Cooperative and Uniform Fish?

Hooi Ling Khaw

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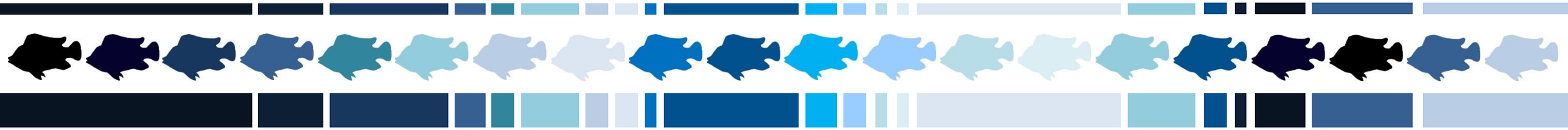

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Hooi Ling Khaw

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Abstract

Khaw, HL. (2014). Cooperative and uniform fish? Social interactions and variability in live body weight in the GIFT strain (Nile tilapia, *Oreochromic niloticus*) in Malaysia. PhD thesis, Wageningen University, the Netherlands.

Social interactions are present everywhere in the living world. Such social interactions may lead to indirect genetic effects (IGE), which are heritable effects of an individual on trait values of the other individuals its interacts with. IGEs may affect the direction and magnitude of response to selection in breeding programs. Moreover, social interactions may affect variability of traits. In aquaculture, competition for resources inflates size variation within populations. In this thesis, we used the Genetically Improved Farmed Tilapia (GIFT; *Oreochromis niloticus*) strain to investigate the genetic basis for social interactions and variability in harvest weight for tropical finfish. Social interaction experiments were established for quantifying the genetic and non-genetic indirect effects on harvest weight in the GIFT strain. We found evidence for IGEs on harvest weight, and a negative direct-indirect genetic correlation, which suggesting heritable competitive interactions for harvest weight in GIFT. Hence, breeding schemes may need to be adapted to avoid an increase in competition. A stochastic simulation study was conducted to examine the effect of BLUP selection on the rate of inbreeding for socially affected traits. The rates of inbreeding for scenarios with IGEs were greater than for scenarios without IGE. Therefore, with IGEs there is a greater need for a selection algorithm that restricts the increase of mean kinship. In aquaculture industry, there is a wide range of commercial production environments, which may leads to genotype by environment (GxE) interaction, for example due to differential social interactions. The GIFT fish were tested in ponds and cages to study the GxE interaction. The genetic correlations between environments (0.73 to 0.85, for harvest weight and body measurements) indicate little GxE-interaction. The data collected from the social interaction experiments were also used to investigate the presence of genetic variation in uniformity for harvest weight. The genetic coefficient of variation for standard deviation of harvest weight (0.17) shows that uniformity of harvest weight is heritable and can be increased by selective breeding. In the General Discussion of this thesis, the uniformity study was extended to incorporate IGE. The result indicates that more cooperative fish are not necessary more uniform for harvest weight. Overall, our results suggest that genetic improvement in fish breeding programs can be increased by accounting for social interactions.

Contents

1

General Introduction

1.1. Introduction

1.1.1. Global aquaculture situation

By 2050, we need to produce enough food to feed 9 billion people. Aquaculture could be one of the solutions in meeting the demand. Within a decade, aquaculture increased its contribution to the world fish production from 25.7% to 42.2% in 2012 [FAO, 2014a]. Seventy four percent of world aquaculture production is destined to food fish, namely 66.6 million tonnes [FAO, 2014b]. Food fish includes fin fishes, molluscs, amphibians, freshwater turtles and other aquatic animals used as food for human consumption. Aquatic algae and non-food products (for examples, pearls and shells) make up the other 26% of the world aquaculture productions. This is the first time aquaculture food fish production exceeded the world beef production [57.62 million tonnes; USDA, 2014]. Out of these 66.6 million tonnes of food fish, 61.6% takes place in freshwater (Figure 1.1).

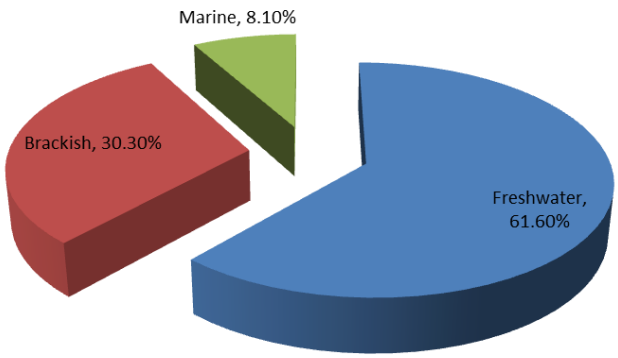

Figure 1.1 Percentage food fish production by water bodies for year 2012 [FAO,2014a].

Inland aquaculture of herbivorous and omnivorous finfish species is the greatest contributor to production [FAO, 2014b]. Due to the increasing demand for healthy and affordable animal protein sources, the freshwater aquaculture industry is running into space and resources constraints [Gjedrem *et al.*, 2012]. Over 70% of the Earth's surface is covered by water and only 1% of that is freshwater that can be used in aquaculture (another 1.5% is currently frozen). To meet the food demand and simultaneously preserve the environment, sheer expansion should be avoided. Instead, more efficient, improved technologies to increase aquaculture production are required. One of the most promising approaches is through genetic

technologies, such as selective breeding programs to develop more productive strains of aquaculture species.

1.1.2. Tilapia and the GIFT strain

Tilapia, native to Africa, is the most widely cultured species in the world. It currently cultured in about 140 countries and territories [FAO, 2014b]. Tilapia is popular for its hardiness, it is easy to breed and tolerant to a wide range of water qualities and temperatures. Back in the 1970s tilapia was labeled as the "aquatic chicken", and at the beginning of the new millennium, it was dubbed as "food fish of the 21st century" [Maclean, 1984; Shelton, 2002]. In 2012, the world tilapia production was about 4.5 million tonnes. Among all the tilapia species, Nile tilapia (*Oreochromis niloticus*) accounts for about 71% of the world production (Figure 1.2).

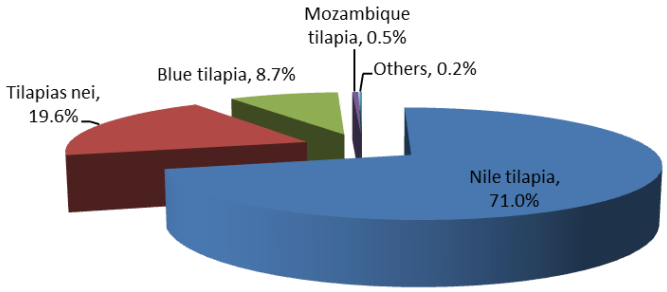

Figure 1.2 World tilapia production by species, in percentage, for year 2012; Tilapias *nei* stands for incompletely identified tilapia species [FAO,2014a].

In the late 1970s, WorldFish (formerly known as International Center for Living Aquatic Resources Management, ICLARM) and its partners found that the expansion and intensification of tilapia farming was faced with an inadequate seed supply and a deteriorating growth performance in many aquaculture systems [Ponzoni *et al*., 2010b]. In response to these problems, in 1987, the status of tilapia genetic resources was reviewed by the team. A year later, WorldFish and its partners from Philippines and Akvaforsk (now known as Nofima) from Norway, together designed a program with the aim of "developing a methodology for the genetic improvement of tropical finfish, using Nile tilapia as test species". This undertaking signaled the birth of the Genetically Improved Farmed Tilapia (GIFT) project, funded by the United Nations Development Programme and the Asian

Development Bank. Details about the establishment of the GIFT project and its genetic progress during its first phase can be found in Gupta and Acosta [2004], and Acosta and Gupta [2010]. After the project ended in 1997, a sample of fish from 63 families of the GIFT strain were received by WorldFish. The fish were later transferred to Malaysia when WorldFish headquarters moved to Penang, Malaysia in 2000. Refer to Ponzoni *et al.* [2005, 2010a] for details about the GIFT strain in Malaysia.

GIFT is one of the most successful examples of a traditional selective breeding program for tropical finfish. It is well known for its high performance [for example, Nguyen *et al.*, 2011]. In the GIFT population received by WorldFish in Malaysia, selection for live weight at harvest time has continued to further improve the growth rate. Figure 1.3 shows the cumulative genetic gain after nine generations of selection in Malaysia. Since 1993, GIFT has been disseminated to more than 10 different countries in Asia and South America [Ponzoni *et al.*, 2010b]. Recently, a sample of 60 families of GIFT from Malaysia was sent to Africa for evaluation purposes. The final aim of this transfer is to disseminate this unique International Public Goods around its continent of origin.

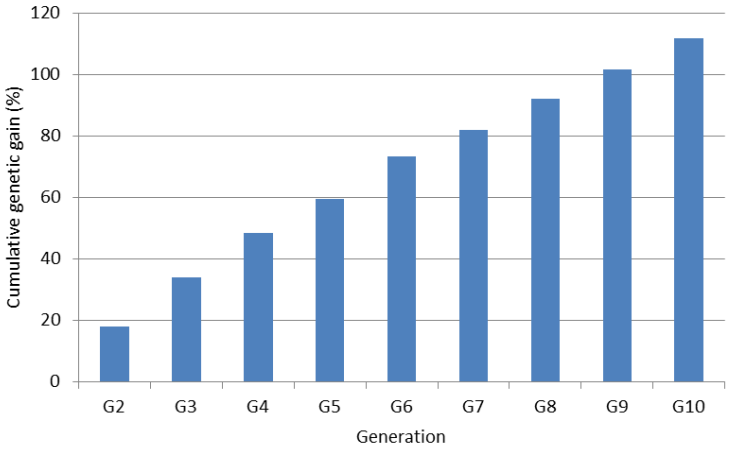

Figure 1.3 Cumulative genetic gain in estimated breeding value (as a percentage of the base population) from generation two to 10 in Malaysia (Unpublished data owned by WorldFish).

1.1.3. Social interaction

Social interactions are the acts, actions or practices of two or more people or animals mutually oriented towards each other's selves [Rummel, 1976]. Social

interactions are present everywhere in the world, including wild and domesticated animals in terrestrial and aquatic environments. Competition is a type of social interaction and it is common in aquaculture environments. It inflates variation in size when the animals compete for resources in the culture environment. In addition, competition also causes a reduction in productivity and it is harmful to animal wellbeing. There is evidence that fish selected for rapid growth rate may be more aggressive and competitive [Lahti *et al.*, 2001; Ødegård and Olesen, 2011].

In general, the coefficient of variation (CV) for live weight in Nile tilapia is around 40 to 60% [Ponzoni *et al.*, 2005; Nguyen *et al.*, 2007; Khaw *et al.*, 2010], which is relatively large compared to values reported in livestock [CV ranging between 7 and 10%; Gjedrem, 1998; Damgaard *et al.*, 2003; Mulder *et al.*, 2009; Wolc *et al.*, 2009]. An increase in CV may indicate inter-individual competition and dominance hierarchy [Jobling, 1995; Adams *et al.*, 2000]. By contrast, a lower CV, or a decrease in CV, may be indicative of less competition and of a good social environment for the animal [Jobling, 1995; Mambrini *et al.*, 2006]. Competition is also an important environmental factor that leads to genotype by environment interactions [James, 2009].

1.1.4. Indirect genetic effects

Within a population, the animals' performance is not solely affected by their genetic makeup. It is also influenced by the environment where the animals grow and socially interact with each other [Waddington, 1960; Hill *et al.*, 2007]. Measuring social interactions and behavioral traits is difficult and expensive. However, in recent decades, scientists and animal breeders have been placing a considerable amount of effort in developing recording techniques, experimental designs and statistical procedures to analyze data on socially affected traits. As a consequence, there have been both major empirical and theoretical contributions [for example, Griffing, 1967; Moore *et al.*, 1997; Muir, 2005; Bijma, 2010, 2014].

The genotype of an individual may affect the trait values of other individuals it interacts with [Griffing, 1967; Muir, 1996]. This heritable effect is known as indirect genetic effect (IGE). Because of their genetic basis, IGEs may affect the direction and magnitude of selection response and the amount of heritable variation available for response to selection [Griffing 1967; Bijma, 2011]. In many livestock species, scientists and animal breeders have shown that implementing a selection strategy that accounts for both direct and indirect genetic effects can increase the

response to selection for socially affected traits [for example, Griffing, 1976; Muir, 1996, 2005; Ellen *et al.*, 2007].

A well-known example of the presence of IGEs is the study by Muir [1996] on cannibalism in laying hens. In this study, Muir [1996] conducted a selection experiment for egg production in cannibalistic laying hens using group selection. He managed to increase egg production from 91 to 237 eggs per hen, largely as result of improved survival. This showed that the survival of an individual depends on its cage mates' genotype for pecking behavior. In addition, there have been successful selection experiments on socially affected traits in quails [Muir and Schinckel, 2002], and flour beetles [Wade, 1976, 1977]. To our knowledge, there have only been two IGE studies in aquaculture species [Brichette *et al.*, 2001; Nielsen *et al.*, 2014).

1.2. Aim and outline of the thesis

Most of the tropical finfish breeding programs have focused on growth rate as their main breeding goal [Gjedrem *et al.*, 2012]. That is certainly the case for the GIFT breeding program in Malaysia, which (at the time of writing this thesis) has undergone 12 generations of selection for harvest weight. In view of its genetic progress and performance, GIFT served as a very good model to study the IGEs of socially affected traits. The large CV in GIFT suggests that there may be competition among individuals in the population. To investigate the prospects of reducing such competition and variability by means of genetic selection, the underlying knowledge of direct and indirect genetic effects of socially affected traits needs to be known. Hence, the main objective of this thesis was to investigate the underlying genetic basis for social interaction and variability in harvest weight in Nile tilapia.

In this thesis, we investigate competition and IGEs in four different but yet connected studies. A large-scale IGE experiment was established to examine the direct and indirect genetic effects for production traits in Nile tilapia. In **Chapter 2**, based on data collected from the experiment, we estimated the genetic and non-genetic indirect effects for harvest weight in the GIFT strain of Nile tilapia. We also conducted a bivariate analysis of harvest weight and survival, fitting different mixed models to investigate the presence of IGEs and other non-genetic effects.

Inbreeding is one the important issues in breeding programs. Its increase may cause a reduction of genetic variance and affect the sustainability of breeding programs on a long-term basis. In **Chapter 3**, we report on a stochastic simulation study was conducted to examine the effect of Best Linear Unbiased Prediction (BLUP) selection for socially affected traits on the rate of inbreeding. The study was conducted in the context of a fish breeding program, but the methodology and results are applicable to other species under a similar design.

In **Chapter 4**, we investigated the genotype by production environment (GxE) interaction between pond and cage culture systems for the GIFT strain in Malaysia. A bivariate animal model was used to estimate (co)variance components, by treating the homologous body traits in pond and cage culture systems as two distinct traits. The GxE interaction was quantified through the estimated genetic correlation between these two traits.

Using the data collected from the IGE experiment, in **Chapter 5**, we conducted a study on the uniformity of harvest weight in the GIFT strain. We investigated and quantified the genetic variation in variability of harvest weight, and the genetic correlation between harvest weight and variability of harvest weight in the GIFT strain of Nile tilapia. For parameter estimation we fitted a bivariate sire and dam model to harvest weight and standard deviation of harvest weight.

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2

Genetic and non-genetic indirect effects for harvest weight in the GIFT strain of Nile tilapia (*Oreochromis niloticus*)

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Submitted to Genetics Selection Evolution

Abstract

Background

Trait values of individuals are affected not only by their genetic makeup, but also by environmental factors and interactions with other individuals. The heritable effect of an individual on the trait values of other individuals it interacts with is known as an indirect genetic effect (IGE). Such IGEs may affect response to selection. Fish selected for high growth rate, for example, have been shown to be more aggressive and competitive, which may reduce the observed response in growth rate. The main objective of this study is to quantify the genetic and non-genetic indirect effects for harvest weight in the GIFT strain of Nile tilapia.

Methods

A total of 6330 fish with harvest weight information were used to estimate genetic and non-genetic parameters. A bivariate analysis of harvest weight and survival was conducted by fitting different mixed models to investigate the presence of IGEs and other non-genetic effects.

Results

The full set of genetic parameters could not be estimated simultaneously with the inclusion of maternal common environmental effects. Models without maternal common environmental effects showed significant IGE on harvest weight, which contributed 48% of total heritable variance. Models with maternal common environmental effects showed suggestive evidence for IGE. The direct-indirect genetic correlation for harvest weight was negative (-0.38±0.19), indicating that traditional selection will increase competition. A strongly negative genetic correlation between direct effects on survival and indirect effects on harvest weight (-0.79±0.30) showed that individuals with better genes for survival suppress growth rate of their social partners. The confounding between maternal environmental effects and genetic effects showed that the common one male to two females mating design in aquaculture has limited power to estimate genetic parameters.

Conclusion

Our results suggest that heritable competitive interactions affect harvest weight in Nile tilapia. Thus breeding schemes may need to be adapted to avoid an increase in competition due to selection for growth rate. The 1:2 mating ratio, which is common in aquaculture breeding programs for reasons of effective population size, limits the statistical power to distinguish between genetic and maternal common environmental effects.

2.1. Background

Trait values of individuals within a population are not only affected by the genetic makeup of individuals, but also by the environmental conditions where the animals develop and socially interact with others [Waddington, 1960; Hill *et al.*, 2007]. With social interactions, the genotype of an individual may affect the trait values of other individuals that it interacts with [Griffing, 1967; Muir, 1996; Moore *et al.*, 1997; Bijma, 2012]. In the past, such social interactions have been ignored by animal breeders. However, in recent decades, social interactions have received increased attention by both evolutionary biologists and animal breeders. This has been mainly due to the increased evidence of heritable effects of individuals on trait values of other individuals, a phenomenon known as indirect genetic effects [IGE; examples: van Vleck *et al.*, 2007; Ellen *et al.*, 2008; Wilson *et al.*, 2011; Peeters *et al.*, 2012; Muir *et al.*, 2013], coupled with advancements in genetic evaluation and statistical analysis for socially affected traits [Muir, 2005; Bijma, 2010; Bijma, 2014]. Because of their genetic basis, IGEs may affect the direction and magnitude of selection response and the amount of heritable variation available for response to selection [Griffing, 1967; Bijma, 2011; Muir *et al.*, 2013]. Investigating the magnitude of IGEs in livestock and aquaculture populations is therefore an important issue.

Competition is a type of social interaction that is very common in aquaculture environments, where fish are reared together in ponds, cages or tanks [Ellis *et al.*, 2002; Ashley, 2007; Volpato *et al.*, 2007]. During the past 30 years, selective breeding for aquaculture species has become increasingly important, producing quality seeds for the aquaculture industry [Gjedrem *et al.*, 2012]. However, empirical evidence suggests that fish selected for high growth rate may be more aggressive and competitive for resources [Lahti *et al.*, 2001]. As a consequence, size variation of fish reared in communal environments is inflated by aggression and competition. Besides causing a reduction in productivity, competition also negatively affects fish welfare in terms of stress, injuries and even death [Ashley, 2007; Volpato *et al.*, 2007]. Hence, phenotypic observations strongly suggest that competition inflates variability and reduces productivity and welfare in aquaculture species. Thus a reduction in competition would facilitate the joint improvement of productivity and welfare. To investigate the prospects to reduce competition and variability by means of genetic selection, knowledge of the direct and indirect genetic parameters underlying those traits is required. However, despite the strong phenotypic indications for competition, very little is known of IGEs in aquaculture [see Brichette *et al.*, 2001; Nielsen *et al.*, 2014].

World aquaculture production has increased at an average rate of 8.8% per annum, and Nile tilapia (*Oreochromis niloticus*) is one of the major freshwater cultured species in the world [FAO, 2012]. Genetically Improved Farmed Tilapia (GIFT) is an improved Nile tilapia strain that until now has undergone 12 generations of selection for growth rate in Malaysia, managed by WorldFish. The coefficient of variation (CV) for harvest weight in GIFT or Nile tilapia in general is around 40 to 60% [Ponzoni *et al.*, 2005; Nguyen *et al.*, 2007; Khaw *et al.*, 2010], which is considered large. Generally, an increase in the CV indicates inter-individual competition and dominance hierarchy [Jobling, 1995; Adam *et al.*, 2000]. Hence, the high CV suggests considerable competition in Nile tilapia. In order to reduce the competition and size variation in harvest weight of Nile tilapia or aquaculture species in general, we need to quantify and select for the IGEs on socially affected traits in those populations. Thus, the main objective of this study was to quantify the heritable variation for growth rate of GIFT, and the contribution of IGEs to this heritable variation. Here we describe the experiment conducted for this purpose, and present estimated parameters of genetic and non-genetic indirect effects on growth rate in the GIFT strain.

2.2. Methods

2.2.1. The environment and the fish

The social interaction experiment was initiated in year 2009 and conducted at the Aquaculture Extension Center (Department of Fisheries), located at Jitra in Kedah State of Malaysia. The first batch of experimental fish was produced from generation seven of the GIFT selection line in year 2009. The other three batches were produced in subsequent years, with fish from generation eight, nine and ten. All the batches were named after the year in which the fish were stocked in the experimental ponds. Table 2.1 shows the reproduction and management schedule for the four batches of the experiment. Refer to Ponzoni *et al.* [2005; 2010] for further details on selection and mating process in the GIFT breeding program.

2.2.2. The experimental design

The common strategy of mating one male to two females (nested mating design) in the GIFT breeding program was also used in the production of the fish for this experiment. The offspring were placed in groups, each consisting of two distinct families. This is the optimal group composition for estimating the indirect genetic

variance [Bijma, 2010]. To allocate the families in groups, we implemented a design with blocks composed of 11 full-sib families per block (Figure 2.1). For better statistical power of parameter estimation, the combination of two paternal half-sib families within the same block was avoided. With the block design, each family was combined precisely once with each of the other ten families in the block, yielding 55 different family-combinations per block. Each group consisted of 16 fish, with both families each contributing eight randomly selected progeny. Thus, for each experimental batch, 80 fish per family were needed. All the fish were individually identified with PIT (Passive Integrated Transponder) tags before stocking in the pond (the tagging size was 2 to 5 grams).

Family no.	12	34	39	42	65	69	85	100	111	121
5	5-12	5-34	5-39	5-42	5-65	5-69	5-85	5-100	5-111	5-121
12		12-34	12-39	12-42	12-65	12-69	12-85	12-100	12-111	12-121
34			34-39	34-42	34-65	34-69	34-85	34-100	34-111	34-121
39				39-42	39-65	39-69	39-85	39-100	39-111	39-121
42					42-65	42-69	42-85	42-100	42-111	42-121
65						65-69	65-85	65-100	65-111	65-121
69							69-85	69-100	69-111	69-121
85								85-100	85-111	85-121
100									100-111	100-121
111										111-121

Figure 2.1 Example of the block design for assignment of two families to each group.

Two earthen ponds of size 0.1 ha were used in this experiment, except in Batch 2010 for which only one pond was used. In Batch 2010 there was high mortality during nursing (fry were over-stressed by high temperature), so that there were not enough fry to fill two ponds. In each pond, an equal number of net-cages (sized 1m x 1.5m, and 1.0m depth) were installed (per pond: 182 units for Batch 2009; 144 units for Batch 2010; 171 units for Batch 2011; 183 units for Batch 2012). The number of net-cages used depended on the number of groups stocked. Table 2.2 shows the number of families, groups and fish involved in the experiment.

During the grow-out period, the fish were fed twice a day, an amount of 3 to 5% of their average live weight, using a commercial dry pellet feed containing 32% of protein. In order to facilitate competition among the fish, the feed was administered at a corner of the net-cage, instead of spreading it all over the surface of the net-cage (see Discussion). The water temperature, pH and dissolved oxygen level were monitored once a week.

2.2.3. Records

The grow-out period in net-cages was about five to eight months to reach a harvest size of 200 to 250 grams on average. The fish were harvested at the end of the grow-out period. Harvesting took about one to three days (Table 2.1). At that time, live weight, standard length, body width, body depth, sex, tag number, net-cage label, and pond number were recorded. The details of body measurement and sexing are described in Khaw *et al.* [2012].

Table 2.1 Schedule of reproduction and management.

Activities	Batch			
	2009	2010	2011	2012
Mating	January to April	January to March	December to April 2011	January to March
Nursing	February to Aug	February to July	January to June	February to July
Stocking	01 to 03 September; 30 September to 01 October[1]	05 July	27 to 28 June	11 to 12 July
Grow-out	September to April 2010	July to December	June to November	July to January 2013
Harvest	25 to 29 April 2010	08 December	21 to 23 November	06 to 07 January 2013

[1] The fry for Batch 2009 were stocked in two batches.

Table 2.2 Number of families and groups used, and the number of fish stocked and harvested, by batch.

Batch	Number of family	Number of group		Number of fish	
		Stocked	Harvested	Stocked	Harvested
2009	68	212	209	3350	2565
2010	31	45	45	720	509
2011	68	248	239	3958	3256
2012	70	196	110	3130	655
Total	237	701	603	11158	6985

The age at harvest of each fish was computed based on the recorded spawning date and harvesting date. A total of 6330 fish with phenotypic information (harvest live weight) over the first three batches of the experiment was used in the statistical analysis. Batch 2012 was excluded from the analysis because of the high mortality during the last phase of the grow-out period, caused by unforeseen weather conditions (Table 2.2). The pedigree data for generation one to ten of GIFT

were combined with the data collected in this study for (co)variance component estimation. The full pedigree consisted of 37,670 individuals. To our knowledge, this is by far the largest experiment for the estimation of IGE in aquaculture to date.

2.2.4. Estimation of phenotypic and genetic parameters

Variance and covariance components were estimated by residual maximum likelihood (REML) fitting an animal model with full pedigree information, implemented in ASReml [Gilmour *et al.*, 2009]. Parameter estimates can be biased when the data are a non-random subset of the entire population [Pollak *et al.*, 1984]. In order to reduce such bias, bivariate analysis of harvest weight and survival was conducted. In a preliminary analysis, univariate models were fitted to find the best model for both harvest weight and survival. To improve the distribution of residuals of harvest weight, we (natural) log-transformed harvest weight. In tilapia breeding programs, maternal common environmental effects are routinely included in parameters estimation to account for non-genetic covariances between full sibs due to the shared environment before communal rearing. However, we had difficulty in estimating the genetic parameters with the maternal common environmental effects in the model. The issue of maternal common environmental effects is further discussed below. Social interactions between group mates were accounted for by including IGEs in the model for harvest weight. For survival, the estimated variance of IGEs was fixed at the boundary of zero and the effect was therefore left out of the model.

For the bivariate analysis, we fitted five different models to investigate the presence of IGEs. Model 1 was a classical animal model extended with random group-effects and random group-by-family effects. The random group-effects account for non-genetic indirect effects between group mates; such non-genetic indirect effects create a covariance between group mates that takes positive values unless groups are very small [Bergsma *et al.*, 2008]. The group-by-family effects account for differential non-genetic interactions between members of the same family versus members of different families within a group. In the following, we refer to the group-by-family effects as non-genetic kin effects. The non-genetic kin effect is further elaborated on in the Discussion section. Thus model 1 was

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix} = \begin{bmatrix}\mathbf{X}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{X}_2\end{bmatrix}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\end{bmatrix} + \begin{bmatrix}\mathbf{Z}_{1_D} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z}_{2_D}\end{bmatrix}\begin{bmatrix}\mathbf{a}_{1_D}\\ \mathbf{a}_{2_D}\end{bmatrix} + \begin{bmatrix}\mathbf{V}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{V}_2\end{bmatrix}\begin{bmatrix}\mathbf{g}_1\\ \mathbf{g}_2\end{bmatrix} + \begin{bmatrix}\mathbf{W}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{W}_2\end{bmatrix}\begin{bmatrix}\mathbf{k}_1\\ \mathbf{k}_2\end{bmatrix} + \begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix},$$

where subscript 1 refers to harvest weight and subscript 2 to survival; $\mathbf{y}$ is the vector of phenotypic observations; $\mathbf{b}$ is the vector of fixed effects; $\mathbf{a}_D$ is a vector of direct random genetic effects, $\mathbf{g}$ is a vector of random group effects; $\mathbf{k}$ is a vector of random non-genetic kin effects, and $\mathbf{e}$ is a vector of random residuals. The $\mathbf{X}$, $\mathbf{Z}_D$, $\mathbf{V}$ and $\mathbf{W}$ are the known design matrices.

Model 2 contained IGE for harvest weight, but without non-genetic kin effects,

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix}=\begin{bmatrix}\mathbf{X}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{X}_2\end{bmatrix}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_{1_D} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z}_{2_D}\end{bmatrix}\begin{bmatrix}\mathbf{a}_{1_D}\\ \mathbf{a}_{2_D}\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_{1_S}\mathbf{a}_{1_S}\\ \mathbf{0}\end{bmatrix}+\begin{bmatrix}\mathbf{V}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{V}_2\end{bmatrix}\begin{bmatrix}\mathbf{g}_1\\ \mathbf{g}_2\end{bmatrix}+\begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix},$$

where $\mathbf{a}_S$ is a vector of indirect random genetic effects, and $\mathbf{Z}_S$ is the corresponding design matrix.

Model 3 contained both IGE for harvest weight and non-genetic kin effects,

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix}=\begin{bmatrix}\mathbf{X}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{X}_2\end{bmatrix}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_{1_D} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z}_{2_D}\end{bmatrix}\begin{bmatrix}\mathbf{a}_{1_D}\\ \mathbf{a}_{2_D}\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_{1_S}\mathbf{a}_{1_S}\\ \mathbf{0}\end{bmatrix}+\begin{bmatrix}\mathbf{V}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{V}_2\end{bmatrix}\begin{bmatrix}\mathbf{g}_1\\ \mathbf{g}_2\end{bmatrix}+\begin{bmatrix}\mathbf{W}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{W}_2\end{bmatrix}\begin{bmatrix}\mathbf{k}_1\\ \mathbf{k}_2\end{bmatrix}+\begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix}$$

Model 4 contained maternal common environmental effects, group effects and non-genetic kin effects,

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix}=\begin{bmatrix}\mathbf{X}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{X}_2\end{bmatrix}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_{1_c} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z}_{2_c}\end{bmatrix}\begin{bmatrix}\mathbf{c}_1\\ \mathbf{c}_2\end{bmatrix}+\begin{bmatrix}\mathbf{V}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{V}_2\end{bmatrix}\begin{bmatrix}\mathbf{g}_1\\ \mathbf{g}_2\end{bmatrix}+\begin{bmatrix}\mathbf{W}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{W}_2\end{bmatrix}\begin{bmatrix}\mathbf{k}_1\\ \mathbf{k}_2\end{bmatrix}+\begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix}$$

where $\mathbf{c}$ is a vector of random maternal common environmental effects, and $\mathbf{Z}_c$ is the corresponding design matrix.

Model 5 contained IGE, maternal common environmental effects, group effects and non-genetic kin effects, but no direct genetic effects (DGE),

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix}=\begin{bmatrix}\mathbf{X}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{X}_2\end{bmatrix}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_{1_S}\mathbf{a}_{1_S}\\ \mathbf{0}\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_{1_c} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z}_{2_c}\end{bmatrix}\begin{bmatrix}\mathbf{c}_1\\ \mathbf{c}_2\end{bmatrix}+\begin{bmatrix}\mathbf{V}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{V}_2\end{bmatrix}\begin{bmatrix}\mathbf{g}_1\\ \mathbf{g}_2\end{bmatrix}+\begin{bmatrix}\mathbf{W}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{W}_2\end{bmatrix}\begin{bmatrix}\mathbf{k}_1\\ \mathbf{k}_2\end{bmatrix}+\begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix}$$

The fixed effects fitted for harvest weight were the interaction of batch (2009, 2010, 2011), sex (male and female) and pond (1 and 2), and the linear covariate age at harvest fitted within this interaction. In addition, we also fitted the non-nested

quadratic effect of age at harvest (to accommodate the non-linear relationship between harvest weight and age) and the linear regression on social age at harvest. Social age at harvest was the average age at harvest of the group mates of an individual. This effect was included to account for age-dependent social interactions. For example, when an individual is accompanied by older group mates it may be smaller than its group mates, which may reduce its growth rate. For survival, we fitted the same fixed effects, except for sex, which was unknown for the dead fish, and the quadratic effect of age at harvest and social age at harvest which were not statistically significant.

The total heritable variance for response to selection in harvest weight, σ^2_{TBV}, for models 2 and 3 was calculated as, $\sigma^2_{TBV} = \sigma^2_{A_D} + 2(n-1)\sigma_{A_{DS}} + (n-1)^2\sigma^2_{A_S}$, where, $\sigma^2_{A_D}$ and $\sigma^2_{A_S}$ denote the direct and indirect genetic variance, respectively; $\sigma_{A_{DS}}$ the direct-indirect genetic covariance, and n the group size [Bijma, 2011]. For model 5, total heritable variance was calculated as $\sigma^2_{TBV} = (n-1)^2\sigma^2_{A_S}$. The heritability, h^2, for harvest weight in model 1 and for survival in all models was calculated as the ratio of $\sigma^2_{A_D}$ and phenotypic variance, σ^2_P. For model 2, 3 and 5, the ratio of total heritable variance and phenotypic variance was calculated, $T^2 = {\sigma^2_{TBV}}\big/{\sigma^2_P}$. Phenotypic variances were calculated as,

model 1, $\sigma^2_P = \sigma^2_{A_D} + \sigma^2_{\mathrm{g}} + \sigma^2_k + \sigma^2_e$;
model 2, $\sigma^2_P = \sigma^2_{A_D} + (n-1)\sigma^2_{A_S} + \sigma^2_{\mathrm{g}} + \sigma^2_e$;
model 3, $\sigma^2_P = \sigma^2_{A_D} + (n-1)\sigma^2_{A_S} + \sigma^2_{\mathrm{g}} + \sigma^2_k + \sigma^2_e$;
model 4, $\sigma^2_P = \sigma^2_c + \sigma^2_{\mathrm{g}} + \sigma^2_k + \sigma^2_e$;
model 5, $\sigma^2_P = (n-1)\sigma^2_{A_S} + \sigma^2_c + \sigma^2_{\mathrm{g}} + \sigma^2_k + \sigma^2_e$.

In principle, phenotypic variance for models 2, 3 and 5 has a term depending on relatedness among group members. However, for the purpose of comparison of phenotypic variances across different studies, we used the standardized phenotypic variance with zero relatedness, following suggestions of Bijma [2012] and Nielsen *et al.* [2014]. Likelihood ratio tests (LRT) were used for comparison of nested models, and Akaike Information Criteria (AIC) for comparison of non-nested models.

2.3. Results

2.3.1. General

A total of 6330 fish with phenotypic information collected over three batches were included in the statistical analysis. Table 2.3 shows the descriptive statistics. The phenotypic means of these three batches were relatively similar (166.55g for batch 2009, 140.34g for batch 2010, and 169.66g for batch 2011). We found a smaller CV of harvest weight, 36%, compared to previous studies of the GIFT strain where fish were communally reared [48% by Ponzoni *et al.*, 2005; 59.8% by Nguyen *et al.*, 2007; 40% by Khaw *et al.*, 2010].

Table 2.3 Number of observations (N), simple mean (μ), minimum and maximum, standard deviation (σ) and coefficient variation (CV,%) of harvest weight (g), standard length (cm), depth (cm), width (cm) and age (days) at harvest for batch 2009, 2010 and 2011.

Variable	N	μ	Min	Max	σ	CV
Harvest weight	6330	166.04	28.9	579.8	58.97	36
Standard length	6330	15.99	8.9	26.0	1.87	12
Depth	6330	6.96	3.9	10.5	0.96	14
Width	6330	2.91	1.4	4.4	0.42	14
Age at harvest	6330	348.62	252	450	58.21	17

Survival was 77% for batch 2009, 71% for batch 2010 and 82% for batch 2011. This is similar to the survival observed in the ordinary GIFT population at Malaysia, which is around 80% on average [Khaw *et al.*, 2010]. Survival was calculated based on the number of fish stocked and number of fish present at harvest time with identification. The unidentified fish were excluded from the data analysis since we were unable to trace back their family. In addition, we cannot be sure that those unidentified fish were the experimental fish or their progeny. This group of fish accounted for about 6% on average of the total number of fish harvested.

Social age at harvest was fitted as a linear covariate in all the models. In all cases the estimated regression coefficient of social age at harvest was -0.001 and was statistically significant, $p<0.01$. The negative regression coefficient indicates that the older the group mates, the greater the reduction in growth rate of an individual within the group. In standard deviation (SD) units for age at harvest (Table 2.3), the magnitude of social age at harvest was -0.058 (calculated as -0.001 x 58.21), indicating that this effect was relatively small. Hence, in spite of being statistically

significant, the effect of social age at harvest resulted in negligible differences in harvest weight, unless the age differences were huge.

To test for robustness of the estimates, we investigated the effect of removing outliers. After the removal of outliers, the parameter estimates either remained very similar (mainly for survival) or changed by 1% to 10%. There was no change in the sign of estimated correlations. Because overall the changes were negligible, all analyses presented here used the complete data set.

2.3.2. Estimation of phenotypic and genetic parameters

Table 2.4 shows the bivariate REML estimates for all the five models and their log-likelihood. Bivariate analysis significantly better fitted the data than univariate analysis, as the difference in log-likelihood of, for example, model 3 versus model 3 with all between-trait correlations fixed at zero equaled 26.5 (result not shown). Therefore results are only shown for the bivariate analysis.

We had difficulty in estimating the genetic parameters when maternal common environmental effects were included in the models. Several different models with maternal common environmental effects included were tested. The ASReml outputs of all the tested models showed the log-likelihood converged. But all the genetic parameters (with or without IGEs) were not properly estimated (parameters did not converge; results not shown).

First we focus on the significance of IGE in models without maternal common environmental effects, which follows from a comparison of model 3 with model 1. A likelihood ratio test (LRT) showed that model 3 had a better goodness of fit than model 1 (Table 2.4, χ^2_{2DF} = 12.52, p = 0.0019). Hence, when maternal common environmental effects were omitted, there was evidence for IGEs on harvest weight. Including IGEs in the model caused a slight increase in the direct genetic variance for both traits (model 3 versus 1 in Table 2.4). Although the estimated indirect genetic variance may seem small, its contribution to the total heritable variation was substantial, about 48%; $\frac{(n-1)^2\sigma^2_{A_S}}{\sigma^2_{TBV}} \times 100\% = 48\%$. However, the negative direct-indirect genetic covariance fully cancelled the contribution of IGE to total heritable variation ($\frac{2(n-1)\sigma_{A_{DS}}}{\sigma^2_{TBV}} \times 100\% = -55\%$). As a consequence, total heritable variance was nearly identical to the ordinary direct genetic variance, so that the T^2 from model 3 (0.32±0.09) was approximately equal to the h^2 from

model 1 (0.31±0.05). Beware that the lack of impact on σ^2_{TBV} does not mean that IGE do not affect response to selection. Instead, the negative direct-indirect genetic correlation will decrease response to ordinary mass or BLUP selection (see discussion).

The negative direct-indirect genetic correlation for harvest weight of -0.38±0.19, indicates a moderate, yet statistically significant, competitive phenomenon in the GIFT population. The estimated direct genetic correlation between harvest weight and survival did not differ significantly from zero, -0.05±0.24. However, the estimated correlation between the direct genetic effect for survival and indirect genetic effect for harvest weight was strongly negative (-0.79±0.30). Thus, genotypes that survive better have a negative effect on the growth rate of their group mates, indicating competition. The estimated parameters for survival were similar to those estimated from model 1.

Subsequently we investigated the evidence for IGEs in models including maternal common environmental effects (models 4 and 5; Table 2.4). A comparison of likelihoods and AIC of models 3 and 4 shows stronger evidence for maternal common environmental effects than for genetic effects. Since the full set of genetic parameters could not be estimated from models including maternal common environmental effects, we only investigated the evidence for IGE (model 5 versus 4). Based on the AIC for models 4 and 5, model 5 has the smallest AIC and was likely the best model. To test for the significance of IGEs, a LRT was performed between model 4 and 5. The test, χ^2_{1DF} = 3.62 with p = 0.057, gives suggestive evidence for IGEs on harvest weight. The non-genetic random effects were robust to the inclusion or exclusion of genetic effects from the model. We also tested for the presence of social maternal common environmental effects, which were effects of the maternal environment of individuals on the growth rate of their group mates, but this effect was not significant (results not shown). Therefore, it was excluded from all models.

Table 2.4 REML estimates (s.e.) from bivariate models of log-transformed harvest weight [log(hw)] and survival

Parameters	Model 1		Model 2		Model 3		Model 4		Model 5	
	log(hw)	survival	log(hw)	survival	log(hw)	survival	log(hw)	survival	log(hw)	survival
$\hat{\sigma}^2_{A_D}$	0.032 (0.006)	0.003 (0.002)	0.050 (0.008)	0.016 (0.004)	0.036 (0.007)	0.004 (0.002)	-	-	-	-
$\hat{\sigma}^2_{A_S}$	-	-	0.00012 (0.00004)	-	0.00007 (0.00003)	-	-	-	0.00004 (0.000026)	-
$\hat{\sigma}_{A_{DS}}$	-	-	-0.0013 (0.0004)	-	-0.0006 (0.0004)	-	-	-	-	-
$\hat{\sigma}^2_c$	-	-	-	-	-	-	0.011 (0.002)	0.003 (0.001)	0.011 (0.002)	0.003 (0.001)
$\hat{\sigma}^2_g$	0.017 (0.002)	0.025 (0.003)	0.021 (0.002)	0.032 (0.003)	0.016 (0.002)	0.025 (0.003)	0.017 (0.002)	0.025 (0.003)	0.017 (0.002)	0.025 (0.003)
$\hat{\sigma}^2_k$	0.010 (0.002)	0.016 (0.002)	-	-	0.010 (0.001)	0.015 (0.002)	0.010 (0.001)	0.014 (0.002)	0.010 (0.001)	0.014 (0.002)
$\hat{\sigma}^2_e$	0.043 (0.003)	0.109 (0.002)	0.037 (0.004)	0.109 (0.003)	0.040 (0.004)	0.108 (0.002)	0.058 (0.001)	0.110 (0.002)	0.057 (0.001)	0.110 (0.002)
$\hat{\sigma}^2_{TBV}$	-	-	0.036 (0.010)	-	0.033 (0.010)	-	-	-	0.009 (0.006)	-
$\hat{\sigma}^2_P$	0.103 (0.004)	0.153 (0.003)	0.109 (0.004)	0.157 (0.004)	0.103 (0.004)	0.153 (0.003)	0.097 (0.003)	0.153 (0.003)	0.096 (0.003)	0.153 (0.003)
$\hat{T}^2$ or $\hat{h}^2$	0.31 (0.05)	0.02 (0.01)	0.33 (0.09)	0.10 (0.02)	0.32 (0.09)	0.03 (0.01)	-	-	0.099 (0.06)	-
$\hat{c}^2$	-	-	-	-	-	-	0.12 (0.02)	0.02 (0.007)	0.12 (0.02)	0.02 (0.008)
$\hat{g}^2$	0.16 (0.02)	0.17 (0.02)	0.19 (0.02)	0.21 (0.01)	0.16 (0.02)	0.17 (0.02)	0.18 (0.02)	0.17 (0.02)	0.17 (0.02)	0.17 (0.02)
$\hat{k}^2$	0.10 (0.02)	0.10 (0.01)	-	-	0.09 (0.01)	0.10 (0.01)	0.11 (0.02)	0.09 (0.01)	0.10 (0.02)	0.09 (0.01)
$\hat{r}_{A_{D_{hw}}S_{hw}}$	-		-0.56 (0.14)		-0.38 (0.19)		-		-	
$\hat{r}_{A_{D_{hw}}D_{surv}}$	-0.28 (0.26)		-0.08 (0.13)		-0.05 (0.24)		-		-	
$\hat{r}_{A_{D_{surv}}S_{hw}}$	-		-0.46 (0.17)		-0.79 (0.30)		-		-	
LogL	9122.97		9018.34		9129.23		9135.27		9137.08	
AIC	1778.06		1987.32		1771.54		1753.46		1751.84	

Based on models 3 and 5, group and non-genetic kin effects were highly significant, and contributed about 17% and 10% of the phenotypic variance for both traits respectively. The comparison of model 2 and 3 served the purpose of testing the non-genetic kin effect. The LRT between model 2 and 3 indicated that model 3 was statistically much better than model 2 (χ^2_{1DF} = 221.78, p < 0.0001). The same result was found when comparing model 5 to model 2 (ΔAIC = $AIC_2 - AIC_5$ = 235.48). Thus non-genetic kin effects were highly significant. This result demonstrates that family members in the same group are more similar than family members in different groups, even after correction for group and family effects (see discussion). The elimination of non-genetic kin effects from the model caused a substantial increase in almost all the (co)variances, except the residual variance for harvest weight. This implies that the genetic parameters may be biased when excluding non-genetic kin effects.

2.4. Discussion

2.4.1. Overall findings

When maternal common environment effects were excluded from the model, our results show evidence for IGEs on harvest weight in Nile tilapia. However, we were unable to estimate all the direct and indirect genetic and non-genetic parameters simultaneously. Though the estimated indirect genetic variance may seem very small, the relevant quantity is the contribution of IGE to heritable variation, which is given by $(n-1)^2\sigma^2_{AS}$, and was large (48% of total heritable variance). Similar results were found in the few other studies on IGE in aquaculture [Brichette *et al.*, 2001; Monsen *et al.*, 2010; Nielsen *et al.*, 2014]. Therefore, a very small estimate of indirect genetic variance should not be interpreted as unimportance of IGEs, particularly when group sizes are large. We did not find IGEs for survival, irrespective of the in- or exclusion of maternal common environmental effect in the model. This may indicate the absence of such effects, but may also be due to the limited statistical power because of the low heritability of survival.

2.4.2. Estimation of direct and indirect genetic effects with nested mating design

In fish breeding programs, it is common practice to include maternal common environmental effect in the animal model for harvest weight [examples, Ponzoni *et al.*, 2005; Nguyen *et al.*, 2007; Rezk *et al.*, 2009]. The newly hatched fry are too

small for individual identification and the fish from the same full-sib family are therefore nursed together until they reach tagging size. However, we were unable to estimate the genetic parameters when maternal common environmental effects were included in the models. Our results suggest that maternal common environmental effects were confounded with the DGEs, because previous analysis of larger data sets of the same GIFT population, where the fish reared communally, indicated significant direct genetic and maternal common environmental effects [Khaw *et al.*, 2010]. Furthermore, we found suggestive evidence of IGEs from model 5 that included maternal common environment effects ($p = 0.057$). These results suggest that the difficulty of separating genetic from maternal common environmental effects was most likely due to the nested mating design, rather than the group structure used for studying IGEs.

The nested mating design of one male to two females is the common strategy in GIFT and other tilapia breeding programs [for examples, Rezk *et al.*, 2009; Attipoe *et al.*, 2013]. A classical animal model with maternal common environmental effects yields large standard errors of genetic (co)variances when a 1:2 mating design is used [Bijma and Bastiaansen, 2014]. In addition, we did not always succeed in having a 1:2 mating ratio in this study. This was because not all sires successfully mated with both dams by the end of the reproduction period. Of the 140 sires involved in the experiment, only 27 sires successfully mated with both dams and produced progeny for the experiment. Hence, many records came from 1:1 matings, in which genetic and maternal common environmental effects are fully confounded. This increased the difficulty to separate both effects. In the ordinary GIFT breeding program population, the number of sires succeeding in mating with both dams was about 12% higher than in this study, and the data set is much larger.

To solve the problem with confounding of genetic and maternal common environmental effects, a more powerful mating structure may needs to be implemented. For example, a mating ratio of 1:5 or a factorial mating design. Most tilapia breeding programs are using a natural reproduction technique, with a pair of "ready to spawn" parents placed in a hapa [for examples, Rezk *et al.*, 2009; Attipoe *et al.*, 2013]. For implementing a more complex mating structure, in vitro fertilization (IVF) and hormone induction techniques could be used [Fernandes *et al.*, 2013]. Alternatively, a group mating design could be used, where one male mates to multiple females under natural spawning conditions [Trọng *et al.*, 2013].

Irrespective of the mating technology used, however, a persistent problem is that designs optimal for parameter estimation may be undesirable for long term genetic improvement. In aquaculture breeding programs, limited facilities often restrict the number of full sib families that can be used. Hence, the use of, for example, a 1:5 mating ratio instead of 1:2 would result in fewer sires per generation, substantially decreasing effective population size and threatening long-term genetic improvement. The 1:2 mating ratio is used in the GIFT program because the main aim is to produce a superior strain, rather than accurate genetic parameters.

2.4.3. Heritable competition

Based on model 3, the estimated direct-indirect genetic correlation for harvest weight indicated moderate competition in the GIFT population. This competition almost completely cancelled the heritable variation contributed by IGE. The fact that presence of IGEs did not alter total heritable variation, $\sigma^2_{TBV} \approx \sigma^2_{A_D}$, does not imply that response to selection is unaffected by IGEs. The negative direct-indirect genetic covariance will reduce the accuracy of selection, which in turn reduces response. For mass-selection, for example, the true accuracy is given by

$$\rho = \frac{\sigma^2_{A_D} + (n-1)\sigma_{A_{DS}}}{\sigma_{TBV}\sigma_P} = 0.46$$

when fish are reared in groups composed at random with respect to family [Griffing, 1967; Ellen *et al.*, 2007]. The perceived accuracy when IGEs are ignored equals $\sqrt{h^2} = 0.59$, which is 29% higher than the true accuracy. Hence, when ignoring IGEs, response to selection will be over-predicted by 29%. Moreover, the negative direct-indirect genetic correlation indicates that selection for individual performance will increase in competition when fish are kept in groups composed at random with respect to family [Ellen *et al.*, 2007]. This increase in competition can be avoided by using groups composed of related individuals [Griffing, 1967; Ellen *et al.*, 2007].

A key question for aquaculture breeding is whether IGEs found here are representative of IGEs occurring in commercial production, where fish are usually reared communally in very large groups. Unfortunately, IGEs cannot be estimated from data coming from a few large ponds; estimation of IGEs requires data on many groups [Bijma, 2012]. Hence, in realistic designs, those groups will be much smaller than ordinary communal rearing ponds. In our experiment, the nutrient

composition and amount of feed were the same as in the GIFT selective breeding population (no restriction on feeding amount). The difference was that fish were kept in small net-cages where feed was deposited at the corner of the cage. In communal rearing, the feed is spread over the surface of the pond, but usually not over the entire surface. It is difficult to judge whether our set-up increased or decreased competition compared to communal rearing. The lower CV found here compared to previous studies of GIFT in communal rearing [examples, Ponzoni *et al.*, 2005; Nguyen *et al.*, 2007; Khaw *et al.*, 2010] suggests that the feeding method did not increase the competition. Moreover, our results agree with those of Brichette *et al.* [2001] and Nielsen *et al.* [2014], who also found a negative direct-indirect genetic correlation for growth traits, and submissive fish were prevented from access to the feed in Nielsen's study [2014].

The ideal test to investigate whether the competition found here reflects the level of competition in commercial practice would be a selection experiment aimed at reducing competition. For example, in the breeding program selection could be based on data from fish kept in many groups of family members, while response could be evaluated in communal rearing. However, this will involve more cost in tagging the fish, manpower in maintaining the extra population and infrastructure to accommodate the fish. It would be interesting to know whether a breeding program taking into account IGEs would be more cost effective than a traditional breeding program. Thus, an economic appraisal would be useful.

Results of model 3 suggest competition. Besides reducing the performance of the fish, competition may also reduce the welfare of the fish. With the empirical evidence from livestock, selection on total breeding values (which includes IGEs) could simultaneously improve productivity and welfare of the animals [Muir, 1996; Ellen *et al.*, 2008; Camerlink *et al.*, 2012; Nielsen *et al.*, 2014]. In addition, Nielsen *et al.* [2014] demonstrated that accounting for IGEs in Atlantic cod breeding will improve selection response for welfare traits. Further study is needed to investigate whether this also applies to Nile tilapia.

2.4.4. Group effects and non-genetic kin effects

Our results showed that group effects contributed about 16 to 21% of the phenotypic variance. This is high compared to other genetic and non-genetic effects in the models. This indicates that group mates have similar trait values, which is probably a result of the common social environment experienced by group mates, as net-cages were physically identical. In addition, we found that the

exclusion of group effects inflated the estimated heritable variation for both traits (results not shown). This is consistent with previous studies showing that the removal of group effects from the model causes an upward bias in the genetic estimates [van Vleck and Cassady, 2005; Bergsma *et al.*, 2008]. Thus allowing for random group effects in the model is essential when fitting IGE (see also Cantet and Cappa, 2008, for a discussion on group effects).

In this study, we also fitted a random effect for the interaction of group by family to account for non-genetic kin effects. This effect was highly significant and explained about 9 to 11% of phenotypic variance. This result indicates that family members in the same group show similar trait values, even after correction for group effects and family effects. This indicates that individuals interact differently with their family members than with the members of the other family in the same group, suggesting kin-recognition [Olsén, 1989; Brown and Brown, 1993; Olsén *et al.*, 1998]. From an evolutionary perspective, preferential behavior towards kin is expected because it increases an individual's so-called inclusive fitness [Hamilton, 1964]. Kin recognition has been found before in salmonids [Olsén, 1989; Brown and Brown, 1993], and also in tilapia (*Sarotherodon melanotheron*; samples from a wild population; Pouyaud *et al.*, 1999). The presence of kin-specific behavior may complicate the selection for IGE, because IGEs on kin may differ from those on unfamiliar individuals [Alemu *et al.*, 2014]. Furthermore, the results showed that the exclusion of non-genetic kin effects caused an upward bias for almost all estimated parameters. Thus, the inclusion of non-genetic kin effects in the model was essential.

2.5. Conclusions

Our study is the first large-scale IGE experiment in an aquaculture species. Unfortunately, confounding between maternal common environmental and genetic effects prevented simultaneous estimation of all parameters. Models without maternal common environmental effects showed significant evidence for IGE on harvest weight in Nile tilapia, while a model with such effects showed suggestive evidence for IGE ($p = 0.057$). In models without maternal common environmental effects, the estimated genetic correlation between direct and indirect genetic effects on harvest weight was negative, indicating that traditional selection will increase competition among individuals. We also found a strongly negative genetic correlation between direct effects on survival and indirect effects on harvest weight, indicating that individuals with better genes for survival

suppress growth rate of their social partners. The confounding between maternal common environmental effects and DGEs indicated that the one male to two females nested mating design has limited power to estimate the genetic parameters. We have to be aware that other mating designs may allow more accurate estimation of genetic parameters, but may be suboptimal for long-term genetic improvement in schemes where the number of families is limited.

Competing interests

Authors declare that they have no competing interest.

Authors' contributions

HLK carried out the statistical analysis with the help from PB and RWP, and drafted the manuscript. PB contributed in designing the study, interpreting the results and in drafting the manuscript. RWP helped in setting-up the experiment, interpreting the results and in drafting the manuscript. HYY and MAA managed the daily routine at the research station. All the authors read, revised and approved the final manuscript.

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3

Indirect genetic effects and inbreeding: consequences of BLUP selection for socially affected traits on rate of inbreeding

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Abstract

Background

Social interactions often occur among living organisms, including aquatic animals. There is empirical evidence showing that social interactions may genetically affect phenotypes of individuals and their group mates. In this context, the heritable effect of an individual on the phenotype of another individual is known as an Indirect Genetic Effect (IGE). Selection for socially affected traits may increase response to artificial selection, but also affect rate of inbreeding.

Methods

A simulation study was conducted to examine the effect of Best Linear Unbiased Prediction (BLUP) selection for socially affected traits on the rate of inbreeding. A base scenario without IGE and three alternative scenarios with different magnitudes of IGE were simulated. In each generation, 25 sires and 50 dams were mated, producing eight progeny per dam. The population was selected for 20 generations using BLUP. Individuals were randomly assigned to groups of eight members in each generation, with two families per group, each contributing four individuals. “Heritabilities” (for both direct and indirect genetic effects) were equal to 0.1, 0.3 or 0.5, and direct–indirect genetic correlations were −0.8, −0.4, 0, 0.4, or 0.8. The rate of inbreeding was calculated from generation 10 to 20.

Results

For the base scenario, the rates of inbreeding were 4.09, 2.80 and 1.95% for “heritabilities” of 0.1, 0.3 and 0.5, respectively. Overall, rates of inbreeding for the three scenarios with IGE ranged from 2.21 to 5.76% and were greater than for the base scenarios. The results show that social interaction within groups of two families increases the resemblance between estimated breeding values of relatives, which, in turn, increases the rate of inbreeding.

Conclusion

BLUP selection for socially affected traits increased the rate of inbreeding. To maintain inbreeding at an acceptable rate, a selection algorithm that restricts the increase in mean kinship, such as optimum contribution selection, is required.

3.1. Background

Aquaculture produces fish at an affordable price that are a valuable source of animal proteins, especially in developing countries [FAO, 2012]. Selective breeding plays an important role in aquaculture and provides high quality seed with better growth rate and survival [Gjedrem *et al.*, 2012]. Genetically Improved Farmed Tilapia (GIFT) in tropical countries [Ponzoni *et al.*, 2005, 2010a], and Atlantic salmon in temperate and cold countries [Gjedrem *et al.*, 2012] are good examples that illustrate the benefits of selective breeding. However, there is evidence that fish with high growth rate may be more aggressive and competitive [Lahti *et al.*, 2001; Ødegård and Olesen, 2011]. Competition is a type of social interaction that is very common in aquaculture environments. It reduces productivity and represents a threat to animal welfare [Ashley, 2007; Volpato *et al.*, 2007]. Thus, fish breeders may need to improve productivity and welfare by taking social interactions into account in their breeding programs.

In the absence of social interactions among individuals, the phenotypic value (P_i) of an individual, say i, can be modeled as the sum of its additive genetic or breeding value (A_i), and a non-genetic component, usually referred to as environment (E_i) [Falconer and Mackay, 1996],

$$P_i = A_i + E_i \quad (1)$$

Using this model, breeders have achieved substantial genetic improvement. However, in some cases, especially for traits related to behavior, populations have not responded as expected, in spite of the presence of heritable variation. For example, selection for survival increased mortality in laying hens [Muir, 1996]. One of the reasons for these unexpected responses may be the presence of indirect genetic effects (IGE). An IGE is a heritable effect of an individual on the trait value of another individual [Griffing, 1967; Muir, 1996; Wolf *et al.*, 1998; Muir, 2005]. For example, in fish, when an individual carries genes that cause it to monopolize the feeder, the growth rate of its group mates will be reduced. Another well-known example is mortality due to cannibalism in laying hens, where the survival of an individual depends on the genes for pecking behavior in its cage mates [Muir, 1996].

For such traits, the model in Equation (1) needs to be expanded with IGE [Griffing, 1967; Muir, 2005; Bijma *et al.*, 2007a],

$$P_i = A_{D,i} + E_{D,i} + \sum_{j \neq i}^{n-1} A_{S,j} + \sum_{j \neq i}^{n-1} E_{S,j} \quad (2)$$

where, P_i is the phenotype of focal individual i; $A_{D,i}$ and $E_{D,i}$ are the direct breeding value and direct non-genetic effect of individual *i*, respectively; $A_{s,j}$ and $E_{S,j}$ are the indirect breeding value and indirect non-genetic effect originating from its group mate j, respectively; and n is the group size. The summations are taken over the $n-1$ group mates of an individual, thus excluding i. This model applies to all n group members. From the perspective of the recipient, each individual's phenotype is the consequence of a direct effect of itself, and the sum of the indirect effects of its $n-1$ social partners. From the perspective of the acting individual, each individual expresses its direct genetic effect once in its own phenotype, and its IGE $n-1$ times, once in each of its $n-1$ group mates. Thus, in addition to the classical (direct) breeding value (Equation 1), each individual affects its $n-1$ group mates, and is also affected by its $n-1$ group mates. Several studies have shown the existence of IGE, in quail [Muir *et al.*, 2013], poultry [Bijma *et al.*, 2007b; Ellen et al, 2007; Peeter *et al.*, 2012], pigs [Bergsma *et al.*, 2008; Chen *et al.*, 2009], cattle [van Vleck *et al.*, 2007], and fish [Nielsen *et al.*, 2014].

Theoretical and empirical studies show that response to selection for socially affected traits can be increased by applying a selection strategy that accounts for both direct and indirect genetic effects, such as kin or group selection [Griffing, 1976; Muir, 1996; Muir and Schinckel, 2002; Muir, 2005; Bijma *et al.*, 2007a; Ellen *et al.*, 2007]. Muir [1996], for example, conducted a selection experiment for egg production in cannibalistic laying hens using group selection, and managed to increase egg production from 91 to 237 eggs per hen, largely as result of improved survival. There have been several other selection experiments on socially affected traits, for example, in quail [Muir, 2005] and flour beetles [Wade, 1976; Wade, 1977]. To our knowledge, there have been no similar studies in aquaculture species.

Theoretical and experimental work on socially affected traits shows that response to selection can be increased by using structured populations with groups composed of related individuals. In such populations, response to selection is

maximized by selecting on the Best Linear Unbiased Prediction (BLUP) of breeding values [Muir, 2005]. However, such selection schemes may lead to high rates of inbreeding, because they increase the probability of co-selection of relatives [Verrier *et al.*, 1993; Bijma and Woolliams, 2000]. High rates of inbreeding cause a reduction of genetic variance and threaten the long-term sustainability of breeding programs [Wang *et al.*,2002; Oldenbroek, 2007; Fessehaye *et al.*, 2009; Ponzoni *et al.*, 2010b].

In this study, we examined the effect of BLUP selection for a socially affected trait on the rate of inbreeding for a fish breeding program using stochastic simulation. However, the methods and results are also applicable to other species with similar breeding designs.

3.2. Methods

3.2.1. Population structure

Data were simulated using the R-language [R Development Core Team, 2011]. The simulated population was a closed nucleus with discrete generations in which base population animals were assumed unrelated. Bivariate normal distributions were used to simulate both the genetic and the non-genetic direct and indirect effects of base animals. Subsequently, in each generation, 25 sires and 50 dams were selected and randomly mated. Each male was mated to two females in a nested design, which is a common mating structure in aquaculture breeding programs. Each dam produced eight progeny and the sex of the progeny was randomly assigned with equal probability.

Direct and indirect breeding values of an offspring were simulated as the average breeding value of its parents plus a Mendelian sampling deviation, sampled from a bivariate normal distribution. Individuals in each generation were assigned to groups of eight members, with a group consisting of two full-sib families and each family contributing four progeny. Subsequently, phenotypes of individuals were constructed according to Equation 1 and breeding values were estimated (see below). We chose a design with two families per group because this scheme is optimal to estimate the indirect genetic variance [Bijma, 2010] and yields greater response to selection than schemes with groups composed at random with respect to family [Bijma *et al.*, 2007a; Ellen *et al.*, 2007]. Schemes with a single family per group would yield an even greater response, but would not allow the estimation of

the direct and indirect genetic variances [Bijma *et al.*, 2007b; van Vleck *et al.*, 2007; Cheng *et al.*, 2009]. Hence, having two families per group appears to be an attractive compromise for response to selection and variance component estimation.

The top 25 male candidates and top 50 female candidates were selected as parents of the next generation based on the BLUP estimate of their total breeding value ($T\hat{B}V$),

$$T\hat{B}V = \hat{A}_D + (n-1)\hat{A}_S \quad (3)$$

where $\hat{A}_D$ and $\hat{A}_S$ are the estimated direct and indirect breeding values, respectively, and n is group size [Bijma *et al.*, 2007a]. For each scenario, 20 generations of selection were simulated.

The BLUP estimated breeding values (EBV) were obtained from the following model [Muir, 2005; Bijma *et al.*, 2007a], using the R-version of ASReml [Butler *et al.*, 2009]:

$$\mathbf{y} = \mu + \mathbf{Z}_D\mathbf{a}_D + \mathbf{Z}_S\mathbf{a}_S + \mathbf{V}\mathbf{g} + \mathbf{e} \quad (4)$$

where $\boldsymbol{y}$ is the vector of phenotypic observations; μ is the overall mean, which was the only fixed effect included; $\mathbf{a}_D$ and $\mathbf{a}_S$ are the vectors of direct and indirect random genetic effects, respectively; $\mathbf{g}$ is the vector of random group effects, and $\mathbf{e}$ is the vector of random residuals. The random group effects in $\mathbf{g}$ occur as a result of the non-genetic indirect effects (E_S in Equation 2), which create a covariance among group members that can be fitted as a group effect. The magnitude of this covariance equals $\sigma^2_{\mathrm{g}} = 2\sigma_{E_{DS}} + (n-2)\sigma^2_{E_S}$, where $\sigma_{E_{DS}}$ is the direct–indirect non-genetic covariance and $\sigma^2_{E_S}$ is the non-genetic indirect variance [Bergsma *et al.*, 2008]. Thus, σ^2_{g} is determined by the phenotypic variances, heritabilities and non-genetic correlations that were used as input values for the simulations (see Table 3.1 below). The $\mathbf{Z}_D$, $\mathbf{Z}_S$, and $\mathbf{V}$ are the known design matrices that assign observations to the levels of the direct genetic effects of the animals themselves, to the IGE of their group mates, and to the random group effects, respectively. The $\mathbf{Z}_S$-matrix has a "1" in the column for each group mate of the individual producing the record. Hence, since the group size was equal to 8 in our data, each row of $\mathbf{Z}_S$

contains seven 1s, each linking the IGE of one group mate to the record of the individual. The covariance structure of the random effects was:

$$\text{var}\begin{bmatrix}\mathbf{a}_D\\ \mathbf{a}_S\\ \mathbf{g}\\ \mathbf{e}\end{bmatrix}=\begin{bmatrix}\mathbf{A}\sigma^2_{A_D} & \mathbf{A}\sigma_{A_{DS}} & \mathbf{0} & \mathbf{0}\\ \mathbf{A}\sigma_{A_{DS}} & \mathbf{A}\sigma^2_{A_S} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma^2_g & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma^2_e\end{bmatrix}$$

In the estimation of breeding values, the true (*i.e.*, simulated) values of the genetic parameters were used. Genetic parameters were not estimated from the simulated data.

Table 3.1 Assumed parameters used in simulation by scenario.

Parameters	Scenarios			
	Base	1	2	3
[a]Magnitude of indirect effect, $(n-1)\sigma^2_{P_S}$	0	0.25	1.0	4.0
Correlations between direct and indirect effects, $r_{A_{DS}} = r_{E_{DS}}$	0	0, -0.8, -0.4, 0.4, 0.8	0, -0.8, -0.4, 0.4, 0.8	0, -0.8, -0.4, 0.4, 0.8
Direct phenotypic variance, $\sigma^2_{P_D}$	1	1	1	1
[b]Heritabilities, $h^2_D = h^2_S$	0.1, 0.3, 0.5	0.1, 0.3, 0.5	0.1, 0.3, 0.5	0.1, 0.3, 0.5

[a]Since an individual interacts with $n-1$ group mates, the term $(n-1)\sigma^2_{P_S}$ reflects the contribution of indirect effects (both genetic and non-genetic) to phenotypic variance; b Heritabilities are the ratio of additive genetic variance to the corresponding "phenotypic" variance; for direct effects $h^2_D = \sigma^2_{A_D}/\sigma^2_{P_D}$, and for indirect effects $h^2_S = \sigma^2_{A_S}/\sigma^2_{P_S}$.

3.2.2. Rate of inbreeding

The inbreeding coefficients of individuals were calculated from the pedigree by using the R-package "pedigree" [Coster, 2011]. For each replicate, the rate of inbreeding (ΔF) was then calculated using the mean inbreeding coefficients of generations 10 and 20:

$$\Delta F = 1 - \sqrt[10]{\frac{1-\bar{F}_{20}}{1-\bar{F}_{10}}} \qquad (5)$$

Rates of inbreeding were averaged over 100 replicates and the standard error was calculated. The first 10 generations were not used in the calculation of the rate of inbreeding, to allow the population to reach equilibrium with respect to the Bulmer effect and the buildup of pedigree information [Bulmer, 1985; Dekker, 1992; Bijma and van Arendonk, 1998]. The Bulmer effect reduces the between-family variance, which reduces the correlation between EBV of relatives. This, in turn, reduces the probability of co-selection of relatives, which reduces the rate of inbreeding. Thus, the Bulmer-effect affects the rate of inbreeding [Woolliams and Bijma, 2000].

3.2.3. Simulated scenarios

A base scenario and three alternatives were simulated (Table 3.1). In all schemes, the direct phenotypic variance was set to 1, $\sigma^2_{P_D} = \sigma^2_{A_D} + \sigma^2_{E_D} = 1$. The base scenario was a reference scenario without indirect effects (genetic and non-genetic), where trait values were generated according to Equation 1. The alternative scenarios considered different magnitudes of indirect effects: mild (scenario 1), intermediate (scenario 2) or strong (scenario 3). The magnitude of indirect effects was measured by their contribution to phenotypic variance in a population in which is given by $(n-1)\sigma^2_{P_S}$ and was equal to 0.25, 1, or 4 ($\sigma^2_{P_S} = \sigma^2_{A_S} + \sigma^2_{E_S}$). Thus, compared to direct effects, the contribution of indirect effects was equal to one quarter to four-fold the direct phenotypic variance ($\sigma^2_{P_D}$) to phenotypic variance. For all scenarios, "heritabilities" of direct and indirect genetic effects were equal to 0.1, 0.3 or 0.5 ($h^2_D = \sigma^2_{A_D}/\sigma^2_{P_D}$ and $h^2_S = \sigma^2_{A_S}/\sigma^2_{P_S}$). Genetic and non-genetic correlations between direct and indirect effects were varied as follows: $r_{A_{DS}} = r_{E_{DS}}$ = −0.8, −0.4, 0, 0.4 or 0.8.

3.3. Results

Across the four scenarios, rates of inbreeding ranged from 2.21 to 5.76%. The standard errors of the rates of inbreeding (average over 100 replicates) were small and ranged from 0.0004 to 0.0014, which indicates that the results were accurate. For presentation purposes, the results were grouped according to the correlation between direct and indirect effects:

a. Neutral, the direct–indirect correlations were equal to zero ($r_{A_{DS}} = r_{E_{DS}}$ = 0);
b. Competition, the correlations were negative ($r_{A_{DS}} = r_{E_{DS}}$ = −0.4 and −0.8);
c. Cooperation, the correlations were positive ($r_{A_{DS}} = r_{E_{DS}}$ = 0.4 and 0.8).

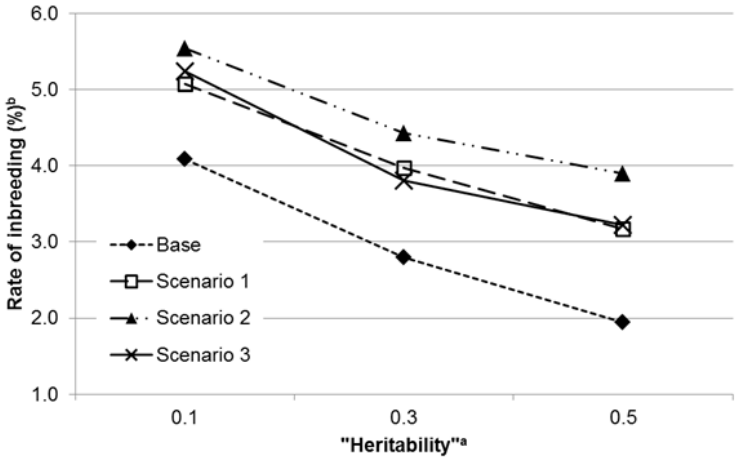
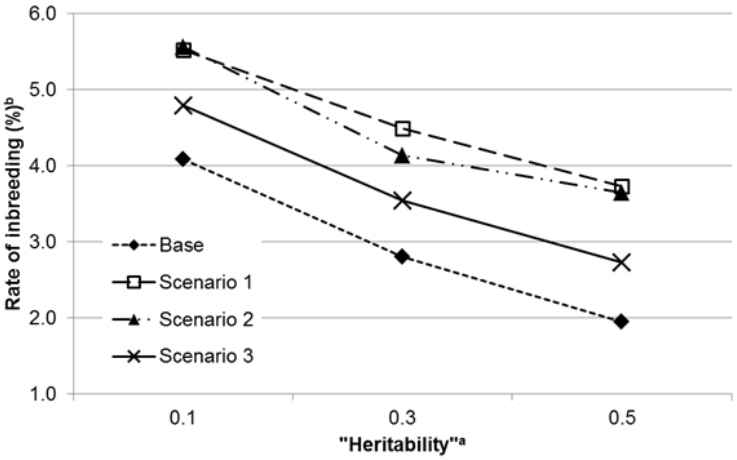


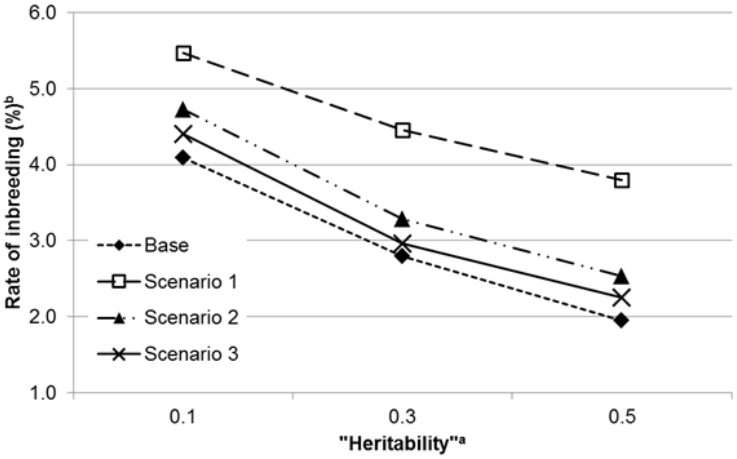
Figure 3.1 Rates of inbreeding (%) for the four scenarios across heritabilities[a] when correlations between direct and indirect genetic effects are equal to 0. [a]Heritabilities are the ratio of additive genetic variance to the corresponding "phenotypic" variance; for direct effects $h_D^2 = \sigma_{A_D}^2/\sigma_{P_D}^2$, and for indirect effects $h_S^2 = \sigma_{A_S}^2/\sigma_{P_S}^2$; [b] the SE of rate of inbreeding ranged between 0.00039 and 0.00125.

Under the neutral situation, the direct effect of an individual on its own trait value is independent of its indirect effect on the trait values of its group mates. Figure 3.1 shows the results for this situation. Rates of inbreeding were always greater for scenarios with IGE than with the base scenario. The range for rates of inbreeding obtained from scenarios 1, 2 and 3 was 3.17 to 5.54%, and from 1.95 to 4.09% for the base scenario. The rates of inbreeding within each scenario were greatest with a low "heritability" (*i.e.*, lower values of $h_D^2 = \sigma_{A_D}^2/\sigma_{P_D}^2$ and $h_S^2 = \sigma_{A_S}^2/\sigma_{P_S}^2$).

In a competitive situation, an individual with positive effects on its own trait value will on average have negative effects on the trait values of its group mates (Figure 3.2a and b). In this situation, the rate of inbreeding was lowest with the base scenario and highest with scenario 1. Rates of inbreeding were almost identical for both direct-indirect correlations with scenario 1. The rates of inbreeding for scenario 2 were between those for scenarios 1 and 3. However, note that in scenario 2, a change in the direct–indirect correlation had a greater effect on rates of inbreeding than in the other scenarios. The lowest rates of inbreeding were obtained from scenario 3 and rates of inbreeding decreased when the correlation changed from −0.4 to −0.8.

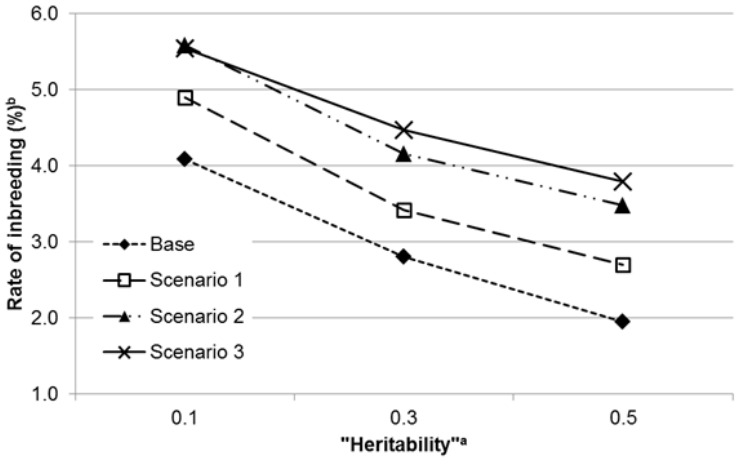


(a)

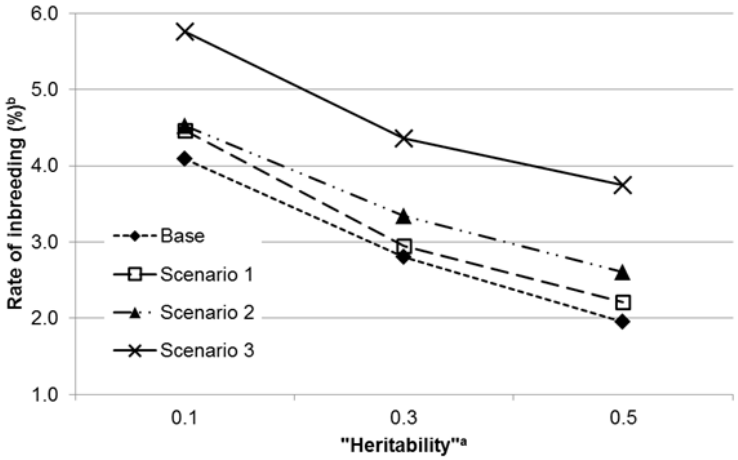


(b)

Figure 3.2 Rates of inbreeding (%) for the scenarios across heritabilities[a] when correlations between direct and indirect genetic effects are equal to −0.4 (a) and −0.8 (b), except for the base scenario. [a]Heritabilities are the ratio of additive genetic variance to the corresponding "phenotypic" variance; for direct effects $h_D^2 = \sigma_{A_D}^2/\sigma_{P_D}^2$, and for indirect effects $h_S^2 = \sigma_{A_S}^2/\sigma_{P_S}^2$; [b] the SE of rate of inbreeding ranged between 0.00046 and 0.00139.



(a)



(b)

Figure 3.3 Rates of inbreeding (%) for the scenarios across heritabilities[a] when correlations between direct and indirect genetic effects are equal to 0.4 (a) and 0.8 (b), except for the base scenario. [a]Heritabilities are the ratio of additive genetic variance to the corresponding "phenotypic" variance; for direct effects $h_D^2 = \sigma_{A_D}^2/\sigma_{P_D}^2$, and for indirect effects $h_S^2 = \sigma_{A_S}^2/\sigma_{P_S}^2$; [b] the SE of rate of inbreeding ranged between 0.00038 and 0.00129.

In the cooperative situation, an individual with positive effects on its own trait value also has positive effects on the trait values of its group mates (Figure 3.3a and b). Apart from the base scenario, which again produced the lowest inbreeding

rate, ranking of scenarios with respect to rate of inbreeding was precisely opposite for this situation to that obtained from the competitive situation. The highest rate of inbreeding was obtained from scenario 3 and the lowest from scenario 1. Scenario 3 also showed the most stable rates of inbreeding across different direct-indirect correlations. As was the case in the competitive situation, scenario 2 was the most sensitive to a change in the value of the direct–indirect correlation.

3.4. Discussion

3.4.1. Overall findings

Our results indicate that BLUP selection on socially affected traits results in greater rates of inbreeding than BLUP selection solely for direct genetic effect, regardless of the genetic correlations between direct and indirect genetic effects. Furthermore, the pattern of the rates of inbreeding for different "heritabilities" was in agreement with BLUP selection theory, with lower heritability yielding higher rates of inbreeding [Verrier *et al.*, 1993; Bijma and Woolliams, 2000].

3.4.2. Rate of inbreeding and BLUP selection

For decades, inbreeding has been identified as an important issue in animal breeding [Robertson, 1961; Falconer and Mackay, 1996; Ponzoni *et al.*, 2010b]. Artificial selection is known to increase the rate of inbreeding because individuals from the best performing families are selected and contribute more to the gene pool compared to those from lower performing families [Robertson, 1961; Wray and Thompson, 1990; Woolliams *et al.*, 1999; Ponzoni *et al.*, 2010b], which is confirmed by our results. Without selection, the expected rate of inbreeding for the simulated population is about 0.75% per generation (using $\Delta F = \frac{1}{8N_m} + \frac{1}{8N_f}$, with N_m = 25 and N_f = 50). In our study, the rates of inbreeding (2.21 to 5.76% with IGE, and 1.95 to 4.09% without IGE) were considerably higher. Furthermore, the highest rates of inbreeding were obtained with low heritabilities. This is as expected with BLUP selection, since information from relatives receives higher weight with low heritabilities, which increases the probability of co-selection of relatives, thus increasing the rate of inbreeding [Verrier *et al.*, 1993; Bijma and Woolliams, 2000].

To investigate whether lower heritabilities can explain the higher rates of inbreeding observed in scenarios with IGE, we calculated the classical (*i.e.* direct) heritability, $\sigma^2_{A_D}/\sigma^2_P$, for the four scenarios under the neutral situation, for values of

$h_D^2 = \sigma_{A_D}^2/\sigma_{P_D}^2 = h_S^2 = \sigma_{A_S}^2/\sigma_{P_S}^2 = 0.3$. Phenotypic variance for groups composed of two families was calculated as $\sigma_P^2 = \sigma_{A_D}^2 + (n-1)\sigma_{A_S}^2 + (n-2)r\sigma_{A_{DS}} + 2\left(\frac{1}{2}n-1\right)\left(\frac{1}{2}n-1\right)r\sigma_{A_S}^2 + \sigma_{E_D}^2 + (n-1)\sigma_{E_S}^2$, where r = 0.5 is the relationship between members of the same family. The three scenarios with IGE had lower classical heritabilities than the base scenario (classical heritability of 0.3). For example, with scenario 2, classical heritability was 0.3/2.39 = 0.13 (see Table 3.1 for the parameters used) and with scenarios 1 and 3, it was equal to 0.22 and 0.05, respectively. When comparing these classical heritabilities to the observed rates of inbreeding, the pattern was different from that observed with classical BLUP selection for direct effects only. The heritability for scenario 2 was in between those with scenarios 1 and 3, yet scenario 2 had the highest rate of inbreeding. Thus, apart from a potential effect working via classical heritability, IGE also affects the rate of inbreeding in other ways.

However, based on these results, we cannot determine whether the increase in rate of inbreeding in scenarios 1 to 3 compared to the base scenario was caused by IGE or by a reduction in classical heritability due to extra variance. Therefore, we simulated an additional scheme with classical heritability fixed at 0.3 by increasing the direct genetic variance ($\sigma_{A_D}^2$) for scenarios 1 and 2, while the genetic and non-genetic indirect effects remained unchanged. A comparison of the rate of inbreeding of this scheme to that of the base scenario (also for h_D^2 = 0.3) reveals the impact of IGE on the rate of inbreeding at a fixed classical heritability. For this additional scheme, the rates of inbreeding for scenarios 1 and 2 were equal to 3.62 and 3.86%, respectively, which was about 1% higher than in the base scenario (2.80%). Based on this result, we can confidently conclude that the indirect effect was a causal factor that contributed to the increase of the rate of inbreeding. Scenario 3 was not included as an additional scheme, because h_D^2 would have to be greater than 1 to achieve a classical heritability of 0.3 for that scenario.

3.4.3. Competition versus cooperation

Comparing the competitive (Figure 3.2) and cooperative (Figure 3.3) situations, we observed a re-ranking of scenarios 1 and 3. To understand the mechanisms behind these results, we calculated the correlations between the estimated total breeding values (ETBV) for full-sibs and half-sibs, for direct–indirect correlations of −0.8 (Figure 3.4a) and +0.8 (Figure 3.4b). The results show that the re-ranking of scenarios observed for the rate of inbreeding was mirrored in the correlation between ETBV of sibs. The highest correlation between ETBV of sibs was obtained

from scenario 1 for a direct-indirect correlation of −0.8 and from scenario 3 for a direct-indirect correlation of +0.8. These results suggest that the correlation between ETBV of sibs is the main cause for the differences in rates of inbreeding. A higher correlation between ETBV of sibs increases the probability of co-selection of sibs, which, in turn, increases the rate of inbreeding because it increases the variance in long-term contributions of ancestors [Wray and Thompson, 1990]. Nevertheless, the correlation between ETBV of sibs did not fully explain the observed pattern of rates of inbreeding. In Figure 3.4a, for example, the correlation between ETVB of sibs was nearly independent of "heritability" for scenario 1, but this trend was not reflected in the rate of inbreeding (Figure 3.1). The observed pattern for the correlation between ETBV of sibs for the base scenario was similar to its pattern for rate of inbreeding.

In traditional selection on BLUP EBV, rates of inbreeding and correlations between EBV of sibs are higher at lower heritability. Thus, it was interesting to investigate whether the same mechanism explains the re-ranking of scenarios 1 and 3 observed here. Therefore, we analyzed the relationship of the ratio of total heritable variance over phenotypic variance, $T^2 = \frac{\sigma^2_{TBV}}{\sigma^2_P}$, with the rate of inbreeding. For the competitive situation, a lower T^2 indeed corresponded to a higher rate of inbreeding. For example, with r_g = -0.8 ($h^2_D = h^2_S = 0.1$), values obtained from scenarios 1 and 3 were T^2_1 = 0.05 and T^2_3 = 0.39, and scenario 1 yielded a greater rate of inbreeding than scenario 3 (Figure 3.2b). However, for the cooperative situation, the rate of inbreeding increased when T^2 increased. For example, with r_g = 0.8 ($h^2_D = h^2_S = 0.1$), values obtained from scenarios 1 and 3 were T^2_1 = 0.37 and T^2_3 = 0.66, and the rates of inbreeding for scenario 3 were greater than for scenario 1 (Figure 3.3b).

The above results on the relationship between inbreeding with classical heritability and T^2 show that patterns observed with selection on classical BLUP-EBV cannot simply be extended to schemes that aim at improving socially affected traits. One reason is that the correlation between EBV of sibs is no longer a simple function of heritability, but depends also on the direct-indirect genetic correlation and on relatedness between group mates. In principle, the theory of long-term genetic contributions [Wray and Thompson, 1990; Woolliams and Bijma, 2000] can be used to predict the rate of inbreeding for socially affected traits using a deterministic approach, similar to its application to selection on traditional animal model BLUP EBV [Bijma and Woolliams, 2000]. However, this requires a pseudo-BLUP selection

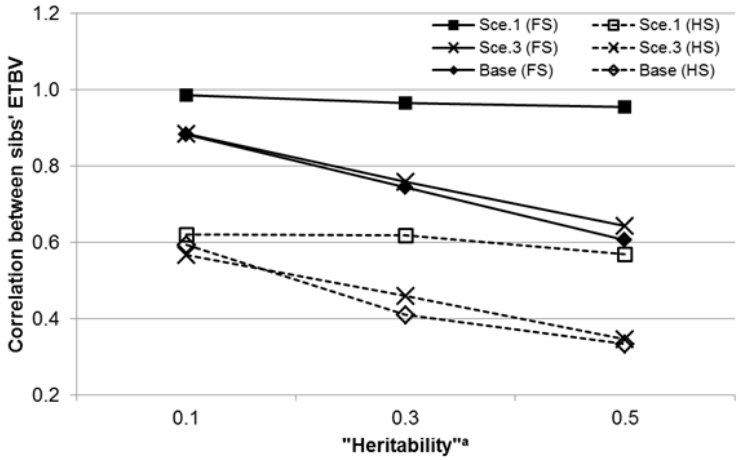
index [Wray and Hill, 1989] for socially affected traits. Although this is relatively straight-forward in principle, the full single-trait pseudo-BLUP selection index for a socially affected trait with sib information has 24 distinct information sources (results not shown). Hence, deterministic prediction of the rate of inbreeding with social effects is feasible but cumbersome, and one may prefer to use stochastic simulations instead.

3.4.4. Relevance of the results to other situations

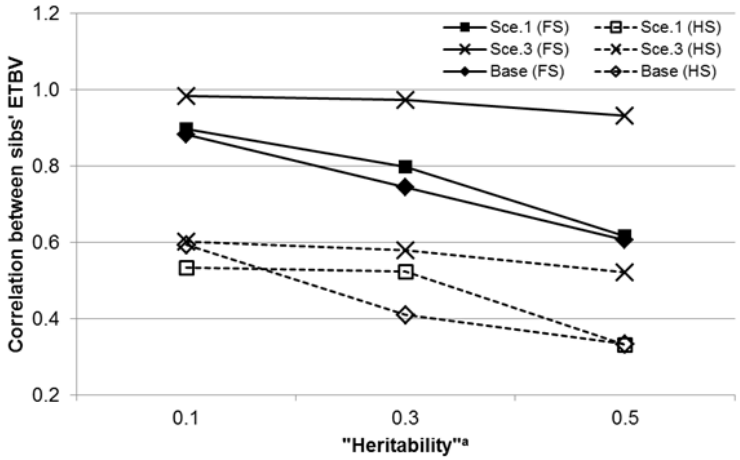
This study focused on breeding schemes in aquaculture. However, the results are also relevant for other species in which selection is based on sib information. In our simulations, we covered a wide-range of values with respect to the magnitude of indirect effects, heritabilities and direct–indirect genetic correlations. For all scenarios, presence of IGE increased the rate of inbreeding. Moreover, our group size of eight individuals is similar to group sizes in pigs and laying hens bred in cage systems. Furthermore, the design with two families per group is optimal for maximizing response to selection while maintaining the opportunity to estimate genetic parameters [Bijma, 2010], and is thus relevant for any breeding scheme for traits affected by IGE. However, the strategy of mating one male to two females is typical for breeding programs applied on some aquaculture species but it is uncommon in livestock. We do not expect that the mating ratio will substantially change the impact of IGE on the rate of inbreeding in sib selection schemes. Hence, we postulate that the main result of this study, which is the presence of IGE increases rates of inbreeding, can be extended to sib selection schemes in other species.

3.4.5. Solutions and future direction to manage rates of inbreeding

Because rates of inbreeding are greater with IGE, breeding programs that aim at improving socially affected traits require greater effort to contain inbreeding, which means that more genetic gain has to be sacrificed. Optimum contribution selection [Meuwissen, 1997] is the best method to restrict the rate of inbreeding while maximizing genetic gain, and implementation to traits affected by IGE is straightforward. Compared to current breeding schemes in aquaculture, which often rely on sib information, the use of genomic selection will decrease the correlation between EBV of sibs. Hence, we anticipate that the cost of restricting inbreeding will be reduced with genomic selection, and, for that reason, aquaculture breeding programs for socially affected traits would also benefit from genomic selection.



(a)



(b)

Figure 3.4 Correlations between estimated total breeding values (ETBV) for full-sibs (FS) and for half-sibs (HS) for a correlation between direct and indirect genetic effects of −0.8 (a) or +0.8 (b). [a]Heritabilities are the ratio of additive genetic variance to the corresponding "phenotypic" variance; for direct effects $h_D^2 = \sigma_{A_D}^2/\sigma_{P_D}^2$, and for indirect effects $h_S^2 = \sigma_{A_S}^2/\sigma_{P_S}^2$.

The feasibility of BLUP and optimum contribution selection, however, depends on the availability of pedigree or genomic information [Meuwissen, 1997]. Aquaculture breeding programs in developing countries are generally faced with

difficulties to maintain a fully pedigreed structure because it is too costly to individually identify the fish. When pedigree or genomic information is not available, breeders have to rely on mass selection. IGE will not affect the rate of inbreeding with mass selection when groups are composed at random with respect to relatedness because IGE do not affect the ranking of individuals in this case [Bijma *et al.*, 2007a]. However, genetic improvement of traits affected by IGE using mass selection is efficient only when the population is structured into groups consisting of families [Griffing, 1976; Bijma *et al.*, 2007a]. Such schemes will also increase the resemblance between phenotypes of relatives and thus lead to increased rates of inbreeding when mass selection is simply by truncation based on the observed phenotype. Hence, in those cases, breeders will have to restrict the contribution of individual families to the next generation, which is less efficient and will yield further reduction in genetic gain compared to full optimal contribution selection.

3.5. Conclusions

Our study shows that BLUP selection for socially affected traits increases the rate of inbreeding compared to traditional BLUP selection. This is at least partly due to the greater resemblance between EBV of relatives when animals are kept in groups consisting of two families. When accounting for IGE in a selection program, measures have to be taken to limit the rate of increase in mean kinship. Such measures may include optimum contribution selection, or limiting the number of candidates selected from each family.

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

HLK carried out the simulations with the help of PB, and drafted the manuscript. PB contributed in designing the study. RWP helped in interpreting the results and in drafting the manuscript. All the authors read and approved the final manuscript.

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4

Genotype by production environment interaction in the GIFT strain of Nile tilapia (*Oreochromis niloticus*)

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Abstract

Three discrete generations of GIFT fish (Nile tilapia strain, *Oreochromis niloticus*; a total of 10,065 fish with pedigree and phenotypic information) were tested in pond and cage culture environments to determine genotype by production environment interaction between both environments in Malaysia. Live weight (selected trait), standard length, body depth and width were recorded. A bivariate animal model was used to estimate variance and covariance components, whereby the homologous body traits in pond and cage environments were treated as genetically distinct traits. The heritabilities estimated for these body traits ranged from 0.19 to 0.40 in the pond environment, and from 0.23 to 0.34 in the cage environment. Across all traits the maternal common environmental effects ranged from 0.14 to 0.26 and were greater for the pond than for the cage environment. The genetic correlations between the pond and cage environments were 0.73±0.09 for live weight, 0.81±0.09 for standard length, 0.78±0.10 for body depth, and 0.85±0.13 for body width. Coupled with the total selection responses for live weight after two generations of selection, being 35% for the pond environment and 45% for the cage environment, we concluded that genotype by environment interaction for GIFT strain between pond and cage environments was not important. Hence, it would not be necessary to have two separate selective breeding programs for the GIFT strain in Malaysia.

Key words: Nile tilapia, selection response, correlated response, genotype by environment interaction, genetic correlation

4.1. Introduction

Tilapia farming has become one of the most important aquaculture industries in Malaysia. In 2008, Nile tilapia (*Oreochromis niloticus*) accounted for 24% of the total tilapia production in Malaysia, the other 76% fell under the category of incompletely identified tilapia species, tilapia *nei* [FAO, 2010]. The Department of Fisheries Malaysia estimates that out of this 24% of Nile tilapia production, 10% belonged to the GIFT (Genetically Improved Farmed Tilapia) strain [Hamzah, 2010, Personal Communication].

The GIFT strain is well known worldwide for its high growth performance and hardiness. In 1989 the GIFT selective breeding project started in Philippines in collaboration with institutes and universities from various countries. The project ended in 1998 after five generations of selection [Bolivar, 1998; Eknath and Acosta, 1998; Eknath *et al.*, 1993; Tayamen, 2004]. As one of the partners in the GIFT project, the WorldFish Center received representatives from the GIFT families to continue the work in Malaysia, where its headquarters are located. In Malaysia, the breeding program continued the selection for live weight at harvest time to improve the growth rate of the strain [Ponzoni *et al.*, 2005]. The GIFT fish in Malaysia are under the care of the WorldFish Center in collaboration with the Department of Fisheries Malaysia. The fish are being disseminated to government and private hatcheries within Malaysia, and also to other Asian and Latin American countries.

In aquaculture breeding programs, selection takes place in a nucleus, which is usually kept in a well-controlled environment, whereas a wide range of commercial production environments usually exist (e.g. cages, canals, reservoirs, lakes, and mining ponds). This diversity of production environments may result in genotype by environment (G×E) interaction. In the context of animal breeding, G×E interaction describes the situation where different genotypes do not respond in the same way to different environments, so that the genetic and environmental effects are not additive. Falconer [1952] suggested that the same phenotype expressed in two different environments can be treated as two genetically different traits, so that the degree of G×E interaction can be quantified from the genetic correlation between the trait expressions in both environments. In aquatic animals genotype by environment interactions were considered as an issue in farmed fish as early as the 1970s [Moav *et al.*, 1975]. Working with the common carp, these authors recognized their importance and identified their presence.

In Malaysia, tilapia farming is mainly conducted in two production systems, namely, cage and pond culture systems [Hanafi and Chua, 2008]. Farmers are increasingly shifting away from pond culture, because cage culture is more economical in terms of land use, only a minimum infrastructure is required, and cages are easier to manage [Hanafi and Chua, 2008]. However, in Asia, most of the selective breeding programs for Nile tilapia have been conducted under pond culture systems, including the GIFT breeding program [Bolivar, 1998; Eknath and Acosta, 1998; Eknath *et al.*, 1993; Tayamen, 2004; Zimmermann and Natividad, 2004]. Thus, it is very important to examine the G×E interaction between cage and pond culture systems, to investigate whether the genetic gain achieved in a pond environment will be realized in a cage environment. Several studies have investigated G×E interaction in Nile tilapia, but the degree of G×E interaction between pond and cage production systems in Malaysia has not been investigated to date for the GIFT strain [see review by Ponzoni *et al.*, 2011]. The estimated genetic correlations for live weight vary among studies and very much depend on the degree of differences between the tested environments [Eknath *et al.*, 2007; Khaw *et al.*, 2009; Luan *et al.*, 2008].

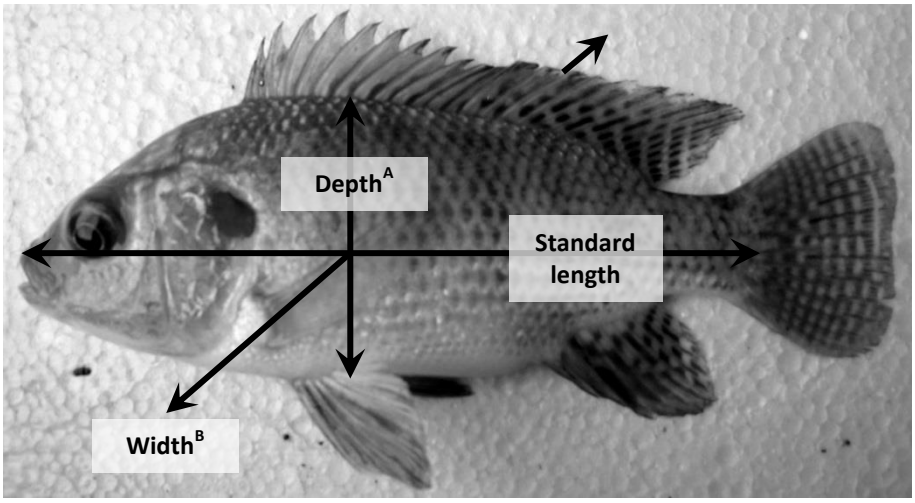

Figure 4.1 The definition of the fish body measurements ([A]Body depth is measured from dorsal to ventral locations at the mid-side of the fish; [B]Body width is measured from left to right lateral across the mid-side of the fish).

The objectives of this study were i) to estimate the genetic parameters for body measurements expressed in cage and pond environments, ii) to evaluate the

response to selection in both environments, and iii) to determine whether there was G×E interaction between both environments. For this purpose, we treated the body measurements at harvest in cage and pond systems as genetically distinct traits. Body measurements of interest were live weight, standard length, body depth, and body width (Figure 4.1).

4.2. Materials and methods

4.2.1. The environment

The GIFT breeding program in Malaysia is being conducted at the Aquaculture Extension Center, Department of Fisheries, Jitra, Kedah State, Malaysia. Details about the environment are provided by Ponzoni *et al.* [2005] and Nguyen *et al.* [2007].

4.2.2. The fish and data structure

The foundation stock of GIFT in Malaysia consisted of 63 full-sib groups (63 males, each mated to a different female) from the sixth generation of GIFT provided by the GIFT Foundation International Inc., Philippines [Ponzoni *et al.*, 2011]. These groups of fish were transferred in batches to Malaysia between the end of year 2000 and the beginning of year 2001. In the spawning season 2002, those fish were mated and produced the base population in Malaysia. No artificial selection took place among the fish producing the base population.

With the progeny produced in the spawning season 2002, two lines were created: the selection line that was selected for high live weight, and the control line that was selected for average live weight. All the tested fish were individually identified with Floy® tags at the size of about 10 g before sending them for communal rearing. The data set consisted of a total of 10,065 observations from three spawning seasons (2002, 2003, 2004, Table 4.1). Each male was mated either to one (control line) or two females (selection line) resulting in the number of sires and dams reported in Table 4.1. Progeny of all the sires and dams were represented in both cage and pond environments, except for a few of the families in the selection line that were not represented in the cage environment for spawning seasons 2003 and 2004, and one family in the control line in 2004 (Table 4.1). This was mainly due to tag losses, mortality and predation during the grow-out period in the cage environment. In the spawning season 2003, 69% of the parents were from the cage environment and the complement (31%) was from the

pond environment. By contrast, in 2004, the situation was almost the mirror image of that in 2003 with 30 and 70% coming from the cage and pond environment, respectively. The reproduction and management schedules are shown in Table 4.2. Note that the data set analyzed in the present study come from the same source [the GIFT selection program, Ponzoni *et al.*, 2011] as the data sets used by Ponzoni *et al.* [2005] and by Nguyen *et al.* [2007]. In this particular instance we analyzed them from a different angle, with a different purpose, hence generating different information. In the earlier work there was no attempt at estimating genotype by production environment interaction, whereas this latter issue is the main focus of the current paper.

Table 4.1 Number of sires, dams and progeny, by spawning season, line and environment.

Spawning season	Line	Environment	Sires	Dams	Progeny
2002	Base population	Cage	52	54	978
		Pond	52	54	706
2003	Selection	Cage	34	61	1524
		Pond	35	65	1036
	Control	Cage	19	19	695
		Pond	19	19	455
2004	Selection	Cage	53	83	1468
		Pond	54	84	2246
	Control	Cage	17	21	421
		Pond	17	22	536
Total			177	244	10065

Table 4.2 Schedule of reproduction and management in pond (Po) and cage (Ca) environment.

Activities	Spawning season		
	2002	2003	2004
Mating	February to March	January to February	November to February
Nursing	February to May	January to April	December to March
Tagging	April to May	March to April	February to March
Grow-out	June to November	April to September	March to September
Harvest	Po: 28 to 31 October	Po: 18 to 25 August	Po: 14 August to 22 September
	Ca: 9 to 13 November	Ca: 2 to 17 September	Ca: 14 August to 20 September

4.2.3. The grow-out system

The tagged fingerlings from each full-sib family were randomly allocated to two groups of equal size (50 fingerlings per group), and then sent for grow-out either in cages or earthen ponds. Growing out the relatives in two different environments enables the estimation of the genetic correlation between the expressions of the body traits in these two environments [Fishback *et al.*, 2002; James, 2009].

The cage culture farm was located at Kodiang, Kedah state, about 22km away from Jitra Aquaculture Extension Center. Four cages, each measuring 3m × 3m × 3m, adjacent to each other were established in an irrigation canal, and the fingerlings were randomly assigned to these four cages. The stocking density for each cage was 55 fish per square meter of surface water. The earthen pond used in this study was located at the Jitra Aquaculture Extension Center itself. The size of the pond was 0.1 ha with a stocking density of three to four fish per square meter. The stocking densities used for both environments were consistent with farming practices in Malaysia. In both environments the fish were fed twice a day, an amount of three to five percent of their average live weight. A commercial dry pellet feed containing 32% of protein was used. The water parameters (temperature, pH, dissolved oxygen) were monitored once a week.

4.2.4. Records

The fish in both environments were harvested after a period of approximately 120 days of grow-out. In the cage system, the fish were harvested by up-lifting the net and using a scoop net to put the fish into aerated tanks, which were later transferred to Jitra Aquaculture Extension Center and conditioned in hapas. In the pond system, a harvesting net was used to pull all the fish together, before drying out the pond to collect any remaining fish. After harvest, the fish were transferred to conditioning hapas. At harvest, records from all the tagged fish were taken for live weight, standard length, body width and depth. The latter two body traits were measured at the mid-side of the fish, where they were greatest (Figure 2.1). Sex of the fish was recorded by examining their genital papilla. Based on the spawning and harvesting dates, the age of each fish was computed. Upon the completion of data recording, the male and female fish were separated and returned to different conditioning hapas to avoid unintentional mating during the conditioning period that could subsequently affect their reproductive performance in the breeding program.

4.2.5. Selection and mating

The selection of parents to breed the next generation was based on the estimated breeding values (EBV) for live weight (live weight square root transformed, $LW^{0.5}$) calculated from a univariate animal model, and fitting environment (cage and pond) as fixed effect. In other words, the choice of parents for the next generation was made independently of the environment in which the fish had grown. The model used for breeding value estimation was as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_1\mathbf{a} + \mathbf{Z}_2\mathbf{c} + \mathbf{e}$$

where, $\mathbf{y}$ is the vector of the observed individual body traits in cage and pond environment; $\mathbf{b}$ is the vector of fixed effects, which were "spawning season, line, environment, sex sub-classes and their interaction with the covariate age at harvest; $\boldsymbol{a}$ is the vector of random additive genetic effects; $\mathbf{c}$ is the vector of random maternal common environment effects (accounting for maternal and common environment shared among the full-sibs before communal rearing); $\mathbf{e}$ is the vector of residual effects. The $\mathbf{X}$, $\mathbf{Z}_1$ and $\mathbf{Z}_2$ are the design matrices assigning observations to the levels of fixed effects, additive genetic effects, and maternal common environment effects, respectively.

For selection purposes all the individuals were ranked on estimated breeding value within each sex. After that, the best male from the best full-sib family was selected to mate with the best females from the best and the second best full-sib families (we mated one male to two females) with the condition that the male and female breeders were not closely related. In other words, the mating of the potential pair of breeders should result in progeny with no inbreeding. The intention was to keep inbreeding low in the population and at the same time select individuals of high genetic merit for growth rate. In addition, we attempted controlling the contribution of each family to the next generation to one male and two females. The adopted selection and mating strategy described above resulted in low between family selection intensity, but high within family selection intensity [refer to Ponzoni *et al.*, 2005, 2010a, for further details on selection and mating process].

4.2.6. Data analysis

4.2.6.1. General

The SAS statistical software [SAS Institute Inc., 1990] was used to obtain descriptive statistics and to remove anomalies, for example, to make sure that the parents were correctly sexed and to check-out duplicate data. Preliminary selection of

statistical models was conducted using PROC MIXED in SAS [SAS Institute Inc., 1997] by treating the homologous body traits in cage and pond as a single trait in univariate analyses within each environment. The fixed effects fitted with PROC MIXED were spawning season (2002, 2003 and 2004), line (selection and control), sex (male and female), and their two-way interactions. Sire and dam nested within sire, were fitted as random effects. Age at harvest was included as linear covariate to correct for variation in age. Note that in these analyses, the base population (spawning season 2002) was treated as part of the established control line.

4.2.6.2. Estimation of phenotypic and genetic parameters

Variance and covariance components of body measurements were estimated by residual maximum likelihood (REML) fitting an animal model with full pedigree information, implemented in ASReml [Gilmour *et al.*, 2002]. In order to quantify the G×E interaction between cage and pond environments, the genetic correlation was estimated by treating each of the homologous body traits in these two environments as two different traits in a bivariate analysis. Due to the fact that one animal can only grow-out in one environment, there would be no environmental (residual) correlation between the homologous traits, and the environmental covariance was set to zero in the bivariate analysis [James, 2009]. This also means that the phenotypic correlation cannot be estimated.

In the preliminary analysis, the two-way interactions among fixed effects were either statistically non-significant or deemed unimportant because they were due to a scale effect and not to a reversal of rankings. Therefore, in ASReml, we fitted the following model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_1\mathbf{a} + \mathbf{Z}_2\mathbf{c} + \mathbf{e}$$

where, $\mathbf{y}$ is the vector of observed individual body traits in cage and pond environment; $\mathbf{b}$ is the vector of fixed effects, which were "spawning season, line, sex" sub-classes and their interaction with the covariate age at harvest; $\boldsymbol{a}$ is the vector of random additive genetic effects; $\mathbf{c}$ is the vector of random maternal common environment effects (solely accounting for maternal and common environment shared among the full-sibs before communal rearing); $\mathbf{e}$ is the vector of residual effects. The $\mathbf{X}$, $\mathbf{Z}_1$ and $\mathbf{Z}_2$ are the design matrices assigning observations to the levels of fixed effects, additive genetic effects, and maternal common environment effects. Age at harvest was fitted as covariate with the spline option in ASReml [Gilmour *et al.*, 2002]. The same statistical model as described was used

for all body traits. The square root transformation improved the distribution of residuals of live weight, but not of the other three body traits. For this reason it was used for live weight only in all the analyses.

The heritability (h^2) and common environmental effect (c^2) were calculated as the ratio of the additive genetic variance (estimated from the animal effect), or the common environmental variance (estimated from the dam effect), and the phenotypic variance, respectively. The genetic correlation and maternal common environment correlation between cage and pond environments were calculated as:

$$r_g = \frac{cov(A_{cage}, A_{pond})}{\sigma_{A_{cage}} \cdot \sigma_{A_{pond}}}; r_c = \frac{cov(c_{cage}, c_{pond})}{\sigma_{c_{cage}} \cdot \sigma_{c_{pond}}}$$

where, A denotes additive genetic effects and c as maternal common environment effects, and, σ_A and σ_c are the corresponding standard deviations.

4.2.6.3. Direct selection response and correlated responses

The selection responses of live weight in both environments were estimated by two different methods. In Method 1, the selection responses were calculated by comparing the estimated breeding value (EBV) of progeny from the selection line between spawning seasons. For Method 2, the comparisons were made based on the EBV of progeny between both lines, selection and control, in each spawning season. The selection responses were expressed as a proportion of the phenotypic least squares mean of the control line in percentage units. The same methods were used to estimate the correlated responses in standard length, body depth and width in cage and pond environments. Correlated response is a change in an unselected trait resulting from genetic selection of another trait that is genetically correlated with the unselected trait.

4.3. Results

4.3.1. General

The phenotypic mean of live weight in the pond environment, 222.9g, was greater compared to cage environment, 146.5g (Table 4.3). On the other hand, the phenotypic means of standard length, body depth and width between cage and pond environments were not much different within each of the homologous traits (Table 4.3). In terms of phenotypic variation, again, standard length, body depth

and width showed similar coefficient of variation (CV) between these two environments. However, for live weight, the CV in cage environment was about 6% greater than the CV in pond environment (Table 4.3).

Tables 4.4a to 4.4d show the statistical significance for the fixed effects and the linear covariate (age at harvest) for cage and pond, respectively, on the four body traits. All the fixed effects and the covariate were statistically significant ($P<0.05$). For the average age at harvest, the difference between cage and pond environments was about 10 days. This could be due to the shortage of manpower and experience during earlier generations, where the harvest had to be done in one environment first, and later in the other environment (Table 4.2). Survival rate was estimated from the number of fish present at harvest time, relative to those that were initially stocked after tagging. The value obtained in this way was about 80% across environments. Out of the surviving fish to harvest time, about half had lost their tag and therefore could not be identified. The unidentified fish were not included in the estimate of selection response because we were unable to ascertain the line (selection or control) they belonged to.

Table 4.3 Number of observations (N), simple mean (μ), minimum and maximum, standard deviation (σ) and coefficient variation (CV,%) of live weight (g), standard length (cm), depth (cm), width (cm) and age (days) at harvest.

Variable	Environment	N	μ	Min	Max	σ	CV
Live weight	Cage	5086	146.5	13.0	591.0	77.8	53
	Pond	4979	222.9	17.0	682.0	104.2	47
Standard	Cage	5086	15.6	7.0	25.0	2.8	18
length	Pond	4979	17.9	8.0	25.0	3.2	18
Depth	Cage	5086	6.5	2.0	11.0	1.3	21
	Pond	4979	7.5	2.0	12.0	1.5	20
Width	Cage	5086	2.8	1.0	5.0	0.61	22
	Pond	4979	3.4	1.0	6.0	0.69	20
Age at harvest	Cage	5086	240	151	289	27.5	11
	Pond	4979	230	125	302	32.7	14

Table 4.4a Analysis of variance of square root of live weight ($LW^{0.5}$): Tests of fixed effects using PROC MIXED.

Effect	Cage		Pond	
	F Value	Prob. > F	F Value	Prob. > F
Spawning season (SS)	53.66	< 0.0001	33.73	< 0.0001
Line (L)	13.90	0.0002	24.52	< 0.0001
Sex (S)	496.59	< 0.0001	440.65	< 0.0001
SS x S x L	3.90	0.0016	20.84	< 0.0001
Age at harvest	72.17	< 0.0001	409.67	< 0.0001
Residual Variance	4.0289		3.4832	

Table 4.4b Analysis of variance of standard length: Tests of fixed effects using PROC MIXED.

Effect	Cage		Pond	
	F Value	Prob. > F	F Value	Prob. > F
Spawning season (SS)	51.71	< 0.0001	46.90	< 0.0001
Line (L)	12.68	0.0004	27.74	< 0.0001
Sex (S)	477.80	< 0.0001	370.88	< 0.0001
SS x S x L	3.07	0.0091	16.75	< 0.0001
Age at harvest	87.03	< 0.0001	573.02	< 0.0001
Residual Variance	3.2226		2.4449	

Table 4.4c Analysis of variance of body depth: Tests of fixed effects using PROC MIXED.

Effect	Cage		Pond	
	F Value	Prob. > F	F Value	Prob. > F
Spawning season (SS)	57.31	< 0.0001	44.26	< 0.0001
Line (L)	10.96	0.0009	22.86	< 0.0001
Sex (S)	433.39	< 0.0001	319.76	< 0.0001
SS x S x L	4.77	0.0002	18.01	< 0.0001
Age at harvest	99.76	< 0.0001	273.86	< 0.0001
Residual Variance	0.8197		0.7032	

Table 4.4d Analysis of variance of body width: Tests of fixed effects using PROC MIXED.

Effect	Cage		Pond	
	F Value	Prob. > F	F Value	Prob. > F
Spawning season (SS)	25.10	< 0.0001	28.08	< 0.0001
Line (L)	8.47	0.0036	17.39	< 0.0001
Sex (S)	163.89	< 0.0001	134.16	< 0.0001
SS x S x L	3.49	0.0038	8.78	< 0.0001
Age at harvest	47.03	< 0.0001	183.24	< 0.0001
Residual Variance	0.2255		0.2053	

4.3.2. Estimation of phenotypic and genetic parameters

The phenotypic and genetic parameters estimated in this study are presented in Table 4.5. The heritabilities (h^2) estimated across the four body traits expressed in two different environments were moderate to high, ranging from 0.19 to 0.40. Within the homologous traits, the h^2 estimated in cage and pond environments were checked for significance of the differences by using z-score and all were found to be not significantly different from each other (P>0.05).

The maternal common environment effects (c^2) estimated for cage environment across four body traits were smaller compared to pond environment. In all the body traits for pond environment, the c^2 accounted for a large proportion of the total variance and it was more variable, ranging from 0.17 to 0.26. The c^2 estimates in cage environment were lower and ranged from 0.14 to 0.18. However, the difference between c^2 of cage and pond for each homologous trait was not statistically significant (P>0.05).

Table 4.5 Phenotypic and genetic parameters for live weight ($LW^{0.5}$), standard length (SL), body depth (D) and body width (W), and their genetic and maternal common environment correlations between cage (Ca) and pond (Po) environments.

Parameter	Env	REML Estimate			
		$LW^{0.5}$	SL	D	W
Phenotypic Variance (σ_P^2)	Ca	7.00[a]	5.36	1.26	0.29
	Po	6.78[a]	4.18	1.15	0.26
Heritability (standard error) [h^2 (s.e.)]	Ca	0.34 (0.061)	0.33 (0.065)	0.27 (0.058)	0.23 (0.056)
	Po	0.40 (0.067)	0.35 (0.066)	0.35 (0.067)	0.19 (0.057)
Maternal common environment (standard error) [c^2 (s.e.)]	Ca	0.18 (0.027)	0.18 (0.028)	0.15 (0.024)	0.14 (0.024)
	Po	0.26 (0.031)	0.21 (0.029)	0.20 (0.028)	0.17 (0.026)
Genetic correlation (standard error) [r_g (s.e.)]		0.73 (0.092)	0.81 (0.094)	0.78 (0.103)	0.85 (0.127)
Maternal common environment Correlation (standard error) [r_c (s.e.)]		0.38 (0.095)	0.29 (0.104)	0.25 (0.113)	0.36 (0.112)

[a] Those values can be back-transformed to the original scale using a first-order Taylor-series approximation: $\mathrm{LW}^{0.5} \approx \overline{\mathrm{LW}}^{0.5} + 0.5\overline{\mathrm{LW}}^{-0.5}(LW - \overline{\mathrm{LW}})$, so that $\mathrm{var}(\mathrm{LW}) = 4\overline{\mathrm{LW}} \cdot \mathrm{var}(\mathrm{LW}^{0.5})$. Using this approach, phenotypic standard deviations on the original scale were 64g for the cage environment and 78g for the pond environment.

The genetic correlations estimated for body traits between cage and pond environments ranged from high to very high with 0.73±0.092 for live weight, 0.78±0.103 for body depth, 0.81±0.094 for standard length and 0.85±0.127 for body width (Table 4.5). All the genetic correlations were significantly different from unity (P<0.05), except for width (P>0.05). The maternal common environment correlations were estimated for all the four body traits (Table 4.5). All the estimates were positive but low. However, all the estimates were significantly different from zero (P<0.05).

As a test for robustness of the estimates, we checked out the effect of outliers in the parameter estimates, based on the output from ASReml. Outliers are defined as residuals that are more than 3.5 standard deviations in magnitude [refer to page 244, Gilmour *et al.*, 2009]. For each of the traits we compared the parameters estimated with the complete data set without removing the outliers, with the results obtained after removal of outliers. After removing the outliers, the variances, h^2 and c^2 estimated either remained the same as before removal (for body depth and width) or increased just by one to two percent compared to the original estimates (for live weight and standard length). Regarding genetic correlations, almost all of the estimates increased by six to nine percent after removal of outliers, except for the estimate for body depth that remained the same. For maternal common environment correlations the estimates either remained the same as with the complete data set (for live weight and depth) or decreased by three to five percent (standard length and body width). Overall the estimates seemed robust to the presence or absence of outliers. All the parameters presented in this paper were estimated from the complete data set without removing the outliers.

4.3.3. Direct selection response and correlated responses

The direct selection and correlated responses estimated in this study were in relatively good agreement both between the estimation methods and between the environments (Table 4.6). For all body traits a trend can be observed whereby the direct selection and correlated responses expressed in the cage environment were always greater than those in the pond environment, ranging from one to five percent more.

With the exception of live weight, the rest of the body traits were analyzed, and are expressed, in their actual units of measurement. For live weight, the selection responses were estimated for both environments in square root transformed units.

The estimates obtained in this way should be doubled to estimate the response in actual units [James, 2007]. The selection responses for live weight presented in Table 4.6 are back-transformed based on James' method [2007]. Hence, in actual units the total selection responses in live weight after two generation of selection were 45% for cage environment and 35% for pond environment (estimates from Method 2, Table 4.6).

Table 4.6 Direct response to selection in live weight (LW) and correlated responses in standard length (SL), body depth (D) and width (W).

Method	Environment	Direct Response (%)[a]	Correlated responses (%)[a]		
		LW	SL	D	W
1. Comparing the estimated breeding values between progeny from the selection line in two consecutive spawning seasons					
Spawning season	Cage	20.2	5.9	6.2	4.6
2002 and 2003	Pond	14.6	3.7	4.6	2.5
Spawning season	Cage	17.6	5.2	5.1	3.7
2003 and 2004	Pond	15.8	3.7	4.5	2.5
2. *Comparing the estimated breeding values between progeny from the control line and selection lines in the same spawning season*					
Spawning season	Cage	25.6	7.5	7.7	5.7
2003	Pond	18.2	4.6	5.6	3.0
Spawning season	Cage	45.0	13.2	13.3	9.6
2004[b]	Pond	34.6	8.4	10.1	5.5

[a] Direct and correlated responses are expressed as a percentage of the overall least squares mean (LSM) of the control line in cage and in pond environments, for each trait. Overall LSMs for cage and pond were 125.9g and 196.8g for LW (back-transformed from the least squares means computed with square root transformed data), 14.12cm and 18.33 cm for SL, 5.81cm and 7.58cm for D, and 2.53cm and 3.41cm for W, respectively.

[b] Note that the responses predicted in spawning season 2004 with Method 2 are equal to two ΔG.

4.4. Discussion

4.4.1. Overall findings

The estimates of both the genetic correlation between environments and the selection responses obtained in each (cage and pond) environment indicate that the magnitude of G×E interaction was small. Genetic correlations between traits expressed in both environments were around 0.8. Response to selection in the cage environment was somewhat greater than in the pond environment.

Heritabilities and maternal common environment effects were similar in both environments, but they were slightly greater in the pond environment. The maternal common environment correlations, in contrast, were considerably lower than the corresponding genetic correlations, suggesting the presence of an interaction between such effect in the cage and pond environments.

4.4.2. Descriptive statistics

The average live weight of the fish in the pond environment was 52% greater than in the cage environment, whereas the other three body measurements were only 15% larger in pond environment. These differences between live weight and body measurement traits make sense when considering the relationship between volume and size, where volume = length × height × depth. Thus, for an increase of 15% on each measurement trait, we should expect the live weight to increase by $(1.15)^3$, which is equal to 1.52, which is consistent with our result. This also explains the smaller differences between cage and pond environments for standard length, depth and width, compared to live weight.

Floy® tags were used to individually identify the fish before placing them in communal rearing. Floy® tag is a flat oval shape plastic disk with family and individual numbers printed on both sides. The tag is attached to the fish by a vinyl thread inserted with the aid of a needle, going through the anterior cartilage tissue under the sixth and seventh spines of the dorsal fin of the fish. During harvest, when bigger and stronger fish have their tags entangled on the fishing net, they pull and break the thread to get away. We observed numerous tag losses during harvest, especially among fish reared in the pond environment where we used a fishing net to harvest the fish. Consequently, we lost many potential breeders. In later generations, PIT (Passive Integrated Transponder) tags have been used, which have a better retention rate.

The overall coefficient of variation (CV) of weight in cage across three generations was greater than the CV of weight in pond. Although the difference in CV was relatively small, it could be interpreted as indicating that there was more competition in the cage environment compared to the pond environment. This would be reasonable, because the stocking density was much greater in cages than in ponds. Generally, an increase in CV indicates inter-individual competition and dominance hierarchy [Adams *et al.*, 2000; Jobling, 1995; McCarthy *et al.*, 1992]. On the other hand, low CV is suggestive of less competition and of a good social environment within population [Jobling, 1995; Mambrini *et al.*, 2006]. According to

James [2009] competition is an important environmental factor leading to G×E interactions.

4.4.3. Phenotypic, genetic parameters and selection responses

The heritabilities of live weight estimated in cage and pond environments were greater than those reported for other tilapia populations in the literature. For example, 0.24 to 0.25 by Gall and Bakar [1999], 0.20 by Gall and Bakar [2002], 0.26 by Rutten *et al.* [2005], 0.24 and 0.19 by Luan *et al.* [2008], and 0.14 by Rezk *et al.* [2009]. However, they were consistent with those earlier reported for the same population of GIFT in Malaysia [0.34 by Ponzoni *et al.*, 2005; 0.35 by Nguyen *et al.*, 2007; 0.30 by Khaw *et al.*, 2010; 0.31 by Nguyen *et al.*, 2010]. Note that in the just cited analyses of the Malaysian GIFT population, cage and pond were treated as a fixed effect, whereas in the present study the expression of weight and other body measurements in cage or pond were treated as if they were different traits.

The estimated heritabilities for standard length in cage and pond environments were in good agreement with those estimated from other Nile tilapia populations [0.25 by Rutten *et al.*, 2005; 0.40 to 0.60 by Charo-Karisa *et al.*, 2007], and consistent with the estimates earlier reported for the GIFT population in Malaysia [0.30 by Nguyen *et al.*, 2007; 0.31 by Nguyen *et al.*, 2010]. There are fewer estimates of heritability for standard length in fish species than for live weight. This could be due to the fact that standard length has a less clear economic value compared to live weight. However, standard length will have value if body shape is an important trait or when measuring live weight is not possible (for example, a proper digital scale may not always be easily available in developing countries). The genetic correlations between live weight and length estimated from a variety of fish species are close to unity indicating that these two body traits are controlled by the same set of genes [0.87 by Rutten *et al.*, 2005; 0.95 by Charo-Karisa *et al.*, 2007; 0.95 by Nielsen *et al.*, 2010].

The heritabilities estimated for body depth and width in cage and pond environments were consistent with previous studies in the Malaysian GIFT population [0.32 for depth and 0.20 for width by Nguyen *et al.*, 2007; 0.29 for depth and 0.20 for width by Nguyen *et al.*, 2010]. To our knowledge, there is no other tilapia or any other fish species for which the heritability for body depth, or for width, or for both have been reported, except the study by Rutten *et al.* [2005] where they estimated a heritability for body width of 0.25. For the GIFT population in Malaysia, the genetic correlations between each pair of analogous body traits

(live weight, standard length, depth and width) have been previously estimated by treating the environmental effect (cage or pond) as a fixed effect fitting a multivariate model; all the estimates reported are close to unity [0.95 to 0.99; Nguyen *et al.*, 2007, 2010].

The maternal common environment effects (c^2) estimated for all the body traits were in good agreement with those earlier reported for the GIFT population in Malaysia [Ponzoni *et al.*, 2005; Nguyen *et al.*, 2007; Khaw *et al.*, 2010], except those estimated by Nguyen *et al.* [2010] where the study was on parameters estimation for fillet traits and also the body traits. In the study by Nguyen *et al.* [2010], a sub-set of the GIFT population from three consecutive generations were grown to an average weight of 700g. A much smaller c^2 (0.04 to 0.08 for four different body traits) was found, compared to those studies with GIFT where the fish were harvested at 250g (on average). The authors concluded that the magnitude of c^2 diminished with the more prolonged grow-out period. This is supported by the study conducted by Winkelman and Peterson [1994] on Chinook salmon (*Oncorhynchus tshawytscha*). These authors suggested that c^2 may not be important for market performance, especially those that grow the fish until 800g and above for fileting, restaurant and export markets. However, when the c^2 is substantial, it is essential to account for this effect in the statistical model in order to have unbiased estimation of parameters and increased accuracy of selection [Maluwa *et al.*, 2006; Nguyen *et al.*, 2007].

The maternal common environment correlations (r_c) obtained in this study between the two different culture environments were low but positive. The low r_c indicates that the maternal common environmental effects resulting from the environment the full-sibs shared early in their life became smaller due to the separation into two environments during the grow-out period. The estimates were consistent with that obtained by Luan *et al.* [2008] when they tested the fish in freshwater and brackish water (r_c=0.36±0.13).

Gjedrem [2000] predicted that genetic gain per generation for aquatic animals may range from 10% to 20%. In the present study, the estimates of genetic gains for live weight in cage and pond environments fell within Gjedrem's prediction. The estimates were also in good agreement with those of genetic gain for live weight reported in the published literature [Bolivar and Newkirk, 2002; Gall and Bakar, 2002] and our previous study [Khaw *et al.*, 2010]. We may conclude that for all the traits, the responses were large enough to indicate that genetic change was being

achieved in both the cage and the pond environments, and in the intended direction. Note also that the amount of tag losses among vigorous and large fish observed in this study may have caused a negative bias on the selection responses.

The estimates of the magnitude and importance of G×E interactions in fish vary across studies, and they depend on the degree of differences between the test environments, as well as on the experimental designs. Eknath *et al.* [2007] conducted the G×E study for the earlier GIFT strain in Philippines in seven different culture environments, which can be separated in two main groups, cage and pond environments. Eknath *et al.* [2007] found genetic correlations for harvest weight ranging from 0.76 to 0.99 within pond environments, and 0.99 within cage environment. By contrast, the genetic correlations estimated between cage and pond environments were lower and more variable, ranging from 0.36 to 0.82. Our estimate of genetic correlation for live weight between cage and pond environments was in agreement with the higher values reported by Eknath *et al.* [2007]. The results reported by Eknath *et al.* [2007] suggest that greater differences between the test environments increase the chances of finding significant and important G×E interactions. This is consistent with the prediction made by Moav *et al.* [1976], in the context of the transition from traditional to modern fish farming, that if a broad range of environments is considered genotype by environment interactions will emerge. This same research group later found [Wohlfarth and Moav, 1990] a poor association between performance in cages and ponds.

The G×E study conducted by Luan *et al.* [2008] has further validated the finding by Eknath *et al.* [2007]. Luan *et al.* [2008] reared Nile tilapia in freshwater and brackish water systems, and they found a relatively low genetic correlation for live weight between these two systems, of 0.45±0.09. In other words, there is G×E interaction between freshwater and brackish water environments. In the study conducted by Dupont-Nivet *et al.* [2010] where European sea bass (*Dicentrarchus labrax*) were tested in four different conditions (recirculation system, raceway with well water, estuarine earthen pond and tropical seawater cage) located in four different countries (France, Israel, Italy and Portugal), no significant G×E interactions for live weight were found (genetic correlation ranged from 0.75 to 0.93). However, they did find significant G×E interaction for daily growth coefficient (genetic correlations ranged from 0.21 to 0.61), with one exception between Italy and Israel (genetic correlation of 0.78). Furthermore, Kause *et al.* [2003], Kolstad *et al.* [2006] and Maluwa *et al.* [2006] recorded performance in relatively distinctive test environments, and did not find any significant G×E interactions in live weight for

rainbow trout (*Oncorhynchus mykiss*), Atlantic Cod (*Gadus morhua*) and *Oreochromis shiranus*, respectively. Kolstad *et al.* [2006] also concluded that live weight is a trait that has low sensitivity or less plasticity to environmental changes. Since G×E interaction varies among species and it is difficult to predict, Iwamoto *et al.* [1986] suggest that the study of G×E interaction should be done on case by case basis.

Despite the numerous studies on G×E interaction in fish species, it is still difficult to draw a general line on the magnitude of G×E interaction that should determine when to decide having separate breeding programs for each environment or a single breeding program for all environments [James, 2009]. Mulder *et al.* [2006] conclude that for genetic correlations of less than 0.7 to 0.8, optimal genetic gain will be achieved by having two separate breeding programs, provided both environments are equally important. This agrees approximately with Robertson's [1959] suggestion that for genetic correlations of 0.8 and above, G×E interactions could be considered unimportant. In addition to the statistical evidence on G×E interaction, Montaldo [2001] suggests that before making the decision to have multiple or single breeding programs, we need to take the following aspects into consideration: economic status of the country where the breeding program is going to be established, the likely future of the breeding program (international strain, intercontinental strain, or local strain), the evolution of the environmental conditions and the available resources of the country in question.

The data used in this G×E interaction study with the GIFT strain were collected during the period 2002 to 2005. Before we conducted the present detailed analyses it had already been decided to continue the selection in pond environment only. The ability to better control the environment and to avoid fish escapes to the natural environment were important considerations in making the decision. Currently, the GIFT population in Malaysia has undergone seven generations of selection and the genetic gain in harvest weight is more than 100% [Khaw *et al.*, 2010]. In addition, from the recent survey that we conducted with the GIFT custodians (within Malaysia, other Asian and Latin American countries with different kinds of culture environments, including cages and ponds), the feedback from the farmers denoted satisfaction with the performance of the fish, and as a consequence the number of farmers culturing GIFT has increased in all countries involved [Ponzoni *et al.*, 2010b]. This may be taken as circumstantial evidence that the strain performs well in a broad range of environments.

4.5. Conclusions

Coupled with the high genetic correlation for live weight (and other body traits) between cage and pond environments observed in the present study, the evidence outlined above regarding worldwide distribution and successful performance of GIFT, leads us to confidently conclude that the G×E interaction between cage and pond environments for the GIFT strain in Malaysia is not important. Consequently, there is no need to have two separate breeding programs.

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5

Genetic variance for uniformity of harvest weight in Nile tilapia (*Oreochromis niloticus*)

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Abstract

Competition for resources is common in aquaculture, which inflates the variability of fish body weight. Selective breeding is one of the effective approaches that may enable reduction of size variability (or increases in uniformity) for body weight by genetic means. The genetic variance of uniformity is commonly known as genetic heterogeneity of environmental variance for particular traits. The data collected from a social interaction experiment were used to investigate the presence of genetic variation in heterogeneity of environmental variance for harvest weight in GIFT strain. A total of 944 records pooled (by family-group) from 6330 individual harvest weights were used in the analysis. For genetic parameters estimation, we used a bivariate sire-dam model between harvest weight and its standard deviation. To normalize the residuals, individual harvest weight was Box-Cox transformed. The heritability (at the family by group level) and genetic coefficient of variation for standard deviation of Box-Cox transformed harvest weight (0.23 and 0.17, respectively) indicated uniformity on harvest weight was partly under genetic control. In addition, we found a very low genetic relationship between Box-Cox transformed harvest weight and its standard deviation, r_A = 0.095±0.183. Hence, these two traits can be selected in different directions using index selection, namely, aiming to increase growth rate while decreasing size variation. We conclude that there is potential to increase harvest weight and its uniformity by selective breeding in the GIFT strain of farmed tilapia.

Keywords: Heterogeneity of environmental variance, uniformity, Nile tilapia, harvest weight, Box-Cox transformation.

5.1. Introduction

Animals of a particular genotype can change their phenotype in response to changes in environmental conditions, and this is known as environmental sensitivity. Conversely, an animal that has the ability to maintain a constant phenotype across different environmental conditions is considered as phenotypically stable or robust. Genetic differences in environmental sensitivity can be studied as genetic heterogeneity of environmental variance, and can be utilized to improve uniformity of traits [SanCristobal-Gaudy *et al.*, 1998; Sorensen and Waagepetersen, 2003]. The increasing demand from consumers and farmers for uniformity of animals and animal products is one of the driving forces for animal breeders to put more attention in selection to improve uniformity for production and reproduction traits. From the point of view of retailers and the food processing industry, uniform animal products make the food processing procedure easier and more consistent in quality. Given adequate market signals, farmers could increase their profit margins by delivering more animals in the preferred weight range to the slaughter house [Hennessy, 2005; Mulder *et al.*, 2008].

Several studies have shown the existence of genetic heterogeneity of environmental variance [reviewed by Hill and Mulder 2010]. Genetic heterogeneity of environmental variance has been demonstrated using selection experiments, for example, in Drosophila [Waddington, 1960; Scheiner and Lyman, 1991], mice [Gutiérrrez *et al.*, 2006], and rabbits [Garreau *et al.*, 2008]. Furthermore, the genetic variance in environmental variance has been estimated, in poultry [Rowe *et al.*, 2006; Mulder *et al.*, 2009], pigs [Damgaard *et al.*, 2003; Sorensen and Waagepetersen, 2003], sheep [SanCristobal-Gaudy *et al.*, 2001], dairy cattle [Rönnegård *et al.*, 2013; Vandeplas *et al.*, 2013], and snails [Ros *et al.*, 2004]. More recently, two studies have shown a genetic basic for heterogeneity of environmental variance for body weight in salmonids [Janhunen *et al.*, 2012; Sonesson *et al.*, 2013].

In aquaculture, competition for resources is one of the main factors inflating the variability of body weight. Generally, a high CV indicates inter-individual competition within the population [Jobling, 1995]. Fish farmers reduce the variability by grading at several different stages during the grow-out phase, and also at harvest time for marketing purposes. Grading during the grow-out phase is harmful to fish welfare [Kubilay and Uluköy, 2002; King *et al.*, 2006], it entails labour costs and requires facilities to house the fish in different size groups.

Tilapia is one of the major species in the aquaculture industry. It has the highest number of breeding programs among the improved aquaculture species [Gjedrem *et al.*, 2012]. In the majority of the tilapia breeding programs, fish are selected for improved growth rate [for examples, see Bolivar, 1998; Eknath and Acosta, 1998; Tayamen, 2004]. One of the most well-known tilapia breeding programs is the Genetically Improved Farmed Tilapia (GIFT) program using Nile tilapia (*Oreochromis niloticus*), which has been through 12 generations of selection in Malaysia. The coefficient of variation (CV) for body weight in the GIFT population is relatively high, 40 to 60% [Ponzoni *et al.*, 2005; Nguyen *et al.*, 2007; Khaw *et al.*, 2010], compared to the salmonids for which the CV ranges from 20 to 40% [Gjedrem, 2000]. Hence, improvement of uniformity of body weight in the GIFT strain is desirable.

When there is a genetic basis for uniformity, selective breeding can be used to increase uniformity [SanCristobal-Gaudy *et al.*, 1998; Sorensen and Waagepetersen, 2003]. To figure out whether this is possible in GIFT, we need to investigate the issue in this strain. Thus, the objectives of this study were to quantify the genetic variation in variability of harvest weight, and the genetic correlation between harvest weight and variability of harvest weight in the GIFT strain of Nile tilapia.

5.2. Materials and methods

5.2.1. The environment and the fish

The experiment was conducted at the Jitra Aquaculture Extension Center of the Department of Fisheries, located at Kedah State of Malaysia. From 2009 to 2012, four batches of experimental fish were produced from the GIFT breeding program, one batch per year. The four batches of experiments were named after the year when the fish were stocked in the ponds. Batch 2012 was excluded because of high mortality due to extreme weather conditions; see Khaw *et al.* [2014] for details. The reproduction and selection methods used in the GIFT breeding program are outlined in Ponzoni *et al.* [2005, 2010].

5.2.2. The experimental design

The experimental design described in this study was established for the purpose of studying indirect genetic effects (IGE) for harvest weight in the GIFT strain [Khaw *et al.*, 2014]. The fry were nursed in full-sib groups in hapas (sized of 1m x 1m x 1m)

until they reached the tagging size of 2 to 5g. Then the fingerlings were individually identified with PIT (Passive Integrated Transponder) tags before they were stocked in net-cages of size 1m length x 1.5m width x 1m depth, installed in earthen ponds of size 0.1 ha. Two earthen ponds were used for grow-out. The distribution of families over net-cages followed the optimum design for estimating IGE described in Bijma [2010; see also Ødegård and Olesen 2011]. The fish were allocated to groups, each consisting of members of two distinct families. Each group consisted of 16 individuals, to which each family contributed eight randomly selected individuals.

During the grow-out phase, the fish were fed twice a day using commercial dry pellet feed containing 32% of protein at a rate of 3 to 5% of their average live weight. The feed was administered at a corner of the net-cage, instead of spreading it all over the surface of the net-cage. This feeding strategy was used to encourage competition among the fish. Water quality was monitored once a week. The fish were harvested at an average size of 200 to 250g. Table 5.1 shows the number of net-cages, families, groups and fish involved in each batch of the experiment. More details can be found in Khaw *et al.* [2014].

Table 5.1 Number of families, groups, and fish harvested, by batch; and the number of family-groups with more than one individual and with less than one individual, by batch.

Batch	No. of families	No. of net-cages installed[§]	No. of groups[Ψ]		No. of family-group[Φ]		No. of fish harvested
			Stocked	Harvested	With >1 fish	With 0-1 fish	
2009	68	364	212	209	401	17	2565
2010	31	288	45	45	83	7	509
2011	68	342	248	239	460	18	3256
Total	167	994	505	493	944	42	6330

[§]The number of net-cages presented refers to two ponds, each with an equal number of net-cages; not all the net-cages installed were used – some were left empty due to families or groups that collapsed before stocking and some were just left empty; [Ψ]Two full-sib families per group. [Φ]Since each group contained members of two families, the number of harvested family-groups is twice the number of harvested groups.

5.2.3. Records

At harvest, we recorded live weight, body length, body width, body depth, sex, tag number, net-cage label and pond number. The spawning and harvesting dates were used to calculate the age at harvest of the fish. A total of 493 groups, each with two families, were harvested over the first three batches, which resulted in 6330 fish

with phenotypic records. The pedigree data collected from this study were combined with those from the GIFT breeding program for (co)variance component estimation. The full pedigree consisted of 37,670 individuals over ten generations from the GIFT population.

To assess the need for data transformation, the skewness and kurtosis of the residual distribution of individual harvest weight were obtained using the R-package "moments" [Komsta, 2012]. Results suggested the need for data transformation. Therefore, the individual harvest weight records were Box-Cox transformed [Box and Cox, 1964] to normalize residuals and equalize residual variance. Box-cox transformation was used because it finds the optimal transformation parameter (the lambda, λ) for skewed data using the maximum likelihood procedure [Sakia, 1992]. The transformed variable is given by $y_i^{(\lambda)} = \frac{y_i^{\lambda} - 1}{\lambda}$. This transformation was performed using the MASS package in the R-program [R Development Core Team, 2011]. For the estimation of λ , the fixed effects fitted for individual harvest weight were the interaction of batch (2009, 2010, 2011), sex (male and female), pond (1 and 2), the linear covariate age at harvest fitted within this interaction term, the non-nested quadratic effect of age at harvest and the linear regression on social age at harvest. These are the same fixed effects fitted in the study by Khaw *et al.*, 2014. Hereafter, the Box-Cox transformed harvest weight is referred to as BC-harvest weight.

We analyzed two traits, the mean of BC-harvest weight of each family in each group, and the standard deviation of BC-harvest weight within each family in each group. We used standard deviation as measure of variability, rather than variance or log-variance, because standard deviation is easier to interpret and more likely to be the trait of interest for fish breeders. Due to mortality, some family-groups consisted of only one fish at harvest, and those were excluded from the analysis (Table 5.1). The final number of records was 944.

5.2.4. Statistical analysis

Variance and covariance components were estimated by residual maximum likelihood (REML) using the ASReml software [Gilmour *et al.*, 2009]. A bivariate analysis of BC-harvest weight and its standard deviation, measured on family by group level, with a sire-dam model was conducted. These two variables were first analyzed separately to obtain the best model for the bivariate analysis.

The model was,

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix}=\begin{bmatrix}\mathbf{X}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{X}_2\end{bmatrix}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_{1_{PAR}} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z}_{2_{PAR}}\end{bmatrix}\begin{bmatrix}\mathbf{a}_{1_{PAR}}\\ \mathbf{a}_{2_{PAR}}\end{bmatrix}+\begin{bmatrix}\mathbf{V}_1\mathbf{g}_1\\ \mathbf{0}\end{bmatrix}+\begin{bmatrix}\mathbf{U}_1\mathbf{m}_1\\ \mathbf{0}\end{bmatrix}+\begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix},$$

where subscript 1 refers to BC-harvest weight and subscript 2 to standard deviation of BC-harvest weight; $\mathbf{y}$ is the vector of phenotypic observations with two records for each trait per group, one record for each family; $\mathbf{b}$ is the vector of fixed effects; $\mathbf{a}_{PAR}$ is a vector of additive genetic parent effects, $\mathbf{g}$ is a vector of random group effects; $\mathbf{m}$ is a vector of random social maternal common environmental effects, and e is a vector of random residuals. The $\mathbf{X}$, $\mathbf{Z}_{PAR}$, $\mathbf{V}$ and $\mathbf{U}$ are the known design matrices. The $\mathbf{Z}_{PAR}$-matrix has a "1" in the columns for the sire and for the dam of the particular family producing the record. Hence, each row of $\mathbf{Z}_{PAR}$ contains two 1s. The random group effect in this model captures the non-genetic covariance between the BC-harvest weight records of the two families in a group. The random effect of the common social maternal environment was included in the model to account for the effect of the maternal environment of a full-sib family on the growth rate of the other full-sib family within the same group. The group and social maternal common environmental effects were not significant for standard deviation of BC-harvest weight, and therefore excluded from the standard deviation model. For the IGE model (on individual level), Khaw *et al.* [2014] included group-by-family effects, to account for differential non-genetic interactions between members of the same family versus members of different families within a group. This effect was not included here because we only have two observations per group so that the group-by-family effect is fully confounded with the residual.

For BC-harvest weight, the fixed effects fitted were the interaction of batch (2009, 2010, 2011), pond (1 and 2), proportion of males in each family per group, and the linear covariate age at harvest fitted within this interaction. The proportion of males (propM) was calculated as the number of males harvested divided by number of fish harvested, by family within a group. For standard deviation of BC-harvest weight, we fitted the same fixed effects, except that propM was replaced by the product of proportion of females and males harvested by family within a group (propFM = propF x propM; where propF is the proportion of females). When males and females differ in weight, this increases the variance within a group by an amount proportional to propFM. We tested the quadratic effect of age at harvest to accommodate a non-linear relationship between harvest weight and age, but

this effect was not statistically significant for both traits (p > 0.05) and was therefore excluded.

The additive genetic variances for BC-harvest weight and its standard deviation were calculated as $\hat{\sigma}_A^2 = 4 \cdot \hat{\sigma}_{A_{PAR}}^2$, where $\hat{\sigma}_A^2$ and $\hat{\sigma}_{A_{PAR}}^2$ denote the estimated additive genetic variance and estimated additive genetic parent variance. Heritability of both traits recorded at family by group level calculated as $\hat{h}_g^2 = \frac{\hat{\sigma}_A^2}{\hat{\sigma}_{P_g}^2}$. The phenotypic variance at family by group level ($\hat{\sigma}_{P_g}^2$) was calculated for BC-harvest weight as $\hat{\sigma}_{P_g}^2 = 2\hat{\sigma}_{A_{PAR}}^2 + \hat{\sigma}_g^2 + \hat{\sigma}_m^2 + \hat{\sigma}_e^2$, and for standard deviation of BC-harvest weight as $\hat{\sigma}_{P_g}^2 = 2\hat{\sigma}_{A_{PAR}}^2 + \hat{\sigma}_e^2$. The $\hat{\sigma}_g^2$, $\hat{\sigma}_m^2$ and $\hat{\sigma}_e^2$ refer to random group variance, social maternal common environmental variance and the residual variance, respectively. To allow comparison with the literature and for practical application, we also calculated the ordinary heritabilities at individual level ($\hat{h}_{ind}^2 = \frac{\hat{\sigma}_A^2}{\hat{\sigma}_{P_{ind}}^2}$), by finding the phenotypic variance at individual level. Phenotypic variance at individual level is given by $\hat{\sigma}_{P_{ind}}^2 = 4\hat{\sigma}_{A_{PAR}}^2 + \hat{\sigma}_g^2 + \hat{\sigma}_m^2 + \hat{\sigma}_E^2$ for BC-harvest weight, and by $\hat{\sigma}_{P_{ind}}^2 = 4\hat{\sigma}_{A_{PAR}}^2 + \hat{\sigma}_E^2$ for standard deviation of BC-harvest weight, where $\hat{\sigma}_E^2$ denotes the residual variance of an individual observation. The residual variance of an individual observation was estimated as $\hat{\sigma}_E^2 = 6.68 \cdot \hat{\sigma}_e^2 - 0.5\sigma_A^2$, where the 6.68 is the average number of members per family-group (Table 5.2).

In addition to heritability, we also calculated the genetic coefficient of variation (GCV) for the BC-harvest weight and its standard deviation. The GCV is defined as the ratio of the additive genetic standard deviation (σ_A) and the mean trait value (μ), $GCV = \sigma_A/\mu$, and is known as evolvability [Houle, 1992]. In this study, the GCV for BC-harvest weight and its standard deviation were calculated as,

$$GCV_{BChw} = \frac{\sigma_{A,BChw}}{\overline{BChw}}\,;\; GCV_{SD} = \frac{\sigma_{A,SD}}{\overline{SD}}\,,$$

where, $\sigma_{A,BChw}$ and $\sigma_{A,SD}$ refer, respectively, to additive genetic standard deviation of BC-harvest weight and its standard deviation; $\overline{BChw}$ and $\overline{SD}$ refer to the phenotypic mean of BC-harvest weight and the mean standard deviation of BC-harvest weight. For ease of interpretation and for comparison with the literature, phenotypic and genetic CVs for BC-harvest weight were back-transformed to the original scale, using (Appendix A),

$$CV_{hw} = \frac{\sigma_{BChw}}{\lambda \cdot \mu_{hw}^{\lambda}}$$

where, CV_{hw} refers to the coefficient of variation at the original scale; σ_{BChw} refers to the standard deviation of the trait value at the Box-Cox transformed scale; λ refers to the lambda value used in the Box-Cox transformation; and μ_{hw} refers to the phenotypic mean at the original scale. A first-order Taylor-series expansion of BC-harvest weight shows that the Box-Cox transformation does not meaningfully change the CV for standard deviation of harvest weight. Therefore, no back-transformation was needed for the standard deviation, and the CV presented in the results may be interpreted as referring to the original scale.

In the study on IGE in the GIFT strain by Khaw *et al.* [2014], results indicated the confounding of maternal common environmental effects and (direct) additive genetic effects. As expected (the same data were used here), those effects were also confounded in the present analysis. To allow estimation of genetic parameters, therefore, we decided to omit maternal common environmental effects from the model.

5.3. Results

5.3.1. Descriptive statistic

Table 5.2 shows the descriptive statistics for harvest weight and its standard deviation on the original and Box-Cox transformed scale, at family by group level. Average size of family-groups at harvest was 6.68 individuals. The phenotypic CV for untransformed harvest weight at family by group level was 26%. As expected, this is smaller than the CV at individual level of 36% reported in Khaw *et al.* [2014]. We also obtained the phenotypic CV within pond by batch for harvest weight, which was 34% at individual level on the original scale. This value was calculated from the standard deviation of the residual of a model with a fixed effect for batch-by-pond.

The phenotypic CV for standard deviation of harvest weight were 42% and 36% on the original and Box-Cox transformed scale, respectively. At first glance, these values may suggest variation in uniformity. However, also without any true variation in uniformity, standard deviations of harvest weight estimated by family

by group will vary considerably due to sampling, because they are based on only 6.68 individuals on average (Table 5.2). To investigate whether there is true variation in uniformity, we calculated the expected CV for estimated standard deviation of harvest weight under the situation where there is no true variation in uniformity. (*i.e.*, where the true standard deviation, y_{SD}, is the same for all family-groups). The standard error of an estimated standard deviation is given by, $\sigma_{\widehat{SD}} = SD * \frac{1}{\sqrt{2(n-1)}}$. Thus for a constant true standard deviation, the expected CV is given by $CV_{SD_{no\ variation}} = \frac{\sigma_{\widehat{SD}}}{SD} = \frac{1}{\sqrt{2(n-1)}} = \frac{1}{\sqrt{2(6.68-1)}} = 0.30$. Our estimated phenotypic CV for standard deviation of harvest weight of 42% and 36% were greater than this value, which indicates that there is variation in the true standard deviation of family-group records.

Table 5.2 Number of observations (N), simple mean (μ), minimum (min) and maximum (max), standard deviation (σ) and coefficient of variation (CV, %) of harvest weight (hw, in gram), standard deviation of harvest weight (SDhw), for original and Box-Cox transformed scale, and the family-group size.

Variable	Transformation	N	μ	Min	Max	σ	CV
Hw[§]	No	944	167.5	60.7	357.83	42.9	26
SDhw	No	944	41.3	2.5	135.6	17.4	42
Hw[§]	Box-Cox[ϕ]	944	5.72	4.07	7.53	0.51	8.9
SDhw	Box-Cox[ϕ]	944	0.49	0.02	1.37	0.18	36
No. of fish per family-group		944	6.68	2	8	1.5	23

[§]The harvest weight (original and Box-Cox transformed scale) refers to the mean of 6.68 observations; [ϕ]The lambda used in Box-Cox transformation was 0.3434.

5.3.2. Data distribution

For harvest weight, the skewness and excess kurtosis were 0.54 and 1.13, respectively. For the Box-Cox transformation, we obtained a maximum likelihood estimate of λ of 0.3434. After the Box-Cox transformation for the individual harvest weight, the skewness and excess kurtosis of the residual distribution were -0.09 and 0.3, which are close to values for a normal distribution (i.e. zero). Skewness and excess kurtosis of the residual distribution of BC-harvest weight averaged by family-group were -0.11 and 0.8, which are also close to values for a normal distribution.

5.3.3. Estimation of phenotypic and genetic parameters

Table 5.3 shows the GCV and REML estimates obtained from the bivariate analysis for BC-harvest weight and its standard deviation. At family by group level, the

estimated heritabilities for BC-harvest weight and standard deviation of BC-harvest weight were 0.59±0.09 and 0.23±0.07, respectively. The estimated heritability at individual level for BC-harvest weight was 0.19±0.04. After applying the back-transformation, the GCV_{hw} was 0.1745, which is considered moderate. Back-transformation was not needed for the GCV_{SD}, which was 0.1695 (Table 5.3).

Table 5.3 The phenotypic mean, genetic coefficient of variation (GCV) and REML estimates (s.e.) from a bivariate model of Box-Cox transformed harvest weight (BChw) and standard deviation (SD) of Box-Cox transformed harvest weight.

Parameters	BChw[§]	SD(BChw)
μ	5.72	0.49
$\hat{\sigma}_A^2$	0.121 (0.024)[#]	0.0069 (0.0022)[#]
$\hat{\sigma}_g^2$	0.059 (0.008)	-
$\hat{\sigma}_m^2$	0.012 (0.004)	-
$\hat{\sigma}_e^2$	0.075 (0.006)	0.0265 (0.0013)
$\hat{\sigma}_{P_g}^2$	0.206 (0.013)	0.0299 (0.0015)
$\hat{\sigma}_{P_{ind}}^2$	0.631 (0.037)[ϕ]	0.180 (0.009)[ϕ]
$\hat{h}_g^2$	0.59 (0.09)	0.23 (0.07)
$\hat{h}_{ind}^2$	0.19 (0.04)[Ψ]	-
$\hat{g}^2$	0.28 (0.04)	-
$\hat{m}^2$	0.06 (0.02)	-
GCV	0.1745[‡]	0.1695
$\hat{r}_e$	-0.050 (0.045)	
$\hat{r}_A$	0.095 (0.183)	

[§] The BC-harvest weight refers to the mean of 6.68 observations; [#] Additive genetic variance was obtained as 4 times the additive genetic parent variance; [ϕ] Estimated phenotypic variance at individual level; [Ψ] Estimated heritability at individual level; [‡] The GCV is presented on the original scale after back-transformation,

$$GCV_{HW} = \sqrt{0.121} / 0.3434 \cdot 167.5^{0.3434}$$

For BC-harvest weight, we found significant random group and social maternal common environmental effects (Table 5.3), which indicated non-genetic indirect effects for harvest weight. The estimated genetic and residual correlation between

BC-harvest weight and its standard deviation were not significantly different from zero, 0.095±0.183 and -0.050±0.045.

To investigate the impact of the Box-Cox transformation on the estimated genetic correlation between harvest weight and its standard deviation, an additional bivariate analysis of untransformed harvest weight and its standard deviation was performed. The genetic correlation estimated from this analysis was positive and highly significant (0.60±0.12). The large difference between the genetic correlation on the original and transformed scale illustrates that the estimated genetic correlation between trait level and trait variability is very sensitive to the distribution of the trait values [Yang *et al.*, 2011; Sonesson *et al.*, 2013].

5.4. Discussion

5.4.1. Overall findings

The estimated heritability and GCV for standard deviation of BC-harvest weight indicate that uniformity for harvest weight in GIFT is partly under genetic control. (See below for a discussion on the confounding between genetic and maternal common environmental effects). Furthermore, the genetic correlation between BC-harvest weight and its standard deviation was not significantly different from zero.

5.4.2. Descriptive statistics

The phenotypic CV for average harvest weight at family by group level strongly depends on group size, and has, therefore, not much biological meaning. Fish breeders and producers should focus on the $CV_{HW_{within\ pond}}$ because, in general, fish farmers harvest and market the fish by pond. The $CV_{HW_{within\ pond}}$ was slightly lower compared to previous studies of GIFT [Ponzoni *et al.*, 2005; Nguyen *et al.*, 2007; Khaw *et al.*, 2010], but still greater than CVs reported in livestock [7 to 10%; Gjedrem, 1998; Damgaard *et al.*, 2003; Mulder *et al.*, 2009; Wolc *et al.*, 2009]. This suggests that there is competition for resources in the GIFT population. For uniformity, the comparison of the estimated phenotypic CVs for standard deviation of harvest weight and the expected CV when there is no true variation in standard deviation indicates that there is phenotypic variation in standard deviation of harvest weight between family-groups.

5.4.3. Data transformation

For the standard deviation of BC-harvest weight, the skewness of the residual distribution was 0.6316. For the purpose of investigating the effects of this skewed distribution, we did a second Box-Cox transformation to the standard deviation of BC-harvest weight (λ = 0.5455), resulting in a skewness of 0.02, which is close to the value for a normal distribution. However, estimated heritabilities were similar, 0.23 and 0.22, for untransformed and Box-Cox transformed standard deviation of BC-harvest weight, respectively. Therefore we only present results for the untransformed standard deviation of BC-harvest weight.

5.4.4. Heritable variation for uniformity and harvest weight

With the development in statistical methods, the evidence for genetic heterogeneity of environmental variance has increased rapidly [for example, SanCristobal-Gaudy *et al.*, 1998; Sorensen and Waagepetersen, 2003; Rönnegård *et al.*, 2010; Mulder *et al.*, 2013]. The majority of the heritabilities reported are low, ranging from 0.02 to 0.05 [Hill and Mulder, 2010]. In this study, we present the estimated heritabilities for standard deviation of BC-harvest weight at family by group level, which was 0.21±0.06. This value is higher than the range reported by Hill and Mulder [2010] because it is measured on family by group level. Heritabilities for BC-harvest weight are presented at family by group level (0.59±0.09) and at individual level (0.19±0.04). The higher values at family by group level is because the σ_e^2 in that model is an average of Mendelian sampling and individual environmental effects over 6.68 individuals on average (Table 5.2). Thus, we obtained a smaller $\sigma_{p_g}^2$ compared to the $\sigma_{p_{ind}}^2$. The $\hat{h}_{ind}^2$ for BC-harvest weight was slightly smaller than values reported earlier for GIFT and Nile tilapia populations [Ponzoni *et al.*, 2005; Rezk *et al.*, 2009; Khaw *et al.*, 2010; Attipoe *et al.*, 2013].

In the data analyzed, as was the in the IGE study by Khaw *et al.* [2014], there was confounding between the genetic and maternal common environmental effects for both traits. As discussed in the IGE study [Khaw *et al.*, 2014], the confounding was most likely caused by the one male to two female nested mating design used in the experiment, where we not always succeeded in having a 1:2 ratio. As consequence, the data collected did not have the design required to disentangle the genetic and maternal common environmental effects. In the heterogeneity of environmental variance study by Sonesson *et al.* [2013], a two sires by two dams factorial mating design was used. These authors showed that that design enable a proper

separation of genetic and non-genetic common environmental effects. Indeed, Sonesson *et al.* [2013] managed to disentangle the genetic and common environmental effects in the mean model of body weight. However, they found difficulty to separate these two effects in variance model of body weight, and excluded common environmental effects from the variance model. In our preliminary analysis, the model for standard deviation of BC-harvest weight caused convergence problems when including maternal common environmental effects. Therefore, maternal common environmental effects were excluded from both the mean and the standard deviation model.

Due to the difficulty in comparing the heritabilities estimated in this study with values reported in the literature, especially for the heterogeneity of environmental variance, we calculated the GCV for both traits. The GCV provides a measure of the genetic variation standardized by the trait mean, which is independent from other sources of variance [Houle, 1992]. This property makes GCV comparable across different experimental designs and statistical methods, with the condition that the results are reported on the original scale. The GCV values reported in the literature on heterogeneity of environmental variance in growth traits for livestock species range from 0.3 to 0.6 [summarized by Hill and Mulder, 2010]. However, those values are on the variance scale (GCV_{V_E}), while the GCV presented here is on standard deviation scale (GCV_{SD}). To allow comparison of our results with values in the literature, we multiplied our GCV_{SD} by a factor of two, yielding 0.17 x 2 = 0.34. (By approximation [James, 2007], $GCV_{SD} \sim 0.5\sigma_{A_v} = 0.5GCV_{V_E}$, where $\sigma^2_{A_v}$ is the genetic variance in environmental variance in the exponential model). This value is close to the 0.37 found by Janhunen *et al.* [2012] in Rainbow trout. In the study by Sonesson *et al.* [2013] on Atlantic salmon, we calculated the GCV_{V_E} based on their reported $\sigma^2_{A_v}$ (untransformed data) as $\sqrt{\sigma^2_{A_v}} = 0.42$. Hence, values from all three studies are similar.

5.4.5. Social interaction for uniformity and harvest weight

The data used in this study came from an experiment established to study social interactions in the GIFT population [Khaw *et al.*, 2014]. In that study, we found evidence for both genetic and non-genetic indirect effects. An indirect effect is an effect of an individual on the trait values of other individuals it interacts with [Griffing, 1967]. Non-genetic indirect effects surface as random group effects in group-structured populations [Bergsma *et al.*, 2008]. We found evidence of non-genetic indirect effects on BC-harvest weight because random group effects and

social maternal common environmental effects were significant, and explained 28% and 6% of phenotypic variance (Table 5.3), respectively. However, despite the high phenotypic CV of the standard deviation of harvest weight, which suggests inter-individual competition (Table 5.2), we did not find any evidence of non-genetic indirect effects for this trait.

Peeters *et al.* [2013] found that the additive genetic variance estimated from data pooled by group is an estimate of the total genetic variance, $\sigma^2_{A_T} = \sigma^2_{A_D} + 2(n-1)\sigma_{A_{DS}} + (n-1)^2\sigma^2_{A_S}$ [Bijma *et al.*, 2007], rather than the direct additive genetic variance. The $\sigma^2_{A_T}$, $\sigma^2_{A_D}$ and $\sigma^2_{A_S}$ refer to the total genetic variance, direct genetic variance and indirect genetic variances, respectively; and the $\sigma_{A_{DS}}$ refers to the direct-indirect genetic covariance. In this study, we analyzed the trait values averaged by family-group, which is akin to pooling data. Thus, the additive genetic variances estimated may also have captured the IGEs from the group mates of same family, since IGEs were not included in the models. The mean phenotype of a family within a group is given by $\bar{P}_{F1} = \bar{A}_{D_{F1}} + \left({}^{n}/_{2} - 1\right)\bar{A}_{S_{F1}} + \left({}^{n}/_{2}\right)\bar{A}_{S_{F2}} + \bar{E}$, where F1 and F2 refer to family 1 and family 2 within a group; $\bar{P}$ refers to the mean phenotype; $\bar{A}_D$ and $\bar{A}_S$ refer to the average direct and average indirect genetic effects; n refers to the group size, which is 16 for this study (8 individuals per family); and $\bar{E}$ refers to all the other non-genetic terms. Therefore, the genetic variance captured in this study was, $\sigma^2_{A_T} = \sigma^2_{A_D} + 2\left({}^{n}/_{2} - 1\right)\sigma_{A_{DS}} + ({}^{n}/_{2} - 1)^2\sigma^2_{A_S}$, which is different from the ordinary direct additive genetic variance when IGE are present ($\sigma^2_A = \sigma^2_{A_D}$). As consequence, the IGEs may have contributed to the estimates of additive genetic variances presented here.

5.4.6. Genetic correlation between harvest weight and its standard deviation

Janhunen *et al.* [2012] found a significant negative genetic correlation between (untransformed) body weight and its residual variance in Rainbow trout. Based on this correlation, the authors concluded that selection for growth may enable simultaneous improvement of uniformity. In the literature on livestock species, the genetic correlations between production traits and their variability are mostly negative [summarized by Hill and Mulder, 2010], except for milk production traits [Mulder *et al.*, 2013]. In the present study, the estimated genetic correlation between BC-harvest weight and its standard deviation was near zero (0.095±0.183). This indicates that there is no relationship between transformed harvest weight and its variability. The strongly positive genetic correlation between

untransformed harvest weight and its variability probably indicates a scaling effect, where higher means are associated with higher variances. This mean-variance relationship is removed by the Box-Cox transformation.

5.4.7. Future implication

In poultry and pig production, there is a penalty system for delivering animals outside the preferred weight range to the slaughterhouse [Hennessy, 2005]. In tilapia production, however, farmers get their incentive indirectly by having a better selling price or more income from selling more fish within the preferred range [personal communication with fish farmers in Malaysia]. Based on the estimated GCV for standard deviation of harvest weight, the within-group standard deviation would be reduced by 17% when changing the trait by one genetic standard deviation through selective breeding. In other words, our results indicate good prospects for selection for uniformity of harvest weight. Not only farmers would benefit from more uniform fish, but increased uniformity will improve welfare of the fish. When the fish are more uniform, farmers do not have to do grading frequently and fish will not get stressed by frequent handling. Furthermore, increased uniformity may result in less competition among fish [Jobling, 1995].

5.5. Conclusions

Based on the heritability and GCV of the standard deviation of Box-Cox transformed harvest weight, our results suggest that there are good opportunities for genetic improvement of uniformity for growth in the GIFT strain of Nile tilapia. The zero genetic correlation between Box-Cox transformed harvest weight and its standard deviation indicates that these two traits can be selected in opposite directions, so as to increase growth rate and decrease its variation.

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Appendix A

For the Box-Cox transformation, $y = x^{\lambda}$ and with approximation, $\bar{y} \approx \bar{x}^{\lambda}$, where y and $\bar{y}$ refer to the transformed harvest weight and the mean of transformed harvest weight; x and $\bar{x}$ refer to the untransformed harvest weight and the mean of untransformed harvest weight; λ refers to lambda that used in Box-Cox transformation.

To back-transform the coefficient of variation (CV) to original scale, we started with taking the first order Taylor-series expansion of Box-Cox harvest weight.

$$y \approx \bar{x}^{\lambda}$$

$$y \approx \bar{x}^{\lambda} + \lambda\bar{x}^{(\lambda-1)}(x - \bar{x})$$

$$y \approx \text{constant} + \lambda\bar{x}^{(\lambda-1)} \cdot x$$

$$x \approx \frac{y - \text{constant}}{\lambda\bar{x}^{(\lambda-1)}}$$

$$x \approx \frac{y}{\lambda\bar{x}^{(\lambda-1)}} + \text{constant}^{*}$$

$$\sigma_x \approx \frac{\sigma_y}{\lambda\bar{x}^{(\lambda-1)}} \quad \text{(A1)}$$

The CV at original scale,

$$CV_x = \frac{\sigma_x}{\bar{x}} \quad \text{(A2)}$$

Substitute the equation A1 to σ_x of equation A2,

$$CV_x = \frac{\sigma_y}{\bar{x}\lambda\bar{x}^{(\lambda-1)}}$$

$$CV_x = \frac{\sigma_y}{\lambda\bar{x}^{\lambda}}$$

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6

General Discussion

6.1. This thesis

Social interactions are present everywhere in the world, including animals in aquatic environments. Within a population, an animal's performance is not solely affected by its genetic makeup. It is also influenced by the environment where the animals grow and socially interact with each other [Sakai, 1955; Waddington, 1960; Lynch, 1987]. The genotype of an individual may affect the trait values of other individuals it interacts with [Griffing, 1967; Muir, 1996]. This heritable effect is known as indirect genetic effect (IGE; Moore *et al.*, 1997). Theoretical and experimental work on social interactions shows that IGEs may affect the direction of selection response and the amount of heritable variation available for response to selection [Griffing, 1967; Bijma, 2011; Muir *et al.*, 2013]. In the IGE experiments (chapter 2), we found evidence suggesting the presence of IGE on harvest weight in the GIFT population. In models without maternal common environmental effects, 48% of the total genetic variance was contributed by the indirect genetic variance. We also found evidence of moderate competition among individuals in the population (direct-indirect genetic correlation of -0.38). This may be attributed to traditional selection for improved growth rate based on individual performance ignoring IGE.

Inbreeding is one of the factors that will affect the long-term sustainability of a breeding program. A stochastic simulation study was performed to investigate the effects of BLUP selection for a socially affected trait on the rate of inbreeding (chapter 3). The results showed that BLUP selection for socially affected traits increased the rate of inbreeding compared to traditional BLUP selection. The increases in rate of inbreeding were partly due to the greater resemblance between EBVs of relatives when animals were kept in groups consisting of two families. This is relevant information to be aware of, especially in cases where the design is similar to the one we investigated.

The environmental conditions where the animals grow affect their performance [Waddington, 1960; Lynch, 1987]. These conditions can be separated into two types, namely macro- and micro-environments [Zhang, 2005; Hill *et al.*, 2007; Mulder *et al.*, 2013]. The macro-environments are those shared by many individuals within a population [Zhang, 2005]. The genetic variance in macro-environmental sensitivity, commonly known as genotype by environment (GxE) interaction, is a measure of the differential response of genotypes to different environments. A genotype by environment interaction study was conducted between pond and cage culture systems (commonly used in Malaysia) for the GIFT

population (chapter 4). In this study, the genetic correlations for harvest weight and body measurements between these two production environments ranged from 0.73 to 0.85. In view of the worldwide distribution and successful performance of GIFT (Ponzoni *et al.*, 2010a), we concluded based on these results that it is not necessary to have two separate breeding programs for GIFT.

On the other hand, the micro-environmental variation represents developmental noise within an individual, which is unique to the individual in question and unpredictable. The genetic variance in micro-environmental sensitivity is also referred to as genetic heterogeneity of environmental variance [Mulder *et al.*, 2013]. In the GIFT population, we found that there was a genetic basis for heterogeneity of environmental variance or uniformity for harvest weight (chapter 5). This finding indicates there is the potential to select for increased uniformity in GIFT, which may benefit farmers and also favor the fish's well-being.

In this chapter, I will discuss the possibility of selecting for cooperative and uniform fish, the relevance of social interactions at the breeding nucleus and commercial levels, and future directions to further improve the tilapia breeding programs and the aquaculture industry as a whole.

6.2. Social interactions

6.2.1. Cooperative and uniform fish?

The data collected from the IGE experiments conducted in the context of this thesis provided the opportunity to estimate the heterogeneity of environmental variance and its relationship with the IGEs for harvest weight in GIFT. Unfortunate, the data did not allow the simultaneous estimation of all the parameters of a model with direct genetic and maternal common environmental effects included as part of the random effects. As discussed in chapters 2 and 5, the maternal common environmental effect was confounded with the genetic effect. The confounding was mostly likely due to the nested mating design used in the GIFT breeding program, which results in limited statistical power to estimate the genetic parameters. To be able to get an indication of the genetic parameters, I performed additional analysis with model in which the maternal common environmental effects were excluded. The results are presented below.

For answering the question whether it is possible to breed cooperative and uniform fish, the relationship between the indirect genetic effect for harvest weight (Box-Cox transformed; referred as BC-harvest weight in the following) and the direct genetic effect for standard deviation of BC-harvest weight needs to be quantified. Thus, the parameter of interest is the genetic correlation between these two effects. A negative value of this correlation would indicate that individuals with positive IGEs on BC-harvest weight of their group mates, *i.e.* cooperative individuals, on average have reduce variability of BC-harvest weight. Hence, a negative correlation would indicate that cooperative fish are more uniform. In order to estimate this genetic correlation, an extended model to the one reported in chapter 5 was fitted with IGEs included for harvest weight, as follows:

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix} = \begin{bmatrix}\mathbf{X}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{X}_2\end{bmatrix}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\end{bmatrix} + \begin{bmatrix}\mathbf{Z}_{1_{PAR}} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z}_{2_{PAR}}\end{bmatrix}\begin{bmatrix}\mathbf{a}_{1_{PAR}}\\ \mathbf{a}_{2_{PAR}}\end{bmatrix} + \begin{bmatrix}\mathbf{Z}_{1_{socPAR}}\mathbf{a}_{1_{socPAR}}\\ \mathbf{0}\end{bmatrix} + \begin{bmatrix}\mathbf{V}_1\mathbf{g}_1\\ \mathbf{0}\end{bmatrix} + \begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix}$$

where, subscript 1 refers to BC-harvest weight and subscript 2 to standard deviation of BC-harvest weight; $\mathbf{y}$ is the vector of phenotypic observations on the family by group level (two records for each trait per group and one record for each family); $\mathbf{b}$ is the vector of fixed effects (same fixed effects as the model in chapter 5); $\mathbf{a}_{PAR}$ is a vector of direct genetic parent effects, *i.e.* this term fits the sire and dam of the animals producing the records; $\mathbf{a}_{socPAR}$ is a vector of indirect genetic parent effects; $\mathbf{g}$ is a vector of random group effects; and $\mathbf{e}$ is a vector of random residuals. The $\mathbf{X}$, $\mathbf{Z}_{PAR}$, $\mathbf{Z}_{socPAR}$, and $\mathbf{V}$ and are known design matrices.

Based on the results from this model, the correlation between indirect genetic effect for BC-harvest weight and direct genetic effect for standard deviation of BC-harvest weight was close to zero, 0.026±0.264. This suggests that selection for more cooperative fish (*i.e.* with estimated social breeding greater than zero) will not necessarily lead to more uniform fish for body weight. However, according to Jobling (1995) suggestion, uniformity of fish at harvest weight was associated with a favorable social environment. In addition, the coefficients of variation for harvest weight obtained from this data set were 36% at individual level (chapter 2), 34% within pond at individual level and 26% at family by group level (chapter 5). These are high value, which suggest that large size variation may be due to competition for resources. The near zero genetic correlation and the CVs presented above, suggest that the relationship between competition and variability may be caused by environmental factors. Note that the standard error of the genetic correlation was large. This indicated that the parameter was not precisely estimated, and

probably due to the limited size of the data set. The exclusion of maternal common environmental effect may be another limiting factor. If financial resources are sufficient, I would suggest collecting more data on social interactions in the GIFT strain to further investigate the relationship between competition and variability. Such an IGE experiment could follow the experimental design described in chapter 2, so as to capture both the direct and indirect genetic effects for studying the relationship between uniformity and competition. Box 1 shows results of such analysis using the present data.

Box 1: Additional analysis

To investigate the presence of IGE on uniformity, an additional model was fitted with IGEs included for both traits – BC-harvest weight and standard deviation of BC-harvest weight,

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix} = \begin{bmatrix}\mathbf{X}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{X}_2\end{bmatrix}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\end{bmatrix} + \begin{bmatrix}\mathbf{Z}_{1_{PAR}} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z}_{2_{PAR}}\end{bmatrix}\begin{bmatrix}\mathbf{a}_{1_{PAR}}\\ \mathbf{a}_{2_{PAR}}\end{bmatrix} + \begin{bmatrix}\mathbf{Z}_{1_{socPAR}} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z}_{2_{socPAR}}\end{bmatrix}\begin{bmatrix}\mathbf{a}_{1_{socPAR}}\\ \mathbf{a}_{2_{socPAR}}\end{bmatrix} + \begin{bmatrix}\mathbf{V}_1\mathbf{g}_1\\ \mathbf{0}\end{bmatrix} + \begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix}$$

The estimated indirect genetic variance for uniformity was 0.002±0.001, and the other estimates from this model were almost identical with those from the above model presented in section 6.2.1. The likelihood ratio test resulted in borderline significance (χ^2_{4DF} = 7.72, p=0.1024), suggested that IGE on uniformity could be present. The borderline significance may be due to the small estimated indirect genetic variance component, and, as mentioned above, to the insufficient size of the data set. See section 6.2.1 for suggestions for future studies.

6.2.2. Incorporating IGE at nucleus level

In livestock breeding, it has been proven that incorporating IGEs increased the survival of the animals, which in turn improved their welfare and production [Muir, 1996; Ellen *et al.*, 2008; Camerlink *et al.*, 2012]. To my knowledge, there has been no fish breeding program incorporating IGE for any socially affected trait.

Our experiment constituted one of the first in aquaculture estimating IGE, using the GIFT strain to investigate a potentially socially affected trait – harvest weight. The genetic parameters estimated from the IGE experiments were presented in chapter 2. Unfortunately, the confounding between direct genetic and maternal common environmental effects meant that all the parameters could not be simultaneously

estimated. Despite of this limitation, the results from the models with and without maternal common environmental effect suggested that the indirect genetic variance for harvest weight was greater than zero. Nevertheless, we did not manage to show clear evidence for the presence of IGEs for harvest weight in the GIFT strain.

Therefore, I suggest that the incorporation of IGE for harvest weight in the GIFT breeding program should be postponed until reliable estimates of the relevant parameters have been obtained. Furthermore, the good reputation of GIFT in more than 10 different countries [Ponzoni *et al.*, 2010a] may be taken as an indication that competitive phenomena occurring in the population have not greatly reduced its performance. Hence, a postponement in the incorporation of IGEs in the GIFT program is justified.

To further investigate and validate the presence of IGE for harvest weight, I suggest conducting a selection program as the one suggested in Box 2. The group composition suggested in Box 2 for the IGE line can only be used to estimate the total genetic variance. For estimating the direct and indirect genetic variances separately, the group composition should follow the experimental design described in chapter 2. In addition, each sire should at least be mated with two dams, and ideally more than two. This is to obtain sufficient statistical power to disentangle the genetic and maternal common environmental effects. The choice of group composition depends on the objective of the study. If financial resources are sufficient, I suggest using groups composed of two families (as described in chapter 2), where more information can be obtained and used for additional studies, for example, the study on the relationship between uniformity and competition.

Box 2: Suggestion for selection plans to incorporate IGEs

If one would like to incorporate IGEs in a fish breeding program, my suggestion would be to establish an additional selection line that incorporates IGE (referred to as the IGE line below), next to the traditional breeding program on own performance (referred to as the growth line below). After a few generations of selection, the performance of both lines can be compared and evaluated on-farm (commercial) or on-station (nucleus) as described in the main text. To minimize any environmental differences, both lines should grow at the same nucleus, use the same feed and feeding regime. The only difference would be that the IGE line would grow in net-cages installed in earthen ponds, and, as usual, the growth line

would be reared in communal ponds. Because the primary objective of breeding is to generate selection response, I suggest using groups composed of full-sib families for the IGE line. Such groups would enable the estimation of the total breeding values. In addition, the design with groups composed of full-sib families gives the most accurate estimate of total breeding values [Bijma, 2012]. Furthermore, this design with full-sib family groups would also reduce the time spent handling the fish when they have to be transferred to net-cages installed in the ponds. As a consequence, the fish would be less stressed compared to the experimental design described in chapter 2, where two families have to be carefully paired and mixed in each net-cage.

6.2.3. Social interaction at the commercial level

One of the main problems in tilapia production is the large size variation presumably caused by competition for resources among the fish in the same rearing environment. It is certainly a practical problem at the commercial level, especially in small to medium sized farms where there is no grading of the fish during the grow-out phase. However, social interactions for tilapia (and for aquaculture species generally) at the commercial level are not documented and their effect is unknown. In my opinion, the lack of documentation on social interactions at commercial level is mainly because this is still a rather new research topic for aquaculture. Furthermore, in the past (before IGE and its estimation methods were introduced), breeding for social interactions on behavioral traits relied on direct observation of the animals' behavior, which are costly and difficult to record even at the breeding nucleus level. For example, to record the aggressiveness of a fish, one will have to set up the experiment in an aquarium where the behavior of a group of fish can be observed by video recording [e.g. Cooper, 2009].

Stocking density differs between the breeding nucleus and commercial farms. The stocking density at commercial farms could be more than 100 times greater than in a breeding nucleus. For example, there may be 1500 fish (of 10 grams fingerlings) per square meter in a commercial intensive system [FAO, 2014] compared to five fish per square meter in the GIFT breeding nucleus (chapter 4). For socially affected traits, the total genetic variance and selection response depend on the relationship between group size and the IGEs [Bijma, 2012]. In a larger group, the social interaction between a particular pair of animals may be less and this phenomenon is named dilution [Bijma, 2012]. So far, there have been no studies on the degree of

dilution for tilapia species. This is mainly because IGEs cannot be estimated from data coming from a few large ponds. It is necessary to have data from many groups of small size [Bijma, 2012]. Thus at present the relevance of IGEs at the commercial level is unknown.

Although social interactions cannot be directly studied at the commercial level, an alternative, indirect method is still possible. To study the social interactions at the commercial level, my suggestion is to conduct a simple line comparison experiment (provided the presence of IGE for harvest weight has been confirmed as suggested in section 6.2.2). For the comparison experiment, an additional GIFT selection line needs to be developed, selected only for high total breeding value to produce fast growing and yet more cooperative fish (as suggested in Box 2). For the high growth line, selection should continue as usual. Once the cooperative fish are ready for testing, both lines of fish would be sent to commercial farms for the comparison of fish resulting from both lines using the same stocking density, feeding regime and length of grow-out period as in the commercial practice. Fish of both lines should be kept in different ponds, so that they can show their optimum performance without interference by the other line. The conclusion about which is the superior line at the commercial level can be drawn from the performance of the fish from both lines upon finishing the experiment. If both lines were equal, with no significant difference, then I would suggest that IGEs are not important at the commercial level. I anticipate that the above mentioned evaluation would take at least four years from selection to completion of the comparison experiment. The design of the comparison experiment should follow the guidelines provided by Ponzoni *et al.* [2013] with respect to, for example, synchronization of the spawning, determinate the test environment, the number of strains involved, and the traits that will be measured.

Until we have scientific evidence of the presence and importance of IGEs at nucleus and commercial level, it is very difficult to predict whether the commercial farms would benefit from the incorporation of IGEs in the methodology used in the selection program. Having said that, my opinion is the commercial hatcheries and farmers in developing countries would be willing to adopt the cooperative strain if it outperformed the traditional improved strain. (Under the condition that the hatcheries and farmers are aware of the existence of such improved strains).

6.2.4. Investment appraisal

Based on my experience in conducting the IGE experiment for this thesis project, I can anticipate that the incorporation of IGEs in tilapia or any other aquaculture breeding programs will be a costly undertaking. Unlike the poultry and swine breeding programs, where the animals are generally grown in small groups, fish are communally reared in earthen ponds or cages in very large groups and at varying densities (e.g. from 4 fish to 1500 fish per square meter). An IGE breeding program as suggested in Box 2 would cost twice as much as a traditional breeding program where selection is on own performance. In my opinion, before one decides to conduct a similar IGE breeding program in aquaculture species, an investment appraisal should be conducted to obtain the benefit/cost ratio and assess possible risks, especially in developing countries, where lack of resources is always a constrain. The investment appraisal should consider different levels of importance of IGE to account for the uncertainty on the presence of IGE. Selection for cooperative fish by incorporating IGEs is an interesting scientific research, which might have positive effect on production and animal welfare. However, a rigorous examination of the feasibility and cost effectiveness are essential before large scale implementation of the approach.

6.3. GIFT and tilapia breeding programs

In Malaysia, GIFT has gone through 12 generations of selection at the time of writing this chapter. The inbreeding and effective population size of GIFT was examined in 2009 on data from seven generations. The results from that study indicated that inbreeding was not a problem (ΔF = 0.0037 per generation) in this population and the effective population size was satisfactory (N_F = 88, calculated from the rate of increase in the co-ancestry) for the sustainability of the selection program [Ponzoni *et al.*, 2010b]. In recent years, the GIFT population has been facing some difficulties in reproduction of new generations and with disease outbreaks (*i.e.* Streptococcus). In this chapter, I will only focus on opportunities to overcome the reproduction difficulties in the years to come.

6.3.1. Reproduction – prolonged mating period

In the GIFT breeding program, each male breeder is mated with two female breeders from different families in a nested mating design. Based on our observation at the research station, in recent years, it has become more difficult to obtain selected females that are “ready to spawn”. The “ready to spawn refers to

the conditions for sexual maturity of female at the time of reproduction [WorldFish Center, 2004]. As a consequence, the breeders need to hold the females for a longer time in the conditioning hapas and this has resulted in an extension of the mating period necessary to produce enough families (about 90-100 full-sib families) for the breeding program. This prolonged mating period will increase the confounding effects of initial size and age in the genetic parameters and breeding value estimation. For the GIFT population, this phenomenon requires a balancing between producing enough families to maintain the program's viability and coping with greater environmental effects from a number of sources (e.g. climate change, water quality and farm management) or both. Note that we also observed the same phenomenon happen in the improved Red tilapia (*Oreochromis spp.*) population in Malaysia managed by WorldFish [Personal Communication].

Hormone induction technique is one of the possible solutions for the prolong mating period in tilapia, where the spawning can be induced and synchronized. Hormone induction is a fast track solution. However, the use of hormone may cause negative effects to the fish in long term application, *i.e.* the fish may become even more difficult to reproduce under natural spawning, especially at farmers' hatcheries. In addition, for human consumption, the effect of the hormone residual in the fish is unknown and the acceptability of consumers could be reduced.

Recently, the relationships between harvest weight and female reproduction traits (*i.e.* weight at spawning, number of eggs, number of fry, total weight of fry and number of dead fry), were studied in the GIFT population [Hamzah *et al.*, 2014]. The results showed that the genetic correlations between female harvest weight and the reproduction traits were 0.01 to 0.31, but not significantly different from zero [standard error ranges from 0.21 to 0.25; Hamzah *et al.*, 2014]. These results by Hamzah *et al.* [2014] coupled with our observation on the difficulty in obtaining "ready to spawn" female, late maturation in female could be one of the causes of prolong mating period. Hence, in my opinion, selection for early female maturation could be the possible solution in tackling the problem of the long mating period in GIFT. This solution requires genetic variation for female maturation, which is unknown at present. People may argue that female maturation is a difficult trait to select for, due to the low heritability for reproduction traits (*i.e.* 0.05 to 0.10) and because it can be measured in female only. But I believe that with the help from the advance genomic techniques (e.g. determination of the quantitative trait loci for female maturation), selection for female maturation will no longer be a problem.

Before selection can take place, a detailed study on the genetic basis of female maturation in GIFT is needed. For the GIFT strain, maturation has never been recorded for the female population. To study the female maturation in GIFT, the trait to be measured needs to be determined and my suggestion is age at maturation. Age at maturation can be calculated by recording the spawning date of the fry and the date when the fish reached the status of "ready to spawn". The main challenge that I foresee is it may not be possible to accurately record this trait. This is mainly because the fish are all reared in earthen ponds and it is not possible to record the trait without sampling the fish from the ponds. In addition, a female may already be matured and have spawned before the sampling (this is a condition that cannot be avoided). In my opinion, a possible solution could be to keep the females in fiberglass tanks at hatchery, instead of in the net-cages installed in the earthen ponds. With this design, the status of female sexual maturation could be checked more frequently and easily.

6.3.2. Reproduction – confounding between genetic and maternal common environmental effects

Due to the lack of paternal half-sib families it was difficult to estimate the genetic parameters accurately as discussed in chapter 2. In that chapter, we did not manage to disentangle the direct genetic and maternal common environmental effects. I want to discuss opportunities to disentangle these effects.

Full and partial factorial mating designs are commonly practiced in cold water fish species [Dupont-Nivet *et al.*, 2006; Busack and Knudsen, 2007]. These designs give better genetic ties between full- and half-sib families compared to nested mating designs. The advantage of factorial mating designs is that the genetic parameters can be estimated more accurately [Gjerde, 2005]. To the best of my knowledge, none of the tilapia breeding programs are currently using factorial mating designs (neither full nor partial). This could be mainly because it is very difficult to conduct factorial mating under natural spawning. Factorial mating designs can be practiced in tilapia if the *in-vitro* fertilization technique can be used, as in the cold water fish species. (Provided that "ready to spawn" females can be obtained easily)

Compared to the nested mating designs, factorial mating designs may not only affect the estimation of genetic parameters, but also the effective population size. This effect is summarized in Table 6.1 from the book chapter by Gjerde [2005]. Table 6.1 compares the three mating designs under the assumption of no selection and random mating. With a limitation of 100 full-sib families in the breeding

nucleus, the full and partial factorial mating designs have no advantage over the 1:2 nested mating designs for the effective population size and the rate of inbreeding. Since the main objective of GIFT breeding program is long term sustainable genetic improvement, in my opinion, GIFT should continue using the 1:2 nested mating designs and apply IVF technique to improve the success rate of one male mated to two females. (Assuming that it is not a problem to obtain "ready to spawn" females, see section 6.3.1).

Table 6.1 The effective population size and rate of inbreeding per generation for three different mating designs [summarized from Gjerde, 2005].

Mating design	Sires (or vice versa)	Dams (or vice versa)	Effective population size	Rate of inbreeding (%)
1:2 nested	50	100	133	0.38
2 x 2 factorial	50	50	100	0.50
2:2 partial factorial	50	50	100	0.50

6.4. Global issues – genetic improvement in aquaculture

In developing countries, the failure of aquaculture breeding programs has been caused by many factors in the area of economy, science, policy, and value chain. In this chapter, I will discuss three issues that I am concerned about, which are the adoption of improved strains, capacity building and the long term sustainability of aquaculture breeding programs.

6.4.1. Adoption of improved strains

Based on the inventory of Gjedrem *et al.* [2012], 8.2% of the aquaculture production in 2010 used genetically improved strains. The authors comment that this is a relatively optimistic estimate. In the majority of the cultured species, the use of genetically improved strains is less than 8.2% of their production. During interactions with farmers in developing countries where at least one fish breeding program exist, we found that farmers were either not aware of the existence of the improved strain or that they did not believe that the improved strain will perform better than the wild or their own strain. This suggests that a better marketing strategy to promote the value of improved strains is essential. Such a strategy could include local campaigns and workshops to introduce the improved strain and illustrate its benefits to the farmers. Besides that, accreditation of local hatcheries that use the improved strains is good strategy that could result in a more effective penetration into the communities. Accreditation is a process where the hatcheries

will be examined by their facilities, financial capacity, technical and managerial competency before they got the certification of authority to multiply and disseminate the improved stock. Accreditation of local hatcheries is promising because the personnel from local hatcheries and local farmers speak the same language – farmer's language. In addition, accreditation can also help in maintaining the quality of the fish that farmers received [Ponzoni *et al.*, 2012].

If the marketing strategies are properly conducted, it would help in increasing the awareness of the farmers about not only the existence but also the benefit of adopting improved strains. No matter how much improvement a breeding program achieved, without significant adoption by the end users, there will be no impact on the industry.

6.4.2. Capacity building

In the aquaculture breeding industry, especially in developing countries, the majority of technicians working at breeding nuclei or multiplication centers have very little or no basic knowledge of animal breeding and quantitative genetics. This contrasts with the dairy cattle or livestock industries in general, where most of the technicians and farm managers have been exposed to, and have participated in genetic improvement programs for decades. Having some basic knowledge of quantitative genetics will be extremely useful when emergencies happen, since technicians can be the first to react on an issue. For example, at the breeding nucleus, if a selected parent due for mating was not available, the technician or farm manager should have enough knowledge to figure out by which fish it may be replaced. Furthermore, it will also benefit the communication and understanding between the scientists or breeders and the farm managers or technicians. Based on my personal experience and observation, for example, when the farm managers or technicians do not understand the objective or the need of recording certain information (e.g. pond numbers where the fish grow), the farm managers will tend not to do it and judge by themselves that it is not necessary. As consequence, we may miss out some useful information that may cause imprecise estimates of the breeding values.

To advance the fish breeding industry, both non-government and government organizations should take the initiative to conduct training courses on basic quantitative genetics and its application to improve the skills of technicians and farm managers working at fish breeding nuclei and multiplication centers. In my opinion, the training course on quantitative genetics should be as basic as possible.

For example, instead of estimating breeding values with advanced statistical software, we have to make sure that the farm and hatcheries personnel understand what estimated breeding value is, and what its function is. I strongly believe that skillful personnel are a prerequisite for the improved strains and other technologies to show the impact they are capable of on the aquaculture production industry.

6.4.3. Long term sustainability of aquaculture breeding programs

Genetic improvement is a continuous process entailing implementation as well as further research and development. GIFT is well known for its high performance. After nine generations of selection, the cumulated genetic gain was 111.7% (Figure 1.3, chapter 1). Furthermore, GIFT has been disseminated to more than 10 different countries in Asia and South America [Ponzoni *et al.*, 2010a]. GIFT demonstrates that genetic improvement to meet the needs of fish farmers can be very effective. It is important to keep in mind that the GIFT breeding program has been receiving financial support from donors from developed countries. In recent years, the funding has been reduced which puts the sustainability at risk. Funding agencies tend to change their focus to genomic research, which has the potential to have impact at shorter time compared to investments in animal breeding which generally require at least five years before resulting in impact.

Financial self-sustainability of a breeding program requires not only a technically sound program but also a business plan that ensures that sufficient revenues are generated to recover the investments and running costs of a breeding program. The GIFT Foundation International Incorporation was established in 1999 with that aim. However, so far it did not prosper. Ponzoni *et al.* [2010a] concluded that self-sustainability was not possible for this particular aquaculture breeding program. This was mainly because the hatchery managers and farmers were not willing to pay higher prices for the genetically improved brood stock. One of the reasons for the unwillingness to pay for the improved stock is that hatcheries and farmers in developing countries still collect fish stock from the wild, which has no cost. Acceptance of the added value of a breeding program is a time consuming process. In the long run, a breeding program needs to be financially sustainable. Until that point is reached, financial support is needed. From my personal point of view, the local government should put more effort in capturing funding for sustaining the breeding program with the help from non-profit organizations. Then, to realize financial sustainability of the breeding program, the government or non-profit

organizations should at an early stage involve the private sector. Private sector involvement could start at the multiplication level. In addition, to make sure the small, subsistence and resource poor farmers also benefit from the superior material from the breeding program, an agreement should be drawn with the private sector that a certain percentage of their annual production should go to the small farmers with no cost in return for the access to the genetic material from the breeding program. The GIFT program has taught us that the different actors in the value chain need to be involved in the design of a breeding program in order to capture all the benefits that can be derived from a genetic improvement program.

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Summary

Summary

Social interactions are present everywhere in the living world, including aquatic environments. In aquaculture environments, competition is common and inflates the size variation among individuals within a population. In fish, there is evidence that individuals selected for rapid growth rate may be more aggressive and competitive. Various social interaction studies show that the genotype of an individual may affect the trait values of other individuals it interacts with. Such heritable effects are known as indirect genetic effects (IGE). IGEs may affect the direction and magnitude of selection response in breeding programs. Genetically Improved Farm Tilapia (GIFT) is one of the well-known and most successful examples of tropical finfish selective breeding programs, and has focused on improving growth rate since it was established in 1980's. In view of the genetic progress and the large coefficient of variation for harvest weight (40 to 60%), GIFT was chosen as the first tropical finfish improved strain for studying the IGEs. The main objective of this thesis was to investigate the genetic basis underlying social interactions and variability in harvest weight in Nile tilapia.

An experiment was established aiming to quantify the genetic and non-genetic indirect effects on harvest weight in the GIFT strain (**chapter 2**). A bivariate analysis of harvest weight and survival was conducted by fitting different mixed models, to investigate the presence of IGEs and other non-genetic effects. We found confounding between direct genetic and maternal common environmental effects. Therefore, the full set of genetic parameters could not be estimated simultaneously. Despite of the confounding, we found evidence suggesting the present of IGE on harvest weight (models with and without maternal common environmental effects). The results also suggest that those heritable interactions are competitive in the GIFT strain (the direct-indirect genetic correlation for harvest weight was -0.38±0.19). Hence, breeding schemes may need to be adapted to avoid an increase in competition due to selection for growth rate.

Though selection accounting for IGEs may increase rates of genetic improvement, it may also affect other aspects of breeding populations, such as the maintenance of genetic variation. To investigate such effects, we conducted a stochastic simulation study to examine the effect of BLUP selection for traits affected by IGEs on the rate of inbreeding (**chapter 3**). A base scenario without IGE and three alternative scenarios with different magnitudes of IGE were simulated using the R-language. We simulated a breeding program for three different "heritabilities" (0.1, 0.3 or 0.5

for direct and indirect effects) and five different direct-indirect genetic correlations (-0.8, -0.4, 0, 0.4, or 0.8). Rates of inbreeding for the three scenarios with IGE ranged from 2.21 to 5.76% and were greater than for the base scenarios. These results show that BLUP selection for socially affected traits increases the rate of inbreeding. To maintain inbreeding at an acceptable rate, therefore, a selection algorithm that restricts the increase in mean kinship is important in breeding schemes for socially-affected traits.

Not only the social environment, but also the physical environment may affect productivity in aquaculture. In aquaculture industry, there is a wide range of commercial production environments. This diversity of production environments may lead to genotype by environment interaction. In Malaysia (where the GIFT breeding program is located), cage and pond culture systems are the two most widely used production systems. Three discrete generations of GIFT fish were tested in ponds and cages to study the genotype by environment interaction for growth rate and body measurements at harvest (**chapter 4**). A bivariate animal model was used to estimate the (co)variances, by treating the traits in cage and pond systems as genetically distinct traits. The genetic correlations between these two systems ranged from 0.73 to 0.85 (standard error ranged from 0.09 to 0.13) for harvest weight and three body measurements. In view of this high genetic correlation and the successful performance of GIFT, we concluded that the genotype by environment interaction between cage and pond systems of limited importance. Thus, there is no need to have separate breeding programs for pond and cage systems for GIFT in Malaysia.

On the one hand, genetic differences between individuals in sensitivity to the macro environment lead to genotype by environment interaction, as discussed above. On the other hand, genetic differences in sensitivity to the micro environment lead to heritable differences in variability between individuals. This phenomenon is also known as “inherited variability” or “genetic heterogeneity of environmental variance”. In aquaculture, this phenomenon is probably linked to social interactions, as there is evidence that competition inflates variability among individuals. Genetic differences in variability can be utilized to improve uniformity of traits, which is desired, for example, for size and body weight in aquaculture. The data collected from the IGE experiments were used to investigate the presence of genetic variation in environmental variance for harvest weight in GIFT strain (**chapter 5**). A bivariate sire-dam model between harvest weight and its standard deviation (data pooled by family- group) was used to estimate genetic parameters.

The genetic coefficient of variation for standard deviation of harvest weight was 0.17, indicating that uniformity on harvest weight was partly under genetic control. As a result, it is possible to increase uniformity of harvest weight by means of selective breeding for the GIFT strain. Furthermore, we found zero genetic correlation between harvest weight and its standard deviation. Thus, these two traits can be selected as two different traits in different directions – increase in growth rate and decrease in size variation.

In **chapter 6**, the possibility of selecting for cooperative and uniform fish is investigated with an extended model to the one reported in chapter 5. The estimated correlation between indirect genetic effect for harvest weight and direct genetic effect for standard deviation of harvest weight (0.026±0.264) suggested that it is unlikely that selection for more cooperative fish will lead to more uniform fish for harvest weight. In the same chapter, the relevance of social interactions at the breeding nucleus and commercial levels were discussed. Furthermore, the suggestions on future directions to incorporate IGEs at different levels of the value chain, to further improve the tilapia breeding programs, and the aquaculture as a whole are also presented in chapter 6.

Samenvatting

Samenvatting

Sociale interacties spelen een belangrijke rol in de natuur, in zowel terrestrische als aquatische milieus. In aquatische milieus komt veel competitie tussen individuen voor, en leidt tot een hogere variatie in grootte tussen individuen in de populatie. In vissen zijn er aan wijzingen dat selectie voor snelle groei leidt tot meer agressie en competitie. Uit een aantal studies is gebleken dat het genotype van een dier invloed heeft op de eigenschappen van de andere dieren waarmee het interacteert. Dergelijke erfelijke effecten worden Indirect Genetische Effecten (IGE) genoemd. IGE beïnvloeden de grootte en richting van de genetische verbetering in fokprogramma's.

De zogenaamde Genetically Improved Farm Tilapia (GIFT) populatie is een van de meest bekende en succesvolste voorbeelden van een selectieprogramma in tropische vinvissen. De GIFT populatie is opgezet rond 1980, en is sindsdien gefokt op een verbetering van de groei. De GIFT populatie vertoont een hoge groei en een grote coëfficiënt van variatie in slachtgewicht, en is om deze reden verkozen voor onderzoek naar IGE. Het hoofddoel van dit proefschrift is het onderzoeken van de erfelijke achtergrond van sociale interacties en fenotypische variatie in slachtgewicht in de GIFT populatie.

Hoofdstuk 2 beschrijft een experiment waarin de genetische en niet-genetische indirecte effecten op slachtgewicht in de GIFT populatie in kaart zijn gebracht. Om de aanwezigheid van zowel erfelijke als niet-erfelijke indirecte effecten te onderzoeken is een bivariate analyse van slachtgewicht en overleving uitgevoerd, met behulp van een zogenaamd mixed model. Uit deze analyse is gebleken dat direct genetische en maternale effecten verstrengeld zijn, waardoor het niet mogelijk was de volledige set van genetische parameters in één analyse te schatten. Ondanks deze verstrengeling zijn er aanwijzingen gevonden voor IGE op slachtgewicht, in zowel modellen met als zonder maternale effecten. De resultaten suggereren dat de erfelijke interacties competitief zijn in de GIFT populatie, hetgeen blijkt uit de negatieve genetische correlatie tussen directe en indirecte effecten op slachtgewicht (-0.38±0.19). Dit resultaat betekent dat het GIFT fokprogramma aangepast zou moeten worden om te voorkomen dat competitie toeneemt als gevolg van selectie voor groei.

Selectieprogramma's waarin rekening wordt gehouden met IGE verhogen niet alleen de snelheid van de genetische verbetering, maar kunnen andere aspecten van fokprogramma's beïnvloeden, zoals het verlies aan genetische diversiteit. Om dit effect te onderzoeken is een simulatiestudie uitgevoerd waarin de gevolgen van BLUP-selectie voor de inteelttoename in kaart zijn gebracht voor een eigenschap die beïnvloed wordt door IGE (**hoofdstuk 3**). Een scenario zonder IGE en drie scenario's met een toenemende grootte van IGE zijn gesimuleerd met behulp van de R programmeertaal. Een fokprogramma is gesimuleerd voor verschillende erfelijkheidsgraden (0.1, 0.3 of 0.5) en direct-indirect genetische correlaties (-0.8, -0.4, 0, 0.4, of 0.8). De inteelttoenames in de drie scenario's met IGE varieerden van 2.21% tot 5.76% and waren hoger dan in het basis scenario zonder IGE. Hieruit blijkt dat BLUP-selectie voor sociaal-beïnvloede eigenschappen leidt tot een hogere inteelttoename. Om de inteelttoename in fokprogramma's voor sociaal-beïnvloede kenmerken te beperken zijn daarom selectie-algoritmen nodig die de toename van de gemiddelde verwantschap beperken.

Niet alleen de sociale omgeving, maar ook de fysieke omgeving heeft invloed op de productiviteit in de aquacultuur. In de aquacultuur komt een groot scala aan productieomstandigheden voor, hetgeen zou kunnen leiden tot genotype-milieu interactie. In Maleisië, waar het GIFT programma is gevestigd, komen kooi en vijver productiesystemen het meeste voor. Om de genotype-milieu interactie tussen kooi- en vijversystemen in kaart te brengen is slachtgewicht van drie generaties GIFT vissen vergeleken in beide systemen (**hoofdstuk 4**). Een bivariaat model, waarin slachtgewicht in beide systemen als twee verschillende kenmerken wordt behandeld, is gebruikt om de genetische verbanden tussen slachtgewicht in beide systemen te bepalen. De genetische correlatie tussen slachtgewicht en lichaamsmaten gemeten in beide systemen varieerde van 0.73 tot 0.85, met een standaard fout van 0.09 tot 0.13. Deze hoge genetische correlaties geven aan dat genotype-milieu interactie tussen kooi- en vijversystemen van beperkt belang is. Er is daarom geen aanleiding tot het opzetten van aparte fokprogramma's voor kooi- en vijversystemen.

Genetische verschillen in gevoeligheid voor productieomstandigheden kunnen aanleiding geven tot genotype-milieu interactie, zoals beschreven in hoofdstuk 4. Daarnaast kan gevoeligheid voor variatie binnen productiemilieus, d.w.z. variatie in gevoeligheid voor het micromilieu, aanleiding geven tot erfelijke verschillen in variabiliteit tussen individuen. Dit fenomeen staat bekend als "erfelijke variabiliteit" of als "genetische heterogeniteit van variatie". Erfelijke variabiliteit is

in de aquacultuur waarschijnlijk gerelateerd aan sociale interacties, omdat competitie tussen individuen de variatie tussen individuen vergroot. Erfelijke verschillen in variabiliteit kunnen benut worden om dieren te fokken met meer uniforme kenmerken. Dit is wenselijk voor bijvoorbeeld grootte en gewicht in de aquacultuur. In **hoofdstuk 5** zijn de gegevens van het IGE experiment gebruikt om de erfelijke variatie in variabiliteit van slachtgewicht in de GIFT populatie te onderzoeken. Om genetische parameters te schatten voor variabiliteit zijn slachtgewicht en standaard deviatie in slachtgewicht op familie-niveau geanalyseerd in een bivariate analyse. De resultaten laten een genetische coëfficiënt van variatie van 17% zien voor standaard deviatie van slachtgewicht, wat betekent dat uniformiteit van slachtgewicht ten dele erfelijk is. Het is dus mogelijk uniformiteit van slachtgewicht te verbeteren door middel van fokkerij in de GIFT populatie. Er is geen relatie gevonden tussen slachtgewicht en de standaard deviatie in slachtgewicht. Dus beide kenmerken kunnen onafhankelijk van elkaar worden verbeterd, om een uniforme populatie met snelle groei te fokken.

In de algemene discussie in **hoofdstuk 6** worden de mogelijkheden besproken om vissen te fokken die uniform en minder competitief zijn. Hiertoe is het statistisch model van hoofdstuk 5 uitgebreid. In deze analyse werd geen verband gevonden tussen het indirect genetische effect van een dier op het slachtgewicht van zijn sociale partners en de erfelijke aanleg van het dier voor variabiliteit van zijn eigen slachtgewicht (geschatte genetische correlatie van 0.026±0.264). Dit resultaat suggereert dat selectie voor dieren die positieve effecten hebben op slachtgewicht van hun sociale partners niet perse samengaat met een verbetering van uniformiteit van slachtgewicht. In hetzelfde hoofdstuk wordt de relevantie van sociale interacties in het fokprogramma en onder productieomstandigheden bediscussieerd en worden aanbevelingen gedaan voor het opnemen van IGE in verschillende stadia van de aquacultuur fokkerij.

Publications

Peer-reviewed papers

Khaw HL, Ponzoni RW, Danting MJC: **Estimation of genetic change in the GIFT strain of Nile tilapia (*Oreochromis niloticus*) by comparing contemporary progeny produced by males born in 1991 or in 2003.** *Aquaculture* 2008, **275:** 64-69.

Khaw HL, Bovenhuis H, Ponzoni RW, Rezk MA, Charo-Karisa H, Komen H: **Genetic analysis of Nile tilapia (*Oreochromis niloticus*) selection line reared in two input environments.** *Aquaculture* 2009, **294:** 37-42.

Khaw HL, Ponzoni RW, Hamzah A, Abu-Bakar KR, Bijma P: **Genotype by production environment interaction in the GIFT strain of Nile tilapia (*Oreochromis niloticus*).** *Aquaculture* 2012, **326-329:** 53-60.

Khaw HL, Ponzoni RW, Bijma P: **Indirect genetic effects and inbreeding: consequences of BLUP selection for socially affected traits on rate of inbreeding.** *Genet Sel Evol* 2014, **46:** 39.

Khaw HL, Ponzoni RW, Yee HY, Aziz MA, Bijma P: **Genetic and non-genetic indirect effects for harvest weight in the GIFT strain of Nile tilapia (*Oreochromis niloticus*).** *Genet Sel Evol* 2014: *Submitted to journal.*

Khaw HL, Ponzoni RW, Yee HY, Aziz MA, Bijma P: **Genetic variance of uniformity for harvest weight in Nile tilapia (*Oreochromis niloticus*).** *In preparation.*

Conference proceedings

Khaw HL, Ponzoni RW, Hamzah A, Abu-Bakar KR, Kamaruzzaman N, Ismail N, Jaafar H, Nguyen NH: **A comparison of gift and Red Tilapia for fillet yield and sensory attributes of flesh quality assessed by a trained panel.** In *Proceedings of 8th World Congress on Genetics Applied to Livestock Production, 13-18 August 2006;* Belo Horizonte. Brazil; 2006: article number 09-05.

Khaw HL, Ponzoni RW, Hamzah A, Kamaruzzaman H: **Genotype by environmental interaction for live weight between two production environments in the GIFT**

strain (Nile tilapia, *Oreochromis niloticus*). In *Proc. Assoc. Advmt. Anim. Breed. Genet*, 28 September - 01 October 2009; Barossa Valley. Australia; 2009: 60-63

Khaw HL, Ponzoni RW, Hamzah A, Nguyen NH, Abu-Bakar KR, Yee HY: **Genetic analysis of the GIFT strain (Nile tilapia, *Oreochromis niloticus*) in Malaysia.** In *Proceedings of 9th World Congress on Genetics Applied to Livestock Production, 1-6 August 2010;* Leipzig. Germany; 2010: article number 0920. (ISBN 978-3-00-031608-1).

Khaw HL, Ponzoni RW, Bijma P: **The effect of BLUP selection for socially affected traits on the rate of inbreeding: A simulation study.** In *Proceedings of Annual Meeting of the European Association for Animal Production (EAAP);* Slovakia; 2012.

Khaw HL, Ponzoni RW, Yee HY, MA Aziz, Bijma P: **Indirect genetic effects for harvest weight in Nile tilapia (*Oreochromis niloticus*).** In *Proceedings of 10th World Congress on Genetics Applied to Livestock Production, 17-22 August 2014;* Vancouver. Canada; 2014: article number 773.

About the author

About the author

On 14th November 1980, Hooi Ling was born in Penang, Malaysia, as the second child of the Khaw family. Upon finishing her post-secondary education, Hooi Ling decided to pursue her bachelor degree in Fisheries Sciences at University Malaysia Terengganu (formerly known as Malaysia Science and Technology University College). In 2004, Hooi Ling successfully obtained her bachelor degree and started her career as research assistant in the Fish Breeding and Genetics Group of WorldFish (formerly known as The WorldFish Center) in Penang. WorldFish is the place where Hooi Ling gained her experience in fish breeding and developed her interests to have a more in depth knowledge in quantitative genetics. After three years with WorldFish, Hooi Ling received the opportunity to participate in the European Master in Animal Breeding and Genetics (EMABG) with the scholarship granted by Erasmus Mundus. Hooi Ling completed two theses during her Master of Science study. The first thesis was on investigating genotype by environment interaction for Nile tilapia reared in low and high input environments. For the second thesis, Hooi Ling studied the adaptation of fish to their environment by investigating the link between phenotypic plasticity and classical welfare measurements. After two years of study, Hooi Ling graduated with double master degree awarded by Wageningen University (The Netherlands) and AgroParisTech (France) in 2009. Upon finishing of EMABG, Hooi Ling resumed her work at WorldFish as research analyst. At the same time, Hooi Ling was offered a sandwich PhD position by Wageningen University to study social interactions in fish using the GIFT strain (Genetically Improved Farmed Tilapia; Nile tilapia) in Malaysia. This was a collaboration project between Wageningen University and WorldFish funded by Technology Foundation STW of Netherlands Organisation for Scientific Research (NWO) and European Union. This thesis resulted from Hooi Ling's study over the past five years. Hooi Ling is continuing her career with WorldFish as research scientist to further pursue her wish of helping the poor through selective breeding and the creation of improved aquaculture species.

"Before anything else, preparation is the key to success" – Alexander Graham Bell

Training and education

Training and education

The Basic Package (3.0 ECTS)	**Year**
WIAS Introduction Course	2012
Ethics and Philosophy in Life Sciences	2012
Scientific Exposure (12.5 ECTS)	
International conferences	
9th World Congress on Genetics Applied to Livestock Production, Leipzig, Germany	2010
Aquaculture America 2012, Las Vegas, United State of America	2012
63rd EAAP Annual Meeting, Bratislava, Slovakia	2012
10th World Congress on Genetics Applied to Livestock Production, Vancouver, Canada	2014
Seminars and workshops	
Seminar for Tilapia Volta Project GCP/FAR/417/SPA, Ghana	2011
The WorldFish Center Science Week, Penang, Malaysia	2011
Presentations	
Oral - 9th WCGALP, Leipzig, Germany	2010
Oral - The WorldFish Center Science Week, Penang, Malaysia	2011
Oral - Aquaculture America 2012, Las Vegas, USA	2012
Oral - 63rd EAAP Annual Meeting, Bratislava, Slovakia	2012
Poster – 10th WCGALP, Vancouver, Canada	2014
In-Depth Studies (8.0 ECTS)	
Disciplinary and interdisciplinary courses	
Armidale Animal Breeding Summer Course, UNE, Australia	2005
Armidale Animal Breeding Summer Course, UNE, Australia	2007
Social Genetic Effect: Theory and Genetic Analysis, Wageningen, The Netherlands	2013

Advanced statistics courses	
Statistical Learning Methods for DNA-based Prediction of Complex Traits, Wageningen, The Netherlands	2011
PhD students' discussion groups	
Quantitative Genetics Discussion Group	2010-2013

Professional Skills Support Courses (3.0 ECTS)

Communication, Confidence and Creativity, Penang, Malaysia	2011
In-house Leadership Course, Penang, Malaysia	2012
In-house Team Building Course, Penang, Malaysia and Cairo, Egypt	2013
The Art of Writing Publishable Scientific Manuscript, Penang, Malaysia	2013

Research Skills Training (5.5 ECTS)

Preparing own PhD research proposal	2010
Special research assignments (apart from PhD project) – ongoing breeding programs for partner countries within WorldFish	2010-2014
Statistical Analysis Using R, Penang, Malaysia	2013

Didactic Skills Training (4.0 ECTS)

Lecturing	
Training Course on Quantitative Genetics Applied to Fish Improvement, Penang, Malaysia	2010
Training Course on Quantitative Genetics Applied to Fish Improvement, Penang, Malaysia	2011
Supervisor theses	
PhD thesis (co-supervise): Azhar Hamzah, University of Science Malaysia, Penang, Malaysia	2010-2013
PhD thesis (co-supervise): Olga Assémien - Université Félix Houphouët-Boigny, Côte d'Ivoire,	2010-2014

Education and Training Total	**36 ECTS**

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"Being happy is the priority of living"

Colophon

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