.176	0.347	-0.082	NS	ss244316504	0.316	407	0.435	-0.016	0.340	-0.009	NS
ss244316570	0.405	384	0.481	-0.104	0.365	-0.050	NS	ss244316640	0.196	404	0.313	-0.051	0.264	-0.025	NS

Locus	MAF	N	HExp	FIS	PIC	F(Null)	HW	Locus	MAF	N	HExp	FIS	PIC	F(Null)	HW
ss244316669	0.378	410	0.471	-0.144	0.360	-0.068	NS	ss244316505	0.212	346	0.333	-0.162	0.278	-0.076	NS
ss244316585	0.477	407	0.499	-0.058	0.374	-0.029	NS	ss244316673	0.325	411	0.441	0.091	0.343	0.046	NS
ss244316586	0.395	398	0.479	-0.071	0.364	-0.034	NS	ss244316457	0.284	358	0.408	-0.027	0.324	-0.014	NS
ss244316470	0.479	339	0.499	-0.218	0.374	-0.099	*	ss244316612	0.293	406	0.410	-0.039	0.325	-0.020	NS
ss244316533	0.268	405	0.393	-0.163	0.315	-0.076	NS	ss244316534	0.397	407	0.479	0.000	0.364	0.000	NS
ss244316471	0.335	354	0.444	-0.227	0.345	-0.103	**	ss244316499	0.314	405	0.434	-0.120	0.339	-0.058	NS
ss244316628	0.277	403	0.397	-0.025	0.318	-0.013	NS	ss244316551	0.384	406	0.474	-0.055	0.362	-0.027	NS
ss244316587	0.353	383	0.458	-0.055	0.353	-0.027	NS	ss244316459	0.198	408	0.317	-0.076	0.267	-0.036	NS
ss244316453	0.497	410	0.501	-0.124	0.375	-0.060	NS	ss244316670	0.216	407	0.337	0.009	0.280	0.003	NS
ss244316630	0.277	390	0.403	-0.030	0.322	-0.015	NS	ss244316644	0.268	411	0.392	-0.166	0.315	-0.077	NS
ss244316590	0.264	290	0.387	-0.034	0.312	-0.017	NS	ss244316507	0.385	404	0.474	-0.200	0.361	-0.093	**
ss244316591	0.369	404	0.467	-0.013	0.358	-0.007	NS	ss244316613	0.337	320	0.451	0.647	0.349	0.477	***
ss244316476	0.438	396	0.493	-0.061	0.371	-0.030	NS	ss244316462	0.497	406	0.501	-0.012	0.375	-0.007	NS
ss244316592	0.436	400	0.492	-0.134	0.371	-0.063	NS	ss244316564	0.411	403	0.486	0.045	0.367	0.022	NS
ss244316594	0.434	342	0.491	-0.214	0.370	-0.098	*	ss244316464	0.432	403	0.490	0.057	0.370	0.029	NS
ss244316478	0.363	401	0.464	-0.101	0.356	-0.049	NS	ss244316645	0.197	406	0.320	0.063	0.268	0.031	NS
ss244316668	0.429	410	0.491	-0.088	0.370	-0.043	NS	ss244316549	0.446	383	0.495	-0.038	0.372	-0.020	NS
ss244316479	0.422	354	0.489	-0.051	0.369	-0.026	NS	ss244316615	0.387	401	0.475	-0.076	0.362	-0.037	NS
ss244316556	0.281	327	0.400	-0.100	0.319	-0.049	NS	ss244316496	0.281	356	0.398	-0.093	0.319	-0.045	NS
ss244316455	0.381	405	0.474	0.053	0.361	0.026	NS	ss244316647	0.246	410	0.369	0.008	0.301	0.004	NS
ss244316472	0.429	388	0.492	0.004	0.370	0.001	NS	ss244316512	0.469	356	0.499	-0.379	0.374	-0.160	***

Locus	MAF	N	HExp	FIS	PIC	F(Null)	HW	Locus	MAF	N	HExp	FIS	PIC	F(Null)	HW
ss244316631	0.407	408	0.483	0.010	0.366	0.004	NS	ss244316446	0.414	399	0.487	-0.080	0.368	-0.040	NS
ss244316524	0.422	407	0.488	-0.037	0.369	-0.019	NS	ss244316651	0.230	402	0.355	-0.045	0.292	-0.023	NS
ss244316633	0.290	407	0.410	-0.102	0.326	-0.049	NS	ss244316574	0.358	386	0.463	0.037	0.355	0.018	NS
ss244316477	0.250	401	0.374	-0.112	0.304	-0.055	NS	ss244316573	0.351	411	0.457	-0.151	0.352	-0.070	NS
ss244316634	0.453	410	0.496	0.075	0.373	0.039	NS	ss244316448	0.220	411	0.352	0.040	0.290	0.019	NS
ss244316665	0.348	407	0.456	0.009	0.352	0.004	NS	ss244316546	0.249	401	0.374	-0.099	0.304	-0.049	NS
ss244316447	0.233	400	0.357	-0.064	0.293	-0.031	NS	ss244316511	0.311	316	0.431	-0.146	0.338	-0.069	NS
ss244316658	0.374	409	0.471	-0.236	0.360	-0.106	***	ss244316624	0.329	408	0.443	-0.063	0.345	-0.030	NS
ss244316595	0.226	323	0.350	-0.097	0.289	-0.046	NS	ss244316545	0.263	408	0.389	-0.028	0.313	-0.015	NS
ss244316483	0.325	353	0.444	-0.149	0.345	-0.070	NS	ss244316646	0.371	405	0.469	-0.200	0.359	-0.091	*
ss244316597	0.422	397	0.490	-0.008	0.369	-0.005	NS	ss244316543	0.222	378	0.336	-0.015	0.279	-0.009	NS
ss244316486	0.233	409	0.354	-0.090	0.291	-0.044	NS	ss244316542	0.437	406	0.492	-0.122	0.371	-0.058	NS
ss244316596	0.379	392	0.469	-0.168	0.359	-0.079	NS	ss244316661	0.403	411	0.484	0.014	0.367	0.007	NS
ss244316531	0.427	402	0.490	-0.184	0.370	-0.085	*	ss244316577	0.302	380	0.432	-0.139	0.338	-0.066	NS
ss244316636	0.256	408	0.385	-0.083	0.311	-0.040	NS	ss244316648	0.290	411	0.406	0.054	0.323	0.026	NS
ss244316599	0.317	411	0.432	0.037	0.338	0.018	NS	ss244316525	0.425	407	0.489	0.031	0.369	0.015	NS
ss244316488	0.421	381	0.488	-0.096	0.369	-0.047	NS	ss244316579	0.434	408	0.491	0.002	0.370	0.000	NS
ss244316559	0.279	406	0.400	0.088	0.320	0.046	NS	ss244316667	0.352	407	0.456	-0.039	0.352	-0.021	NS
ss244316558	0.251	397	0.377	-0.188	0.306	-0.087	*	ss244316625	0.426	331	0.490	-0.220	0.369	-0.100	**
ss244316639	0.418	404	0.486	-0.091	0.367	-0.044	NS	ss244316552	0.366	409	0.466	-0.013	0.357	-0.007	NS
ss244316660	0.331	403	0.444	0.045	0.345	0.022	NS	ss244316517	0.411	408	0.485	0.039	0.367	0.020	NS

ss244316491	0.350	407	0.459	-0.194	0.353	-0.089	*	ss244316518	0.236	382	0.358	-0.184	0.294	-0.085	NS
ss244316526	0.480	372	0.500	-0.220	0.375	-0.100	**	Average	0.346	393	0.440	-0.055	0.341	-0.024	
ss244316655	0.279	404	0.403	-0.032	0.322	-0.016	NS								

- SNP names taken from SNPexp database (http://app3.titan.uio.no/biotools/tool.php?app=snpexp)
- All loci had call-rate > 0.8
- **N**: number of animals typed at the locus, **MAF:** Minor allele frequency, **HExp**: Expected heterozygosity, $\mathbf{F_{IS}}$: (H expected – H observed)/H expected, **PIC**: Polymorphic Information Content, **F(Null)**: Estimated null allele frequency, **HW**: Significance of deviation from Hardy-Weinberg equilibrium. NS: Not significant, ***: Significant at the 0.1% level.

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3

Genetic parameters for reproductive traits in Nile tilapia (*Oreochromis niloticus*): I. Spawning success and time to spawn

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Submitted to Aquaculture (2012)

Abstract

Breeding programs for Nile tilapia typically use nested mating designs with 2 females mated to 1 male to produce paternal half-sib families. This mating design can take up to 3 months or longer to produce the desired number of half-sib family groups. Prolonged family production increases common environmental effects, and negatively affects estimation of genetic parameters. In this paper we investigated the hypothesis that prolonged family production is a consequence of selection for growth in Nile tilapia. We compared two mating systems: multiple males, multiple females group mating (7M:15F) and single male, multiple females mating (1M:10F), to estimate heritability for 'spawning success' and 'time to spawn', and their genetic correlations with harvest weight, in a selected population of GIFT 12. Spawning success was modelled as a threshold trait (SPAWN) using a linear repeatability animal model and a generalised logit linear repeatability model. All animals that spawned before 32 days were labelled 'spawn' (1) and animals that did not spawn after 32 days were considered as 'no-spawn' (0). We then changed the threshold and estimated heritability at each threshold point; e.g. with a threshold at 20 days, all animals with 'spawn' records after 20 days are considered 'no-spawn'. For SPAWN, estimates for heritability, repeatability and genetic correlations were consistent between linear and logit models. Heritability estimates for SPAWN were 0.20 to 0.22 for linear model and 0.14-0.18 for logit model with thresholds from 20 to 32 days. Estimates for "time to spawn" were not different from zero (0.01 ± 0.02). Genetic correlations of SPAWN with harvest weight were positive and ranged from 0.48-0.52 for thresholds of 20-32 days. Overall, the 'multiple females, multiple males' experiment yielded a higher proportion of successful spawn records than the single male, multiple female experiment. However, in both experiments 85% of the successful spawns were produced within 21 days. We conclude that Nile tilapia favour mating in groups and that spawning success as defined here is a heritable trait. Our results show that selection for harvest weight in GIFT should improve spawning success of Nile tilapia, provided the mating period is limited to 20-32 days. To facilitate the rapid production of paternal half-sibs, we recommend using a mating design of multiple females with a single male in a Nile tilapia breeding program.

Key words: Nile tilapia, spawning success, time to spawn, harvest weight, heritability, genetic correlation.

3.1 Introduction

In Nile tilapia (*Oreochromis niloticus*), the Genetic Improvement of Farmed Tilapia (GIFT) project has been conducted for ten years, from since 1988 until 1997 (Bentsen *et al.*, 2012; Gjedrem, 2012). Thereafter the GIFT stock has been further improved until present, mainly by the WorldFish Center (Ponzoni *et al.*, 2011). In the GIFT technology manual, a nested mating design with 2 females mated to 1 male is used to produce paternal half-sib families (WorldFish Center, 2004). In practice, one female is stocked in a spawning unit with one male. After spawning, the female is replaced by a second female to produce a half-sib group. This nested mating design can take up to 3 months or longer to produce the desired number, typically 50, of half-sib groups (Ponzoni *et al.*, 2011). Prolonged family production increases common environmental effects to full-sibs, and negatively affects estimation of genetic parameters like heritability or genetic correlations between traits (Bentsen *et al.*, 2012).

It can be argued that the difficulty with producing full and half-sib families within a shorter time-span is a consequence of the natural mating system of Nile tilapia. In Nile tilapia, natural spawning behaviour resembles that of other lekking animals (Turner and Robinson, 2000), that is, groups of males occupy a spawning area and each male defends a "nest" as a site for mating and oviposition. Females enter the spawning area when they are ready to ovulate and mate with one of more males. Fessehaye *et al.* (2006) showed that mating systems in Nile tilapia are diverse, including not only single pair mating but also polygamous mating.

In commercial Nile tilapia seed production, group mating (i.e. multiple females and multiple males) is normally used. The stocking sex ratio is often 2 females to 1 male (Barman and Little, 2006) , but sometimes 3 to 1 or even 4 to 1 (Mires, 1982). A sex ratio of 2 females to 1 male produced the highest seed/m^2/day and seed/female/day (Hughes and Behrends, 1983) and today many small-scale tilapia seed production systems use a ratio of 2 to 1 (Barman and Little, 2011; Bhujel *et al.*, 2007). Traditionally, the reproduction period is about 3 to 4 weeks. In the Mekong Delta of Vietnam, Nile tilapia hatcheries normally use a stocking ratio of 4 females to 1 male or 5 to 1, during a reproduction period of about 21 days. The fact that group mating for 21-30 days is used to produce large numbers of fry suggests that single pair mating is perhaps not optimal for the production of offspring, and that group-mating designs could be more successful.

An alternative explanation for the long reproductive period in GIFT tilapia (up to 3 months or more, (e.g. Ponzoni *et al.*, 2011)) is given by the observation that selection for growth can lead to unwanted correlated responses in reproduction. In many livestock species, long-term selection for high production efficiency resulted in physiological, immunological and reproductive problems (Rauw *et al.*, 1998). Typical reproductive problems are defective eggs and poor semen quality in chicken, delayed age at puberty and farrowing in pigs, and low success rates after insemination in dairy cattle (Rauw *et al.,*(1998). In Atlantic salmon and rainbow trout, there seems to be no strong unfavourable relationship between growth rate and age at maturity (Gjerde, 1986). In Nile tilapia, Longalong *et al.* (1999) reported undesirable genetic association between body weight and early maturation, that is, selection for larger body weight leads to early maturing females. The GIFT strain has been selected for harvest weight for more than 15 generations at the WorldFish Center. However, to our knowledge, there is no study on the genetics of reproduction, in terms of spawning success, in female tilapia.

In this paper we investigate the hypothesis that prolonged family production is a consequence of selection for growth in Nile tilapia. We compare two mating systems: multiple male, multiple female group mating (7M:15F) and single male, multiple female mating (1M:10F), to investigate the female "spawning success" and "time to spawn". The objective of this study was to estimate heritabilities for these two spawning traits and to estimate the genetic correlation of these spawning traits with harvest weight, the main selection trait in the GIFT program.

3.2 Materials and methods

3.2.1 Broodstock

Broodstock were from the Research Institute for Aquaculture No. 2 (RIA2) stock (G12), which was the second generation in the Mekong Delta of Vietnam and originated from the GIFT 10^{th} generation obtained from the WorldFish Center, Penang, Malaysia (Ponzoni *et al.*, 2010). All broodstock were from G12, which were produced in September 2008 through February 2009, and had complete pedigree. Fish had been reared according to standard procedures: they had been grown in hapas to the age of 56–224 days, after which they were tagged and communally grown in a pond to the age of 246–552 days when they were harvested. Harvest weight was recorded and females and males were separately stocked in conditioning hapas until further use. The broodstock were conditioned in 3×5×1 m hapas (mesh size = 5 mm) installed in a 1,000 m^2 pond for approximately four

weeks and fed twice a day on a commercial floating pelleted feed (brand AFIEX), with about 30% crude protein and 6% fat, at a feeding rate of 3% body weight daily.

3.2.2 Experiments

We conducted two experiments that differed in the mating design, namely 'Multiple males, multiple females' (MM) and 'Single male, multiple females' (SM). The two mating designs differed in terms of (i) the number of males and females stocked and (ii) the mating ratio for female to male, as described hereafter.

'Multiple males, multiple females' experiment

The aims of the MM experiment were (i) to mimic natural group spawning conditions of Nile tilapia, as reported in the literature (see e.g. Turner and Robinson, 2000), and (ii) to obtain as many spawning records for females as possible. In this experiment, 15 females and 7 males were stocked into a 15 m^3 (3×5×1 m) concrete tank, equivalent to a stocking ratio (female to male) of about 2 to 1. Prior to stocking, all tanks were cleaned with water, then disinfected with sodium hypochlorite (Chlorine) (30 ppm), and thereafter washed again with water to remove chlorine residue. The tanks were then fully filled with water and stocked with fish. After four days, the first check for spawning was conducted, and checking for spawning was repeated at four-day intervals. The males remained in the spawning tanks, and only dead males were replaced by new males. Females that spawned were removed from the spawning tanks and replaced by a new female. Females were allowed to stay in the spawning tank until 32 days. Females that did not spawn at 32 days were removed from the spawning tanks, and recorded as 'no-spawn'.

In total, we used 7 tanks for this experiment. The experiment was conducted from December 2009 through August 2010. In total 771 unique females were tested, corresponding to 862 records. Of these, 675 were first 'spawn' records and 91 were first 'no-spawn' records. Some females were used twice during the course of the experiment which resulted in 26 repeated 'spawn' records and 70 repeated 'no-spawn' records.

'Single male, multiple females' experiment

The SM experiment was designed to resemble more the nested mating design used in the GIFT program. For this experiment, 10 females and 1 male were stocked in a spawning tank, equivalent to a stocking ratio (female to male) of 10 to 1. The tank size and tank preparation were the same as with the MM experiment. However, every four days, i.e. after each check for spawning, the male was removed from the spawning tank and replaced by a new male. Therefore, each male stayed in a spawning tank only for four days. Similar to the MM experiment, females that spawned were removed from the spawning tanks and replaced by a new female. Females were allowed to stay in the spawning tank for a maximum of 32 days and females that did not spawn at 32 days were removed from the spawning tanks and recorded as 'no-spawn'.

The SM experiment was conducted twice, from January 2010 through August 2010 (SM-1), and again from April to June 2011 (SM-2). For SM-1 we used five tanks, and 18 tanks for SM-2. In total for SM-1 and SM-2 combined, 401 unique females were tested, corresponding to 478 records. Of these, 82 were first 'spawn' records and 66 were first 'no-spawn' records. Many females were used twice during the course of the experiment, resulting in 221 repeated 'spawn' and 109 repeated 'no-spawn' records.

3.2.3 Data collection

The age of the fish at the start of the experiments ranged from 321–693 (MM and SM-1) and from 760–987 (SM-2) days. For each female, tagging weight (TW) was recorded at the time of tagging, growing age (GA) was calculated as number of days from stocking into grow-out pond until harvest, harvest weight (HW) was recorded at harvest, and body weight at spawning (WSP) was recorded at the time of egg/fry collection. The time from stocking until spawning (time to spawn, TS) was calculated for each spawned female. For females that spawned, the spawning age (SA) was calculated from the date that the female was born until the date the first "spawn" record was obtained. For females that did not spawn, SA was calculated from the date that the female was born until she was removed from the spawning tank.

3.2.4 Statistical analysis

Genetic parameters were estimated using ASReml version 3 (Gilmour *et al.*, 2009). For the analysis of spawning success, data from MM, SM-1, and SM-2 experiments were pooled for estimating genetic parameters. In addition, SM-1 and SM-2 were considered as duplicates of SM. Fixed effects fitted were experiment, tank, and age of fish at spawning (for 'spawn' records) or when removing from the tank (for 'no-spawn' records). Experiment was fitted to account for the differences in the designs of MM and SM experiment. The MM and SM-1 were conducted at the same time, but the SM-2 was conducted 8 months later. Therefore, SA was fitted to account for this difference. Tank was fitted because several tanks were used in each experiment, and each tank has its own location in the hatchery.

Spawning success was analysed as a threshold trait, termed SPAWN. All animals that spawned before 32 days were labelled 'spawn' (coded as 1) and animals that did not spawn after 32 days were considered as 'no-spawn' (coded as 0). Heritability for SPAWN was calculated at each threshold point, e.g. with a threshold at 20 days, all animals with 'spawn' records after 20 days are considered 'no-spawn' as well. We first fitted a linear repeatability animal model (LIN). The model was:

$$y_{ijk} = \mu + \beta_1 SA_{ijk} + EXP_i + (TANK(EXP))_j + ANIMAL_k + PE_k + e_{ijk} \quad \text{(Model 1)}$$

where y_{ijk} is spawn/no spawn (1/0); μ is the population mean; β_1 is the regression coefficient of spawning age, SA_{ijk}; EXP_i is the fixed effect of the experiment i (MM, SM-1, SM-2) (i = 1, 2, 3); $\left(TANK(EXP)\right)_j$ is the fixed effect of the spawning tank j (j = 1, 2,…, 23) nested within experiment i; $ANIMAL_k$ is the random additive genetic effect of the individual k with $\mathrm{N}(0, \mathbf{A}\sigma_a^2)$, PE_k is the random permanent effect on the individual k with $\mathrm{N}(0, \mathbf{I}_{\boldsymbol{PE}}\sigma_e^2)$ where $\mathbf{I}_{PE}$ is the identity matrix of the appropriate dimension, and e_{ijk} is the random residual term with $\mathrm{N}(0, \mathbf{I}_{\boldsymbol{e}}\sigma_e^2)$ where $\mathbf{I_e}$ is the identity matrix of the appropriate dimension and σ_e^2 is the residual variance.

There is a concern that animal threshold models applied to binary data can give a biased estimation of genetic parameters, particularly if there is more than one fixed effect (see e.g. Odegard *et al.*, 2010). Therefore, we also fitted a generalised linear

repeatability animal model (LOGIT), to estimate variance component and genetic parameters for SPAWN (spawn = 1, no-spawn = 0). The model was:

$$\eta_{ijk} = \mu + \beta_1 SA_{ijk} + EXP_i + (TANK(EXP))_j + ANIMAL_k + PE_k + e_{ijk} \quad \text{(Model 2)}$$

where η_{ijk} is the linear predictor on logit scale and other effects are the same as in Model 1, except that the residual variance e_{ijkl} is fixed at $\frac{\pi^2}{3} \approx 3.28987$ under the logit scale according to the ASReml 3 User Manual (Gilmour *et al.* (2009).

For "time to spawn" (TS), fish that did not spawn were not included in this analysis. Initially SA was fitted as a fixed effect for TS, but this led to convergence problems. Replacing SA with body weight at spawning (WSP) solved the problem. Therefore, WSP was fitted as fixed effect in the final analysis. The trait was square root-transformed, and the following model was used:

$$Y_{ij} = \mu + \beta_1 WSP_{ij} + EXP_i + ANIMAL_j + PE_j + e_{ij} \quad \text{(Model 3)}$$

where Y_{ij} is the square root of TS; β_1 is the regression coefficient of the co-variable body weight at spawning, WSP_{ij}; EXP_i is the fixed effect of the experiment i (*i* = 1, 2, 3); $ANIMAL_j$ is the random additive genetic effect of the individual *j;* PE_j is the random permanent effect on the individual *j*, e_{ij} is the random residual term with $N(0,\mathbf{I}\sigma_e^2)$ where **I** is the identity matrix and σ_e^2 is the residual variance.

For SPAWN (in both LIN and LOGIT) and TS, heritability (h^2) and repeatability (*r*) were calculated as:

$$h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_{E_p}^2 + \sigma_{E_t}^2}, r = \frac{\sigma_A^2 + \sigma_{E_p}^2}{\sigma_A^2 + \sigma_{E_p}^2 + \sigma_{E_t}^2}$$

where σ_A^2 is the additive genetic variance, $\sigma_{E_p}^2$ is the permanent environmental variance, and $\sigma_{E_t}^2$ is the temporary environmental variance.

With LIN, the heritability on the observed binary scale for SPAWN was transformed to an underlying continuous scale according to Dempster and Lerner (1950), in order to be compared with results from LOGIT:

$$h_{und}^2 = h_{obs}^2 \frac{[p(1-p)]}{z^2},$$

where h_{und}^2 is the heritability on the underlying continuous scale, h_{obs}^2 is the heritability on the observed binary scale, *p* is the fraction of 'spawned' female, and

z is the ordinate of a standard normal distribution at the threshold point corresponding to the fraction *p*. The heritability of SPAWN on the underlying scale can be interpreted as the heritability presuming a continuous scale.

For HW, fixed effects were birthday, tagging weight, and growing age. Birthday (BD) was calculated as the number of days from January 1st until the date that the fish was born. The effect was fitted as class variable, because more than one family can be collected in one day. Full-sibs were nursed in separate family nursing hapas until tagging. Tagging weight (TW) was fitted to account for differences in nursing conditions among families. Growing age (GA) was fitted to account for the difference in grow-out period (when all tagged fish were grown communally in one pond). Although all families were stocked into the grow-out pond at the same time, harvesting was conducted over many days because of practical reasons. The following mixed animal model was used:

$$Y_{ijk} = \mu + BD_i + \beta_1 TW_{ijk} + \beta_2 GA_{ijk} + \beta_3 GA_{ijk}^2 + ANIMAL_j + DAM_k + e_{ijk} \quad \text{(Model 4)}$$

where Y_{ijk} is the phenotypic value of HW for the k^{th} fish; μ is the population mean; BD_i is the fixed effect of the i^{th} birth date for the fish, calculated as the number of dates from January 1st until the date that the fish was born ($i = 1, .., 23$); β_1 is the regression coefficient of the co-variable tagging weight, TW_{ijk}; β_2 is the regression coefficient of the co-variable growing age, GA_{ijk}; β_3 is the regression coefficient of the co-variable growing age squared, GA_{ijk}^2; $ANIMAL_j$ is the random effect of the j^{th} fish with $\mathrm{N}(0, \mathbf{A}\sigma_a^2)$ where $\mathbf{A}$ is the additive genetic relationship matrix among the animals and σ_a^2 is the additive genetic variance; DAM_k is the random environmental effect common to full-sibs with $\mathrm{N}(0, \mathbf{I}\sigma_c^2)$; e_{ijk} is the random residual term with $\mathrm{N}(0,\mathbf{I}\sigma_e^2)$ where $\mathbf{I}$ is the identity matrix and σ_e^2 is the residual variance.

For HW, heritability (h^2) and common environmental to full-sibs (c^2) was calculated as:

$$h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_c^2 + \sigma_e^2}, \; c^2 = \frac{\sigma_c^2}{\sigma_A^2 + \sigma_c^2 + \sigma_e^2}$$

where σ_A^2 is the additive genetic variance, σ_c^2 is the common environmental to full-sibs variance, and σ_e^2 is the residual variance.

Bivariate analyses were used to estimate genetic correlations (i) between SPAWN and HW, and (ii) between TS and HW. The fixed effects (i) for SPAWN as in Model 1

(LIN) or 2 (LOGIT), (ii) for TS as in Model 3, and (iii) for HW were the same as in Model 4. For the bivariate model between SPAWN (with LOGIT) and HW, residual covariance was set to zero to overcome convergence problems. Therefore, for this particular analysis, phenotypic correlation could not be estimated.

3.3 Results

3.3.1 Experiments

The MM experiment produced 862 records from 771 unique females, while the SM experiments produced 478 records from 401 unique females. Overall, the total proportion of 'spawn' records was three times that of 'no-spawn' records (75% versus 25%).There were considerable differences in proportions of 'spawn' and 'no-spawn' records between the two experimental designs. The proportion of spawn (1) records for MM was 1.3 times higher than that of SM experiments (83.1 versus 63.4%). Consequently, the proportion of no-spawn (0) records for SM was twice that of MM experiment (36.6 versus 18.7%) (Table 3.1).

Table 3.1 Number of records (percentage) of spawning success for G12 females.

Experiment	Spawning success					
	# of records			# unique females		
	No spawn	Spawn	Total	No spawn	Spawn	Total
MM	161 (18.7)	701 (81.3)	862 (100)	96 (12.5)	675 (87.5)	771 (100)
SM-1	46 (28.0)	118 (72.0)	164 (100)	45 (27.6)	118 (72.4)	163 (100)
SM-2	129 (41.1)	185 (58.9)	314 (100)	121 (42.9)	161 (57.1)	282 (100)
Total	336 (25.1)	1004 (74.9)	1340* (100)	262 (21.5)	954 (78.5)	1216** (100)

*from a total of 914 unique females.

**some females were used in more than one experiment.

MM: 'multiple males, multiple females' (7 male and 15 females per spawning tank) experiment, SM-1 and SM-2: 'single male, multiple females' (1 male and 10 females per spawning tank) experiments.

Interestingly, the distribution of animals that spawned over time was very similar between two experiments (Table 3.2). For both experiments, about ¾ of the females that spawned, spawned in the first three weeks after the females were stocked into the tanks. From the total number of 'spawn' records collected in both experiments, about 85% were obtained within 21 days (Figure 3.1).

Table 3.2 Numbers and percentage of 'spawn' records (spawn = 1) for the females that spawned at different time point, from stocking until the female spawned, for each experiment.

	Time to spawn (days)								No spawn	Total
	4	8	12	16	20	24	28	32		
MM										
#	183	140	115	80	62	56	44	21	161	862
%	26.1	20.0	16.4	11.4	8.8	8.0	6.3	3.0		100*
SM-1										
#	52	16	16	14	9	6	4	1	46	164
%	31.7	9.8	9.8	8.5	5.5	3.7	2.4	0.6		100*
SM-2										
#	35	30	36	29	21	17	10	7	129	314
%	11.1	9.6	11.5	9.2	6.7	5.4	3.2	2.2		100*

MM = 'Multiple males, multiple females' experiment, 7 male and 15 females per spawning tank, SM-1 and SM-2 = 'Single male, multiple females' experiments, 1 male and 10 females per spawning tank.* Do not include 'no spawn' (=0) records.

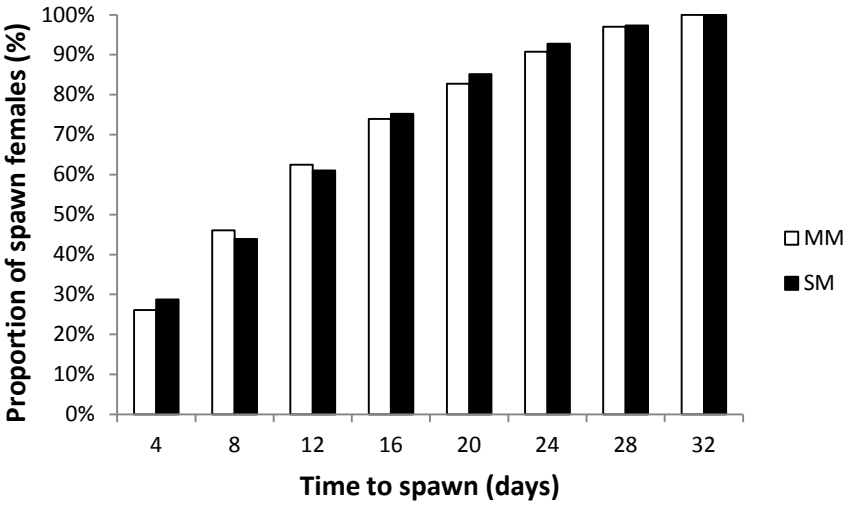

Figure 3.1 Cumulative percentage of 'spawn' records at each time point, from stocking until the female spawn. MM = 'Multiple males, multiple females' experiment: 7 male and 15 females per tank, SM = 'Single male, multiple females' experiment: 1 male and 10 females per tank.

3.3.2 Fixed effects

For HW, all fixed effects (BD, TW, and the first and second polynomial of GA) were significant ($P < 0.01$). In general, fish with smaller BD (i.e. born earlier) were heavier at harvest as estimates for HW decreased when BD increased. Similarly, fish with larger TW or longer GA were heavier at harvest, as estimates for TW and GA were positive (results not shown).

For SPAWN, similarly, all fixed effects (SA, experiment, and tank) were significant ($P<0.01$). Spawning age reflects the age differences among fish within and between experiments. As the age of a female increased, the ability of a female to spawn until a designated threshold day (12 – 32 days) decreased, as estimates of SA were negative (-0.0026 to -0.0051, results not shown). The effect of experiment (MM, SM-1, and SM-2) was significant ($P<0.01$), because the two experiments differ in female to male ratio and number of fish per tank. Effect of tank (nested within experiment) was significant ($P = 0.005$), a consequence of the fact that the tanks are in different locations in the hatchery.

For ST, all fixed effects (WSP and experiment) were significant ($P<0.01$). Heavier females tend to take longer time to spawn. Females in MM experiment tend to spawn quicker than those in SM experiment.

3.3.3 Genetic parameter estimates

Harvest weight
For harvest weight, 910 records were available for females. Heritability (se) was estimated at 0.32 (0.18). Common environmental effect among full-sibs was estimated at 0.07 (0.07).

SPAWN
There was a large variation in proportion of spawn/no-spawn records between families, as illustrated in Figure 3.2, for thresholds of 8 and 32 days. When increasing the threshold for time to spawn from 12 to 32 days, heritability estimates for SPAWN with both LIN and LOGIT models increased (Table 3.3 and Figure 3.3). In general, heritability estimates (underlying scale) from LIN were 20 – 50% higher than heritability estimates from LOGIT. With LIN, heritability estimates for SPAWN increased from 0.02 (12 days) to 0.07 (16 days), and thereafter changed little from day 20 (0.14) through day 32 (0.11) on the observed scale. On the

underlying continuous scale, heritability estimates for SPAWN increased from 0.03 (12 days) to 0.10 (16 days), and thereafter remained stable: 0.20–0.22 (20–32 days). With LOGIT, the same trend was observed: heritability estimates for SPAWN increased from 0.02 (12 days) to 0.07 (16 days), and thereafter ranged from 0.14 – 0.18 (20–32 days).

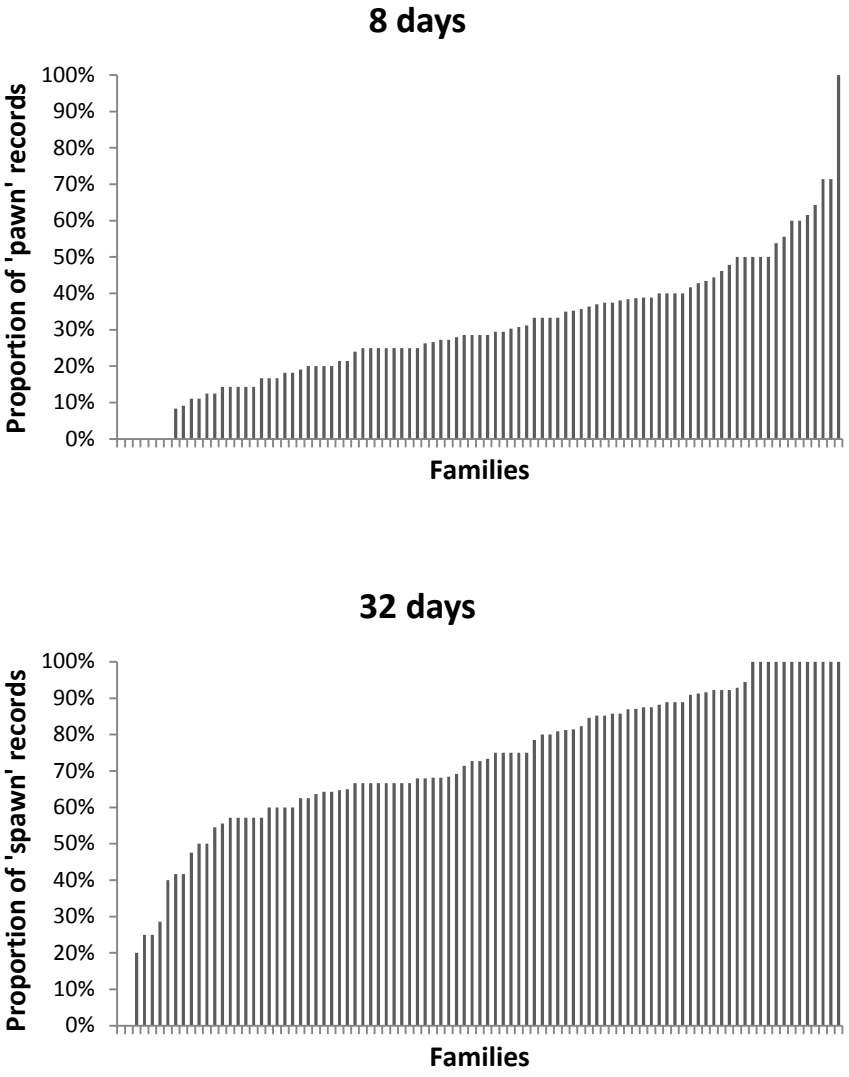

Figure 3.2 Proportion of 'spawn' records (= females) within each full-sib family at two thresholds: 8 days (above) and 32 days (below).

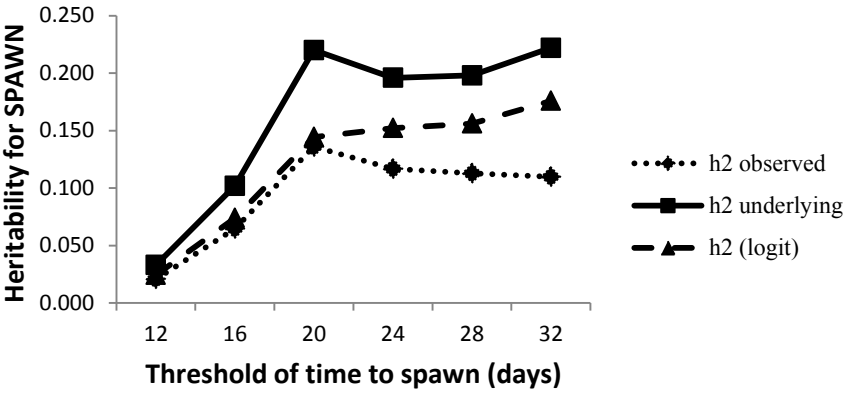

Figure 3.3 Heritability on the observed binary scale (h^2 observed) (LIN), heritability on the underlying continuous scale (h^2 underlying) (LIN), and heritability on the logit scale [h^2 (logit)] (LOGIT) for SPAWN at different thresholds for time to spawn, that is, females that spawned after the threshold date were labelled as 'no-spawn'.

Table 3.3 Estimates of heritability for SPAWN at different thresholds (12–32 days). For LIN: heritability on the observed binary scale ($h^2_{obs} \pm se$), heritability on the underlying continuous scale ($h^2_{und} \pm se$). For LOGIT: ($h^2 \pm$ se).

Threshold (days)*	*p*	LIN		LOGIT
		h^2_{obs}	h^2_{und}	h^2
12	0.43	0.02 ± 0.02	0.03 ± 0.04	0.02 ± 0.03
16	0.53	0.07 ± 0.03	0.10 ± 0.05	0.07 ± 0.03
20	0.66	0.14 ± 0.05	0.22 ± 0.08	0.14 ± 0.04
24	0.66	0.12 ± 0.05	0.20 ± 0.08	0.15 ± 0.04
28	0.71	0.11 ± 0.05	0.20 ± 0.08	0.16 ± 0.06
32	0.80	0.11 ± 0.04	0.22 ± 0.09	0.18 ± 0.07

* females that spawned after the threshold date were recorded as 'no spawn';

For LIN: $h^2_{und} = h^2_{obs} \frac{[p(1-p)]}{z^2}$: heritability after transformation, $r_{und} = r_{obs} \frac{[p(1-p)]}{z^2}$: repeatability after transformation, $se_{und} = se_{obs} \frac{[p(1-p)]}{z^2}$: standard error after transformation, *p* is the fraction of 'spawned' female, and *z* is the ordinate of a standard normal distribution at the threshold point corresponding to the fraction *p*, according to Dempster and Lerner (1950).

Repeatability estimates for SPAWN with both LIN and LOGIT models are presented in Table 3.4. Repeatability estimates from LIN were lower than from LOGIT for 12 and 16 days but comparable from 20–32 days (Table 3.4). With LIN, on the observed binary scale, repeatability estimates for SPAWN increased from 0.04 (12 days) to 0.07 (16 days), and thereafter remained stable: 0.15 (20 days) to 0.17 (24–32 days). On the underlying continuous scale, repeatability for SPAWN increased from 0.06 (12 days) to 0.34 (32 days). With LOGIT, repeatability estimates for SPAWN were fairly similar from 0.31 (12 days) to 0.26 (20-32 days) (Table 3.4).

Table 3.4 Estimates of repeatability for SPAWN at different thresholds (12 – 32 days). For linear model: repeatability on the observed binary scale ($r_{obs} \pm se$), repeatability on the underlying continuous scale ($r_{und} \pm se$). For LOGIT model: repeatability ($r \pm$ se) is presented.

Threshold*(days)	*p*	LIN		LOGIT
		r_{obs}	r_{und}	*r*
12	0.43	0.04 ± 0.04	0.06 ± 0.07	0.31 ± 0.04
16	0.53	0.07 ± 0.03	0.10 ± 0.05	0.28 ± 0.01
20	0.66	0.15 ± 0.05	0.25 ± 0.08	0.26 ± 0.01
24	0.66	0.17 ± 0.05	0.29 ± 0.08	0.26 ± 0.01
28	0.71	0.17 ± 0.04	0.30 ± 0.08	0.26 ± 0.06
32	0.80	0.17 ± 0.04	0.34 ± 0.09	0.26 ± 0.06

* females that spawned after the threshold date were recorded as 'no spawn';
$h^2_{und} = h^2_{obs} \frac{[p(1-p)]}{z^2}$: heritability after transformation, $r_{und} = r_{obs} \frac{[p(1-p)]}{z^2}$: repeatability after transformation, $se_{und} = se_{obs} \frac{[p(1-p)]}{z^2}$: standard error after transformation, *p* is the fraction of 'spawned' female, and *z* is the ordinate of a standard normal distribution at the threshold point corresponding to the fraction *p*, according to Dempster and Lerner (1950).

Genetic correlations between SPAWN and HW, with both LIN and LOGIT models, are presented in Table 3.5. Genetic correlation between SPAWN and HW was very similar between LIN and LOGIT models. With LIN, on the observed binary scale for SPAWN, genetic correlation between SPAWN and HW was initially high (0.83) at 12 days, and reduced to 0.63-0.48 for the period between 20 and 32 days. With LOGIT, estimates for genetic correlation between SPAWN and HW were very similar to those obtained with LIN. Phenotypic correlations (estimable only with LIN) were low (range 0.07- 0.11).

Table 3.5 Genetic correlation ($r_g \pm se$) and phenotypic correlation ($r_p \pm se$) from the linear model (LIN) between SPAWN (on the observed binary scale) and harvest weight (on the continuous scale) at different thresholds for time to spawn (12–32 days), and from the LOGIT model ($r_g \pm$ se) between SPAWN (on the logit scale) and harvest weight (on the continuous scale).

Threshold* (days)	LIN		LOGIT
	r_p	r_g	r_g
12	0.08 ± 0.035	0.83 ± 0.50	0.83 ± 0.48
16	0.10 ± 0.04	0.63 ± 0.30	0.63 ± 0.22
20	0.11 ± 0.04	0.50 ± 0.24	0.51 ± 0.17
24	0.10 ± 0.04	0.48 ± 0.26	0.45 ± 0.17
28	0.07 ± 0.04	0.58 ± 0.27	0.41 ± 0.19
32	0.09 ± 0.04	0.52 ± 0.25	0.47 ± 0.18

* females that spawned after the threshold date were recorded as 'no spawn'

Time to spawn

Heritability estimate for TS was close to zero when records from both MM and SM experiments were combined (0.01 ± 0.02). Using only records from the MM experiment gave similar low estimates (0.04 ± 0.05). Genetic correlation between TS and HW was not estimable, because of low heritability estimate.

3.4 Discussion

3.4.1 Experiments

Spawning success, measured as the proportion of spawn/no-spawn records, was higher in the MM (81/19) than in the SM experiments (63/37). High stocking density (>10 fish/m^2) has been reported to inhibit spawning in tilapia (review of Coward and Bromage, 2000; Guerrero, 1982). In our study, the stocking density was 1.5 fish/m^2 for MM and 0.7 fish/m^2 for SM. This density was much lower than in other experiments, with Nile tilapia, where they used 5 to 10 fish/m^2 (Hughes and Behrends, 1983). In hybrid tilapia (*O. niloticus × O. aureus*), a stocking ratio of 2 fish/m^2 was found to be optimum for fry production (Siddiqui and Al-Harbi, 1997). Based on these observations and the fact that stocking density was higher in the MM experiment than in the SM experiment, we do not think that the stocking densities in our study had a negative effect on spawning success of the females.

The experimental designs differed in terms of number of male(s) in one spawning tank. For MM experiment, there were 7 males in one spawning tank; while for the SM experiments there was only 1 male in one tank. Females in MM experiment therefore had the choice to mate with different males, while females in SM experiments had only one choice. Biologically, the spawning success in tilapia may depend on many factors such as temperature, photoperiod, social interaction, etc. Social interaction can be through visual, audible or chemical (pheromone) stimulation from conspecifics (Coward and Bromage, 2000). Chemical stimuli was found to be more important than visual stimulation in *O. mossambicus* (Silverman, 1978), whereas contacts between female and male Nile tilapia was though as the most important factor for successful spawning (Srisakultiew, 1993). In the MM experiment, a female had contact with more males than in SM experiments (7 versus 1) at any time. In the SM experiments, only females that did not spawn or spawned at 28 – 32 days had sequentially contact with 8 males. Our results suggest that the MM design stimulated spawning more as it shows more resemblance to the natural spawning conditions. Natural spawning behaviour in Nile tilapia is polygamous, where females can choose among many male candidates in the 'lek" as seen in most mouth brooding cichlids (Barlow, 1991).

The two experiments also differed in female to male ratio. In Nile tilapia, Hughes and Behrends (1983) reported that a female to male ratio of 2 to 1 gave better spawning results (in term of percentage of females spawned) than any higher female to male ratio, while Bautista *et al.* (1988) found the best spawning results for female to male ratio of 4 to 1. In this aspect, the MM experiment (ratio 2 to 1) was more similar to the reported ratio than the SM experiment (ratio 10 to 1). The MM experiment used the same mating ratio (2 to 1) that Fessehaye *et al.* (2006) used successfully in a study on natural mating of Nile tilapia. On the other hand, the SM experiments resemble more the pair mating (one female to one male) design that is recommended in the GIFT manual. However, the SM experiments showed an improvement of female spawning success over the conventional GIFT methodology, because at many occasions, more than one female was found to spawn with a single male after 4 days. For family production, this implies that at least two half-sib families can be produced in just 4 days, thereby shortening considerably the time needed to produce half-sib groups. Based on the results of our study, we therefore recommend the use of a SM design with multiple females and a single male in a spawning tank for family production of Nile tilapia.

As experiments differed in their designs, which resulted in different proportion of 'spawn' (spawning success=1) records, we fitted experiment as a fixed effect, to account for all the differences (number of fish, female to male ratio, and timing). This allowed us to analyse combined data from two experiments to increase the number of records, thereby improving estimation of genetic parameters. As expected, the effect of experiment on SPAWN was found to be significant ($P<0.01$), confirming that correction for experiments leads to more precise estimation of genetic parameters in our study.

The effect of age at spawning for females was significant, as the two experiments were conducted over a long period (9 months for MM, 8 months for SM-1 and 3 months for SM-2), and that the SM-2 was conducted 8 months after the SM-1. Smaller and/or younger Nile tilapia spawn more frequently (Guerrero and Guerrero, 1985). In commercial seed production, broodstock are normally discarded when they are heavier than 300 g, because larger fish are more difficult to handle when harvesting for eggs/fry (Bhujel, 2000), and probably because they are more costly to feed. Our results showed that older females had a slightly reduced ability to spawn, as estimates of the regression of SA on HW were negative (-0.0026 to -0.0051).

3.4.2 Heritability

Heritability estimates for spawning success, defined as SPAWN in our study, increased rapidly from 12 to 20 days and became fairly stable from 20 to 32 days. The trend was similar with both LOGIT and LIN models, as illustrated in Figure 3.3. This result indicates that genetically SPAWN is expressed most clearly from day 20 (SPAWN_20) up to 32 days (SPAWN_32). Results for SPAWN_20 indicate that the duration that is needed to measure SPAWN reliably should be at least 20 days. Moderate heritability (0.22 with LIN and 0.17 with LOGIT, both at 32 days) indicates good prospects for selection for spawning success when the mating period is limited to 3-4 weeks.

In general, animal threshold models for binary data give biased estimations of genetic parameters, particularly if there are several fixed effects (Hoeschele and Tier, 1995; Stock *et al.*, 2007). Therefore, binary data are often analysed using a sire or a sire-dam threshold model (Ødegård *et al.*, 2006; Ødegård *et al.*, 2007), or linear model (Schurink *et al.*, 2009). Heringstad *et al.* (Heringstad *et al.*, 2003) and Ødegård *et al.* (Ødegård *et al.*, 2007) showed that predicted breeding values from

linear and threshold models were in good agreement. In the current study, results from LIN and LOGIT models were also in good agreement (Table 3.3 and 3.4, and Figure 3.2). We are therefore confident that both animal models (linear and logit) give reliable estimates.

Heritability estimates for TS were close to zero. This might be due to the biological complexity of ovarian development in tilapia, and the fact that time to spawn is strongly influenced by biological and social factors as discussed before. In coho salmon (*Oncorhynchus kisutch*), Gall and Neira (2004) found genetic correlations between spawn day (calculated as the number of days from 31st December to the date of spawning within years) and four measurements of fish body size (spawn weight, spawn length, harvest weight, post-spawn weight) to be not significantly different from zero. The authors suggested that, within spawning season, the genetic control of spawn date and body size are independent. In the present study, we selected ready-to-spawn females that had been pre-conditioned and showed external signs of maturation, i.e. a reddish and swollen genital papilla (GIFT manual; WorldFish Center (2004). These ready-to-spawn females were a selected subset of our female GIFT 12th population, which probably explains why we could not detect a genetic component for TS.

That spawning success of female Nile tilapia is heritable was also strongly indicated by the substantial variation in proportion of 'spawn' records among families of female origins, as illustrated in Figure 3.2 (data from MM and SM experiments combined). This between-family variation can be exploited by selection, e.g by excluding families with poor spawning success from the breeding program.

Genetic correlation

Favourable genetic correlation ($r_g = 0.52$ with LIN and 0.47 with LOGIT) between SPAWN and HW implies that selection for HW would result not only in heavier fish, but also in increased spawning success. As the GIFT-origin lines of Nile tilapia have been selected for harvest weight for more than 15 generations (under the GIFT project and at the WorldFish Center), a favourable genetic correlation between harvest weight and SPAWN would be valuable, as selection for heavier fish will also result in females that have the highest chance to spawn. This has a great advantage, because successful spawning reduces the number of fish needed to produce families. The question is then whether SPAWN should be included in a Nile

tilapia breeding program or whether selection could still rely on HW only, given that the correlation is only 0.5. Based on the results of this study, we recommend to use a single male-multiple female mating design with a 4-day rotation of males, and to limit the time that a female is allowed to produce offspring to 21 days. If the female does not spawn after 21 days, she should be removed from the breeding stock as the chance that she will spawn later is small. This would result in quick testing of males and females and allow family production to be conducted in as short time as possible.

3.5 Conclusion

We can conclude that spawning success is a heritable trait in the studied GIFT 12th female population. Spawning success, defined as SPAWN ('spawn' or 'no spawn') should be best measured over a period of 20 days. SPAWN at 20 days gives higher heritability than time to spawn and, based on our analysis, is the preferred trait for genetic selection. Equally important is that SPAWN has a favourable genetic correlation with harvest weight. Selection for harvest weight in GIFT did not affect spawning success of Nile tilapia. We recommend the system of multiple females with a single male for family production in a Nile tilapia breeding program. Relationships to other fertility traits need to be quantified before these finding can be implemented in a breeding scheme aimed at improving the spawning success of females.

Acknowledgements

This study is part of the first author's sandwich PhD study, funded by Wageningen University. We would like to thank Dr. Raul W. Ponzoni and Dr. Nguyễn Hồng Nguyên of the WorldFish Center for supplying the GIFT generation 10th, and for financial support for field work from 2007 to date. We thank Dr. Henk Bovenhuis for his helpful suggestions for data analysis. We thank the tilapia team at RIA2, especially Phạm Đăng Khoa, for their field work and collection of data.

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4

Genetic parameters for reproductive traits in Nile tilapia (*Oreochromis niloticus*): II. Fecundity and fertility

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Submitted to Aquaculture (2012)

Abstract

Harvest weight is the main trait in Nile tilapia (*Oreochromis niloticus*) breeding programs. The effects of selection for harvest weight on female reproductive traits are unknown. In this paper we estimate genetic parameters for reproductive traits and their correlation with harvest weight using females from the 12th generation of GIFT in the Mekong Delta of Vietnam. Spawning records were obtained from single pair mating as well as group mating experiments. The traits were categorised into two groups: fecundity-related traits and fertility-related traits. Fecundity traits were: number of eggs (NEGG), relative fecundity as the ratio of number of eggs to female spawning weight (RFEC), egg weight (EGGW) and egg diameter (EGGD); fertility traits were: number of fertilised eggs (FEGG), number of hatched eggs (HAT), number of swim-up fry (SWUP), and fertilisation rate (FER, in %). Heritability estimates for fecundity traits were low, ranging from 0.02–0.08. Heritability estimates for fertility traits were also low, ranging from 0.06–0.12. Genetic correlations for HW with NEGG and TEGGW were positive (0.51 and 0.42, respectively), while correlations for HW with RFEC, EGGW, and EGGD were negative (−0.72, −0.48, and −0.50, respectively). The same trend was observed for body weight at spawning (SPW), but genetic correlations between SPW and fecundity traits were higher than those between HW and fecundity traits. Genetic correlations between HW and fertility traits were all moderate to high (0.46 to 0.69), except for FER (0.15 ± 0.24). Genetic correlations between SPW and fertility traits were even higher (0.69 to 0.93). We conclude that both HW and SPW have favourable genetic correlations with NEGG, RFEC, and SWUP, which are the desired characteristics for Nile tilapia seed production. Selection for HW does not affect these traits. On the other hand, Nile tilapia females selected for large HW tend to produce smaller and lighter eggs. We recommend monitoring the phenotypic and/or genetic trend in this trait, as smaller eggs might, on the longer term, lead to lower fry survival.

Key words: Nile tilapia, harvest weight, reproductive traits.

4.1 Introduction

The Genetic Improvement of Farmed Tilapia (GIFT) project has been conducted for ten years (1988–1997) to realise genetic improvement in Nile tilapia (*Oreochromis niloticus*) (Bentsen *et al.*, 2012; Gjedrem, 2012). Thereafter, the Nile tilapia of GIFT origin has further been selected, mainly by the WorldFish Center, until present (Ponzoni *et al.*, 2011). Harvest weight has been the main trait of interest, with genetic gains ranging from 10 to 15 per cent per generation over six generations (Ponzoni *et al.*, 2011). In addition to harvest weight, genetic parameters of other traits have been studied in different generations of the GIFT stocks including body dimension (Nguyen *et al.*, 2007), fillet yield (Nguyen *et al.*, 2010; Thodesen *et al.*, 2012), flesh composition (Ponzoni *et al.*, 2011), and shape (Trọng *et al.*, 2013). In other Nile tilapia strains, genetic parameters of body dimension, gut length, visceral weight/index, and low-temperature tolerance were studied by Charo-Karisa *et al.* (2007), and fillet yield by Rutten *et al.* (2005).

There are few studies on reproductive traits in Nile tilapia. Phenotypically, fecundity (calculated as the ratio of number of eggs to weight of ovary) varied more with body length (r = 0.860) and body weight (r = 0.806) than with age (r = 0.604) (Babiker and Ibrahim, 1979). Genetic parameters for gonado-somatic index (GSI) in Nile tilapia were estimated by Charo-Karisa *et al.* (2007). Heritability of GSI was 0.25 for females and 0.03 for males. The authors also estimated genetic correlation between harvest weight and GSI was 0.27 for females and 0.01 for males. In Nile tilapia, Longalong *et al.* (1999) reported undesirable genetic association between body weight and early maturation, that is, selection for larger body weight leads to early maturing females. The GIFT strain has been selected for harvest weight for 15 generations at the WorldFish Center. However, to our knowledge, the genetics of female reproductive traits in GIFT Nile tilapia have not been studied. Therefore, the effects of selection for harvest weight on female reproductive traits are unknown.

Reproductive traits in fish are usually are expressed as fecundity-related traits: number of eggs, egg weight, egg diameter, and egg volume (Gjerde, 1986). Genetic parameters for fecundity-related traits in fish have been studied mainly in salmonid species. In coho salmon (*Oncorhyncus kisutch*), heritability and genetic correlation between number of eggs, egg weight, spawn days, harvest weight, female pre-spawning and post-spawning weight were reported by Gall and Neira (2004). In

wild brown trout (*Salmo trutta*), descriptive statistics of fecundity (expressed as number of eggs in the ovary), and egg diameter were reported by L'Abee-Lund and Hindar (1990) and by Hao and Chen (2009). In a random mating population of rainbow trout (*Oncorhynchus mykiss*), heritability estimate was 0.15 for female post-spawning weight, 0.32 for egg number and 0.28 for egg size (Gall and Huang, 1988). In selected populations of rainbow trout, heritability estimate was low (0.14) for spawning weight, but medium to high (0.52–0.65) for spawning date, egg size, number of eggs, egg volume, and for fertility-hatchability (Su *et al.*, 1997).

In this paper we investigate the effect of selection for harvest weight on reproductive traits for female Nile tilapia. The traits are categorised into two groups: fecundity-related traits (fecundity traits) and fertility-related traits (fertility traits). We first report heritability for fecundity traits: number of eggs, relative fecundity as the ratio of number of eggs to female spawning weight, egg weight and egg diameter; and fertility traits: number of fertilised eggs, number of hatched egg (hatchling), number of swim-up fry, and fertilisation rate. Secondly, we report the genetic and phenotypic correlations between the two groups of reproductive traits and body weight at harvest and at spawning.

4.2 Materials and methods

4.2.1 Broodstock

Broodstock were from the Research Institute for Aquaculture No. 2 (RIA2) stock, which was the second generation in the Mekong Delta of Vietnam and originated from the GIFT 10^{th} generation obtained from the WorldFish Center, Penang, Malaysia (Ponzoni *et al.*, 2010). Generation 12 had been produced in September 2008 through February 2009 and all fish had complete pedigree. Fish had been reared according standard procedures: they had been grown in hapas to the age of 56–224 days, after which they were tagged and communally grown in a pond to the age of 246–552 days when they were harvested. Harvest weight was recorded and females and males were separately stocked in conditioning hapas until further use. The broodstock were conditioned in 3×5×1 m hapas (mesh size = 5 mm) installed in a 1,000 m^2 pond for approximately four weeks and fed twice a day on a commercial floating pelleted feed (brand AFIEX), with about 30% crude protein and 6% fat, at a feeding rate of 3% body weight daily.

4.2.2 Experiments

Fertility records were available from three experimental designs that differed in the mating ratios, namely 'family' (FAM), 'Multiple males, multiple females' (MM) and 'Single male, multiple females' (SM). The three mating designs differ in terms of (i) the number of males and females stocked and (ii) the ratio for female to male, as described hereafter.

'Family' experiment

The aim of FAM experiment was to produce families for the 13th generation. The design follows the GIFT protocol described by the WorldFish Center (WorldFish Center, 2004). In this experiment, one female and one male were stocked into a 2.0×1.5×1.0 m spawning hapa (meshed size 1 mm). After the female spawned, the same male was mated with a second female to produce a second half-sib family. Checks for spawning were conducted four days after stocking, and again at eight days. The experiment was conducted from September 2010 through January 2011. In total, 114 records of spawns were collected from 104 unique females that successfully mated with 50 males.

'Multiple males, multiple females' experiment

The aim of MM experiment was to mimic natural group spawning conditions of Nile tilapia, as reported in literature (see e.g. Turner and Robinson, 2000), and to obtain as many spawning records for females as possible. In this experiment, 15 females and 7 males were stocked into a 15 m^3 (3×5×1 m) concrete tank, equivalent to a stocking ratio (female to male) of about 2 to 1. Prior to stocking, all tanks were cleaned with water, and disinfected with sodium hypochlorite (Chlorine) (30 ppm). Checks for spawning were conducted four days after stocking, and thereafter at four day intervals. The males remained in the spawning tanks, and only dead males were replaced by new males. Females that spawned were removed from the spawning tanks and replaced by a new female. Females were allowed to stay in the spawning tank until 32 days. Females that did not spawn at 32 days were removed from the spawning tanks. We used in total 7 tanks for this experiment. The experiment was conducted from December 2009 through August 2010. In total, 740 records of spawns were collected, corresponding to 711 unique females. Of these, 711 were first spawn records. Some females were used twice during the course of the experiment, resulting in 29 repeated spawn records.

'Single male, multiple females' experiment

The SM experiment was designed to resemble more the nested mating design used in the GIFT program. For this experiment, 10 females and 1 male were stocked in a spawning tank, equivalent to a stocking ratio (female to male) of 10 to 1. The tank size and tank preparation were the same as with the MM experiment. After each check, the male was removed from the spawning tank, and was replaced by a new male. Therefore, a male stayed in a spawning tank for only 4 days. Similar to the MM experiment, females that spawned were removed from the spawning tanks and replaced by a new female. Females were allowed to stay in the spawning tank until 32 days. Females that did not spawn at 32 days were removed from the spawning tanks. The SM experiment was conducted twice, from January 2010 through August 2010 (SM-1), and again from April to June 2011 (SM-2). In total, 327 records of spawns were collected, corresponding to 272 unique females. Of these, 237 were first spawn records. Some females were used twice during the course of the experiment, resulting in 90 repeated spawn records. Some females were also used in the MM experiment, and their spawn records were included for MM.

4.2.3 Data collection

Harvest weight and spawning weight (females only)
The age of the fish at the start of the experiments age ranged from 321–693 (MM and SM-1), 620–855 (FAM), and from 760–987 (SM-2) days. Body weight of spawned females (SPW) was recorded at the time of egg/fry collection. The spawning age (SA) was calculated from the date that the female was born until the date she spawned. In total, 804 records were collected for harvest weight (HW) and 1181 records (including 377 repeated records) were collected for SPW.

Fecundity traits
At collection, the stage of eggs or fry (STAGE) was identified and eggs/fry were counted. Egg stages were identified as: 'egg-1' (1 day old), 'egg-2' (2 days old), and 'egg-3' (three days old). In some cases, yolk sac fry were collected from the females. These were recorded as 'fry' (NFRY). The total number of eggs per female (NEGG), was counted for each spawn. Relative fecundity per female (RFEC) was calculated as $\frac{NEGG}{SPW}$.

Eggs were incubated in 0.5 litre plastic down-dwelling bottles with a constant flow-through of water. For each spawn, a sample of thirty eggs was measured, and the mean diameter for these thirty eggs was used in calculations. Total weight of eggs per female (TEGGW, in g) was calculated as $\frac{EGGW}{30} \times NEGG$, with EGGW being the weight of thirty eggs (in g). Egg diameter was measured for each single egg under microscope (Olympus SZX7) with 25X magnification (EGGD, in mm).

Fertility traits

Total number of fertilised eggs per female (FEGG) was counted on date 3 and fertilisation rate (FER, %) was calculated as $100 \times \frac{FEGG}{NEGG}$. The numbers of newly hatched fry (HAT) were counted shortly after all eggs hatched. Hatchlings were then transferred to 30×40×5 cm plastic trays and number of swim-up fry (SWUP) were counted after all fry had their yolk-sac completely absorbed.

4.2.4 Statistical analysis

Genetic parameters were estimated using ASReml version 3 (Gilmour *et al.*, 2009). For HW, fixed effects were birthday, tagging weight, and growing age. Birthday (BD) was calculated as the number of days from January 1st until the date that a family was collected from the mouth-brooding female. The effect was fitted as class variable, because usually more than one family was collected in one day. In total there were 23 collection days; all fish in the same family were given the same BD. As full-sibs were nursed in separate family nursing hapas, tagging weight (TW) was fitted to account for difference in nursing conditions. Growing age (GA) was fitted to account for the difference in length of grow-out period (when all tagged fish were grown communally in one pond). Although all families were stocked into the grow-out pond at the same date, harvesting was conducted over many days because of practical reasons. The following mixed animal model was used:

$$Y_{ijk} = \mu + BD_i + \beta_1 TW_{ijk} + \beta_2 GA_{ijk} + \beta_3 GA_{ijk}^2 + ANIMAL_j + DAM_k + e_{ijk}\,, \qquad \text{(Model 1)}$$

where Y_{ijk} is the phenotypic value of HW for the k^{th} fish; μ is the population mean; BD_i is the fixed effect of the i^{th} birth day for the fish (i = 1, .., 23); β_1 is the regression coefficient of the co-variable tagging weight, TW_{ijk}; β_2 is the regression coefficient of the co-variable growing age, GA_{ijk}; β_3 is the regression coefficient of the co-variable growing age squared, GA_{ijk}^2; $ANIMAL_j$ is the random effect of the

j^{th} fish with $\mathrm{N}(0, \mathbf{A}\sigma_a^2)$ where **A** is the additive genetic relationship matrix among the animals and σ_a^2 is the additive genetic variance; DAM_m is the random environmental effect common to full-sibs with $\mathrm{N}(0, \mathbf{I}\sigma_c^2)$; e_{ijklm} is the random residual term with $\mathrm{N}(0,\mathbf{I}\sigma_e^2)$ where **I** is the identity matrix and σ_e^2 is the residual variance.

Because for spawning weight, the ages of fish were different from those at harvest, the following model was used for SPW:

$$Y_{ijk} = \mu + BD_i + \beta_1 SA_{ijk} + \beta_2 SA_{ijk}^2 + ANIMAL_j + PE_j \pm DAM_k + e_{ijk}\,, \qquad \text{(Model 2)}$$

where Y_{ijk} is the phenotypic value of SPW; β_1 is the regression coefficient of the co-variable age at spawning, SA_{ijk}; β_2 is the regression coefficient of the co-variable age at spawning squared, SA_{ijk}^2; PE_j is the random permanent effect on the individual *j* with $\mathrm{N}(0, \mathbf{I}_{\boldsymbol{PE}}\sigma_e^2)$ where $\mathbf{I}_{PE}$ is the identity matrix of the appropriate dimension; BD_i, $ANIMAL_j$ and DAM_k were the same as in Model 1.

For HW, heritability (h^2) and common environmental effect to full-sibs (c^2) was calculated as:

$h^2 = \frac{\sigma_A^2}{\sigma_a^2 + \sigma_c^2 + \sigma_e^2}$, $c^2 = \frac{\sigma_c^2}{\sigma_a^2 + \sigma_c^2 + \sigma_e^2}$,

where σ_A^2 is the additive genetic variance, σ_c^2 is the common environmental to full-sibs variance, and σ_e^2 is the residual variance.

For SPW, heritability (h^2), repeatability (*r*), and common environmental effect to full-sibs (c^2) was calculated as:

$h^2 = \frac{\sigma_A^2}{\sigma_a^2 + \sigma_c^2 + \sigma_{E_p}^2 + \sigma_{E_t}^2}$, $r = \frac{\sigma_A^2 + \sigma_{E_p}^2}{\sigma_a^2 + \sigma_c^2 + \sigma_{E_p}^2 + \sigma_{E_t}^2}$, $c^2 = \frac{\sigma_c^2}{\sigma_a^2 + \sigma_c^2 + \sigma_{E_p}^2 + \sigma_{E_t}^2}$,

where σ_A^2 is the additive genetic variance, σ_c^2 is the common environmental to full-sibs variance, $\sigma_{E_p}^2$ is the permanent environmental variance, and $\sigma_{E_t}^2$ is the temporary environmental variance.

For fecundity traits (NEGG, RFEC, EGGW, TEGGW, and EGGD) the following model was used:

$$Y_{ijkl} = \mu + \beta_1 SA_{ijkl} + STAGE_i + EXP_j + (TANK(EXP))_k + ANIMAL_l + PE_l + e_{ijkl} \qquad \text{(Model 3)}$$

,

where Y_{ijkl} is the phenotypic value for NEGG, RFEC, EGGW, TEGGW, and EGGD for the l^{th} fish; μ is the population mean; β_1 is the regression coefficient of the co-variable spawning age, SA_{ijkl}; $STAGE_i$ is the fixed effect of stage of eggs at collection (i = 1, 2, 3, 4); EXP_j is the fixed effect of the experiment j (FAM, MM, SM-1, SM-2) (j = 1, 2, 3, 4); $\left(TANK(EXP)\right)_k$ is the fixed effect of the spawning tank k (k = 1, 2,…, 23) nested within experiment j; $ANIMAL_l$ is the random additive genetic effect of the individual l with $\mathrm{N}(0, \mathbf{A}\sigma_a^2)$, PE_l is the random permanent effect on the individual l with $\mathrm{N}(0, \mathbf{I}_{\boldsymbol{PE}}\sigma_e^2)$ where $\mathbf{I}_{PE}$ is the identity matrix of the appropriate dimension, and e_{ijkl} is the random residual term with $\mathrm{N}(0, \mathbf{I}_{\boldsymbol{e}}\sigma_e^2)$ where $\mathbf{I_e}$ is the identity matrix of the appropriate dimension and σ_e^2 is the residual variance.

For fertility traits (FEGG, HAT, SWUP, and FER) the same Model 3 was used. Values for FER were square root-transformed for analysis.

For fecundity and fertility traits, heritability (h^2) and repeatability (r) were calculated as:

$$h^2 = \frac{\sigma_A^2}{\sigma_a^2 + \sigma_{E_p}^2 + \sigma_{E_t}^2}, r = \frac{\sigma_A^2 + \sigma_{E_p}^2}{\sigma_a^2 + \sigma_{E_p}^2 + \sigma_{E_t}^2}$$

where σ_A^2 is the additive genetic variance, $\sigma_{E_p}^2$ is the permanent environmental variance, and $\sigma_{E_t}^2$ is the temporary environmental variance.

Bivariate analyses were used to estimate genetic correlations between HW and fecundity/fertility traits. The fixed effects for HW were the same as in Model 1, for SPW as in Model 2, and for fecundity and fertility traits as in Model 3.

4.3 Results

4.3.1 Experiment

The number of records for each egg stage at collection are presented in Table 4.1. In total, there were 1181 records. Of these, 740 were from the MM experiment, 327 were from the SM experiment, and 114 were from the FAM experiment. At collection, four stages of spawns could be distinguished: egg-1, egg-2, egg-3, and fry (hatchling). Within each experiment, the proportion of each stage varied. For the FAM experiment, the proportion of 'egg-1' and 'egg-2' was similar, and when combined they accounted for 73% of the total number of batches. For MM experiment, about 50% of the total number of batches was 'egg-1', while 32% were

'fry'. For SM, the corresponding proportions were 64% for 'egg-1', and only 3.7% were 'fry'. Overall, when data of all experiments were combined, half of the batches were 'egg-1', 20–22% were 'egg-2' or 'fry', and only 5% were 'egg-3' (Table 4.1).

Table 4.1 Number of records in each experiment, by stages of eggs/fry at collection.

Experiment	Stage of eggs at collection				Total
	Egg_1	Egg_2	Egg_3	Fry	
FAM	42	41	13	18	114
MM	367	120	14	239	740
SM	210	77	28	12	327
Total	619	238	55	269	1181*

* from a total 923 unique females.

FAM: 'family' (1 female and one male per spawning hapa) experiment, MM: 'multiple males, multiple females' (7 males and 15 females per spawning tank) experiment, SM: 'single male, multiple females' (1 male and 10 females per spawning tank) experiment.

4.3.2 Fecundity traits

Descriptive statistics for fecundity traits in each experiment are presented in Table 4.2. Females in SM experiment, on average, produced more eggs than those in FAM and also more eggs than those in MM experiment. Females in SM-2 produced the highest NEGG, followed by females in FAM, while females in MM and SM-1 produced similar and lowest NEGG. Females in MM and SM-1 had, on average, the highest relative fecundity (RFEC). Mean EGGW and mean EGGD were similar among experiments (Table 4.2).

Table 4.2 Mean and coefficient of variation (CV) for fecundity* and fertility** traits by experiment (N = number of records).

Experiment		Fecundity traits						Fertility traits			
		NEGG	NFRY	RFEC	EGGW	TEGGW	EGGD	FEGG	HAT	SWUP	FER
FAM	N	96	18	96	82	82	82	82	82	99	82
	Mean	1535.8	1204.7	2.3	0.14	218.9	1.89	1400.5	1268.7	1135	87.5
	CV	48.8	49.2	51.3	15.71	49.5	6.68	51.1	54.6	54.7	18.7
MM	N	501	239	501	458	458	458	458	458	694	458
	Mean	1367.2	1181.4	3.5	0.15	205.5	1.94	1184.3	1057.1	942.9	86.7
	CV	42.2	45.2	43.9	19.57	46.8	6.11	47	51.3	50.2	18.5
SM-1	N	134	8	134	78	78	78	78	78	86	78
	Mean	1382.2	934.4	3.4	0.14	195.3	1.97	1100.3	942.9	793	81.6
	CV	31.4	45.8	34.5	13.30	33.1	5.26	32.7	34.5	40.2	17.4
SM-2	N	181	4	181	126	126	126	126	126	130	126
	Mean	1884.3	1445.3	2.9	0.17	335	2.05	1391.5	1214	1105.4	76.8
	CV	50.3	25.9	49.4	18.12	49.6	6.14	50	57.3	60.7	30.5

FAM: 'family' experiment (1 female and one male per spawning hapa), MM: 'multiple males, multiple females' experiment (7 males and 15 females per spawning tank), SM-1 and SM-2: duplicates of 'single male, multiple females' experiment (1 male and 10 females per spawning tank).

* total number of eggs at collection per female (NEGG), Relative fecundity = total number of eggs/female body weight at spawning (RFEC), number of fry at collection per female (NFRY), weight of 30 eggs (EGGW, in g), total weight of eggs per female (TEGGW, in g), mean dimension of 30 eggs (EGGD, in mm).

** number of fertilised eggs (FEGG), number of hatchling (HAT), number of swim-up fry (SWUP), fertilisation rate (FER, in %).

4.3.3 Fertility traits

Descriptive statistics for fertility traits in each experiment are presented in Table 4.2. The total number of fertilised eggs per female (FEGG), on average, ranged from 1100–1400. In general, fertilisation rate (FER) was good, ranging from 77–87%. As the females were getting older, the number of fertilised eggs per female increased: females in FAM and SM-2, on average, produced more eggs than those in MM and SM-1. The number of hatched fry (HAT) and swim-up-fry (SWUP) followed the same trend, although the average numbers for each development stage were getting lower because of mortality (Table 4.2).

4.3.4 Genetic parameters

Heritability estimates for HW and SPW were 0.30 and 0.68 respectively (Table 4.3). Estimates for common environmental effects for HW were 0.07 (+/−0.10). Estimates for c^2 on SPW were not different from zero.

Estimates for additive genetic variance, repeatability variance, heritability and repeatability for fecundity and fertility traits are presented in Table 4.3. In general, heritability estimates for fecundity traits were low, ranging from 0.02–0.08. Heritability estimates for fertility traits were low as well: ranging from 0.06 for SWUP to 0.12 for FEGG and HAT. Repeatability estimates for fecundity traits ranged from 0.05–0.08, only repeatability of RFEC, was higher than 0.10 (0.17). Repeatability estimate for fertility traits was 0.06 for SWUP, and 0.12 for FEGG and HAT.

Table 4.3 Estimated parameters for body weight*, fecundity and fertility traits**: additive genetic variance (σ_a^2), permanent environment variance (σ_{Ep}^2), residual variance (σ_e^2), heritability ($h^2 \pm$ se) and repeatability ($rep \pm$ se).

Trait	σ_a^2	σ_{Ep}^2	σ_e^2	$h^2 \pm se$	$rep \pm se$
HW	1194.30	–	2468.90	0.30 ± 0.19	–
SPW	10785.30	15.69	4942.03	0.68 ± 0.10	0.69 ± 0.03
NEGG	33316.80	0.00	403193.00	0.08 ± 0.03	0.08 ± 0.03
RFEC	0.11	0.23	1.66	0.05 ± 0.04	0.17 ± 0.05
EGGW***	0.04	0.00	0.75	0.05 ± 0.03	0.05 ± 0.03
TEGGW	780.00	264.00	9976.00	0.07 ± 0.04	0.08 ± 0.07
EGGD***	0.07	0.07	1.30	0.05 ± 0.03	0.05 ± 0.03

FEGG	41387.00	0.00	291538.00	0.12 ± 0.05	0.12 ± 0.05
HAT	36380.80	0.00	278806.00	0.12 ± 0.05	0.12 ± 0.05
SWUP	15158.00	0.00	233087.00	0.06 ± 0.03	0.06 ± 0.03
FER	0.07	0.00	0.78	0.08 ± 0.05	0.08 ± 0.05

* HW = harvest weight, SPW = female body weight at spawning

** NEGG = total number of eggs at collection per female, RFEC = relative fecundity (total number of eggs/female body weight at spawning), EGGW = weight of 30 eggs in g, TEGGW = total weight of eggs per female, EGGD = mean dimension of 30 eggs in mm, FEGG = number of fertilised eggs, HAT = number of hatchling, SWUP = number of swim-up fry, FER = fertilisation rate (number of fertilised eggs/total number of eggs, in %).

*** variance component are scaled up by 1000.

4.3.5 Genetic correlations

Genetic correlations between body weight (HW and SPW) and fecundity traits (NEGG, RFEC, EGGW, TEGGW, and EGGD) are presented in Table 4.4. Between the two body weights, genetic correlation was close to unity (0.96), despite that the time between two measurements was far apart.

Genetic correlations between HW and fecundity traits were moderate to high. For HW with NEGG and TEGGW, genetic correlations were positive (0.51 and 0.42, respectively), while the correlations for HW with RFEC, EGGW, and EGGD were negative (-0.72, -0.48, and -0.50, respectively). Similarly, genetic correlations between SPW and NEGG and between SPW and TEGGW were positive (0.72 and 0.71, respectively), while those for SPW with RFEC, EGGW, and EGGD were negative (-0.88, -0.63, and -0.43, respectively). In general, genetic correlations between SPW and fecundity traits (except for EGGD) were higher than those between HW and fecundity traits.

Table 4.4 Estimated genetic correlations (above diagonal) and phenotypic correlations (below diagonal) between body weights* and fecundity traits** (estimates ± se, NE = not estimable).

	HW	SPW	NEGG	RFEC	EGGW	TEGGW	EGGD
HW		0.96 ± 0.05	0.51 ± 0.29	–0.72 ± 0.14	–0.48 ± 0.41	0.42 ± 0.30	–0.50 ± 0.64
SPW	0.70 ± 0.02		0.72 ± 0.16	–0.88 ± 0.10	–0.63 ± 0.24	0.71 ± 0.21	–0.43 ± 0.27
NEGG	0.17 ± 0.04	0.25 ± 0.04		0.99 ± 0.01	–0.74 ± 0.50	0.93 ± 0.13	–0.40 ± 0.52
RFEC	–0.20 ± 0.04	–0.33 ± 0.03	NE		0.25 ± 0.51	0.08 ± 0.54	–0.07 ± 0.81
EGGW	–0.05 ± 0.05	–0.05 ± 0.04	–0.18 ± 0.04	–0.13 ± 0.04		–0.40 ± 0.52	0.79 ± 0.60
TEGGW	0.16 ± 0.05	0.25 ± 0.04	0.88 ± 0.10	0.68 ± 0.02	0.19 ± 0.04		–0.96 ± 0.53
EGGD	0.04 ± 0.05	–0.02 ± 0.04	–0.22 ± 0.04	NE	0.61 ± 0.02	0.02 ± 0.04	

* HW = harvest weight, SPW = female body weight at spawning.

** NEGG = total number of eggs at collection per female, TEGGW = total weight of eggs per female, RFEC = relative fecundity (total number of eggs/female body weight at spawning), EGGW = weight of 30 eggs in g, EGGD = mean dimension of 30 eggs in mm.

Among fecundity traits, genetic correlations varied widely, and most of the estimates came with high standard errors, except for the correlation between NEGG – RFEC (0.99 ± 0.01) and between NEGG – TEGGW (0.93 ± 0.13).

Genetic correlations between two body weights (HW and SPW) and fertility traits (FEGG, HAT, SWUP, FRY, and FER) are given in Table 4.5. Genetic correlations between HW and fertility traits were moderate to high (0.46 to 0.69), except for the correlation with FER which was low with high standard error (0.15 ± 0.24). Genetic correlations between SPW and fertility traits were moderate to high (0.69 to 0.93), except for the correlation with FER which was low (0.32 ± 0.21). Among FEGG, HAT and FRY, genetic correlations were close to unity (0.94 to 0.99). However, between those three traits (FEGG, HAT and FRY) and FER, genetic correlation ranged from 0.38 to 0.64 with high standard errors (Table 4.5).

Table 4.5 Genetic correlations (above diagonal) and phenotypic correlations (below diagonal) between two body weight* and fertility traits**. Values = estimates ± se.

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	HW	SPW	FEGG	HAT	SWUP	FER
HW		0.96 ± 0.05	0.51 ± 0.29	0.46 ± 0.21	0.69 ± 0.23	0.15 ± 0.24
SPW	0.70 ± 0.02		0.69 ± 0.15	0.72 ± 0.16	0.94 ± 0.16	0.32 ± 0.21
FEGG	0.17 ± 0.04	0.24 ± 0.04		0.99 ± 0.01	0.94 ± 0.08	0.64 ± 0.26
HAT	0.13 ± 0.05	0.22 ± 0.04	0.95 ± 0.01		0.96 ± 0.08	0.64 ± 0.27
SWUP	0.10 ± 0.04	0.21 ± 0.04	0.88 ± 0.01	0.92 ± 0.01		0.38 ± 0.41
FER	0.02 ± 0.05	0.04 ± 0.04	0.38 ± 0.03	0.39 ± 0.03	0.36 ± 0.03	

*HW = harvest weight, SPW = body weight at spawning

** FEGG = number of fertilised eggs, HAT = number of hatchling, SWUP = number of swim-up fry, FER = fertilisation rate (number of fertilised eggs/total number of eggs, in %).

4.4 Discussion

4.4.1 Experiments

The three main experimental designs differed with respect to the spawning units used. The tanks (15 m^2, used in MM and SM experiments) were five times larger than the hapas (3 m^2, used in FAM experiment). The stocking density was similar in FAM and SM (0.7 fish/m^2), which was half the density for MM (1.5 fish/m^2). The experiments also differed in number of male(s) with respect to the number of male(s) stocked in a single spawning tank/hapa. For MM experiment, there were always seven males in one spawning tank, while for FAM and SM there was only a

single male in any tank during eight and four days, respectively. Females in MM experiment therefore had more mate choices and, especially, more time to mate with different males, while females in the SM and FAM experiments had only 4-8 days to mate with a single male. This probably explains the very high proportion of 'fry' batches found among spawns in MM (32.2% of the total 740 records) compared to SM (3.7% of the total 327) and FAM (15.8% of the total 114), and suggests that many successfully matings occurred three days before the spawning checks.

High stocking density (>10 fish/m^2) was reported to inhibit spawning in tilapia (review of Coward and Bromage, 2000; Guerrero, 1982). In our study, the stocking density was 1.5 fish/m^2 for MM and 0.7 fish/m^2 for both FAM and SM. These densities are lower than in other published experiments with Nile tilapia, stocking 5 to 10 fish/m^2 (Hughes and Behrends, 1983). In hybrid tilapia (*O. niloticus* × *O. aureus*), a stocking ratio of 2 fish/m^2 was found to be optimum for fry production (Siddiqui and Al-Harbi, 1997). Therefore, we do not think that stocking densities in our study have had a negative effect on fertility and fecundity of the females.

Biologically, spawning of tilapia depends on many factors such as temperature, photoperiod, and social interaction (as reviewed by Coward and Bromage, 2000). Contacts between females and males Nile tilapia is thought of as at the most important factor for successful spawning (Srisakultiew, 1993). In the MM experiment, a female had contact with more males than in SM experiment (7 versus 1) at any time. Thus, the MM experiment show more resemblance to the natural spawning conditions of Nile tilapia, which is polygamous species as seen in mouth brooding cichlids (Barlow, 1991). The condition in the FAM experiment was completely different as each female had the opportunity to mate with only one male during a course of eight days.

The effect of age at spawning for females was significant, which is not surprising as the experiments were conducted over a long period (04 December 2009 through 05 June 2011); the SM-2 was conducted 8 months after the SM-1. Smaller and/or younger Nile tilapia spawn more frequently than older tilapia (Guerrero and Guerrero, 1985). In our experiment we found that age at spawning affected NEGG and RFEC: as females grew older, NEGG increased but less so than the weight of females. These observations corroborate the practice of commercial seed producers, to discard broodstock when they are heavier than 300 g, because larger

fish are more difficult to handle when harvesting for egg/fry (Bhujel, 2000), and because they are more costly to feed.

4.4.2 Heritability

In our study, heritability estimates for NEGG, EGGW and EGGD were much lower than those reported for rainbow trout by Gall and Huang (1988): 0.32 $\pm$ 0.12 for number of eggs, 0.28 $\pm$ 0.16 for egg size (# of eggs per 30 ml), and 0.30 $\pm$ 0.15 for egg volume. Gall and Huang (1988) also reported a heritability estimate of 0.15 $\pm$ 0.14 for post-spawning weight (equivalent to spawning weight-SPW- in our study). The estimates in our study were based on additive genetic variance obtained from a linear mixed animal model. In contrast, Gall and Huang (1988) estimated heritability, for post-spawning weight, egg volume, egg size (#/30 ml) and number of eggs, based on sire components. However, heritability estimates for egg volume and number of eggs in the same species reported by Su *et al.* (1997) were in good agreement with those of Gall and Huang (1988), even when an animal model was used. More recently, Gall and Neira (2004) used a sire-dam model for coho salmon and obtained similar results as Gall and Huang (1988) and Su *et al.* (1997) for number of green eggs and the weight of ova (equivalent to EGGW in our study). In general, animal model is recommend over sire model (see, for example, Sun *et al.*, 2009). In our opinion, the differences in heritability between our study and studies by Gall and Huang (1988), Su *et al.* (1997), and Gall and Neira (2004) are most likely species-specific. In tilapia eggs mature in batches and spawned eggs represent only a fraction of the total number of oocytes in the ovary, as described by Coward and Bromage (2000). In contrast, salmon and trout release all eggs in the ovary at once. Charo-Karisa *et al.* (2007) reported heritability estimates for gonado-somatic index (ovary weight to body weight ratio, GSI) in Nile tilapa of 0.25 $\pm$ 0.10 for females. This value is higher than our heritability estimates for RFEC (number of eggs spawned to spawning weight ratio). This might indicate that GSI and RFEC are two genetically different traits in Nile tilapia. As Nile tilapia is a multiple spawner, the number of eggs (NEGG) represent only a fraction of the true reproductive potential of the species (i.e. total number of eggs in the ovary) (Macintosh and Little, 1995; Rana, 1988).

As collected eggs entered the hatchery, non-genetic factors, such as management during incubation, are expected to affect estimates of heritability for fertility traits (FEGG, HAT, SWUP, and FER). Heritability estimates for fertility traits were highly

variable. Estimates for FEGG and HAT were higher compared to NEGG, though the standard errors increased as well. On the other hand, estimates for SWUP were lower compared to NEGG. For FER, the estimate was not significantly different from zero, if two standard errors were considered. Derived traits, such as FER, can be difficult to work with, because of accumulated errors in the phenotypic estimates of the component traits (FEGG and NEGG in this case) (Sokal and Rohlf, 1981). We were not able to obtain estimates for hatching rate (i.e. FRY/NEGG*100%), again most likely due to the high measurement/environmental error.

4.4.3 Genetic correlations

In this study, the estimated genetic correlation between two body weights (HW and SPW) and fecundity traits were highest with RFEC (−0.72 and −0.88 respectively). Interestingly, the genetic correlation estimates between female body weights and NEGG and TEGGW were positive, while negative for RFEC, EGGW, and EGGD. Biologically, it would mean that heavier females tend to produce more eggs and higher egg mass, but the eggs are getting smaller and the females have lower relative fecundity. In seed production practice, it would mean that a female selected for high harvest weight tend to produce more eggs, which, combined with high fertilisation and hatching rate, will result in higher fry production. In *Tilapia zillii*, Coward and Bromage (1999) reported a highly significant phenotypic relationship between number of eggs and maternal weight, but no relationship between egg size and maternal weight. Our results are in agreement with results reported for rainbow trout (Gall and Gross, 1978; Gall and Huang, 1988; Huang and Gall, 1990; Su *et al.*, 1997; Su *et al.*, 2002) and coho salmon (Gall and Neira, 2004) where genetic correlation between spawning weight and egg size, number of eggs and egg volume were positive and moderate. A negative genetic correlation between NEGG and EGGW and between NEGG and EGGD was also reported in rainbow trout by Huang and Gall (1990).

Genetic correlation between two body weights (HW and SPW) and fertility traits were all positive (Table 4.5), but correlations with FER were estimated with high error. Biologically, it would mean that heavier females tend to produce more fertilised eggs, hatched eggs and swim-up fry. However, larger females would not necessarily produce eggs with a higher fertilization rate. This is understandable since fertilization is the combined result of egg and sperm quality. Unfortunately the current dataset did not allow us to estimate the effect of males (for example, sperm quality). The effectiveness of the incubation system also plays an important

role here. Estimates were getting higher and had smaller standard errors as the time between two measurements are closer, as genetic correlations for SPW were higher than those for HW. This might be because the time between recording spawning weight and fecundity/fertility was short (few days), while the time between recording harvest weight and fecundity/fertility was much longer. Nevertheless, the genetic correlation between HW and SPW was close to unity (0.96), suggesting that HW can be used to select for females with good reproductive performance.

Conclusion

We conclude that harvest weight and spawning weight both have favourable genetic correlations with number of eggs per females, relative fecundity, and number of swim-up fry, which are the desired characteristics for Nile tilapia seed production. Selection for harvest weight will not affect these traits in a negative way. On the other hand, Nile tilapia females selected for large harvest weight do tend to produce smaller eggs. In the longer term, this trait might affect survival of fry and we therefore recommend that the phenotypic and/ or genetic trend in this trait is monitored in the GIFT program.

Acknowledgements

This study is part of the first author's sandwich PhD study, funded by Wageningen University. We would like to thank Dr. Raul W. Ponzoni and Dr. Nguyễn Hồng Nguyên of the WorldFish Center for supplying the GIFT generation 10th, and for financial support for field work from 2007 to date. We thank the tilapia team at RIA2, especially Phạm Đăng Khoa, for their field work.

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5

Heritability and genotype by environment interaction estimates for harvest weight, growth rate, and shape of Nile tilapia (*Oreochromis niloticus*) grown in river cage and VAC in Vietnam

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Aquaculture 384–387 (2013): 119–127

Abstract

Harvest weight has been the main selected trait for the GIFT strain of Nile tilapia (*Oreochromis niloticus*). However, growth rate and body shape are traits of increasing interest. In the Mekong Delta of Vietnam, intensive river-cage culture and low-input ponds are the most important production environments, whereas the breeding program is conducted in an intensive nucleus pond. The first objective was to estimate heritability and phenotypic and genetic correlations for harvest weight (HW), growth rate expressed as daily growth coefficient (DGC), condition factor (K), and shape expressed as ellipticity: mid-sagittal plane (E_{L-H}), transverse plane (E_{L-T}), and frontal plane (E_{H-T}). The second objective was to estimate genotype by environment interactions (G x E), expressed as the genetic correlation (r_g) between the nucleus and two production environments, cage and VAC (Vietnamese acronym for garden, pond and livestock pen), for these traits. Data were obtained from the 13th generation of selection of GIFT. Within the breeding nucleus, heritability was high for HW, DGC, but low for K, E_{L-H}, E_{L-T}, and E_{H-T}. DGC was positively correlated with condition factor K ($r_g = 0.59$), while the r_g of HW with K was non-significant. This suggests that selection for harvest weight alone will not result in fish with higher condition factor. Genetic correlations between HW and body dimensions (L, H, T) were 0.89 – 0.98, but genetic correlations of DGC with ellipticity showed that fish selected for high growth rate will become more rotund rather than simply larger. GxE was minor for harvest weight and for growth, but substantial for shape traits. For DGC, genetic correlation was 0.77 between cage and VAC, but higher between the breeding nucleus and cage or VAC. For E_{L-H}, substantial GxE (r_g 0.54) was found between cage and nucleus pond. GxE was also found for E_{L-T} between cage – VAC (r_g 0.51), and for E_{H-T} across all three environments, although with high standard errors of estimates. We conclude that selection in nucleus ponds will produce desired correlated responses in Nile tilapia grown in river-cages as they are expected to develop a more rotund and thicker body shape at the same length compared to fish grown in ponds.

Key words: Nile tilapia, harvest weight, growth rate, condition factor, ellipticity, genotype by environment interaction.

5.1 Introduction

Nile tilapia (*Oreochromis niloticus*) of GIFT origin is currently the most important cultured tilapia strain worldwide (Bentsen *et al.*, 2012; Neira, 2010; Ponzoni *et al.*, 2011). In Vietnam, Nile tilapia is cultured in three production environments: in river cages, in monoculture in ponds and in low-input integrated polyculture in ponds with a mix of other fish and manure from livestock species (e.g. pigs, chickens, or ducks) and gardening (VAC[1]). Most tilapia production is conducted in cages in the Mekong River. Tilapia production from VAC ponds is mainly for household consumption and domestic market. In the Mekong Delta of Vietnam, GIFT tilapia of 10^{th} generation has been selected for three generations since its introduction from the WorldFish Center (WFC) to Research Institute for Aquaculture No. 2 (RIA2) in 2007.

As the breeding program of GIFT is conducted in ponds, it is important to estimate genetic parameters and to investigate whether G×E exists for GIFT tilapia between the nucleus environment and either cages or VAC.

Most estimates for genetic parameters focus on harvest weight. However, on-growers are often more concerned with growth rate during the grow-out period, because high growth rate is associated with higher feed efficiency, especially at more restricted feeding regimes (Henryon *et al.*, 2002). Good prediction of growth is important for an efficient production and contributes to a more profitable and sustainable aquaculture (Dumas *et al.*, 2010; Jobling, 2003). Feed costs are highest during grow-out time and this is the main investment cost in most aquaculture systems (Edwards *et al.*, 2000; El-Sayed, 2006; Parker, 2012). Growth rate can be expressed as average daily gain (ADG), specific growth rate (SGR) (Dumas *et al.*, 2010), thermal growth coefficient (TGC) (Jobling, 2003), or daily growth coefficient (DGC) (Cho, 1990). Daily growth coefficient is a simplified form of TGC, where water temperature during grow-out period is assumed constant (Cho, 1990). Of these growth rates, TGC and DGC are preferred because they are simple and flexible growth models for harvest weight prediction and production planning under various conditions (Jobling, 2003) and have been used for estimating growth rate in a wide range of species including tilapia (Bureau *et al.*, 2000). To our knowledge, no

[1] This integrated farming system term is an acronym for three Vietnamese words, namely 'vườn', 'ao' and 'chuồng' meaning garden, pond and livestock pen in English.

genetic parameters for growth rate, expressed as DGC, have been reported for Nile tilapia.

Genetic parameters for body size measurements can be used to predict correlated responses in fillet- weight and fillet-yield (Nguyen *et al.*, 2010; Rutten *et al.*, 2004). A few studies in Nile tilapia have reported genetic parameters for body length, height and thickness. In general, genetic correlations of these traits with harvest weight and fillet weight are high while genetic correlations with yield are much lower (Charo-Karisa *et al.*, 2006; Gjerde *et al.*, 2012; Nguyen *et al.*, 2007; Rutten *et al.*, 2005).

Fish body shape is becoming of increasing interest to consumers and producers (Blonk *et al.*, 2010; Kause *et al.*, 2003a). Consumers are willing to pay higher prices for well-shaped fish, which is especially true for live fish and un-gutted fish. Condition factor, defined as weight/length^3, is the most common parameter used to express shape in fish. Condition factor has been studied in many species, e.g. rainbow trout (*Salmo gairdneri*) (Gjerde, 1989), gilthead seabream (*Sparus auratus*) (Navarro *et al.*, 2009) and olive flounder (*Paralichthys olivaceus*) (Kim *et al.*, 2011), but not in tilapias. However, condition factor describes only the relationship of the weight with a given length; it does not clearly describe the appearance of fish. To better describe shape, appearance, described as 'slender', 'medium' and 'rotund', has been proposed (Kause *et al.*, 2003a). Heritability estimate of this shape trait in rainbow trout was 0.33 (Kause *et al.*, 2003a). More recently, ellipticity was used to describe shape in common sole (*Solea solea*). Blonk *et al.* (2010) reported a heritability of 0.34 and showed that selection for harvest weight would lead to a undesired correlated response in shape. To our knowledge, there are no estimates of genetic parameters for ellipticity and growth in Nile tilapia.

Genotype by environment interaction (G×E) has been studied extensively in Nile tilapia (Bentsen *et al.*, 2012; Khaw *et al.*, 2009; Khaw *et al.*, 2012; Thodesen *et al.*, 2011). Most G×E studies with Nile tilapia found no evidence of biologically important G×E for body weight as indicated by genetic correlations ranging from 0.73 to 0.99. Genotype by environment interaction has also been studied for other important cultured species, e.g. common carp (*Cyprinus carpio*) (Moav *et al.*, 1975), European seabass (*Dicentrarchus labrax*) (Dupont-Nivet *et al.*, 2010), rainbow trout (Kause *et al.*, 2003a; Sylvén *et al.*, 1991), and other salmonids (Hutchings, 2011). Interestingly, in European seabass, substantial G×E was found for growth rate while no G×E was found for body weight at harvest (Dupont-Nivet *et al.*, 2010).

The objectives of this paper were two fold. First, we estimated heritability and phenotypic and genetic correlations for harvest weight, growth rate, condition factor and shape, defined as ellipticity, in the breeding nucleus. Second, genotype by environment interactions, expressed as the genetic correlation between the nucleus and two production environments, cage and VAC, for each trait were investigated.

5.2 Materials and methods

5.2.1 Candidate parents

Candidate parents were from the 12th generation of GIFT-VN strain, which was the second generation in the Mekong Delta of Vietnam, produced from the GIFT 10th generation obtained from WFC, Penang, Malaysia (Ponzoni *et al.*, 2010). Parent fish were conditioned, in single sex cohorts, in 4×8×1 m hapas (mesh size 5 mm) for approximately four weeks and fed twice a day on a commercial floating pelleted feed (brand AFIEX), with approximately 30% of crude protein and 6% fat, at a feeding rate of 3% of body weight daily.

5.2.2 Production of G13 families

For family production, a 2000 m^2 earthen pond with fifty spawning hapas (1.5×2.0×1.0 m) was used. Each female breeder was stocked into one single hapa with a single male. Fry and fertilized eggs were collected from the mouth of the spawned females. First check for eggs was conducted four days after stocking and continued at four day intervals. In total, there were 24 checks, equivalent to 24 dates of egg collection. Females that spawned were removed from the spawning hapas, and replaced by a second female to produce a half-sib family. In total 92 full-sib families were produced, i.e. the offspring of 47 sires and 92 dams, over a period of 105 days (from 29 September 2010 to 23 January 2011). From these families, six families did not have half-sibs, because each of the six sires was only mated to a single dam.

Fertilized eggs were incubated until hatching in 0.5 litre plastic down-dwelling bottles with a constant flow-through of water, and after hatching transferred to 30×40×5 cm plastic trays with a constant flow-through of water. Dead eggs and fry were removed twice daily.

5.2.3 Nursing of families and tagging

After yolk-sac absorption, swim-up fry were moved into nursing hapas (1.5×2.0×1.0 m, mesh size 1 mm) suspended in a 2,000 m^2 earthen pond. For each family, roughly 350 fry were stocked into a single nursing hapa, equivalent to a nursing density of 120 fish per m^2. Fry were fed on a fine powdered feed (35% crude protein) given *ad libitum* three times per day. The family rearing period in nursing hapas ranged from 41 to 186 days. Due to differences in egg collection dates and tagging dates, fingerlings were tagged at the age of 48–202 days (2 – 82 g), using PIT (Passive Integrated Transponder) tags. For each family, 50–60 fingerlings were randomly chosen and tagged for the nucleus environment; similarly on average 14 fish were randomly chosen and tagged for cage and VAC environments respectively. All individuals in one family were tagged at the same time. Each family was stocked in a randomly assigned 2.0×2.5×1.5 m post-tagging hapa with a density of 100 fish/m^2, prior to stocking into three grow-out environments.

5.2.4 Grow-out environments

'Nucleus' environment was a 2,000 m^2 earthen pond at the National Breeding Centre for Southern Freshwater Aquaculture, located in Cai Be, Tien Giang province, Vietnam, under the auspices of RIA2. 'Cage' environment consisted of two 100 m^3-cages, acting as duplicates, located in the Mekong River. VAC environment was an 800 m^2 earthen pond belonging to a farmer. Weather conditions were considered similar for all three environments. Water temperature ranged from 28 to 33°C in ponds and from 29 to 31°C in the river cages. During the grow-out period from March 23rd to October 12th 2011, water temperature stayed relatively constant in all environments. Prior to stocking, all fish were weighed to obtain stocking weight. Stocking density on average was 38.4 fish/m^3 for cage, 2.9 and 2.9 fish/m^2 for nucleus and VAC respectively. For cage, tagged fish were stocked together with non-tagged fish (2000 in cage 1 and 3000 in cage 2), to provide a stocking density that is representative for normal on-growing conditions. For VAC, tagged fish were stocked together with a mix of 6 other fish species (1200 fish in total), in number and proportion typical of the VAC pond system. Details for stocking are given in Table 5.1.

Fish were fed twice a day on commercial floating pelleted feed, brand UP (Uni-President Vietnam) for cage and Afiex (AFIEX, Vietnam) for nucleus, both with 30%

crude protein and 5% fat. For VAC, pig manure (from five sows and 10 young pigs) and aquatic morning glory (*Ipomoea aquatic*) (approximately 5 kg per day) were the main fertiliser and feed sources respectively. Grow-out period ranged from 140 to 176 days for cages and pond, and from 163 to 195 days for VAC (Table 5.1).

Table 5.1 Stocking age, grow-out time, number of tilapia at stocking and at harvest, and survival rate at harvest in cage, nucleus, and VAC.

Environment	Stocking age	Grow-out time	# of fish		Survival
		(days)	Stocking	Harvest	(%)
Cage	63 – 208	140 – 173			
Cage 1			1164	340	29
Cage 2			1514	418	28
Nucleus	77 – 194	140 – 176	3775	1946	52
VAC*)	71 – 183	163 – 195	1118	760	68

*) tagged tilapia were stocked with 1200 other fish, including climbing perch (*Anabas testudineus*), common carp (*Cyprinus carpio*), giant gourami (*Osphronemus gouramy*), grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*) and non-tag Nile tilapia. Per species, two hundred fish were stocked.

5.2.5 Data collection and description of traits

5.2.5.1 Data collection

At harvest, weight in g (HW), standard body length (L), body height (H) and body thickness (T) all in mm (Figure 5.1) was recorded. Standard body length was measured at the maximum horizontal distance; body height was measured at the maximum vertical distance, both using a ruler. Body thickness was measured at the maximum thickness, using a vernier calliper with long jaws to accommodate the height of the fish. At harvest, representatives of all 92 families were recovered in nucleus and VAC. There was high mortality in both cages (Table 5.1). In cage 1, 87 families were recovered, and 90 families were recovered in cage 2.

5.2.5.2 Calculation of growth rate

Daily growth coefficient (DGC) was calculated as (Dabrowski *et al.*, 1986):

$$DGC = \frac{HW^{\frac{1}{3}} - SW^{\frac{1}{3}}}{days} \times 100 \quad (1)$$

where SW is body weight at stocking, HW is harvest weight, and 'days' is the time between stocking and harvest.

5.2.5.3 Calculation of shape

We used ellipticity as a measure for Nile tilapia shape, as the contours of the cross-sectional planes are close to an ellipse (all fins removed). We calculated ellipticity of the mid-sagittal plane ($E_{L\text{-}H}$), transverse plane ($E_{L\text{-}T}$) and frontal/coronal plane ($E_{H\text{-}T}$), using harvest body length (L), harvest body height (H) and harvest body thickness (T) as follows (Merigot, 2007) (Figure 5.1):

$$E_{L-H} = \frac{(L-H)}{(L+H)} \quad (3)$$

$$E_{L-T} = \frac{(L-T)}{(L+T)} \quad (4)$$

$$E_{H-T} = \frac{(H-T)}{(H+T)} \quad (5)$$

From Eqs (3) to (5), it can be derived that larger values (maximum = 1) reflect more elongated shapes whereas smaller values represent more circular shapes (Figure 5.1). In a perfect circle, ellipticity is zero.

Condition factor (K) was calculated as (Weatherley *et al.*, 1987):

$$K = \frac{HW}{L^3} \times 10^5 \quad (6)$$

with HW in grams and L in mm.

Figure 5.1 Calculation of ellipticity, from left to right: mid-sagittal plane (E_{L-H}), transverse plane (E_{L-T}), and frontal plane (E_{H-T}).
E_{L-H} = (Length − Height)/(Length + Height), E_{L-T} = (Length − Thickness)/(Length + Thickness), E_{H-T} = (Height − Thickness)/(Height + Thickness).
Sollid ellipse = Ellipticity, solid arrow = length measured at maximum horizontal distance from the mouth until the end of the peduncle, dotted arrow = height measured at maximum vertical distance, dashed arrow = thickness measured at the maximum thickness distance.

5.2.6 Statistical analysis

5.2.6.1 General

Significant levels of fixed effects were tested using the qualifier '!DDF' in ASReml version 3 (Gilmour *et al.*, 2009). All traits had normal distribution (not shown). Date of egg/fry collection ("collection date"), was calculated as the number of days from January 1st to the date that a family was collected, and modelled as class variable (n=24). Collection date, sex, post-tagging hapa, age (and stocking age), age squared (and stocking age squared), the interactions of sex×hapa, and the effect of age nested within sex were found significant (P<0.05) and therefore were fitted in the models for genetic analysis. The pedigree used for analysis included 6 generations, i.e. back to the 8th generation in the WorldFish Center.

5.2.6.2 Estimation of phenotypic and genetic parameters

Phenotypic and genetic parameters were estimated using ASReml version 3. For harvest weight (HW), length (L), height (H), thickness (T), ellipticity ($E_{L\text{-}H}$, $E_{L\text{-}T}$, and $E_{H\text{-}T}$) and condition factor (K) the following mixed animal model was used:

$$Y_{ijklm} = \mu + CL_i + (SEX \times HAPA)_j + \left(AGE(SEX)\right)_k + \left(AGE^2(SEX)\right)_l + ANIMAL_m + e_{ijklm}$$

where Y_{ijklm} is the phenotypic value of the traits of interest (HW, L, H, T, $E_{L\text{-}H}$, $E_{L\text{-}T}$, $E_{H\text{-}T}$, and K) for the m^{th} fish; μ is the population mean; CL_i is the fixed effect of the i^{th} collection date (1,..,24), $(SEX \times HAPA)_j$ is the fixed effect of the j^{th} combination of sex (2 sexes) and post-tagging hapas (1, …, 21); $\left(AGE(SEX)\right)_k$ is the fixed regression on total age (from the date of egg/fry collection until harvest, *AGE*), nested within sex k; $\left(AGE^2(SEX)\right)_l$ is the fixed regression on total age squared nested within sex k; $ANIMAL_m$ is the random effect of the m^{th} fish with $N(0, \mathbf{A}\sigma_a^2)$ where $\mathbf{A}$ is the additive genetic relationship matrix among the recorded animals and σ_a^2 is the additive genetic variance; e_{ijklm} is the random residual term with $N(0,\mathbf{I}\sigma_e^2)$ where $\mathbf{I}$ is the identity matrix and σ_e^2 is the residual variance. For data from the cage environment, a fixed effect of cage (1, 2) was fitted.

For growth rate trait, by definition, DGC already account for growing time from stocking until harvest [see Eq(1)]. The model used for DGC (Model 2) was the same

as Model 1, but with stocking age (SA) instead of AGE. For data from the cage environment, a fixed cage effect was fitted as before.

Initially, for all traits, models 1 and 2 were also fitted with common environmental effects to full-sibs (c^2). However, the model yielded ambiguous results. In many cases the models did not converge, or when models did converge, results were not significantly different (P>0.05) from models without a common environment effect fitted (based on likelihood ratio test, results not shown).

Phenotypic and genetic correlations (r_g) between traits within the nucleus environments were estimated using bivariate models. For all these bivariate models, the same fixed effects in Model 1 were fitted for HW, L, H, T, $E_{L\text{-}H}$, $E_{L\text{-}T}$, $E_{H\text{-}T}$, and K, while the same fixed effects in Model 2 were fitted for DGC. The animal effects were distributed as $N(0, \mathbf{A} \otimes \mathbf{G})$ with the additive genetic variance-covariance matrix ($\mathbf{G}$) is $\begin{bmatrix} \boldsymbol{\sigma}^2_{A,1} & \boldsymbol{r}_{A,12}\boldsymbol{\sigma}_{A,1}\boldsymbol{\sigma}_{A,2} \\ \boldsymbol{r}_{A,12}\boldsymbol{\sigma}_{A,1}\boldsymbol{\sigma}_{A,2} & \boldsymbol{\sigma}^2_{A,2} \end{bmatrix}$ where $\sigma^2_{A,1}$ ($\sigma^2_{A,2}$) is the additive genetic variance of trait 1 (trait 2), and $r_{A,12}$ is the additive genetic correlation between trait 1 and trait 2. The residuals were distributed as $N(0, \mathbf{I} \otimes \mathbf{R})$ with residual variance-covariance matrix ($\mathbf{R}$) is $\begin{bmatrix} \boldsymbol{\sigma}^2_{e,1} & \boldsymbol{r}_{e,12}\boldsymbol{\sigma}_{e,1}\boldsymbol{\sigma}_{e,2} \\ \boldsymbol{r}_{e,12}\boldsymbol{\sigma}_{e,1}\boldsymbol{\sigma}_{e,2} & \boldsymbol{\sigma}^2_{e,2} \end{bmatrix}$ where $\sigma^2_{e,1}$ ($\sigma^2_{e,2}$) is the residual variance of trait 1 (trait 2), and $r_{e,12}$ is the residual correlation between trait 1 and trait 2.

Genotype by environment (G×E) interactions for HW, DGC, $E_{L\text{-}H}$, $E_{L\text{-}T}$, and $E_{H\text{-}T}$ were quantified by estimating genetic correlations (r_g) between the traits of interest in cage, nucleus and VAC using a trivariate model. For this trivariate model the additive genetic variance-covariance matrix is $\begin{bmatrix} \boldsymbol{\sigma}^2_{A,C} & \boldsymbol{r}_{A,CN}\boldsymbol{\sigma}_{A,C}\boldsymbol{\sigma}_{A,N} & \boldsymbol{r}_{A,CV}\boldsymbol{\sigma}_{A,C}\boldsymbol{\sigma}_{A,V} \\ \boldsymbol{r}_{A,CN}\boldsymbol{\sigma}_{A,C}\boldsymbol{\sigma}_{A,N} & \boldsymbol{\sigma}^2_{A,N} & \boldsymbol{r}_{A,NV}\boldsymbol{\sigma}_{A,N}\boldsymbol{\sigma}_{A,V} \\ \boldsymbol{r}_{A,CV}\boldsymbol{\sigma}_{A,C}\boldsymbol{\sigma}_{A,V} & \boldsymbol{r}_{A,NV}\boldsymbol{\sigma}_{A,N}\boldsymbol{\sigma}_{A,V} & \boldsymbol{\sigma}^2_{A,V} \end{bmatrix}$ where $\sigma^2_{A,C}$ is the additive genetic variance for the traits in cage, $\sigma^2_{A,N}$ is the additive genetic variance for the traits in nucleus, $\sigma^2_{A,V}$ is the additive genetic variance for the traits in VAC, $r_{A,CN}$ is the additive genetic correlation between cage and nucleus, $r_{A,CV}$ is the additive genetic correlation between cage and VAC, and $r_{A,NV}$ is the additive genetic correlation between nucleus and VAC. The correlation of residuals between environments was set to zero, as a fish performed in only one environment. Therefore, the residual

variance-covariance matrix is $\begin{bmatrix} \boldsymbol{\sigma^2_{e,C}} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{\sigma^2_{e,N}} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \boldsymbol{\sigma^2_{e,V}} \end{bmatrix}$ where $\sigma^2_{e,C}$ is the residual variance for the trait in cage, $\sigma^2_{e,N}$ is the residual variance for the trait in nucleus, and $\sigma^2_{e,V}$ is the residual variance for the trait in VAC. The phenotypic correlations between environments could, therefore, not be estimated. Due to lack of convergence, genetic correlations of K between three environments were estimated based on bivariate models.

5.3 Results

5.3.1 Descriptive statistics

Descriptive statistics are presented in Table 5.2 and Figure 5.2. Fish grown in cages had highest mean harvest weight (HW), while fish in VAC had smallest mean HW. The CV in cage was larger than in nucleus and VAC. DGC showed a similar trend across environments, i.e. mean DGC was highest in cage, followed by nucleus and VAC. At comparable lengths, fish in cages were heavier compared to those in nucleus and VAC. The average condition factor (K) was 4.41 in cage, 3.99 in nucleus and 3.97 in VAC. The same trend was observed when regressing log(HW) against log(L), as the slope was highest in cages and similar in nucleus and VAC (Figure 5.2). Shape traits ($E_{L\text{-}H}$, $E_{L\text{-}T}$, and $E_{H\text{-}T}$) were similar across environments, with the lowest values observed in cages, suggesting that fish in cages were thicker.

Table 5.2 Mean and coefficient of variation (CV) of stocking weight (SW), harvest weight (HW), daily growth coefficient (DGC), condition factor (K), and ellipticity: mid-sagittal plane ($E_{L\text{-}H}$), transverse plane ($E_{L\text{-}T}$), frontal plane ($E_{H\text{-}T}$) in three production environments.

Trait	Cage		Nucleus		VAC	
	Mean	CV	Mean	CV	Mean	CV
SW	132.8	27.1	137.5	31.4	134.1	29.0
HW	743	32.3	510	27.7	294	21.4
DGC	3.85	12.7	3.11	19.0	2.20	11.9
K	4.41	13.5	3.99	15.4	3.97	9.3
$E_{L\text{-}H}$	0.35	9.1	0.38	8.2	0.39	5.7
$E_{L\text{-}T}$	0.64	4.8	0.68	4.5	0.69	2.3
$E_{H\text{-}T}$	0.38	13.4	0.41	12.6	0.41	7.5

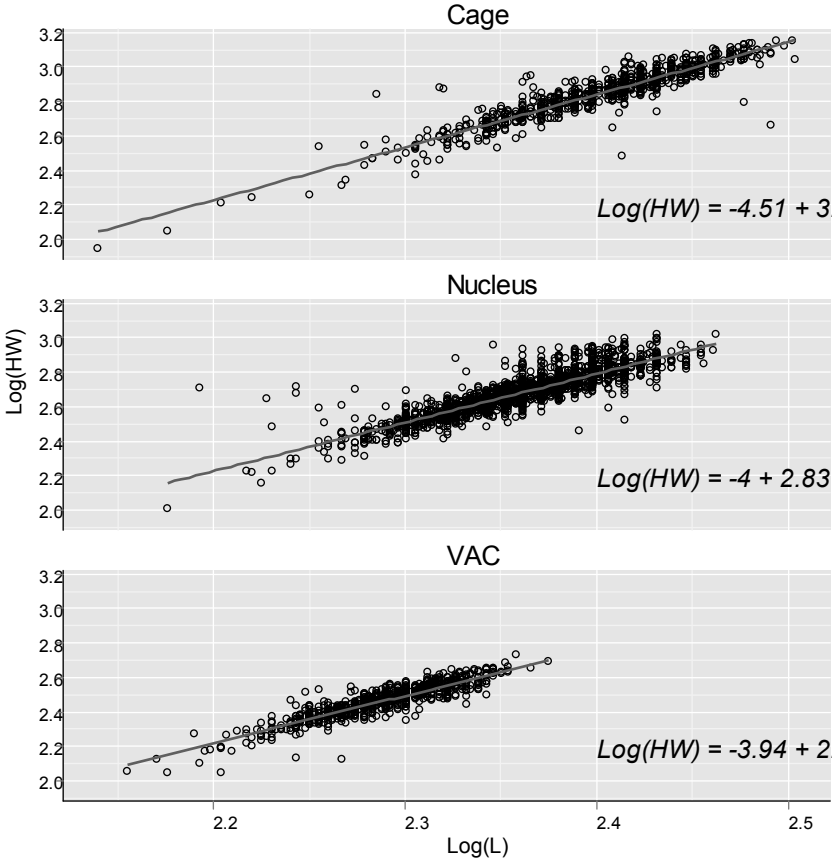

Figure 5.2 Log_{10}(HW) plotted against Log_{10}(L) in three environments: cage, nucleus and VAC. HW = harvest weight, L = length.

5.3.2 Genetic parameters within the breeding nucleus

Within the breeding nucleus, heritability estimates are presented in Table 5.3, on the diagonal. The heritability for HW was moderate (0.55). For body dimensions, heritability for length (L) was moderate (0.60), while the estimate for height (H) and thickness (T) was two to three times lower. Heritability for condition factor (K) was low (0.04) with high standard error. Heritability estimate for daily growth coefficient was close to that of harvest weight (0.47). For ellipticity, heritability ranged from 0.08–0.14.

Genetic correlations between HW and body dimensions in the nucleus were close to unity (Table 5.3, above diagonal). The corresponding phenotypic correlations were lower: 0.75, 0.49 and 0.59 respectively (Table 5.3, below diagonal). Genetic correlation was negative (−0.12) between HW and K with high standard error, but phenotypic correlation was positive (0.36). Genetic correlations between HW and shape traits ($E_{L\text{-}H}$, $E_{L\text{-}T}$, and $E_{H\text{-}T}$) were low to moderate, with high standard errors. Genetic correlations were positive (0.47) between HW and $E_{L\text{-}H}$, negative (−0.15) between HW and $E_{L\text{-}T}$ and also negative between HW and $E_{H\text{-}T}$ (−0.42). All corresponding phenotypic correlations were low, with small standard errors (Table 5.3).

Between DGC and HW, the genetic correlation was 0.94, and the phenotypic correlation was 0.92 (Table 5.3). The genetic correlation between DGC and $E_{L\text{-}H}$ was low (0.15), whereas genetic correlation between DGC and $E_{L\text{-}T}$ was −0.42, and between DGC and $E_{H\text{-}T}$ was −0.52, indicating that selection for faster growing fish will result in a correlated response in shape, with heavier fish being thicker and more rotund (Table 5.3).

Heritability estimates for HW, DGC, $E_{L\text{-}H}$, $E_{L\text{-}T}$, and $E_{H\text{-}T}$ in cages and VAC are presented in Table 5.4. For HW, heritability estimates were similar among three environments. For K, heritability estimates for cages and VAC were higher than that of the nucleus. For DGC, heritability estimates in cages was lower than that of nucleus, but the estimate for VAC was higher. For ellipticity, results were mixed: estimates for $E_{L\text{-}H}$, and $E_{H\text{-}T}$ were similar among three environments (except for $E_{L\text{-}H}$ which in cage was higher), while the estimate for $E_{L\text{-}T}$ in VAC was much higher than those in nucleus and in cages.

Table 5.3 Heritability (in bold, on the diagonal), genetic (above diagonal) and phenotypic (below diagonal) correlations within nucleus for harvest weight (HW), length (L), height (H), thickness (T), condition factor (K), daily growth coefficient (DGC), and ellipticity: mid-sagittal plane (E_{L-H}), transverse plane (E_{L-T}), and frontal plane (E_{H-T}). Values = estimates ± standard errors.

Trait	HW	L	H	T	K	DGC	E_{L-H}	E_{L-T}	E_{H-T}
HW	**0.55 ± 0.09**	0.98 ± 0.01	0.97 ± 0.03	0.89 ± 0.05	–0.12 ± 0.33	0.94 ± 0.02	0.47 ± 0.21	–0.15 ± 0.22	–0.42 ± 0.21
L	0.75 ± 0.02	**0.60 ± 0.10**	0.93 ± 0.04	0.83 ± 0.07	–0.30 ± 0.29	0.41 ± 0.14	0.62 ± 0.17	–0.23 ± 0.27	–0.37 ± 0.21
H	0.49 ± 0.03	0.61 ± 0.02	**0.21 ± 0.06**	0.84 ± 0.08	0.12 ± 0.33	0.70 ± 0.11	0.29 ± 0.27	–0.17 ± 0.23	–0.29 ± 0.25
T	0.59 ± 0.02	0.53 ± 0.03	0.32 ± 0.03	**0.27 ± 0.07**	0.42 ± 0.29	-0.01 ± 0.18	0.24 ± 0.26	–0.56 ± 0.15	–0.77 ± 0.11
K	0.36 ± 0.03	–0.29 ± 0.03	–0.08 ± 0.03	0.16 ± 0.03	**0.04 ± 0.03**	0.59 ± 0.16	–0.86 ± 0.26	–0.92 ± 0.18	–0.47 ± 0.35
DGC	0.92 ± 0.01	0.59 ± 0.04	0.44 ± 0.03	0.36 ± 0.04	0.57 ± 0.02	**0.47 ± 0.09**	0.15 ± 0.24	–0.42 ± 0.18	–0.52 ± 0.18
E_{L-H}	0.12 ± 0.03	0.22 ± 0.03	–0.63 ± 0.02	0.11 ± 0.03	–0.20 ± 0.02	0.11 ± 0.03	**0.08 ± 0.04**	0.41 ± 0.27	–0.21 ± 0.31
E_{L-T}	–0.17 ± 0.03	0.05 ± 0.04	0.03 ± 0.03	–0.80 ± 0.01	–0.41 ± 0.02	–0.30 ± 0.03	0.04 ± 0.03	**0.14 ± 0.04**	0.81 ± 0.10
E_{H-T}	–0.22 ± 0.03	–0.08 ± 0.03	0.40 ± 0.03	–0.73 ± 0.01	–0.22 ± 0.02	–0.33 ± 0.03	–0.20 ± 0.02	0.79 ± 0.01	**0.08 ± 0.04**

Table 5.4 Heritability within environment three environments (nucleus pond, cages, and VAC pond) for harvest weight (HW), condition factor (K), daily growth coefficient (DGC), and ellipticity: mid-sagittal plane (E_{L-H}), transverse plane (E_{L-T}), and frontal plane (E_{H-T}). Values = estimates ± standard error.

Trait	Environment		
	Nucleus	Cage	VAC
HW	0.55 ± 0.09	$0.52 + 0.12$	0.49 ± 0.12
K	0.04 ± 0.03	0.20 ± 0.07	0.19 ± 0.08
DGC	0.47 ± 0.09	0.21 ± 0.08	0.77 ± 0.12
E_{L-H}	0.08 ± 0.04	0.14 ± 0.08	0.07 ± 0.06
E_{L-T}	0.14 ± 0.04	0.15 ± 0.07	0.26 ± 0.09
E_{H-T}	0.08 ± 0.04	0.09 ± 0.06	0.09 ± 0.06

5.3.3 Genetic correlations of traits between environments

Genetic correlation for HW across environments was very high, ranging from 0.86 to 0.94, indicating that G×E interaction for harvest weight between environments was negligible (Table 5.5). For DGC, genetic correlation was high between nucleus and cage ($r_g = 0.95$) and between nucleus and VAC ($r_g = 0.83$). However, between cage and VAC, the genetic correlation for DGC was 0.77 (Table 5.5).

For body dimensions (L, H and T), genetic correlations across environments were also very high (0.85 to 0.99), except between cage and nucleus for H ($r_g = 0.55$) and T ($r_g = 0.77$).

For ellipticity, genetic correlation between nucleus and cage was 0.54 for E_{L-H}, 0.90 for E_{L-T}, and 0.60 for E_{H-T}. These estimates are in line with the genetic correlation of 0.55 for height between nucleus and VAC, which is the common component for E_{L-H} and E_{H-T}. Genetic correlation between nucleus and VAC was 0.82 for E_{L-H}, 0.72 for E_{L-T} , and 0.64 for E_{H-T}. Genetic correlation between cage and VAC was 0.85 for E_{L-H}, 0.51 for E_{L-T}, and -0.15 for E_{H-T} (Table 5.5).

For K, genetic correlation was 0.96 between nucleus and cage, but was lower and with higher standard error between nucleus and VAC ($r_g = 0.76$,se = 0.35), and between cage and VAC ($r_g = 0.75$, se = 0.20).

Table 5.5 Genetic correlation between environments (cage, nucleus and VAC) for harvest weight (HW), length (L), height (H), thickness (T), condition factor (K), daily growth coefficient (DGC), and ellipticity: mid-sagittal plane (E_{L-H}), transverse plane (E_{L-T}), and frontal plane (E_{H-T}). Values = estimates ± standard errors.

Trait	Nucleus – Cage	Nucleus – VAC	Cage – VAC
HW	0.86 ± 0.07	0.94 ± 0.05	0.94 ± 0.05
L	0.85 ± 0.09	0.97 ± 0.05	0.99 ± 0.06
H	0.55 ± 0.19	0.95 ± 0.10	0.87 ± 0.12
T	0.77 ± 0.13	0.86 ± 0.10	0.85 ± 0.10
K	0.96 ± 0.33	0.76 ± 0.35	0.75 ± 0.20
DGC	0.95 ± 0.09	0.83 ± 0.07	0.77 ± 0.13
E_{L-H}	0.54 ± 0.30	0.82 ± 0.31	0.85 ± 0.30
E_{L-T}	0.90 ± 0.20	0.72 ± 0.17	0.51 ± 0.26
E_{H-T}	0.60 ± 0.37	0.64 ± 0.29	-0.15 ± 0.44

5.4 Discussion

5.4.1 Experiment

In fish selective breeding practices, it is recommended to tag and stock fish as early as possible, to reduce the impact of common environmental effects (Gjedrem, 2005). In this study, family production took place in 105 days, prolonging nursing period and creating a large difference in stocking age among families (Table 5.1). The problem is common for tilapia family production and was observed in GIFT breeding program elsewhere (e.g. in Khaw *et al.*, 2012). Prolonged nursing period (i) increases the impact of environmental effects common to full-sibs (c^2) and (ii) increases the genetic correlation between grow-out environments, especially if the common grow-out period is short relative to the nursing period (Dupont-Nivet *et al.*, 2010). To account for these problems, we fitted collection date and the effect of post-tagging hapa into the models used as fixed effects. These effects were highly significant in most cases.

Models for estimating genetic parameter estimates of GIFT tilapia typically include common environmental effects (Bentsen *et al.*, 2012; Khaw *et al.*, 2012; Ponzoni *et al.*, 2011). However, common environmental effects were difficult to disentangle from genetic effects, due to the low number of dams (2 or 1) mated to each sire, and absence of half-sibs for six of the 92 families. In addition comes that the effect

of date of egg collection was partly confounded with the common environmental effect, as in many occasions only one dam spawned at a given day. Maluwa *et al.* (2006) reported that, in *Oreochromis shiranus*, multivariate models including common environmental effects did not converge or common environmental effects took up all (co)variances of the trait (harvest weight). In this study, estimates for heritability and common environmental effects for harvest weight across environments (nucleus, cage and VAC) were low and not significant different from zero, because of high standard errors. Close to zero estimates and high standard errors were also observed in our study when fitting bivariate models with the common environmental effect included.

Survival rates across environments in our study were lower than those observed by Thodesen *et al.* (2011) on Nile tilapia in China where mean survival rate over five generations (G1 – 5) was 76% at harvest, but was similar to the survival rate in G6 (55%). Average number of fish per family at harvest was 8.3 in cage, 24.3 in nucleus and 8.3 in VAC (data not shown), which is in the acceptable range for a reliable estimation of genetic correlations between environments (Sae-Lim *et al.*, 2010). However, selective mortality might have biased our estimates, by affecting ranking of families. We did try to include stocking weight of all tagged fish, including the ones that did not survive at harvest, as a reference trait into a bivariate model, to correct for bias (Kause *et al.*, 2011), but encountered convergence problems.

5.4.2 Genetic parameters within nucleus

Heritability estimate of HW in the nucleus was high (0.55), compared to those previously reported on the earlier generations of GIFT (0.14 – 0.34) (Bentsen *et al.*, 2012; Khaw *et al.*, 2012; Ponzoni *et al.*, 2011), from which the G13 fish descent. Estimates in those studies were however based on models with common environmental effect included. However, high heritability estimates for Nile tilapia have also been reported when common environmental effect was accounted for: 0.38 – 0.60 (Charo-Karisa *et al.*, 2006) or 0.36 – 0.71 (Khaw *et al.*, 2009). Omitting the common environmental effect from the model might bias the heritability upwards, but given that common environmental effect was partly taken into account by 'collection date', the bias in heritability is likely to be small. In general, heritability estimates of body dimension (L, H, T) were in line with other studies which used models with common environmental effect included.

In our study, heritability of daily growth coefficient was greater than what was found in seabass (Dupont-Nivet *et al.*, 2010). In rainbow trout, heritability estimate of thermal growth coefficient (TGC) ranged from 0.06 – 0.27 (Sae-Lim *et al.*, submitted for publication). In our study, genetic correlation between HW and DGC was high (0.94), similar to results in rainbow trout, where genetic correlation between bodyweight and TGC was 0.89 – 0.92 (Le Boucher *et al.*, 2011; Sae-Lim *et al.*, submitted for publication). Our result indicate that faster growing fish realised greater harvest weight and that harvest weight can be predicted using DGC (Bureau *et al.*, 2000). Daily growth coefficient measures growth rate from the time of stocking onwards. Final harvest weight is the cumulated growth during the entire lifetime. Improving DGC during the grow-out period, which has very high feed costs, likely improves feed efficiency. High harvest weight can, however, result due to factors occurring early in life. Therefore, harvest weight and DGC are complementary traits. One assumption for the precise use of TGC/DGC is that the weight-length relation is assumed to be Weight = a×Length^b in which the power 3 is an accepted estimation of *b* for most fish species (Weatherley *et al.*, 1987). Changes in *b* will affect the estimate of TGC (Jobling, 2003). In our study, the estimate of *b* was 3.08 for cage, 2.83 for nucleus, and 2.80 for VAC (Figure 5.2) which is sufficiently close to 3 to justify its use.

In our study, DGC was positively correlated with condition factor K ($r_g = 0.59$), while the r_g of harvest weight HW with K was −0.12, although with high standard error (0.33). This suggests that selection for harvest weight will not necessarily result in fish with higher condition factor. On the other hand selecting for faster growing fish will produce heavier fish at the same length (or shorter fish at the same weight). This is further supported by the negative genetic correlations between DGC and $E_{L\text{-}T}$ ($r_g = -0.42$), and between DGC and $E_{H\text{-}T}$ ($r_g = -0.52$) which predict that fish selected for faster growth become more rotund. Our results differ from Kause *et al.* (2011) who found genetic correlation of 0.60 between harvest weight and condition factor in European whitefish (*Coregonus lavaretus*). Low heritability estimates and high standard errors of r_g between harvest weight and K were also reported by Fishback *et al.* (2002) in rainbow trout. Similar to the correlation between HW and K, HW was not correlated with $E_{L\text{-}T}$ ($r_g = -0.15$), but heavier fish at harvest were thicker, because the genetic correlation between HW and $E_{H\text{-}T}$ was −0.42. Our results support the observations of Nguyen *et al.* (2007), who reported that GIFT, selected for harvest weight would become longer, relative to their height and thickness. In our study, genetic correlations between harvest

weight and body dimensions (L, H, T) were all very high. However, using ellipticity we show that fish selected for high growth rate will become more rotund and thicker.

Our heritability estimates of ellipticity were lower than those from Blonk *et al.* (2010) (0.34) in common sole, or results for subjective categorical scoring from Kause *et al.* (2004) (0.31 – 0.40) in rainbow trout. Moderate genetic correlations of ellipticity with harvest weight and growth rate imply that selection for weight or growth rate will improve shape in the desired direction (i.e. result in thicker fish). Our results are different from Blonk *et al.* (2010), who found that for E_{L-H} selection for harvest weight results in more circular shape in common sole.

Our results support the general observation that selection for fast growth will lead to more rotund fish (Elvingson and Johansson, 1993; Gjerde and Schaeffer, 1989; Kause *et al.*, 2003a). In our population of GIFT, growth rate can be used to select for higher condition factor and better shape (E_{L-T} and E_{H-T}). This is important for Nile tilapia culture in the Mekong Delta of Vietnam, where farmers and processors prefer thick fish, because a thicker fish looks nice and gives more meat percentage.

5.4.3 Genotype by production environment interaction

In our study, fish were grown in environments with different characteristics and management regimes. We found some evidence for genotype by environment (G × E) interaction between the two most contrasting environments, cage and VAC. There was minor genotype by environment interaction between the breeding nucleus and the two production environments.

Genotype by environment (G×E) interaction for harvest weight has been reported to be varied in Nile tilapia, depending on the magnitude of differences among environments. Eknath *et al.* (2007) reported genetic correlations which were high within one culture environment (0.76–0.99 for within ponds and 0.99 within cages), but which were lower between ponds and cages (0.36–0.82). Bentsen *et al.* (2012) reported that G×E interactions were not important across the pond, rice fish and extensive cage environments tested, but substantial G×E interactions occurred in the cages that used commercial pelleted feed (i.e. similar to the cage environment in this study) compared to other test environments. However, our results are in agreement with Thodesen *et al.* (2011) and Khaw *et al.* (2012) who reported that G×E interaction was not important for harvest weight in Nile tilapia in China

(Thodesen *et al.*, 2011) and in Malaysia (Khaw *et al.*, 2012). In our study, very high genetic correlations for HW were found between the cage, nucleus and VAC environments. This observation confirms the results from the GIFT base population (Eknath *et al.*, 2007), from Nile tilapia grown in low and high input pond environments (Khaw *et al.*, 2009) and from two recent G×E studies in GIFT (Khaw *et al.*, 2012; Thodesen *et al.*, 2011). Non-siginificant G×E interactions have also been reported for weight of Atlantic cod (*Gadus morhua*) (Kolstad *et al.*, 2006), *O. shiranus* (Maluwa *et al.*, 2006), rainbow trout (Kause *et al.*, 2003b), European sea bass (Dupont-Nivet *et al.*, 2008) and Pacific white shrimp (*Litopenaeus vannamei*) (Gitterle *et al.*, 2005).

There was no evidence of G×E interaction for DGC between the breeding nucleus and either two production environments (Table 5.4). However, genetic correlation for DGC between cage and VAC was 0.77, which indicates G×E. Considering the magnitude of the genetic correlation, this might be an indication of environmental sensitivity. More specific, in this study, fish had been selected in the breeding nucleus pond (using pelleted feed) and seemed to perform better in the high-input environment (cages, pelleted feed diet) than in the low-input environment (VAC, phytoplankton diet). In European sea bass (Dupont-Nivet *et al.*, 2010), significant G×E interaction for DGC but not for harvest weight was found. A prolonged nursing period in the same environment increases genetic correlations of harvest weight between environments while DGC accounts for only the growth period, allowing more accurate estimates of G×E for growth instead of harvest weight (Dupont-Nivet *et al.*, 2010).

To our knowledge, there are no studies on genetic correlations of shape traits as defined in this study, making comparison of our results difficult. Genetic correlations of L between three environments were high, indicating that there was no G×E interaction. However, between cage and nucleus, G×E was found for height ($r_g = 0.55$). This was in line with the observed genetic correlations for $E_{L\text{-}H}$ (0.54) and $E_{H\text{-}T}$ (0.60). The genetic correlation of K between nucleus and cage was 0.96 but the regression coefficients of Log(HW) on log (L) (Figure 5.2) suggest that fish in cages had a different shape compared to those in nucleus and ponds. In fact, the lowest genetic correlations for K, $E_{L\text{-}H}$ and $E_{H\text{-}T}$ were observed between the two most contrasting environments: cage and VAC. However, these G×E interactions for K between nucleus – VAC and between cage – VAC are debatable, considering the magnitude of the genetic correlation and high standard errors of the estimates

(Table 5.5). The same argument can also be used for G×E interactions between nucleus and cages (E_{L-H}), VAC and nucleus/cage (E_{L-T}), and among three environments (E_{H-T}) (Table 5.5). These low genetic correlations show that the breeding program in the nucleus may need to use sib information from VAC and cages to increase genetic gain in ellipticity and DGC in VAC and cages (Mulder and Bijma, 2005).

Shape of fish can be affected by several environmental factors, such as water current, feeding and management (Pakkasmaa and Piironen, 2000; Swain *et al.*, 1991). In term of water current, the cage installed in river was subjected to constant water movement with different water velocities, while water movement in ponds (either nucleus or VAC) was very limited. The water velocity in the Mekong river is around 2.5 metres/second outside a cage and less than 1 metre/second inside a cage (Ly, 1988). This suggests that fish in cage were subjected to more active swimming than fish in nucleus and in VAC. Swimming activity appears to increase growth in many fish species (Davison, 1997) and specific growth rate in gilthead sea bream (Ibarz *et al.*, 2011). Effects of water current on fish shape are different depending on species (Burns *et al.*, 2009). In Mozambique tilapia (*O. mossambicus*), steady swimming fish were oblong, while fish swimming in bursts were rotund (Firmat *et al.*, 2012). Juvenile salmon (*S. salar*) reared in fast water current showed higher height, as were juvenile rainbow trout (*S. trutta*) reared in slow water current (Pakkasmaa and Piironen, 2000). Our results seem to indicate that Nile tilapia grown in cages with water velocity of less than 1 metre/second may develop a more rotund and thicker body shape at the same length compared to fish grown in ponds.

Conclusion

Ellipticity is a useful parameter to describe shape in Nile tilapia. Our results show that genotype by environment interaction was minor for harvest weight and for growth, but substantial for shape. Nile tilapia grown in cages with water velocity has a higher condition factor, and develop a thicker body shape at the same length compare to fish grown in ponds. Selection in the nucleus pond should target growth rate and harvest weight and include sib information on shape from the other environments. The expected correlated selection responses for fish grown in cages are positive, as they are expected to become more rotund and thicker.

Acknowledgements

This study is part of the first author's sandwich PhD study, funded by Wageningen University. We would like to thank Dr. Raul W. Ponzoni and Dr. Nguyễn Hồng Nguyên of the WorldFish Center, for supplying the GIFT generation 10th, and for financial support for field work from 2007 to date. We thank Panya Sae-Lim for his constructive discussions. Trọng thanks his team at RIA2, two tilapia cage famers (Mr. Đặng Quang Hiển and Mr. Lê Minh Nghiệm), and a VAC farmer (Mr. Phạm Văn Tòng) for their field work. We thank three reviewers who comments help to improve the manuscripts. Pictures in Figure 5.1 were drawn by Trần Công Thuận.

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6

General discussion

6.1 Background

The aim of the research described in this thesis was to optimise the breeding program for Nile tilapia (*Oreochromis niloticus*) in the Mekong Delta of Vietnam, with harvest weight as the main trait of interest. The main bottleneck in any GIFT (Genetic Improvement of Farmed Tilapia) breeding program is the prolonged time required for family production, which increases environmental effect common to full-sibs (c^2) and reduces the accuracy of estimated breeding value (EBV). The GIFT method also requires separate nursing of families and tagging of individuals, both of which are labour intensive and costly.

In this thesis, several potential improvements to the GIFT Nile tilapia breeding program were investigated (Figure 6.1). The alternative breeding method was based on natural mating in groups, in combination with a rotational cohort mating scheme (rotational mating) (Block A, Figure 6.1). The idea of the rotational mating scheme was to shorten family production time, and to avoid separate nursing of families and tagging of individuals. Fish in the rotational mating scheme were selected on own performance, and pedigree re-construction (for R10 and R11, Block A, Figure 6.1) was conducted to monitor the rate of inbreeding. In the "classic" GIFT breeding program, genetic parameters for harvest weight, growth rate, shape, and reproductive traits were estimated in generation 12 (Block B, Figure 6.1). Genotype by environment interaction was investigated in generation 13, because fish were selected in the breeding nucleus pond, while the major production was conducted in intensive river cages and (to a lesser extent) in traditional low-input integrated ponds (VAC; Block B, Figure 6.1). Special attention was given to methods to reduce the time needed to reproduce tilapia. We investigated whether natural mating in small groups could reduce the time needed to produce progeny. For this purpose, two designs, namely 'single male, multiple females' and 'multiple males, multiple females', were tested (Block C, Figure 6.1).

To date, there are a few breeding programs using BLUP selection for Nile tilapia. These programs are operated by government institutions such as the Research Institute for Aquaculture No.2 (RIA2) in Vietnam[1], non-government organisations (NGOs) such as the WorldFish Center (WFC) (Ponzoni *et al.*, 2011), or large companies such as Hainan Progift Aqua-Tech Co. Ltd (Thodesen *et al.*, 2012).

[1] Partly funded by the WorldFish Center, and was reported in this study.

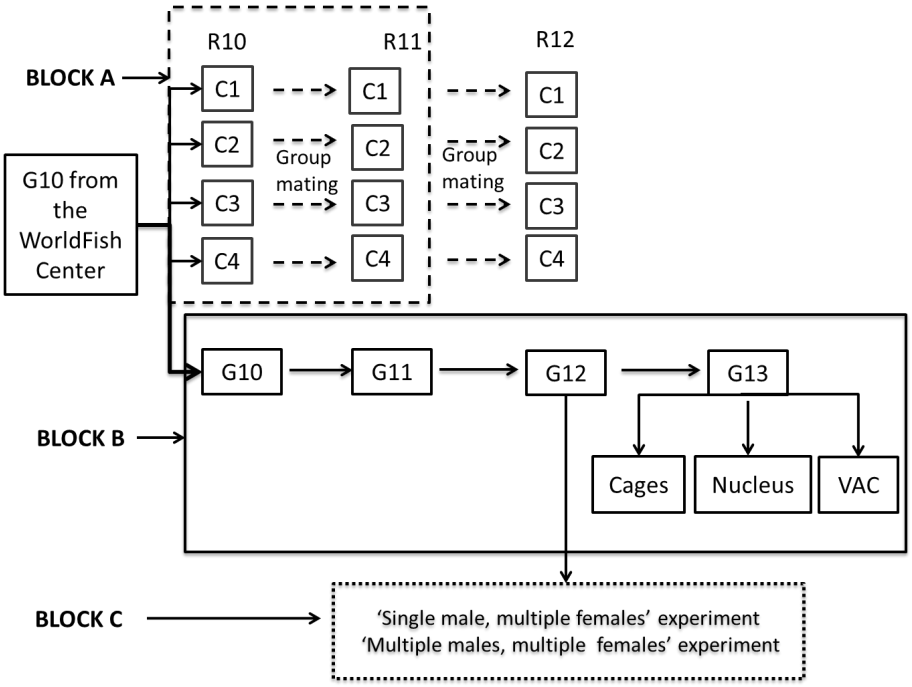

Figure 6.1 Diagram of the study.
R = Rotational mating, C = cohorts in R, G = GIFT breeding program. Numbers following R and G indicate generations. Numbers following C indicate cohort number.
Block A: Rotational mating for 3 generations (R10, R11, and R12), Block B: GIFT breeding program for 4 generations (G10 to G13), Block C: reproductive experiment for G12.
G10 was the base population obtained from the WorldFish Center, Penang, Malaysia.

In many countries of the developing world, such as in Africa, tilapia culture still relies on seed which shows deteriorated performance mainly caused by inbreeding, and as a result genetically improved seed are in great demand (Brummett and Ponzoni, 2009). Very few hatcheries can afford sophisticated selective breeding programs, which require reliable pedigree recording and complex statistical analysis. Therefore, simple, alternative solutions (smaller-scale breeding programs) to produce high-quality seed while avoiding inbreeding as much as possible are required. Even in developed countries, small-scale, tailor-made breeding programs sometimes are required for specific purposes (e.g. to improve a specific trait for a specific market).

Although rotational mating schemes can delay inbreeding to a certain degree, it is still of suitable use for broodstock management purposes in aquatic hatcheries and applications for simple breeding programs in aquaculture. For species that spawn naturally in groups like Nile tilapia, rotational mating can reduce the mating of related individuals within each cohort or hatchery, as the males from one cohort/hatchery are mated with females from another cohort or from another hatchery.

In section 6.2 of the general discussion, reproduction in the "classic" GIFT breeding program is discussed. In section 6.3, optimisation of the GIFT breeding program for the Mekong delta in Vietnam is discussed. In section 6.4, the relation between the breeding nucleus and the dissemination system is presented. In the final part of the general discussion rotational mating is evaluated and suggestions for its use in conventional GIFT breeding programs are given.

6.2 GIFT breeding program

The GIFT method applies single pair mating, followed by separately rearing of full-sib groups (families), individual tagging, and combined family selection (Ponzoni *et al.*, 2011; WorldFish Center, 2004). The single pair mating is practiced in a so-called 1 male to 2 females 'nested mating design'. First, each female breeder is stocked with a single male into one single hapa. Next, females that have spawned are removed from the spawning hapas, and a replacement female is stocked in order to produce a paternal half-sib family.

The nested mating design allows separation of genetic and environmental variances (see e.g. Gjerde, 2005). Controlled mating, separately rearing of full-sib groups until the fish have reaching tagging size and individual tagging provides reliable pedigree information. The combined family selection is based on the Best Linear Unbiased Prediction (BLUP) procedure for selection, and controlled mating (Ponzoni *et al.*, 2011). The BLUP procedure allows estimation of individual's genetic merit for the trait of interest, and builds on recorded pedigrees and phenotypes.

Family production

In Nile tilapia, controlled pair mating as proposed by the WorldFish Center (2004) is labour intensive and requires costly hapa investments (Bentsen and Olesen, 2002).

Nile tilapia is a group-spawning species (Turner and Robinson, 2000), that is, spawning occurs among multiple males and multiple females. Controlled pair mating results in an undesirable side-effect: a prolonged time for family production, especially the time needed to produce paternal half-sib groups. For estimation of genetic parameters for harvest weight, prolonged time for family production reduces accuracies of estimated breeding values, and increases the impact of environmental effects common to full-sibs (c^2) (Bentsen *et al.*, 2012). More importantly, prolonged time for family production can increase the generation interval by about 3–4 months. For GIFT generation 1 (G1) to G5, the time for family production ranged from 40–101 days (Bentsen *et al.*, 2012). Thereafter for G6 to G13, the time for family production ranged from 60–182 days (Khaw *et al.*, 2012; Ponzoni *et al.*, 2011). In this study, setting up one male with one female in a spawning hapa did not stimulate spawning as quickly as expected. The family production time was 136 days for G11, 165 days for G12, and 105 days for G13.

The main cause of prolonged time for family production was that paternal half-sib groups were required for precise estimations of genetic parameters. In the GIFT protocol, per generation, 50 paternal half-sib groups are produced sequentially: after a first (half-sib) family is obtained, a second half-sib family is produced. For unknown reasons, it was time consuming to obtain the second half-sib families. We noticed that family production (to obtain 100 families) could be done within a relatively short time (about 30 days), if only full-sibs families are required and no corresponding half-sib groups are needed.

In many livestock species, long-term selection for high production efficiency can result in reproductive problems such as defective eggs and poor semen quality in chickens, delayed age at puberty and farrowing in pigs, and low success rates after insemination in dairy cattle (Rauw *et al.*, 1998). Genetically, prolonged times for family production was suspected as an undesirable correlated response to selection for harvest weight in GIFT Nile tilapia. In chapter 3, we compared two mating schemes, one in which 1 male was stocked with 10 females (single male, multiple females), and one in which 7 males were stocked with 15 females (multiple males, multiple females), to estimate heritability for spawning success (spawn/no-spawn) and its genetic correlation with harvest weight. Heritability estimates for spawning success ranged from 0.14 to 0.22, depending on the model used and with spawning thresholds from 20 to 32 days. More importantly, genetic correlations of spawning success with harvest weight were positive and ranged

from 0.48 to 0.52 for thresholds of 20 to 32 days. From these results it can be concluded that selection for harvest weight should improve spawning success of female Nile tilapia, provided the mating period is limited to 20 to 32 days.

Considering that the genetic correlations of spawning success with harvest weight were positive, it can be hypothesized that the reasons for prolonged reproduction time were biological/technical, i.e. caused by the controlled pair mating scheme itself. In practice, keeping one male and one female broodstock in a confined space of the spawning hapa often leads to injury or mortality of either broodstock during family production. The major reason is that the males are aggressive. To prevent this, males often have their upper jaw removed, which can lead to additional mortality. The injured or dead candidate broodstock must be replaced with another one, which often has a lower EBV. The direct consequence is that selection intensity is reduced, and that the best EBV individuals do not necessarily successfully contribute offspring to the next generation. In addition, if a male broodstock dies after producing just one family, then no half-sib group can be produced from that male. This results in an incomplete design and confounding of common full sib effects with additive genetic effects.

Family rearing

Extended family production leads to extended family rearing periods, as all animals need to be tagged at the same time before stocking for communal rearing. The family rearing period in hapas is typically modelled as a common environmental effect, termed 'hapa effect'. Prolonged family rearing results in considerable common environmental effects for full-sibs (c^2), which will reduce the reliability of their breeding value estimation (Bentsen *et al.*, 2012). Estimates for c^2 ranged from 0.04–0.16 during the first five generations of GIFT (Bentsen *et al.*, 2012), 0.34 (Ponzoni *et al.*, 2011) or 0.14–0.17 (Khaw *et al.*, 2012) in the subsequent generations. However, in some cases, multivariate models including c^2 either did not converge, or c^2 took up all (co)variances of harvest weight, as reported by Maluwa *et al.* (2006) and as was also observed in our study in G13 in the breeding nucleus and the two production environments (Block B, Figure 6.1). The c^2 is however made up not only by the hapa effect, but also by non-additive genetic and maternal effects. As Nile tilapia are female mouth brooders, maternal effects are normally completely confounded by the hapa effect.

6.3 Optimisation of breeding program in Nile tilapia

From the previous section it follows that the hapa effect should be minimised in a Nile tilapia breeding program to enable accurate genetic evaluation (Bentsen *et al.*, 2012). This can be realised by shortening the time for family production to a minimum, and/or by tagging fingerlings as early as possible.

As shown in chapter 3, the 'single male, multiple female' scheme gave higher spawning success (81.3% tested females spawned) than the GIFT scheme (55.6%, Table 6.1). In addition, in the 'single male, multiple females' nearly half of the males produce at least two paternal half-sib after only 4 days (Box 6.1). Therefore, the GIFT scheme can be altered to the 'single male, multiple females' scheme. This 'single male, multiple females' scheme should reduce the time needed to produce half-sib groups, which is the ultimate objective for Nile tilapia family production, considerably (see Box 6.1 and Table 6.1). More importantly, identification of the sire is easily obtained, because males are used sequentially, one at the time.

Table 6.1 Estimated numbers of female and male broodstock, spawning hapas/tanks, duration for family production of 50 half-sib groups for different mating ratio: 1 male to 1 female, 1 male to 5 females, and 1 male to 10 females, and selection intensity (proportion selected). See Box 6.1 for calculations.

Scheme	# of candidate broodstock		# of tanks/ hapas	Duration (days)	Selection intensity [d] (Proportion selected, %)	
	Male	Female			Male	Female
1:1 [a]	90 [c]	203	50	105	1.8 (9)	1.4 (20)
1.5 [b]	110	190	10	44	1.7 (11)	1.4 (19)
	111	186	15	28	1.7 (11)	1.4 (19)
1:10	110	240	10	44	1.7 (11)	1.3 (24)
	111	261	15	28	1.7 (11)	1.2 (26)

[a] GIFT scheme (pair mating); [b] the 1:5 ratio is assumed to have similar reproduction performance as the 1:10 ratio, based on unpublished data; [c] 50 males successfully reproduced, data from family production of G13; [d] Based on a total of 2000 fish harvested, half of them are males and the other half are females. Values for selection intensity were obtained from Appendix A, page 379, Falconer and Mackay (1996).

In theory, fifty males and one hundred females are needed to produce 50 half-sib groups (one hundred full-sib families). In practice, to account for broodstock mortality and for females that do not spawn, more males and females need to be

selected. These additional fish will have lower EBV than the 150 originally selected fish.

Box 6.1 Calculation of number of broodstock and duration for family production in Table 6.1

The objective was to produce 50 paternal half-sib groups (HSG), equivalent to 100 full-sib families, in a short a time as possible. The pair mating scheme of 1 male to 1 female followed the GIFT method described by WFC (WorldFish Center, 2004). For calculation, actual data from family production of G13, as described in chapter 4, was used for the pair mating. The '1 male and 10 females' scheme is described in detail in chapter 3 and 4. We back-calculated based on the assumption that every 4 days, a male successfully mates with 2 females and produces 2 paternal half-sib families. A period of 20 days, equivalent to 5 checks for spawns, was set (see chapter 3). However, only 45.2% (data from experiment in chapter 3 and 4, not shown) of the males tested in the 'single male, multiple females' experiment produced 2 paternal half-sib families after 4 days. Therefore, the actual number of males needed was $\frac{\text{number of males with 2 HSG}}{0.452}$.

The calculation was as follows: Number of half-sib groups produced per tank was $\frac{50\ \text{HSG}}{10\ \text{tanks}} = 5$. This was equal to the number of males used per tank, because each male needed to produce only one HSG. However, six more males were needed because 54.8% produced only 1 family. Therefore, there were $\frac{5}{0.452} = 11$ males needed per tank. In total, 110 (11*10) male candidates were required. For females, in each tank, 10 females were stocked at the beginning. For 4 checks, two were removed and two were added. For the other 6 checks, only one was removed and one was added. The number of added females was then 14 (= 8+6). In total, 240 $(= (10 + 14) \times 10)$ females were required. Assuming that the 1:5 scheme performed similar to the 1:10, with a calculation similar to above, 111 males and 190 females were needed. The same calculation was done if the number of tank increased from 10 to 15.

Selection intensity was calculated as $i = \frac{z}{p}$, with *i* is the selection intensity, *p* is the proportion of selected individuals, and *z* is the height of the ordinate at the point of truncation (Falconer and Mackay, 1996).

In Table 6.1, the 1:1 (1 male to 1 female) breeding scheme represents the GIFT pair mating, the 1:10 is the design used in this study, and the 1:5 is the "reduced" version of the 1:10. The 1:1 scheme results in the highest selection intensity for both males and females, but requires the most spawning hapas and takes the

longest time to produce 50 half-sib groups. Having more females in a spawning tank, as in the 1:5 and 1:10 schemes, better resembles the group-spawning condition of Nile tilapia (Turner and Robinson, 2000). This has been confirmed as the 1:5 and 1:10 schemes reduce the duration of family production by 2.4 to 3.8 times compared to the 1:1 scheme.

We recommend the '1 male to 5 females' (1:5) breeding scheme with 15 tanks as this scheme requires the least number of males and females, has shortest time required for family production (only 28 days), and has just slightly lower selection intensity than the GIFT scheme (Table 6.1). The 1:5 scheme indirectly improves genetic gain in the following ways. The expected genetic gain per year ΔG is calculated as $\Delta G = \frac{i \times r \times \sigma_A}{L}$ (Falconer and Mackay, 1996), with i the selection intensity, r the accuracy of selection, σ_A the additive genetic standard deviation for the trait of interest, and L the generation interval in years. Shortening the time for family production will increase r, however this increase is currently unknown. In this study, shortening reproduction time reduced L by 61–77 days (Table 6.2), or about 2 months, compared to the GIFT scheme. Assuming that the generation interval of Nile tilapia is 12 months, a shortened interval obtained with the 1:5 scheme is 10 months (or 0.83 year) (Table 6.2). Assuming that the i) selection intensity, ii) accuracy of selection, and iii) genetic standard deviation are the same as with the GIFT scheme, this shortened time interval will result in 20% genetic gain (or $\frac{1.00}{0.83}$) extra compared to the classical GIFT scheme.

Table 6.2 Time schedule in a year (starts in January) for two different mating schemes, '1 male to 1 female' (the GIFT scheme) and '1 male to 5 females', in a Nile tilapia breeding program.

Activities	Time	
	GIFT (1 male to 1 female)	1 male to 5 females
Family production	01 January – 30 March	01 – 31 January
Family rearing	15 January – 30 April	15 January – 28 February
Tagging	01 – 15 May	01 – 15 March
Grow-out	16 May – 15 October	16 March – 15 August
Harvest	16 October – 30 October	15 August – 31 August
Data analysis	01 – 15 November	01 – 15 September
Selection and conditioning of broodstock	16 November – 31 December	16 September – 31 October

6.4 Dissemination

Genetically improved fish produced in the nucleus need to be disseminated to multipliers (i.e. hatcheries), so that genetic improvement can quickly reach farmers/on-growers (Gjedrem, 2005; Ponzoni *et al.*, 2007).

The breeding work in the nucleus is normally funded by the government (e.g. for the pangasius catfish (*Pangasianodon hypophthalmus*) in Vietnam) or by NGOs (e.g. partly by the WFC for this study). Financial support for this is normally given for a designated period. For this study, the WorldFish Center funded the RIA2 breeding program in the Mekong Delta from 2007 to 2012 inclusive. Improved material has been distributed free of charge to local hatcheries and other regional breeding stations.

In this study, relative fecundity, calculated as number of eggs per gram of female, ranged from 2.3–3.5, which was lower than values reported by Ponzoni (4–10; Ponzoni *et al.* (2007)). The reason for this was that, at the time of the experiments described in chapter 4, the females were much older than those reported in Ponzoni *et al.* (2007), and the females in other studies were mostly feral Nile tilapia. In Nile tilapia, relative fecundity decreases with female age, weight, and length (Rana, 1988), which agrees well with the practice of commercial seed producers who discard broodstock that are heavier than 300 g.

Here, we applied the same approach as Ponzoni *et al.* (2007), that is, assuming a breeding program from a national perspective. Surplus fry from selected families need to be disseminated to multipliers for every generation of selection. The number of fry produced per year in the nucleus was calculated using the following parameters. In the breeding nucleus, 100 female broodstock, each on average produced 700 two day-old swim-up fry[2]. Survival rate from fry to sexual maturity was 60%, and each female broodstock spawned a maximum of 10 times per year. Fry from the first spawn was for selection. Fry from the remaining 9 spawns was used to produce sexual mature females for hatcheries. This number was therefore 189,000 (= [100×700×9×0.60]/2). In contrast to Ponzoni *et al.* (2007), who used all the mature females for hatcheries, we proposed considering only mature females

[2] standard figure, which differs from data in chapter 4 where each female produced on average 1,000 fry, because at the time of the experiment the females were already larger than those used for seed production

that are offspring of the best 10% of female broodstock (10). The multipliers then used these matured females to produce fry for grow-out. The estimated the number of fry available for grow-out would be 79,380,000 per year (Table 6.3). This is enough to stock 3,969 cages (two crops/cage/year, each cage 100 m^3, stocking density 100 fish/m^3). In the Mekong Delta of Vietnam, the total number of tilapia cages was estimated at 2,000 (2012). Therefore, per year, our Nile tilapia breeding program can provide sufficient improved fry for the whole Mekong Delta production.

Table 6.3 Reproductive efficiency in the breeding nucleus and hatcheries/multipliers.

Sources	Nucleus				Hatchery					
	N	F_{Nu}	Spw_{Nu}	Sur_{Nu}	$0.5Prg_{Nu}$	10% best of $0.5Prg_{Nu}$	F_{Ha}	Spw_{Ha}	Sur_{Ha}	Prg_{Ha}
Ponzoni *et al.* (2007)*	100	250	16	0.65	130,000		250	16	0.65	338,000,000
This study**	100	700	9	0.60	189,000	18,900	700	10	0.60	79,380,000

All values are per year

* system of hapa in nucleus and hatcheries

** see section 6.4 for calculations

N = Number of females

Nu = Breeding nucleus

Ha = hatcheries/multipliers

F_{Nu} = Number of fry produced per spawning in the nucleus

Spw_{Nu} = Number of spawning per female per year in the nucleus

Sur_{Nu} = Survival of fry from spawning until sexual maturity in the nucleus

Prg_{Nu} = Number of progeny produced by the nucleus

F_{Ha} = Number of fry produced per spawning in the hatcheries/multipliers

Spw_{Ha} = Number of spawning per female per year in the hatcheries/multipliers

Sur_{Ha} = Survival of fry from spawning until sexual maturity in the hatcheries/multipliers

Prg_{Ha} = Number of progeny produced by the hatcheries/multipliers

In the previous section, we ignored the costs to maintain the surplus fry in the breeding nucleus and the costs for setting up of an efficient and reliable multiplier channel. Commercially successful strategies for dissemination are however still unclear. To sustain a long-term breeding program for red tilapia in the Southern region of Vietnam, there were initiatives to create a multiplier system, in which key local hatcheries received improved seed free of charge, but had to pay a royalty

based on the amount of seed they sell to on-growers. Hatcheries would receive newly improved material every selected generation from the breeding nucleus. Alternatively, hatcheries could buy improved seed at a fixed, premium price. In this system, however, hatcheries depend on the breeding nucleus to supply new seed. At that time, the royalty system was too advanced a concept for the red tilapia industry in Vietnam. Farmers were unprepared to pay extra for improved seed, and the market was unwilling to pay more for table fish, thus the multipliers were unable to pay the royalty. More importantly, the whole red tilapia industry in the Mekong Delta of Vietnam experienced a serious downfall in prices for marketable fish in 2012, rendering the royalty system impossible. As the red tilapia industry is recovering, it has been realised that a fixed price system might be more appropriate. In a fixed price system, hatcheries will buy improved seed at a premium price, and would not pay a royalty.

6.5 Rotational mating

In this thesis we tested an alternative to BLUP selection which was selection based on own performance, termed 'mass selection' (Falconer and Mackay, 1996). In this method, pedigree information is ignored and fry can be produced by natural spawning in groups. However, Nile tilapia has relative large numbers of offspring as shown in chapter 4, and there is a highly unequal contribution of the males which might result in unacceptable levels of inbreeding (Fessehaye *et al.*, 2006). To restrict the increase of inbreeding, a mating scheme based on rotating cohorts was proposed. This mating scheme is termed 'rotational mating'. and aims to avoid high rates of inbreeding or to maintain the rate of inbreeding at an acceptable level in a closed population (Alderson, 1990a; b; 1992; Maijala *et al.*, 1984; Nomura and Yonezawa, 1996; Yamada, 1980). In the following discussion, we will use 'scheme' to refer to mating schemes, and 'cohort' to refer to sub-populations or sub-groups of broodstock fish. Rotational mating can be applied either at the individual level (within populations) or at the cohort level (between sub-populations). With rotational cohort mating, a population is first divided into a number of groups or sub-populations (cohorts). Thereafter individuals are exchanged between groups in a systematic way. Based on the pattern of exchange, the schemes can be categorized as circular or cyclical mating. In circular mating, the direction of exchange is the same for all generations, independent of the number of cohorts. With cyclical mating, the direction of exchange differs between generations, but

the pattern of exchange is repeated after m-1 generations, with m = number of cohorts (Figure 6.2).

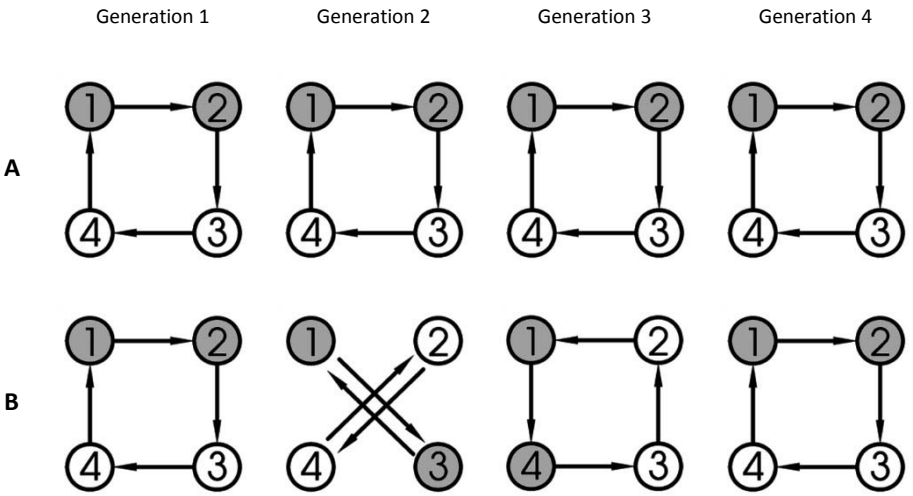

Figure 6.2. Diagram of rotational mating with 4 cohort and 4 generations. A: circular mating, B: cyclical mating. The arrows show the direction of male transfers in four generations. Grey circles show the direction of male transfers from and to cohort No. 1.

Although rotational mating schemes are theoretically promising for the reduction of inbreeding rates in closed populations, related publications in aquaculture are rare. To reduce inbreeding accumulation in rainbow trout, Kincaid (1977) proposed forming three broodstock groups, namely group A, B, and C. Transfer of males was similar to a circular scheme (male A to B, male B to C and male C back to A), and this mating pattern was repeated for each generation. The author suggested having a minimum of fifty fish of each sex in each generation, and a ratio of one male to one female. There was, however, no data on the actual rate of inbreeding for each generation. Using the same rotational mating method as described by Kincaid (1977), Bolivar and Newkirk (2002) applied individual, within-family selection for bodyweight at 16 weeks for Nile tilapia. McPhee *et al.* (2004) applied a rotational mating scheme for four generations of red claw crayfish. The authors pointed out that individual identification of the selected candidates was still needed in order to control inbreeding effectively.

In this study, we tested a breeding scheme based on mass selection and natural spawning using rotating cohorts for two generations (R11 and R12) (Figure 6.1).

Parents for R10 and R11 were used to re-construct the pedigree using microsatellites and SNPs, so that the inbreeding level in the studied population could be monitored. The parents were divided in 4 cohorts and allowed to produce offspring by natural group mating. With 122 SNPs, and allowing up to 15 mismatches, eighty four per cent of the R11 offspring were uniquely assigned to a parent pair. With 12 microsatellites, only 38% offspring got unique assignments. The low assignment rates were related to the missing parents (13.8% of the total potential parents) and the low exclusion power of the microsatellites set (68%). These results emphasize the importance of keeping DNA from all parents, as missing parents make parentage assignment highly ambiguous.

Rotational mating applies group mating, which resembles natural mating conditions of Nile tilapia. Therefore, it was expected to be more efficient in production of offspring, as discussed in chapter 4. In this study, the time to produce full-sib families was indeed shorter than that for the GIFT breeding program: for R11 it was 25–43 days. However, for R12 it was 89 days. This relatively long time for family production was not fully understood, though we suspect that unfavourable weather conditions (prolonged high temperature) and unfavourable water conditions (difficult to exchange water) at the breeding centre might have been the main causes.

For rotational mating, we underestimated the logistics for the rearing period. In the first generation of offspring (R11), we reared each family in a single hapa, similar to family rearing procedures in the GIFT breeding program. The reason was to standardise the number of fry in each family (offspring obtained from one female, though the male identification was unknown) for grow-out. In other words, the families equally contributed representatives for grow-out. The total number of rearing hapas used was 96 (24 hapas for 24 families in each cohort times 4 cohorts). This number was equal to the number of rearing hapas needed for the BLUP scheme (100 hapas for 100 full-sibs families). Consequently, costs for labour, hapas and feed during the rearing period for rotational breeding scheme came close to those of the GIFT breeding program. For R12, we first standardized the number of fry for each spawned female, and thereafter stocked all 24 batches of fry of one cohort into a single, large (5×10×1 m) rearing hapa. In this way, families in one cohort can be communally stocked at an early age, which greatly reduces the costs for rearing hapas. The mortality at the end of the rearing period might be unequal among families, thus the number of fish in each family when stocking for grow out could be different. However, this effect was expected to be negligible,

because mortality mostly happened during the grow-out period, even when the number of fish in each family was equal at stocking. We therefore recommend pooling families (in each cohort) as soon as possible (7–10 days after full yolksac absorption) to reduce hapa costs and environmental effects common to full-sibs.

We have learned that there are a few other key issues that determine the success of practical rotational mating. First, cohort identification is recommended, by means of a token system, e.g. labelling of the holding ponds, tanks or hapas. Preferably simple, cheap tagging methods, such as fin clipping, marking or Floy® tags, should be used, to ensure animals are assigned the right cohort. Animals from different cohorts could then be kept in a common environment, resulting in less environmental effects. Also, the cost of several separately holding facilities, such as ponds, tanks or hapas can be greatly reduced. This approach can be particularly useful for small hatcheries with few broodstock and limited holding facilities. Second, if tagging is not possible, separately housing of cohorts is required. In this way, animals of different cohorts should be kept strictly in separate ponds, tanks or hapas. The disadvantage is that several holding facilities are required. Also there should be marking, as it is important to keep track of identification of the cohorts, and avoid accidentally mixing of animals from different cohorts. Third, the number of cohorts is important. In general, it is recommended to have at least 4 cohorts in each hatchery, because having less than 4 cohorts results in a rapid increase of inbreeding. For example, inbreeding will first occur in generation 2 with three cohorts in a circular mating scheme. Fourth, cohort sizes and number of families in each cohort is important, because these factors determine the probability that a male (or female) is chosen to be mated with the other sex. In general, a cyclical scheme is advised, because its system of exchanging animals is quite straight forward and fairly simple to practice.

Rotational mating can also be incorporated into a GIFT breeding program, to reduce inbreeding. The typical mating strategy in GIFT is to mate fish that do not share a common grandparent, to avoid mating of relatives. This can result in reduced selection intensity. With rotational mating, the fish population in a GIFT breeding program can be divided into 4 sub-populations, and rotational mating is applied among those to further reduce inbreeding. This approach is also useful to improve bio-security of a breeding program: the sub-populations can be kept at different locations (e.g. ponds or stations) so that they are less prone to losses caused by diseases, disaster or unforeseen accidents. The obvious disadvantage of this scheme is that more ponds are needed. However, testing more ponds would

also allow for estimating pond effects, thereby reducing a potential bias in the breeding value estimation.

In conclusion, the GIFT breeding program is a proven means to genetically improve harvest weight in Nile tilapia. The BLUP selection allows multiple-trait selection, which is the ultimate trend of any selective breeding program, giving it an edge over mass selection (with or without rotational mating) which allows only single trait selection. Selection for harvest weight in GIFT does not have a negative effect on female spawning success and key fecundity/fertility traits (number of eggs, relative fecundity, and number of swim-up fry), which is a very significant finding, because most breeding programs in aquatic species focus on body weight. For hatcheries, females that are selected for harvest weight are predicted to be prolific broodstock. The key constraint of prolonged time for family production in GIFT breeding program can be overcome by applying 'single male, multiple females' mating. A rotational mating scheme can be used in combination with GIFT selection to further reduce inbreeding, estimate pond effects and to secure the breeding material. For hatcheries who want to apply a small-scale, simple breeding program, mass selection combined with rotational mating is recommended.

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Summary

Summary

The aim of this thesis was to improve the selective breeding program for GIFT Nile tilapia conducted by RIA2 in the Mekong Delta region of Vietnam. This breeding program is an extension of the GIFT (Genetically Improved Farmed Tilapia) program conducted by the WorldFish Center in Malaysia and is based on Best Linear Unbiased Prediction (BLUP) breeding value estimation using individual information and information from relatives. The BLUP scheme builds on controlled single pair mating to produce full- and half-sib families, and reliable pedigree identification via tagging. The GIFT project has resulted in considerable genetic gain for harvest weight. However, the recommended nested mating design of one sire mated to two dams sequentially often results in prolonged reproduction periods of 3–4 months. This prolonged time for family production increases family rearing effect, reduces accuracy of estimated breeding values, and increases generation interval. It is hypothesised that this prolonged time is a consequence of a correlated response to selection for harvest weight. A second, alternative hypothesis is that the difficulty to produce full- and half-sib families within a reasonable time-span is a consequence of the natural group mating and spawning behaviour of Nile tilapia. In this thesis we investigate two alternative breeding schemes. The “classic” BLUP scheme followed the GIFT method as originally proposed by the WorldFish Center and was conducted for four generations. An alternative breeding method, which was based on own performance selection, natural group spawning and rotational mating was investigated thereafter for three generations.

The aim of a rotational mating scheme was to mimic natural spawning conditions in Nile tilapia, thereby reducing the time for family production. In this method, reconstruction of the pedigree to monitor inbreeding is required. In **chapter 2**, we evaluated two different methods to re-construct the pedigree using two types of molecular markers, microsatellites and Single Nucleotide Polymorphisms (SNPs). Parental assignment was conducted using an exclusion-based (Vitassign) and a likelihood-based (Cervus) method. For the experiment, G10 parents were divided into 4 groups (cohorts) and allowed to produce offspring by natural group mating. In total, 173 offspring were tested against 238 parents, using either 12 microsatellites or 122 SNPs. Missing parents (13.8%) and relatedness among candidate parents resulted in low assignment rates, irrespective of method or the marker type used. Low exclusion power of the microsatellite set resulted in low assignment rates and multiple parent pair assignments when offspring were

assigned to parents in the same cohort. Using Vitassign and SNPs (up to 15 mismatches allowed), 83.8% offspring got unique assignments, 13.9% got multiple assignments, and only 2.3% were not assigned. Assignment rates were higher when cohort offspring were assigned to all parents combined, irrespective of method (Vitassign or Cervus) or the marker type used. Between markers, consistency of assignments was low: 28% with Vitassign and 16% with Cervus respectively. Between methods, consistency of assignments was high with SNPs (65%), but was low with microsatellites (31%). It was concluded that exclusion methods and likelihood-based methods can be equally good for parental assignments, providing that good marker sets with high exclusion power are available and that all parents are sampled.

In **chapter 3**, we investigated the hypothesis that prolonged family production is a consequence of selection for harvest weight in Nile tilapia. We estimated genetic parameters for 'spawning success' and 'time to spawn', and their genetic correlations with harvest weight in a selected population of Generation (G) 12. Two mating systems, namely 'multiple male, multiple female' group mating (7 males and 15 female) and 'single male, multiple female' mating (1 male and 10 females), were compared. In both experiments 85% of the females spawned within 20 days. For 'spawning success', estimates for heritability, repeatability and genetic correlations were consistent between linear and logit models. The heritability estimate was 0.20 to 0.22 for a linear model and 0.14 to 0.18 for a logit model with thresholds from 20 to 32 days. Genetic correlations of 'spawning success' with harvest weight ranged from 0.48 to 0.52 for thresholds of 20 to 32 days. Heritability estimates for 'time to spawn' were not different from zero (0.01 to 0.02). We conclude that Nile tilapia favour mating in groups, and that selection for harvest weight in GIFT should improve spawning success of Nile tilapia, provided that the mating period is limited to 20-32 days.

In **chapter 4**, we estimated genetic parameters for fecundity and fertility traits, and their correlation with harvest weight using females from G12. Heritability estimates for fecundity traits were low, ranging from 0.02 to 0.08. Heritability estimates for fertility traits were also low, ranging from 0.06 to 0.12. Genetic correlations for harvest weight with number of eggs and total egg weight were positive (0.51 and 0.42, respectively), while correlations for harvest weight with relative fecundity, egg weight, and egg dimension were negative (-0.72, -0.48, and -0.50, respectively). The same trend was observed for body weight at spawning. Genetic

correlations between harvest weight and fertility traits were all moderate to high (0.46 to 0.69), except for fertilisation rate (0.15). Genetic correlations between body weight at spawning and fertility traits were higher (0.69 to 0.93). We conclude that both harvest weight and body weight at spawning have favourable genetic correlations with number of eggs, relative fecundity, and number of swim-up fry, which are the desired characteristics for Nile tilapia seed production. However, Nile tilapia females selected for large harvest weight tend to produce smaller and lighter eggs.

In the Mekong Delta of Vietnam, Nile tilapia intensive river-cage culture and low-input VAC ponds are the most important production environments. Growth rate and body shape of fish are the main traits of interest to grow-out producers. In **chapter 5**, with data from G13, we estimated heritability and phenotypic and genetic correlations for harvest weight, daily growth coefficient, condition factor, and shape expressed as ellipticity. We also estimated genotype by environment interactions between the nucleus and two production environments, cage and VAC for these traits. Within the breeding nucleus, heritability was high for harvest weight (0.55) and daily growth coefficient (0.47), but low for condition factor (0.04) and ellipticity (0.08 to 0.14). Genetic correlations of daily growth coefficient with ellipticity showed that fish selected for high growth rate will become more rotund rather than simply larger. Genotype by environment interaction was minor for harvest weight (0.86 to 0.94) and for daily growth coefficient (0.77 to 0.95), but substantial for ellipticity (0.51 to 0.90). We conclude that selection in nucleus ponds will produce desired correlated responses in Nile tilapia grown in river-cages as they are expected to develop a more rotund and thicker body shape at the same length compared to fish grown out in ponds.

In chapter 6, we discuss different ways to improve the GIFT breeding program. First, using 'single male, multiple females' mating could shorten the time for family production by about 2 months compared to single pair mating. This will reduce the generation interval by 2 months; thereby increasing genetic gain by about 20%. Second, a rotational mating scheme, with at least 4 cohorts, can be incorporated into the GIFT breeding scheme to further reduce inbreeding, to estimate pond effects and to secure the breeding material. Third, a reliable multiplier system is important for a sustainable Nile tilapia breeding program. Assuming that multipliers buy genetically improved fry for a one-time, premium payment, with this system, the RIA2 GIFT breeding program can provide sufficient improved fry (>50 million per year) for the whole Mekong Delta cage-culture production.

Samenvatting

Samenvatting

Het doel van het in dit proefschrift beschreven onderzoek was het verbeteren van het fokprogramma voor Nijl tilapia, uitgevoerd door het onderzoeksinstituut voor aquacultuur 'RIA2' in the Mekong delta van Vietnam. Dit fokprogramma is een extensie van het GIFT (Genetically Improved Farmed Tilapia) programma, uitgevoerd door het World Fish centrum in Maleisië. Het GIFT fokprogramma is gebaseerd op BLUP fokwaarde schattingen en gebruikt hiervoor individuele informatie en stamboom informatie van verwanten. Een essentieel onderdeel van het GIFT programma is het paringssysteem, waarbij 1 mannetje achtereenvolgens met 2 vrouwtjes gepaard wordt zodat de ouders van de nakomelingen exact bekend zijn. Elke familie wordt vervolgens apart opgekweekt tot een gewicht waarop ze individueel gemerkt kunnen worden zodat individuele prestaties aan stamboom gegevens gekoppeld kunnen worden. Op deze manier heeft elk individu naast full-sib familie informatie ook paternale half-sib familie informatie en kan de fokwaarde optimaal geschat worden.

Het GIFT fokprogramma heeft geresulteerd in een aanzienlijke genetische verbetering van het gewicht op slachtleeftijd. Een groot probleem in het uitvoeren van het programma is echter de tijd die nodig is voor de reproductie en de productie van de families, die soms wel 3-4 maanden kan bedragen. Hierdoor worden de fokwaarde schattingen onnauwkeuriger en neemt het generatie interval toe, wat de genetische vooruitgang per jaar nadelig beïnvloedt. In dit proefschrift wordt veronderstelt dat de langdurige reproductie periode een gevolg zou kunnen zijn van langdurige en eenzijdige selectie op slachtgewicht. Een tweede, alternatieve, hypothese die onderzocht wordt is dat de problemen met voortplanting een gevolg kunnen zijn van het niet kunnen uiten van het natuurlijke paaigedrag van de Nijl tilapia.

In dit proefschrift worden twee fokprogramma's vergeleken. Het eerste is het klassieke GIFT programma, waarbij gebruik wordt gemaakt van de methodologie, zoals oorspronkelijk beschreven door het Worldfish Centrum in de GIFT manual. Met dit programma zijn vier generaties van selectie uitgevoerd. Het alternatieve fokprogramma was gebaseerd op rotatie kruising, natuurlijke voortplanting, en selectie op eigen prestatie (dus zonder stamboom informatie). Met dit programma werden drie generaties van selectie uitgevoerd. In beide gevallen was het uitgangsmateriaal hetzelfde: ouderdieren van generatie 10 afkomstig uit het GIFT fokprogramma van World Fish in Maleisië.

Het doel van het rotatie kruisingsschema was om de natuurlijke condities voor voortplanting van de Nijl tilapia na te bootsen zodat de tijd benodigd voor voortplanting verkort zou kunnen worden. Om de inteelt te beperken is echter stamboom reconstructie van de ouders nodig. In hoofdstuk 2 worden twee methoden geëvalueerd om de stamboom te reconstrueren: "Vitassign", een methode gebaseerd op uitsluiting op basis van mendeliaanse overerving, en "Cervus", een methode gebaseerd op statistische waarschijnlijkheid (likelihood). Tevens werden twee typen DNA merkers vergeleken: een set van 96 SNP's (single nucleotide polymorfisme) en een set van 12 microsatellieten. Voor het experiment werden ouders van generatie 10 (G10) verdeeld over vier groepen welke vervolgens door natuurlijke groepsvoortplanting nakomelingen produceerden. Deze werden opgekweekt tot slachtgewicht waarna uit elke groep de zwaarste dieren werden geselecteerd als ouder voor de volgende generatie. In totaal werden 173 van deze nakomelingen getest tegen 238 mogelijke G10 ouders. Ontbrekende ouders (13.8 %) en verwantschap tussen mogelijke ouders resulteerden in lage percentages toewijzing, ongeacht het type merker of de gebruikte methode. De set van 12 microsatellieten had een te laag onderscheidingsvermogen waardoor meerdere ouders aan dezelfde nakomeling konden worden toegewezen. Binnen groepen werd het beste resultaat bereikt met Vitassign en de SNP set: van alle nakomelingen kreeg 83 % 1 uniek ouderpaar toegewezen, 13.9 % kreeg meerdere mogelijke ouders toegewezen, terwijl bij 2.3 % van de nakomelingen geen ouderpaar kon worden gevonden. Als de ouders van de nakomelingen uit alle groepen werden samengevoegd werden de toewijzings-% beter, ongeacht de methode of merker type. De consistentie van toewijzing van ouders bij vergelijking van beide merker typen was laag: 28% met Vitassign en 16% met Cervus. Beide methoden gaven in 65% van de gevallen dezelfde ouderschap toewijzing wanneer SNP merkers werden gebruikt. Bij gebruik van microsatelliet merkers was de overeenstemming nog steeds laag (31%). De conclusie van het onderzoek is dat beide methoden goede resultaten kunnen geven mits er goede merkers gebruikt worden en alle potentiele ouders gesampled zijn.

In hoofdstuk 3 onderzochten we de hypothese dat de lange duur om families te produceren een consequentie was een gebrekkig reproductie vermogen van GIFT tilapia, veroorzaakt door een langdurige en eenzijdige selectie op slachtgewicht. Om dit te onderzoeken werden de genetische parameters voor de kenmerken "paai succes", en "tijdsduur tot paaien" en de genetische correlaties met slachtgewicht in vrouwtjes van generatie G12 geschat. Hierbij werden twee groepspaai systemen vergeleken: 15 vrouwtjes en 7 mannetjes (15/7) in een paai tank of 10 vrouwtjes en 1 mannetje (10/1) in een paai tank. In beide systemen

paaiden 85 % van de vrouwtjes binnen 20 dagen af. Schattingen van de genetische parameters voor "paai succes" waren consistent ongeacht de gebruikte methode, een logit model of een lineair model. Schattingen van de erfelijkheidsgraad varieerden van 0.14 tot 0.18 (logit model) en van 0.20 tot 0.22 (lineair model). Hierbij varieerde de drempelwaarde van 20 tot 32 dagen. Genetische correlaties tussen paai succes en slachtgewicht varieerden van 0.48 tot 0.52 bij drempelwaarden van 20 tot 32 dagen. In tegenstelling tot "paai succes" waren de schattingen voor "tijdsduur tot paaien" niet verschillend van nul (0.01-0.02). We concluderen dat Nijl tilapia vrouwtjes zich goed voortplanten in groepen, ongeacht het aantal mannetjes, en dat selectie op slachtgewicht in de GIFT populatie tot een positieve gecorreleerde respons in paai succes zou moeten leiden, mits de paai duur beperkt wordt tot 20-32 dagen.

In hoofdstuk 4 werd dezelfde experimentele data set uit hoofdstuk 3 gebruikt om genetische parameters voor fecunditeit en fertiliteit en de correlatie met slachtgewicht van vrouwelijke Nijl tilapia te schatten. Schattingen van de erfelijkheidsgraad voor fecunditeit kenmerken waren laag, variërend van 0.02 tot 0.08. Schattingen van de erfelijkheidsgraad voor fertiliteit kenmerken waren minder laag, tussen 0.06 en 0.12. Genetische correlaties tussen slachtgewicht en "aantal eieren" en "totaal gewicht aan eieren" waren positief (0.51 en 0.42 respectievelijk) terwijl correlaties tussen slachtgewicht en relatieve fecunditeit, ei-gewicht en ei-diameter negatief waren (respectievelijk -0.72, -0.48 en -0.50). Dezelfde trend werd waargenomen bij correlaties tussen deze kenmerken en "lichaamsgewicht bij afpaaien".

Genetische correlaties tussen slachtgewicht en fertiliteit kenmerken waren redelijk sterk (0.49-0.69) met uitzondering van bevruchtings-% (0.15). Genetische correlaties tussen "lichaamsgewicht bij afpaaien" en fertiliteit waren hoog (0.69-0.93). We concluderen dat de genetische correlaties tussen slachtgewicht en aantal eieren per paai, relatieve fecunditeit en aantal larven per paai positief zijn en dat selectie op slachtgewicht geen negatieve gevolgen voor deze kenmerken zal hebben. Selectie op slachtgewicht zal echter wel resulteren in de selectie van vrouwtjes die kleinere eieren zullen produceren.

In de Mekong delta van Vietnam wordt Nijl tilapia vooral gekweekt in kooien in de Mekong rivier en in kleine vijvers op geïntegreerde bedrijven (VAC). Groei en lichaamsvorm zijn de voornaamste kenmerken voor de producenten van tilapia. Terwijl teelt in kooien zeer intensief is met hoge dichtheden en energierijk voer, is de teelt in VAC boerderijen juist laag productief, met laagwaardig voer, in combinatie met natuurlijke productie van voedsel in de vijvers zelf. Dit roept de vraag op of tilapia, geselecteerd op het proefstation (RIA2) in intensieve vijvers ook

optimaal zullen presteren in kooien of in laag productieve VAC vijvers (de commercieel relevante milieus). In hoofdstuk 5 beschrijven we een experiment waarbij vissen van generatie 13 in 3 verschillende milieus werden opgekweekt: op vijvers van het proefstation, in kooien, en op VAC vijvers. Voor de vissen, opgekweekt op het proefstation werden voor de kenmerken slachtgewicht, groei, lichaamslengte, conditiefactor en lichaamsvorm, uitgedrukt als ellipticiteit, erfelijkheidsgraden en genetische correlaties tussen kenmerken geschat. De mate van "genotype-milieu interactie" (GxE) werd voor elk kenmerk geschat aan de hand van de genetische correlaties gemeten tussen de drie milieus.
De erfelijkheidsgraad voor groei en slachtgewicht was hoog (resp.0.47 en 0.55). Schattingen voor conditiefactor en ellipticiteit waren laag (0.04 en 0.08-0.12). Schattingen van genetische correlaties tussen groei en ellipticiteit lieten zien dat vissen geselecteerd op hoge groeisnelheid niet alleen langer worden maar ook ronder. Er was weinig GxE getuige de hoge genetische correlaties voor slachtgewicht (0.86-0.94) en groei (0.77-0.90). Er was echter substantiële GxE voor lichaamsvorm, gemeten als ellipticiteit (0.51-0.90). We concluderen dat selectie op groei en slachtgewicht in het nucleus milieu sterk gecorreleerd is met prestatie in kooien en VAC vijvers. Tevens zullen vissen in kooien een rondere en dikkere lichaamsvorm ontwikkelen in vergelijking met vissen, opgekweekt in vijvers.
In hoofdstuk 6 worden een aantal manieren besproken om het GIFT fokprogramma te verbeteren. De voortplanting van tilapia kan met 2 maanden verkort worden als een paaisysteem van 1 man met 5-10 vrouwtjes gebruikt wordt in plaats van het huidige systeem waarbij 1 man steeds met 1 vrouwtje gepaard wordt. Door de verkorting van het generatie interval zal de genetische respons met 20% per jaar toenemen. Door het gebruik van rotatie kruising, met ten minste 4 groepen, kan de inteelt verder beperkt worden. Daarnaast is het gebruik van meerdere groepen, mits opgekweekt in verschillende vijvers ook een goede manier om vijver effecten te schatten en hiervoor te corrigeren in de fokwaarde schattingen. Daarnaast biedt het gebruik van meerder vijvers extra bescherming tegen calamiteiten.
Elk fokprogramma heeft een goed vermeerderings- en distributie netwerk nodig om te zorgen dat het genetisch verbeterde materiaal ook bij de producenten komt. Berekeningen laten zien dat een systeem waarbij vermeerderaars van elke generatie van selectie broedvissen van het proefstation kopen, de gehele Mekong delta tilapia kooi teelt van vislarven kan voorzien (> 50 miljoen per jaar).

Publications

Publications

Trịnh Quốc Trọng, Nikkie van Bers, Richard Crooijmans, Bert Dibbits, Hans Komen, 2013. A comparison of microsatellites and SNPs in parental assignment in the GIFT strain of Nile tilapia (*Oreochromis niloticus*): The power of exclusion. Aquaculture 338-391: 14-23.

Trịnh Quốc Trọng, Han A. Mulder, Johan A.M. van Arendonk, Hans Komen. Heritability and genotype by environment interaction estimates for harvest weight, growth rate, and shape of Nile tilapia (*Oreochromis niloticus*) grown in river cage and VAC in Vietnam. Aquaculture 384-387: 119-127.

Trịnh Quốc Trọng, Johan A.M. van Arendonk, Hans Komen. Genetic parameters for reproductive traits in Nile Tilapia (*Oreochromis niloticus*): I. Spawning success and time to spawn. Submitted for publication in Aquaculture.

Trịnh Quốc Trọng, Johan A.M. van Arendonk, Hans Komen. Genetic parameters for reproductive traits in Nile tilapia (*Oreochromis niloticus*): II. Fecundity and fertility. Submitted for publication in Aquaculture.

Conference proceedings

A rotational mating cohort for Nile tilapia in the Mekong Delta of Vietnam. Asia Pacific Aquaculture conference (World Aquaculture Society chapter conference), Ha Noi, Vietnam, August 5-8, 2007.

A comparison of microsatellites and SNPs in pedigree reconstruction of GIFT strain of Nile tilapia (*Oreochromis niloticus*). The International Symposium Genetics in Aquaculture XI, Auburn, Alabama, USA, June 25- 29, 2012.

Heritability estimates and genotype by production environment interaction for body weight, growth rate and shapes of Nile tilapia (*Oreochromis niloticus*) in the Mekong Delta of Vietnam. AQUA 2012, Prague, Czech Republic, September 01-05, 2012.

About the author

Curriculum vitae

Trịnh Quốc Trọng was born 3rd of January 1976 and raised in Hồ Chí Minh City, Viet Nam. He studied for his bachelor degree at the University of Agriculture and Forestry in Thủ Đức, Hồ Chí Minh City where he specialized in aquaculture, and conducted his thesis on masculinisation of Siamese fighting fish (*Betta splendens*) and successfully obtained the degree of Aquaculture Engineer in 1998. After graduation Trong joined the Research Institute for Aquaculture No. 2 (RIA2) in April 1999. In 2002, he started a master education at Agriculture University of Norway (NLH), Ås, Norway (now known as the Norwegian University of Life Science, UMB), with sponsorship from the Norwegian government and specialized in intensive fish culture. His master thesis was on the simulation of fish breeding programs comparing "walkback" selection and within-family selection schemes. He graduated in 2004 and returned to work at RIA2. In 2007, he commenced a sandwich PhD study on breeding programs for Nile tilapia (*Oreochromis niloticus*) at the Animal Breeding and Genomics Centre, Wageningen University, in the Netherlands, with funding from Wageningen University. All experiments were done at the National Breeding Centre for Southern Freshwater Aquaculture in the Mekong Delta of Viet Nam, under the auspices of RIA2, with funding for field work from the WorldFish Center, in Penang, Malaysia. The results of his PhD research are described in this thesis. Currently, Trong is working as a director of the National Breeding Centre for Southern Freshwater Aquaculture in Cái Bè, Viet Nam. Trong is happily married to Thu Hường and has a daughter named Thu Phương, and a son named Khôi Nguyên.

Training and Education

Training and education

	Year	Credits
The Basic Package (3 ECTS)		
WIAS Introduction Course	2009	1.5
Course Ethics and Philosophy of Animal Science	2009	1.5
Scientific Exposure (9.6 ECTS)		
International conferences		
Asia Pacific Aquaculture conference (World Aquaculture Society chapter conference), Ha Noi, Vietnam, August 5-8, 2007	2007	1.2
The International Symposium Genetics in Aquaculture XI, Auburn, Alabama, USA, June 25- 29, 2012	2012	1.5
AQUA 2012, Prague, Czech Republic, September 01-05, 2012	2012	1.5
Seminars and workshops		
Long-term response to selection and QTL-mapping, Wageningen, the Netherlands, April 27, 2007	2007	0.2
Current Theme in Ecology 12: Darwinian agriculture: the evolutionary ecology of agriculture symbiosis, Wageningen, the Netherlands, April 13, 2007	2007	0.2
Presentations		
A rotational mating cohort for Nile tilapia in the Mekong Delta of Vietnam, September, 2007, Ha Noi, Vietnam, oral	2007	1.0
A comparison of microsatellites and SNPs in pedigree reconstruction of GIFT strain of Nile tilapia (*Oreochromis niloticus*), June 26, 2012, Auburn University, Auburn, Alabama, USA, oral	2012	1.0
Heritability estimates and genotype by production environment interaction for body weight, growth rate and shapes of Nile tilapia (*Oreochromis niloticus*) in the	2012	1.0

Mekong Delta of Vietnam, September 03 2012, AQUA 2012, Prague, Czech Republic, oral		
A comparison of microsatellites and SNPs in pedigree reconstruction of GIFT strain of Nile tilapia (*Oreochromis niloticus*), WIAS Science Day 2012, Wageningen, poster	2012	1.0
Selective breeding for Nile tilapia in the Mekong Delta of Vietnam, December 23, 2008, Scientific Workshop, Research Institute for Aquaculture No. 2, Vietnam, oral	2008	1.0

In-Depth Studies (23.5 ECTS)		
Advanced statistics courses		
Modern Statistic for the Life science	2009	6.0
WIAS Course Design of Animal Experiments	2008	1.0
WIAS course Statistics for the Life Science	2012	2.0
Fish Immunology Workshop, WIAS, Wageningen, the Netherlands, April 22-26 2012	2012	1.5
PhD students' discussion groups		
Quantitative Discussion Group, ABG	2007 - 2012	1.0
MSc level courses		
Genetics improvement of livestock	2008	6.0
Population and quantitative genetics	2007	6.0

Professional Skills Support Courses (2.6 ECTS)		
Course Techniques for Writing and Presenting a Scientific Paper	2012	1.2
Information Literacy PhD including EndNote Introduction	2012	0.7
Effective behaviour in your professional surroundings	2012	0.7

Research Skills Training (0.9 ECTS)	year	credits
Getting started in AS-Reml	2009	0.3
Introduction to R for Statistical Analysis	2012	0.6

Didactic Skills Training (8 ECTS)	year	credits

Lecturing		
MSc course "Selective Breeding in Aquaculture", 2010 and 2011, 5 days/course	2010, 2011	1.0
Supervising practicals and excursions		
Pratical and excursion for aquaculture students at Cai Be, Vietnam	2007 - 2011	1.0
Supervising theses		
Two MSc.	2008 - 2011	4.0
Two BSc.	2008 - 2011	2.0

Management Skills Training (3 ECTS)

Membership of boards and committees		
Member of Research Insittue for Aquaculture No. 2 Scientific Board	2007 - to date	3.0

Training and education total **50.6 ECTS**

Acknowledgements

Acknowledgements

The thesis work was conducted at the Research Institute for Aquaculture No. 2 (RIA2) in Viet Nam, and at the Animal Breeding and Genomics Centre, in the Netherlands, in collaboration with the WorldFish Center, Penang, Malaysia. Many people helped me along the way; I would like to thank some specifically by name.

The project idea was formed during a visit of my co-promoter, Dr. ir. Hans Komen, to RIA2 in 2006. Hans initiated a sandwich PhD application the same year, and in March 2007 I commenced my PhD project in Wageningen. Hans has always been the architecture of the project since. I am extremely grateful for his enthusiasm, inspiration, and guidance. Thanks to my promoter, Prof. Johan van Arendonk for believing in me and keeping a bird's-eye view on my project, and for his overall support. Dr. Raul Ponzoni and Dr. Nguyễn Hồng Nguyên of the WorldFish Center provided the base population (GIFT 10 in Penang) in 2006 and financial support for field work from 2007 to date. I am grateful for their kind support over the years. Thanks to my managers (Dr. Nguyễn Văn Hảo and Dr. Nguyễn Văn Sáng) and all my colleagues at RIA2, especially my tilapia team-mates: Khoa, Phúc, Đỉnh, Tùng, and Minh.

During the first two year of my project, Dr. ir. Henk Bovenhuis was my co-supervisor, and I thank him for his guidance. Thanks to Piter Bijma for his useful instructions. Thanks to the co-authors of my papers: Nikkie, Richard, Bert, and Han (Mulder). Thanks to Hamed and Atal for their help with genotyping of my tilapia blood samples. Thanks to my office-mates: Robbert, Anouk, Panya, Han, Gabriel, Hamed, and to all ABG colleagues. To my paranymphs Albart and Robbert, many thanks. To the Aquaculture and Fisheries group, thanks to Roel, Marc, and Johan Verreth. Thanks to Ms. Marianne Bruining for her advise with my TSP. Thanks to Ms. Marion Rodenburg for her kind help with the paper work. Thanks to Ms. Ada, Lisette, and Monique for their kind help with paper work, logistics, and finalisation of my thesis. Thanks to Gerry and Joop for accommodating me at the Nude 58.

My friend Don Griffiths proof-read the General Introduction, General Discussion, and Summary sections. I am grateful for his help. Thanks to my brother-in-law Trần Công Thuận for his help with the design of the cover, and for Figure 5.1 in Chapter 5.

To my kids, and to my wife who have helped me in many ways, I am deeply grateful.

Colophon

The research described in this thesis was financially supported by Wageningen University, the Netherlands and the WorldFish Center, Penang, Malaysia.

This thesis was printed by GVO drukkers & vormgevers B.V. | Ponsen & Looijen, Ede, the Netherlands.